



'ગુજરાતનો નાથ' નવલકથામાં પાત્ર નિરૂપણ કલા

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કનૈયાલાલ માણેકલાલ મુનશી ગાંધીયુગના પ્રભાવક સર્જક તરીકે ગુજરાતી સાહિત્યમાં ઓળખાયા છે. અન્ય ગુજરાતી સાહિત્યકારોની અસર છે એવી જ રીતે જ ક.મા. મુનશીની ગુજરાતી સાહિત્ય પર અસર જોવા મળે છે. એમાં સાહિત્ય- સર્જન અંગ્રેજી પ્રશ્નિકરણ સાહિત્યના વિદ્વાન નવલકથાકાર ડુમાની અસર ક.મા.મુનશી પર વધુ અસરકારક બને છે. એના પ્રભાવ અસર નીચે નાટક, નવલકથા, નવલિકા, આત્મકથા, નિબંધો, વિવેચનો, વ્યાખ્યાનો, ગદ્ય કાવ્યો, પત્રકારત્વના સામાયિકો વગેરે સાહિત્ય સ્વરૂપો સમૃદ્ધ રીતે ખેડ્યા છે. અને સાહિત્ય કૃતિઓ દરેક સ્વરૂપ- પ્રકારોમાં સમૃદ્ધિ વધારી આપે છે. ગુજરાતી સાહિત્યમાં સર્જકે બહુમુખી વ્યક્તિત્વ સર્જકત્વમાં ઘણી ઊંડી અને અસરકારક છાપ પાડ્યા વગર રહ્યા નથી. ગુજરાતના ગદ્ય સર્જકોમાં ક.મા.મુનશીનું સ્થાન અનેક અને બહુ મૂલ્યવાન મોટા પ્રમાણમાં રહેલું જોવાં મળે છે.

ગુજરાતી સાહિત્યના ઐતિહાસિક અને સાહિત્ય ક્ષેત્રે સૌ પ્રથમ ઈ.સ. ૧૮૬૬માં કરણચેલો પ્રગટ રહી, તેનો પ્રભાવ ક.મા.મુનશી, ગોવર્ધનરામ, ર.વ.દેસાઈ, ધૂમકેતુ, પન્નાલાલ પટેલ, ઈશ્વર પેટલીકર વગેરે સર્જકોએ ઝીલ્યો છે. પરંતુ ક.મા.મુનશી જેવાં સર્જક ગુજરાતી ગદ્ય સાહિત્યમાં મોખરે રહ્યા છે.

ક.મા.મુનશીએ ગોવર્ધનરામ ત્રિપાઠીની જેમ રાજકીય સામાજિક - સાંસ્કૃતિક જીવનને આલેખવા ઐતિહાસિક દ્રષ્ટિ નાખીને સોલંકીયુગનું સામ્રાજ્યને પોતાની કલા વિશેની નૂતન દ્રષ્ટિ અને કલ્પના પદ્ધતિનાં સહારે ગુજરાતી ઐતિહાસિક નવલકથાઓ વિશે સર્જન કર્યું છે. જેવી કે, 'પાટણની પ્રભુતા' (૧૯૧૬), 'ગુજરાતનો નાથ' (૧૯૧૭), 'પૃથ્વીવલ્લભ' (૧૯૨૧), 'રાજધિરાજ' (૧૯૧૮), 'જય સોમનાથ' (૧૯૪૦) વગેરે ઐતિહાસિક નવલકથામાં સોલંકીકાળનું ગુજરાતનું જીવન આલેખાયું છે. એમાં માનવતા રંગોથી પાત્રોમાં અદ્ભૂત વિવિધતા દર્શાવેલી જોવા મળે છે.

'ગુજરાતનો નાથ' નવલકથામાં ઐતિહાસિક પ્રસંગો ઘટના- પરિવેશના અનુસંધાને કેટલીક કલ્પનાઓ લઈને કૃતિ રચી છે. ક.મા.મુનશીએ નવલકથામાં કલ્પનાનો છૂટદોર વધુ લીધો છે એ બાબત નોંધનીય બને છે. એની સાથે સાથે ઐતિહાસિક પાત્રો જેવા કે સ્ત્રી પાત્રો મુખ્ય હોય કે ગૌણ, એને કેન્દ્રમાં રાખી ગુજરાતનું વાતાવરણ સર્જકે ભૂત-ભવિષ્ય-વર્તમાનકાળને ધ્યાનમાં રાખીને કૃતિ રચી છે.

'ગુજરાતનો નાથ' નવલકથામાં સ્ત્રી-પાત્રો, પુરુષ-પાત્રો, ગૌણ પાત્રોનું કળા દર્શન અનેક બની રહે છે. એ અંગની જેટલી વધુ સમૃદ્ધિ એટલી જ નવલકથાની વધુ સિદ્ધિ કારણ કે આખરે તો પાત્રો ન હોય તો સર્જક કથા રચી જ ન શકે. પાત્રો હોય તો કથા સહિત્ય રચાય છે. તેથી જ સર્જકનો સંબંધ પાત્રો સાથે 'પ્રાણવાયુ' બની રહે છે. એ બાબત પણ નવલકથા માટે ધ્યાનાર્કર્ષક બની રહે છે. પાત્રો ન હોય તો કથા- કૃતિ કલા બની શકતી નથી. પાત્રાલેખનની સમર્થ કલા જ નવલકથાને જીવંતરૂપ બનાવી મુકે છે. ક.મા.મુનશીએ 'ગુજરાતનો નાથ' નવલકથા સર્જકને પાત્રકલાનું શુદ્ધ સ્વરૂપ દર્શન ભાવકોને કરાવ્યું છે. એમાં પુરુષ- પાત્રો મુંજલ, કાક, કીર્તિદેવ, ખેંગાર, પ્રતાપી નરપુંગવો, ઉદો, ત્રિભુવનપાલ,

સોમ, કાશ્મીર દેવી વગેરે છે. તેમજ આ સ્ત્રી પાત્રોએ ક.મા.મુનશીની કલ્પના વિહાર શક્તિને વધુ બળ આપી આત્મસિધ્ધી વધારી છે. અને બીજી રીતે પુરુષ પાત્રો, સ્ત્રી પાત્રો કરતાં થોડાં ઓછાં ચડિયાતાં દર્શાવ્યાં છે. જ્યારે સ્ત્રી-પાત્રોને વધુ ગુણવાન, વિવેકી, નમ્રતાપાણું, તેમજ પ્રતાપી, ચતુરાઈ ભરી બુદ્ધિશાળી તરીકેની સક્ષમ બતાવી છે. એ પણ સત્ય છે કે, પુરુષો, મહર્ષિઓ, યોધાઓ, મુન્સદીઓ અને પ્રતાપી રાજપુરુષત્વનું વ્યક્તિપાણું ધરાવતા સમાજમાં પોતાનો મોભો જળવાય રહે એ રીતે પોતાની આબરૂં સાચવવાનું વ્યક્તિત્વ ધરાવતા પુરુષ પાત્રો સર્જકે ચીતર્યાં છે. એ બાબત પણ મહત્વની ગણાય છે.

ક.મા.મુનશીએ સૌથી વધુ સ્ત્રી - પાત્રોને 'ગુજરાતીનો નાથ' નવલકથામાં ધ્યાનાકર્ષણ ચિત્ર દર્શાવી આપ્યું છે. સમાજને હંફાવતી પુરુષ પ્રધાન સત્તા હોવા છતાં સ્ત્રીઓનું ચરિત્ર ઉપર આંચ આવવા દીધી નથી. તેથી ક.મા.મુનશીએ સ્ત્રી-પાત્રો દ્વારા ચરિત્ર-ચિત્રણમાં વૈવિધ્યતા બતાવી આપીને સ્ત્રીનું આદર્શપાણું જગત આગળ આલેખી આપ્યું છે. એવી અનુભૂતિનો અહેસાસ ભાવક સમક્ષ મૂકી આપ્યો છે.

ક.મા.મુનશીનાં જીવનમાં જે સ્ત્રી સ્વપ્નદર્શી બનીને આવી પરંતુ એમને તો બાલ્યાવસ્થામાં જે સ્ત્રી-પરીનું સ્વપ્ન હંમેશા માટે સ્વપ્ન બની રહ્યું તેની કલ્પના સૃષ્ટિથી નારીત્વ ભાવના વધુ સમજી હોય એમ લાગ્યા વગર રહી નથી. એમણે પોતાના આત્મચરિત્રમાં આ બાબત અંગે સ્પષ્ટતા પણ કરી છે જે બાબત સાચી નીવડે છે. અતિલક્ષ્મી પત્ની બનીને આવી પરંતુ એના પ્રત્યેય પ્રેમની અનુભૂતિ પણ મોડી જાગી. જ્યારે લીલાવતી શેઠે એમના જીવનમાં પ્રવેશતા જ પ્રેમની અનુભૂતિ-સંવેદનાની જ્યોતિ જગાવી બેઠી. ત્યારે પોતે કબુલ કરે છે કે જે બાળપણમાં જે બાળા જોઈ તેની કલ્પના વિહારમાં ડૂબી ગયેલા અને અતિલક્ષ્મી મૃત્યુ પામી, ત્યાર બાદ લીલાવતી શેઠ એટલે કે પત્ની અને પ્રેમિકા વચ્ચે ક.મા.મુનશીનું મનોસંઘર્ષ થતું જોવાં મળે છે. અને પત્ની પ્રત્યે પોતાની લાગણી પ્રત્યેયનો અક્સોસ થયો એ નોંધનીય ઘટે છે.

'ગુજરાતનો નાથ' નવલકથામાં પાત્રોની અદ્ભુતતા વિવિધતાભરી વિશાળ પટની હારમાળા જેવી લાગી છે. સર્જકે પોતાના મનોમંથન કલ્પના દ્વારા દરેક પાત્રોની ચારિત્ર્ય પણ સુંદર શૈલીમાં નિરૂપી આપ્યું છે. એમની આ નવલકથામાં પણ પ્રેમના તત્વનાં બીજા કૂટાડયા ન હોય એમ સર્જક ચિત્રે છે પરંતુ આ રચનામાં પ્રેમનું તત્વ તેમજ એકબીજાના સંબંધો સહજીવન ચર્ચા સાથે એકબીજા પ્રત્યે સંવેદનશીલ બની રહ્યા હોય એવી વૃત્તિ ભાવકને રહ્યા વગર જોવા મળી નથી. અને પ્રેમના મિલન તથા વિપ્રલભ એવા બે સ્વરૂપ રીતે લેખકે આલેખ્યા છે. એમાં તો વળી, 'ગુજરાતનો નાથ' માં જરા પણ વિયોગપાણું બતાવ્યું નથી. જીવનની અન્ય રસ્તે જે પોતાના હૈયાને આઘાત પંડોચે એવો વિરહ જરા પણ દર્શાવ્યો નથી. એમનો વિરહ તો ઊંડો અને મર્મસ્પર્શી છે. એમાં મીનળ અને મુંજલનો પ્રેમ, કાક અને મંજરીનો પ્રેમ, સોમસુંદરી જેવા પાત્રોનું હૈયાની હોડમાં પુરાયેલો રહે છે, તેવી ઊંડી છાપ ભાવકો ઉપર પડી છે. એક રીતે જોઈએ તો પ્રેમને શુદ્ધ બતાવ્યો નથી વિકૃતિ બતાવી છે. છતાં પણ સમગ્ર ક્ષેત્રે એટલે કે સામાજિક-સાંસ્કૃતિક રાજકીય રીતે દરેક પાત્રો પસાર થઈને લાગણીભેર યર્થાથને ચીતર્યાં છે. ખરેખર જે સ્ત્રી પ્રત્યેની અભિલાષા પડી હોય પુરુષમાં એ અભિલાષા પોતાની અપેક્ષા જ પ્રતિબિંબિત થઈ હોવાનું વરતાયું હોય એમ લાગે છે.

'ગુજરાતનો નાથ' માં મુનશી કેવળ કથાકાર બની રહ્યા નથી પરંતુ અહીં ચિત્રણ પણ એવું પ્રયોજે છે. જે નવલકથામાંથી ઊંચી અપેક્ષા રાખનાર ભાવકોને સારી રીતે સંતોષ આપ્યો છે. મુનશીએ પોતાની આગવી શૈલીથી આરંભાય ગોવર્ધનરામ ત્રિપાઠીની વિશાળ નવલકથા કરતાં પણ ઐતિહાસિક નવલકથામાં સાહિત્ય રત્નકારીનું ચિત્ર ઉપસાવી આપ્યું છે. અને સફળતાના વિશિષ્ટ ગુણો 'ગુજરાતનો નાથ' નવલકથાનાં સમગ્ર પાત્રોસૃષ્ટિમાં કળા દર્શન કરાવ્યા છે. એમાં ક.મા.મુનશીએ રાજકીય તરંગોનાં બીજા વાવીને સામાજિક દૃષ્ટિએ પ્રેમભાવના વિકસાવી પાત્રો દ્વારા સચોટ શબ્દો

સૃષ્ટિથી ચિત્રણ કર્યા છે. ઈ.સ.૧૯૧૬માં 'પાટણની પ્રભુતા' રચીને આગળ વધીને હજી 'ગુજરાતનો નાથ' નવલકથા એક ઐતિહાસિક રૂપે પૂરવાર કરી પાત્રોની કળા દર્શનથી વાચકોને આતુરતાની દ્રષ્ટીએ ભરી દે છે. કે જે વાચકોને પાત્રોના સંવાદો દ્વારા પળ- પળની વૈવિધ્ય સાથે જકડાય રહેવાની ઈચ્છા થાય છે. અને સર્જકતાનો સર્વોચ્ચ ઉન્મેષ પ્રગટ થયેલા જોવા મળે છે. એટલે જ નરસિંહરાવ જેવાં વિવેચકોએ ક.મા.મુનશીની નવલકથા સર્જન કૃતિ 'ગુજરાતનો નાથ'માં પાત્રોની ઘણા પ્રમાણમાં પ્રશંસા કરી છે ને કહે છે કે ગોવર્ધનરામ કરતાં ક.મા.મુનશી 'સવાયા' છે. છતાં ગોવર્ધનરામનો પ્રભાવ ક.મા.મુનશીમાં જોવા મળ્યો છે એ સ્વાભાવિક છે.

'ગુજરાતનો નાથ' નવલકથા પાટણ એટલે કે સોલંકીયુગમાં અણહિલનું પાટણ તરીકે ઓળખાતું છતાં સર્જક પાટણ વિશેની રજૂઆતનો ઉલ્લેખ જોવા મળ્યો નથી. એ નોંધનીય બાબત બને છે. પાટણનાં સિદ્ધરાજ જયસિંહનાં શાસનકાળનાં ગુજરાતની યશગાથા વર્ણવી છે. મુનશીએ કરણદેવના અવસાન દરમ્યાન સિદ્ધરાજ જયસિંહ નાનો હતો. તેથી રાજમાતા તરીકે મીનળદેવીની નિમણુંક કરવામાં આવી. અને એની એ સત્તા ચલાવી હતી. છતાં, ખરી સત્તા મુંજલ જ ચલાવે છે તે ક.મા.મુનશી કલામયતાથી કલ્પનામય બનીને એ સમયને, આપણા વાચક સમક્ષ પ્રત્યક્ષ રીતે નિહાળવો જોઈએ એવો મૂર્તિમંત કરવામાં આવે છે.

ક.મા.મુનશીએ સોલંકીયુગના પ્રસંગો, વસ્તુકથન ઘટના બાબતે ઇતિહાસના દાયરામાં નાખી કલામય રીતે કલ્પનવૃત્તિ દાખવીને પોતાની સૂઝ પ્રમાણે પાત્રો દ્વારા ફેરફાર કરીને કેટલીક મહત્વની ઐતિહાસિક ઘટનાને સ્વીકારીને વર્ણન કર્યું છે. મુંજલ અને મંજરીનું પાત્રસૃષ્ટિની છબી લોકહૃદયમાં અંકિત થયેલી જોવા મળે છે. તેનાથી એવો ભ્રમ થયો છે. ઐતિહાસિક ઘટનાઓનાં મહત્વના ચાલકબળ તરીકે નિરૂપીને સર્જકે ઐતિહાસિક વિકૃતિ આણી એ વાત પણ ચોક્કસ છે.

ગુજરાતી અસ્મિતાના નિયામક અને અપૂર્વતાના ઉપાસક મુનશીએ પાત્રોને સજીવ સબળ પ્રમાણમાં શક્તિશાળી નરપુંગવો અને તેજસ્વી સ્ત્રીઓની સૃષ્ટિ સર્જી છે. તેથી 'ગુજરાતનો નાથ' એક પ્રભાવશાળી પાત્ર વિધાનની 'દર્શન કળા' તરીકે વિશેષ નોંધ લેવાય છે.

આ નવલકથાના પાત્રો ગુજરાતી સાહિત્યમાં ચિરંજીવ બની રહ્યા છે. સજીવતાની સબળ છાપ મૂકી જતાં આ પાત્રો પોતાની બુદ્ધિમત્તાથી અને વાણી-વર્તનથી આપણને આંજી નાંખે છે. અને મુંજલનું પાત્ર પાટણની સત્તા માટે ખુમારી ભર્યું છે. એ સમયની મુત્સદ્દીગીરીનું સચોટ પરિચય કરાવતું જોવાં મળ્યું છે. મુંજલ અને મીનળ વચ્ચે સત્તા માટે તથા પ્રજા સાથે પણ વધુ સંઘર્ષ કર્યાં તે છતાં મુંજલનું વ્યક્તિત્વ આગવું તરી આવે છે. એકલતા, કરુણા અને ભવ્ય રાજકીય જીવનમાં અનેકવિધ મહત્તા સાધનાર, અતૂટ રહેનાર મુંજલનાં જીવનમાં નિર્જનતા પ્રસરે છે. મુંજલ અને મીનળના આંતર વ્યક્તિત્વમાં રાજ્યનો કારભાર હૃદયસ્પર્શી બને રહ્યો છે. મુંજલ પ્રતાપી હોવાં છતાં પણ જ્યારે સોમ મૃત્યુ પામે છે ત્યારે તેની આંખમાંથી આંસુ સારે છે. ત્યાં મુંજલનું હૃદય સંવેદનાભર્યું પણ રહ્યું છે. પોતાના દીકરાના વિરહમાં સહિષ્ણુતા બતાવે છે. મુંજલનું ગૌરવ પ્રતાપી, સત્તા, હીર, જેવાં ગુણોથી ભરેલું છે. છતાં એની એકલતા કોઈપણ વ્યક્તિત્વ હૃદયને હલાવી નાંખે એવી છે. પણ પાટણનાં ગૌરવ માટે તે પોતાનું આખું જીવન ત્યજી દે છે. અને પોતાના શૌર્યને વિકસાવી તેમજ ધીરજ પૂર્વક પોતાની ચતુરાઈથી જયદેવને ચક્રવર્તી થવા માટેની તાલીમ આપે છે. મુંજલની સુટેવ છે કે 'મોહક હાસ્યથી બીજા વ્યક્તિઓનું દિલ તરત જ જીતી લેતું' અને કોઈ અન્ય વ્યક્તિનાં સ્વભાવ, મનને પારખી લેવો. એને કોઈ બીજા માર્ગ તરફ દોરી જાય એવી કોઈનામાં હિમત ન હતી. અખિલ આર્યાવર્તને એકઠા કરી, યુવાનોને હંફાવવાના કાર્યમાં સામેલ થવા નમ્ર કરતો પણ મુંજલને તો પાટણની ચિંતા વધુ હતી. જે પાટણની સત્તા સાચવવાને જ મહત્વ આપ્યું. અને પાટણનો મધ્યાહન સોળે કળાએ ખીલે એ જ એનો પરમ ધર્મનો આદર્શ છે. આદર્શ

અને પોતાનું સ્વમાન જાળવવાં કદી પણ ભોગ આપવાં અચકાવો નથી. એનામાં શારીરિક બળ અને યુદ્ધ કૌશલ્ય ઘણાં છે. એનામાં શૌર્ય તેમજ વિવેક પણ છે.

આમ, મુંજલનું પાત્ર મહામાનવ સમાન હતું છતાં ક.મા.મુનશીએ આદર્શ માનવી તરીકે 'ગુજરાતનો નાથ' માં ન ધબકતું રાખ્યું. તેથી ક.મા.મુનશીએ પાત્રસૃષ્ટિને ક્ષમતાહીન બનાવી એ હકીકત નોંધનીય બની રહે છે. મુંજલનાં પાત્ર દ્વારા ગુજરાતમાં જે પરિસ્થિતિ સર્જાય એની સામે યુવાનો ખડા પગે રહે એવો સંદેશો આપ્યો છે. અને એની મહત્તા ક.મા.મુનશીએ સાબિત કરી આપી છે. સર્જક તરીકેની ભવ્યતા મહોરી ઊઠે છે.

મીનજનાં પાત્રને સ્ત્રી તરીકેનો દરજ્જો અને રાજકર્તા તરીકેની અપાર ભૂમિકા 'ગુજરાતનો નાથ' માં સોળે કળાએ ખીલી ઊઠે છે. પોતાના લગ્નજીવનને કલંકિત ન બનાવીને પ્રેમ -સંબંધો પ્રત્યે પોતાના લગ્નના સંબંધોમાં બલિદાન આપી એક સ્ત્રીત્વનું માન રાખી આદર્શ સ્ત્રી તરીકે પ્રગટ થાય છે. બંને એકબીજાને પ્રૌઢવયે પણ ચાહે છે એ પ્રેમને અશુદ્ધ બનતો અટકાવવાની બંને જણાની પ્રામાણિકતા બતાવી. કુદરતી છે એટલે પવિત્ર છે, એમ માનીને ધર્મશાસ્ત્ર અને રૂઢિની રીતે મિનજ મુંજલ જેવા જ અપવિત્ર સ્નેહનું નવલકથામાં મુખ્ય અંગ તરીકેના પાત્રો રૂપે ગુજરાતી સાહિત્યમાં વિશાળતા પામ્યા છે.

આ નવલકથા બીજા સબળ પાત્રો કાક અને મંજરી છે. કાક યોધ્યા તરીકે અને ત્રિભુવનપાળનો માનીતો વડાદાર સેવક રાજનીતિના દાવપેચ ખેલવામાં કુશળ છે. કાકનો સ્વભાવ પરાક્રમી અને બ્રાહ્મણ હોવાથી પૌરાણિક પ્રાણલિકામાં ઉછરેલો છે. તેથી યુસ્ત શ્રાવકો તરફ તિરસ્કારની નજરે જોવાવાળો ક.મા.મુનશીએ પાત્રનું ચીત્રણ કરેલ છે. છતાં કાકમાં જેટલું સાહસ અને શૌર્ય બતાવ્યું છે. તેટલી ધર્મની ઉચિત ધર્મવૃત્તિ કે આર્યપણું નથી. એ બુદ્ધિવંત છે, પ્રેમી છે, તેજસ્વી છે, પરાક્રમી છે, બળવાન યોધ્ધો, પરિસ્થિતિ ને કાબૂમાં રાખી પ્રભુત્વ જમાવી દીર્ઘ દ્રષ્ટિથી ધાર્યો વળાંક આપી શકે છે. 'કાક' 'ગુજરાતનો નાથ' નું શ્રેષ્ઠ પાત્ર અને નાટક બને છે. એજ રીતે મંજરી પણ તેજસ્વી, સંસ્કારી, પ્રતાપી છે. તેમની પાત્રલેખનની કળા સિદ્ધિ છે. મંજરી આત્મસૌંદર્ય, આત્મબળ, અને આત્મશ્રદ્ધા ધરાવતી છતાં તેનું સર્વસ્વ નથી. મંજરી કાક ઉપર વિશ્વાસ મૂકીને તેની સાથે લગ્ન કરવા તૈયાર થઈ જાય છે. કારણ કે, મંજરી ઉદાથી બચવા માટે કાકને ન છૂટકે પરણ્યા સિવાય ના રહેવાય, એવા સંજોગોમાં તે પરણે છે. ત્યારે કાક કહે છે:

"કાક-મહારાજ, દેવડી કોની થવા માંગે છે:"

ઉપરોક્ત વિધાન પ્રમાણે કાક પોતાના જીવનનું જોખમ સમજતો નથી. અને કાક ફરી મંજરી વિષે કહે છે: "મંજરી! ખરો નર તો મે એક એ જોયો છે. પ્રભુએ પૂરો બત્રીશ લક્ષણો ઘડ્યો છે." ત્યારે આ બધા કાકને તાગી પામી રહેલી મંજરીનું તરત પ્રમાણપત્ર મળે છે: "અત્યારે એનામાં બત્રીશ લક્ષણ હોય તો એના આ મિત્રમાં બાવન લક્ષણ છે."

કાકમાં બ્રાહ્મણત્વના ગુણો, સંસ્કારોનો પ્રભાવથી અસર પડી છે. એટલે કાકમાં ચારિત્ર્યવાનની મધુરતા દીપી ઊઠે છે. એ સર્વોપરી તરીકે અલગ તરી આવે છે. 'ગુજરાતનો નાથ'માં જે નવજીવનના વિશિષ્ટ ગુણોનું સૌંદર્ય છે તે વાચકો સુધી આસાનીથી અસર પડે છે. કદાચ નવલમાં એમાં પાત્રથી બ્રાહ્મણત્વ જ ન હોત તો ક.મા.મુનશીની સર્જકતામાં ખોટ પડી હોત એમ માની શકાય છે. નવલકથામાં ઘણા પ્રસંગો સાથે સંકળાયને ભાગ ભજવતા આ પાત્ર સિવાય કથાનું ચાલી ન શકે એવી પ્રદાનતા મુનશીએ આપી છે. અને રા.વિ.પાઠક જેવા વિવેચક 'કાક' ને શ્રેષ્ઠ તરીકેનું પાત્ર ગણ્યું છે. તેઓ કહે

છે: 'એ જાણે પરાક્રમો કરવાને જ સર્જાયો છે. આખા ગુજરાતમાં ફરી બળનારો, ગમે ત્યાં કૂટી નીકળનારો, ગમે તેવા ફાંસલામાંથી છટકી જનારો, અનેકની યોજનાઓ ખોટી પાડનારો, અભાણ છતાં વિઘ્નાપ્રિય મંજરીને જીતનારો, બધા પાત્રોમાં એ સૌથી વધારે જીવંત છે.' મિનળ મુંજલના છાયામાંથી મલત્તા જ બતાવવાં માંગે છે. એની મલત્તા મુંજલમાંથી જ આવેલી છે. કાક બહાદુર, વફાદાર સૈનિક તરીકે પણ સુંદર કામ બજાવે છે. કિર્તિદેવ એક ઉમદા પાત્ર છે. પણ તે મોખરે આવી શકે એવું છે છતાં એવું પાત્ર દર્શાવણું નથી. પરંતુ મંજરી અને કાકના પાત્રને પ્રવૃત્તિશીલ ચીત્રણો છે. ખરેખર, કાકને પોતાનું સર્વસ્વ અર્પણ કરતી પ્રેમિકા અને નાયિકા તરીકે લાયક છે.

આમ, આ નવલકથામાં કાક અને મંજરી કથાના ચિરંજીવી પાત્રો વર્ણવ્યા છે. મુનશીનું ઉત્તમ પાત્ર અને ઉત્તમ કાર્ય કરનાર કાક છે. કાકના ચારિત્ર્ય અને બુદ્ધિની ઉત્કૃષ્ટતા એણે ઘડેલી રાજકીય યોજનામાં નજરે પડે છે. તેથી કાકનું તેજસ્વી પાત્રનું અદ્ભુત આલેખન મુનશીએ કર્યું છે.

મુનશીએ પુરુષ પાત્રો કરતાં સ્ત્રી-પાત્રો વધુ તેજસ્વી, પ્રતાપી ચતુરાઈ ભર્યા પાત્રો તરીકે નિરૂપણ કર્યા છે અને તે તેમની પાત્રા લેખનની કળા સિધ્ધિ છે. તેમના સ્ત્રી- પાત્રો બળવાન અને સશક્તિકરણ ધરાવતા હોય એમ મુનશીએ ગર્વભરે રીતે વર્ણન કર્યું છે. અને એ જ રીતે આપણે મંજરીના પાત્રને જોઈએ છીએ. કાકને વધુ બુદ્ધિશાળી હોય એવી રીતે લેખક નિરૂપ્યું છે. મંજરીએ જેવાં કલ્પેલાં એવાં પતિ તરીકે પથરાયાં નથી. તેથી મંજરીની નિખાલસતા, નિષ્ઠાવાન જેવાં ગુણો પણ ઉપસી આવે છે.

આમ, 'ગુજરાતનો નાથ'માં મિનળદેવીના પાત્રને તેમજ મંજરીના પાત્રને ક.મા.મુનશીએ સર્વશ્રેષ્ઠ તરીકેની સ્ત્રીત્વની ભાવના બતાવી છે. તથા મુંજલ, કાક પુરુષ- પાત્રોને પરાક્રમી, શૌર્યશીલ પાત્રો ક.મા.મુનશીએ આલેખ્યા. અને ગૌણપાત્રો પણ એટલા જ મહત્વના ભાગ ભજવેલ છે.

ક.મા.મુનશીએ ગુજરાતી સાહિત્યમાં પ્રેમની વિભાવના, ઝળહળતી રોશની ફેલાવતી અસ્મિતા, તત્કાલીન-સમકાલીન સમયની જ્યોતિ બદલાતા સમાજનું સામાજિક- સાંસ્કૃતિક, રાજકીય દર્શન આ પાત્રો દ્વારા કરાવ્યું છે. જે મુનશીએ પોતાના સર્જક વ્યક્તિત્વના આધારે સાહિત્ય –સર્જન નદીના પ્રવાહની જેમ વહેતું મૂક્યું છે. એથી મુનશીએ રંગદર્શી, રસદર્શી, સર્જક કહેવાયા. એ જ એમની લોકપ્રિયતા ગણી છે.

ક.મા.મુનશીએ સમાજને ધ્યાનમાં રાખી મનોવૈજ્ઞાનિકની દ્રષ્ટિ અપનાવી વ્યક્તિત્વ શારીરિક – માનસિક, સુખ દુઃખના પાસા, હાથમાં – નિરાશા જેવી અનેક પ્રકારની સમસ્યાને પોતાની દ્રષ્ટિમાં, જીવનમાં ઉતારીને એની ક્ષમતા કક્ષામાંથી ઉદ્ભવતા જ પ્રશ્નો સમસ્યાને સમાજમાં સામાજિક રીતે અસ્તિત્વને ટકાવી રાખવા કદાચ સાહિત્ય સર્જન શાબ્દિક અંશોથી રચ્યું હોય એમ માની શકાય છે. તેથી જ ક.મા.મુનશીએ પોતાની કલમ ઉપાડીને ગુજરાતની ગૌરવગાથા સાહિત્ય દ્વારા વધારી હશે.

ગુજરાતી સાહિત્યમાં એક વિરલ પ્રતિભાશાળી ધરાવતા ક.મા.મુનશીનો પ્રવેશ થતાં ગુજરાતની અસ્મિતા ઐતિહાસિક નવલકથાઓને આધારે વધારી એમ જણાય આવે છે. એમાં એ પોતે ઈતિહાસકાર અને નવલકથાકાર હોવાના સબંધો

જાળવી રાખીને નવલકથાઓ રચી હશે. ગુજરાતના સમયને, ગુજરાતની પ્રજાને જોઈ ગુજરાતની પરિસ્થિતિ નિહાળી, ગુજરાતનું પ્રતિબિંબ પ્રગટ કર્યું છે. એવી નવલકથાઓમાંથી ઐતિહાસિક નવલકથા 'ગુજરાતનો નાથ' રચના દ્વારા સ્ત્રી-પાત્રોના સંબંધોનું સહજીવન કેવું ટકી રહ્યું, એનું દર્શન આપણને એટલે કે ભાવકોને એ સાહિત્ય રચના દ્વારા દર્શાવ્યું છે. આમ, ક.મા.મુનશીએ પ્રાચીન સંસ્કૃતિથી માંડીને આજ સુધીની સંસ્કૃતિને સાહિત્યના પદ્ધતિમાં લઈને સાહિત્ય કૃતિઓનો સમન્વય સાંધીને ગુજરાતી સાહિત્ય ગદ્યમાં કંડારી સુવિખ્યાત કર્યું છે.

સમગ્ર દ્રષ્ટિએ જોતા આ નવલકથામાં વીર, શૃંગાર, કરૂણ અને અદ્ભુત રસની આહલાદક સૃષ્ટિ પાત્રોમાં સર્જી છે એમ કહી શકાય છે. અને 'ગુજરાતનો નાથ' એ ઐતિહાસિક નવલકથાના સર્જક મુનશીને સર્વોત્તમ કૃતિ છે. સહજ પાત્ર વિધાનની દ્રષ્ટિએ ધારદાર સંવાદ રચનાની દ્રષ્ટિએ અને શૈલીની સાર્થકતાની દ્રષ્ટિએ આ નવલકથા મુનશીની સર્જકતાના સર્વોચ્ચ ઉન્મેષ મુખરિત કરે છે, અને એ કૃતિ એક સીમાચિહ્ન રૂપ ગણી શકાય છે.

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લોકગુર્જરી



સળંગ અંક : બાસઠ



(ત્રૈમાસિક : દસમું વર્ષ, ત્રીજો અંક, ડિસેમ્બર-૨૦૨૧)

સંપાદક : ડૉ. બળવંત જાની



શ્રી ઝવેરચંદ મેઘાણી લોકસાહિત્ય કેન્દ્ર
સૌરાષ્ટ્ર યુનિવર્સિટી, રાજકોટ-૫

લોકગુર્જરી

સળંગ અંક : બાસઠ

(ત્રૈમાસિક : દસમું વર્ષ, ત્રીજો અંક, ડિસેમ્બર-૨૦૨૧)

: સંપાદક :

ડૉ. બળવંત જાની

શ્રી ઝવેરચંદ મેઘાણી લોકસાહિત્ય કેન્દ્ર

સૌરાષ્ટ્ર યુનિવર્સિટી

રાજકોટ-૫

અનુક્રમણિકા

વિભાગ - ૧ : લોકશાસ્ત્ર

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પરંપગત વાદ્ય અને આસ્થા અરવિંદ બારોટ ૧૮

વિભાગ - ૨ : સંતશાસ્ત્ર

૪. ભારતીય સંતપંથ :
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૬. કવિ ઈસરદાસનું 'હરિરસ' :
તત્ત્વ અને તંત્ર હરેશ ધોળકિયા ૬૪
૭. 'હરિરસ'માં રામકથા : સ્વરૂપ અને સંદર્ભ ડૉ. બળદેવ પ્રજાપતિ ૭૮
૮. ગુજરાતમાં ગોદડિયા પંથ :
પરિચય અને સાહિત્ય ડૉ. રાજેશ મકવાણા ૫૧
૯. લોકસંતપરંપરા : સામાજિક ઉત્તરદાયિત્વ ડૉ. કૌશિકકુમાર પંડ્યા ૯૯

વિભાગ - ૩ : લોકગીતશાસ્ત્ર

૧૦. લોકગીતનાં સંશોધન-સંપાદનક્ષેત્રે
સાંપ્રત બે દાયકાનું ચિત્ર ડૉ. ભરત પંડ્યા ૧૧૫
૧૧. 'હાલારની માલધારી જાતિના રાસડા' :
દષ્ટિપૂત વર્ગીકરણ અને તર્કપૂત સ્વાધ્યાય ડૉ. પ્રતિભા પંડ્યા ૧૨૮
૧૨. 'કાગ'ની કવિતામાં કર્મની ફિલસૂફી ડૉ. વિનોદ જોશી ૧૩૫



‘હાલારની માલધારી જાતિના રાસડા’ : દષ્ટિપૂત વર્ગીકરણ અને તર્કપૂત સ્વાધ્યાય ડૉ. પ્રતિભા પંડ્યા

પીએચ.ડી. પદવીપ્રાપ્તિ માટેના સંશોધન-પ્રવાસ-ક્ષેત્રકાર્ય દ્વારા ઘણુંબધું પ્રાપ્ત થતું હોય છે. પણ નિબંધલેખન પૂર્ણ કર્યા પછી એ પ્રાપ્ત સામગ્રી દ્વારા શોધકાર્ય અવિરતપણે સતત ચાલુ રહેતું હોય છે. સુચેતાબહેન ભાડલાવાળાએ પીએચ.ડી. નિમિત્તે સંશોધન કરતી વેળાએ અન્ય કેટલુંક રસરુચિ અનુસાર સંગ્રહિત કરેલું તે ઈ.સ. ૧૯૮૨માં ‘હાલારની માલધારી જાતિના રાસડા’ ગ્રંથ રૂપે પ્રકાશિત થયેલું છે. આ પુસ્તકસંદર્ભે સુચેતાબહેન આ રીતે નોંધે છે. “માલધારી જાતિઓનાં લોકસાહિત્ય અને લોકસંસ્કૃતિને જાણવા માટે મેં પ્રયત્ન શરૂ કર્યો. પ્રસ્તુત સંગ્રહ આ જાતિવિશેષ લોકસાહિત્યના એક અંશરૂપ રાસડાઓમાં પ્રતિબિંબિત થતી તેમની લોકસંસ્કૃતિનો પરિચય આપવા માટેનો એક નૂતન અને મૌલિક પ્રયાસ છે.” (પૃ. ૦૫)

પ્રસ્તુત સંગ્રહને સાત પ્રકરણો અને પરિશિષ્ટોમાં વિભાજિત કરવામાં આવ્યો છે. જેમાં પ્રારંભે પ્રસ્તાવના રૂપે ‘મોરલી રી માયા લાગે...’ શીર્ષકથી સંગ્રહિત રાસડા અને માલધારી જાતિની લોકસંસ્કૃતિ વિશે અભ્યાસપૂર્ણ ચર્ચા કરે છે. રાસડા વિશે સમજ આપતાં નોંધે છે : “ગુજરાતના રાસડામાં ગુજરાતની વિવિધ લોકસંસ્કૃતિ પ્રતિબિંબિત થાય છે. રાસડાનો ઉગમ શ્રીકૃષ્ણ સાથે સંકળાયેલો

છે. અદ્ભુત રસભરી મોરલીના સૂરથી ગોકુળ અને વૃંદાવનની ગોપીઓને મુગ્ધ કરતા શ્રીકૃષ્ણની રાસલીલા સુપ્રસિદ્ધ છે. શ્રીકૃષ્ણે ઉત્તર હિંદમાંથી દ્વારિકા આગમન કર્યું અને યાદવોની રાજધાની દ્વારિકા સ્થાપી. આ કુશળ યોદ્ધા, અદ્ભુત રાજનીતિજ્ઞ અને શ્રેષ્ઠ તત્ત્વચિંતક, રાસનૃત્ય-રાસલીલાની કળાના એક અગ્રિમ પ્રતિષ્ઠાયક બની રહ્યા છે.”

આ પ્રકારની સમજ આપીને માલધારી જાતિ અને કાનુડો-રાસડા કેવાં ઓતપ્રોત છે તેની વાત કરતાં કહે છે, “એમનો મુખ્ય ઉત્સવ છે જન્માષ્ટમીનો. હાલારના ભોપા રબારીઓ એ ઉત્સવને ‘કાનુડો’ કહે છે. ભરવાડ જાતિ એને ‘ગોકુળ આઠમ’ કહે છે. આ જન્માષ્ટમીનો ઉત્સવ એ માલધારી જાતિનું મહાન પર્વ છે. આઠ આઠ દિવસથી આ ઉત્સવની તૈયારી થાય છે. સોનાં-ચાંદીનાં ઘરેણાંથી લયી પડતી લોકનારીઓ રાસડાની રમઝટ બોલાવવા લાગે છે. શ્રાવણ મહિનાની ભીની ભીની રાતો રાસડાની રમઝટે રૂપાળી અને રસીલી બને છે.” (પૃ. ૦૮). સુચેતાબહેને આ રાસડાઓ પ્રસ્તુત સંગ્રહમાં સંગ્રહિત કર્યા છે.

પ્રથમ પ્રકરણ : ‘માલધારી જાતિના લોકોત્સવ અને લોકનૃત્યમાં સુચેતાબહેન પ્રારંભે લોકમેળાનો ઉદ્દેશ સ્પષ્ટ કરીને જુદા જુદા પેટા મુદ્દાઓમાં સઘન ચર્ચા માંડે છે. જેમ કે : ‘કાનુડો ઉત્સવની વિધિ’, ‘કાનુડાની કથા અને લોકશ્રદ્ધા’, ‘લોકનૃત્ય’, ‘રાસનૃત્ય’, ‘રાસનૃત્યના પ્રકારો’, ‘દાંડિયારાસ’, ‘ગોફગૂંથન’, ‘મટકીરાસ’, ‘રાસડા’ અને ‘ઊર્મિગીતો’ની ઝીણવટથી વાત કરે છે. આ બધી ચર્ચા કરતાં સુચેતાબહેન રાસડા અને શ્રીકૃષ્ણની સાથે જોડાયેલ કથા-લોકકથાનાં તત્ત્વો પણ નોંધે છે. જેમ કે, “આ ઉત્સવ પાછળની કથા એવી છે કે કંસ શ્રીકૃષ્ણને મારવા માટે પાછળ પડ્યો હતો ત્યારે શ્રીકૃષ્ણ રબારીવાસમાં સંતાઈ ગયા હતા. રબારીઓએ શ્રીકૃષ્ણને પોતાનાં વસ્ત્રાલંકારો પહેરાવીને રાસડામાં સામેલ કરી લીધા હતા. તેથી કંસ શ્રીકૃષ્ણને ઓળખી ન શક્યો, તેથી જ આ ઉત્સવમાં શ્રીકૃષ્ણની મૂર્તિને સ્ત્રીના વસ્ત્રાલંકારો પહેરાવીને વચ્ચે સ્થાપવામાં આવે છે. તેમજ આ ઉત્સવ પાછળની આ લોકજાતિની એ માન્યતા છે કે મૂર્તિને નદીમાં પધરાવ્યા પછી થોડી વારમાં મૂર્તિ નદીમાં હિલોળા લેતો કાળ નાગ બની જાય છે. બાર મહિના સુધી એ નાગ નદીના પાણીમાં હિલોળા લ્યે છે. કોઈને કનડગત નથી કરતો પણ બધાંનું રક્ષણ કરે છે. આ માન્યતાને કાલિયમર્દનની કથા સાથે જોડી શકાય. શ્રીકૃષ્ણે કાલિનાગને નાથીને વશ કર્યો હતો અને વચન લીધું હતું કે તે હવે કનડગત નહીં કરે. એ કથાના અંશરૂપ આ લોકમાન્યતા હોવી જોઈએ.” (પૃ. ૦૬, ૦૭). ઉક્ત ચર્ચા-વિચારણામાંથી લોકોત્સવ, રાસડા, શ્રીકૃષ્ણ સાથેનો અનુબંધ

અને માલધારી જાતિની લોકસંસ્કૃતિનો સઘન સ્વાધ્યાય પ્રાપ્ત થાય છે.

બીજું પ્રકરણ : 'કાનુડાનાં લોકગીતોના રાસડા'માં ગોપનારીએ શ્રીકૃષ્ણને એક સામાન્ય માનવીય પાત્ર તરીકે વણી લઈને લોકગીતો પ્રસ્તુત કર્યાં છે. માલધારી લોકજાતિમાં કૃષ્ણ અને ગોપીનાં અનેક લોકગીતો મળે છે. ગોપ એટલે માલધારી ગોવાળિયા અને ગોપી એટલે માલધારી ગોપનારી. એટલે સુચેતાબહેન એવું અનુમાન કરે છે કે ભરવાડ નારીઓ અને રબારી નારીઓ શ્રીકૃષ્ણની ગોપીઓ હશે. અહીં 'મોરલી' શીર્ષકથી રાસડા આપ્યા છે. કૃષ્ણ સાથે મોરલી તો અંગભૂત હોય. આવા ઘણા લોકગીત રૂપે રાસડા આ બીજા પ્રકરણમાં છે જેમાંથી કૃષ્ણજન્મના રાસડા, ગોપી-કૃષ્ણ પ્રેમવિહ્વળતા, મહી-માખણની ચોરી, મોરલી સાંભળીને નિદ્રાનો ત્યાગ કરી કૃષ્ણ પાસે દોડી જતી ગોપી, ઘરનાં કામ ભૂલીને કૃષ્ણમય થઈ જતી ગોપીના ભાવો, રિસામણાં, મનામણાં, કૃષ્ણ-રુદ્ધિમણીના રાસડા — આપણે જાણીએ છીએ કે રાધા એ કૃષ્ણની પ્રિયતમા છે. પત્ની બની શકતી નથી પણ અહીં રાસડામાં કાના-રાધાનું દામ્પત્યજીવન નિરૂપાયેલું પણ જોવા મળે છે. જુઓ અષાઠ મહિનાના ધોધમાર વરસાદમાં ગોપીઓ છાણાં વીણવા જાય છે. છાણાં વીણતાં રાધાજીને કાળો નાગ કરડે છે આથી એના પતિ કૃષ્ણને તેડાવવામાં આવે છે. કૃષ્ણ લોકવૈદ્ય જાણતા હોય છે. તે કડવો લીમડો વાટીને રાધાજીને પિવરાવે છે :

‘વરસ્યા વરસ્યા અષાઢુના મેહ જો,
પાદરિયેથી સરવરિયા ફરી વળ્યા જો...
ગોપીઓ હળીમળીને છાણાં વીણવા જાય જો...
છાણાં તે વીણતાં વીણતાં રાધા જો...
એમાં તે રહેતા શીંગડિયા શેષ નાગ જો...
વીણતલીને કરડ્યો કાળો નાગ જો...
લહેરિયા આવે રે જવજા જીવનાં જો...
તેડાવો એના કરશનજી ભરથાર જો...
આવિયા આવિયા કરશનજી ભરથાર જો..
કરશનજીએ મંગાવ્યો કડવો લીમડો જો...
વાટી ઘંટી રાધાજીને પાયો જો...
લીમડિયો પીશે ને રાધાજી જીવશે જો...
વરસ્યા વરસ્યા અષાઢુના મેહ જો...’ (પૃ. ૩૯)

ત્રીજા પ્રકરણમાં સામાજિક લોકગીતના રાસડા આપવામાં આવ્યા છે. લોકજીવનમાં જે જે નવા પ્રવાહો ભળતા ગયા તેની નોંધ પણ લોકોએ લીધી છે.

તેની પ્રતીતિ આપણને આ રાસડાઓ કરાવે છે. આમાં દારૂ, કજોડાં લગ્ન, પરકીયા પ્રેમ, વતનપ્રેમ અને તત્ત્વજ્ઞાન પ્રગટ કરતાં લોકગીતો રાસડા રૂપે મળે છે. રામનરેશ ત્રિપાઠીનું વિધાન આ સંદર્ભે નોંધવા જેવું છે, “લોકગીત એક એવી તિજોરી જેવાં છે જેણે સમય સમયની ઘટનાઓની પ્રતિક્રિયા સ્વરૂપ અને જનતાના હૃદયની ભાવરાશિને સુરક્ષિત રાખવામાં સહાય કરી છે અને કરતી રહે છે. એ ઈતિહાસનો એક અમૂલ્ય ભંડાર છે.”^૧ આ પ્રકારના રાસડાનાં થોડાં ઉદાહરણો જોઈએ :

દારૂ :

‘એ રે... સાંઢણીને દરિયાના બેટમાં ઝુકાવો માણારાજ
દારૂડો એક જ પિયાલો હો રાજ
એ રે.. સાંઢણીને રૂપામાં મઢાવો માણારાજ
દારૂડો એક જ પિયાલો હો રાજ...’ (પૃ. ૪૭)

પુરુષને આકર્ષવા માટે માત્ર નારીત્વ પર્યાપ્ત નથી. સૌંદર્ય પર્યાપ્ત નથી. દારૂની સહાય લેવી પડે છે. આ કરુણતાને પ્રગટ કરતો આ રાસડો છે. માલધારી લોકસમાજમાં દારૂના દૂષણ જેવી જ બીજી કુરૂઢિ છે — તે બાળલગ્નની — પરિણામે કજોડાં લગ્ન થાય છે. તેનું ઉદા. જુઓ :

કજોડા લગ્ન :

વીહ વરહની નાર બાઈ, પાંચ વરસનો કાન બાઈ કેમ કરી રે...
એ દૈયાણાં દળું તો પીટયો બાજરો જ માંગે કેમ કરી રે...
પાટલિયે સમજાવું, મારા હૈડામાં દાજે બાઈ કેમ કરી રે...
— વીહ વરહની નાર...’ (પૃ. ૪૯)

દારૂ અને કજોડા જેવું બીજું દૂષણ આ લોકસમાજમાં પરકીયા પ્રેમનું પણ હશે એટલે સોરઠિયા રબારીઓમાં પ્રચલિત એક રાસડો અહીં નોંધ્યો છે. જુઓ, પરકીયા પ્રેમ :

‘પૈણો લાવે પાલખી, ઓલો મગન લાવે ઊંટ...
પાલખીએ બેહું તો પડી મરુ, ઓલો હલકે હાલે ઊંટ’ (પૃ. ૫૫)

તો વતનપ્રેમને વ્યક્ત કરતા રાસડા મળે છે અગર વચ્છા ચારણોમાં એક રાસડો પ્રચલિત છે...

વતનપ્રેમ :

‘ઓ રે ઓ રે સે ઝાલું ને ઝૂમણાં
હોરે.. હોરે... મણિયારા

મારા દેશજા માડુ શામલી સુરતનાં
હોરે... હોરે.. મણિયારા.' (પૃ.૫૭)

જીવનના તત્વજ્ઞાનને સહજ રીતે પ્રગટ કરતા રાસડા પણ સુચેતાબહેને અહીં નોંધ્યા છે. સોરઠિયા રબારીઓમાં પ્રચલિત રાસડાની એક પંક્તિ જુઓ,

'કાગળ હોય તો વાંચીએ વણઝારા જી રે
કરમ વાંચિયા કીમ જાય રે.' (પૃ. ૫૯)

ચોથા પ્રકરણમાં પારિવારિક લોકગીતોના રાસડા આપ્યા છે. ગૃહસ્થ જીવનથી પરિવાર સાકાર થાય છે. આ જીવનમાં સુખ-દુઃખ, તડકા-છાંયા, હર્ષ-શોક, નીતિ-અનીતિ, પારિવારિક સંબંધો — આ સંબંધોમાં કેટલાક ગમતા અને અણગમતા સંબંધો પણ હોય. લોકે જે જે ભાવ અનુભવ્યા તેને ગીતો રૂપે વ્યક્ત કર્યા છે. આ માલધારી સમાજનાં પારિવારિક લોકગીતોના જે કેટલાક રાસડાઓ મળે છે તેમને સુચેતાબહેને દામ્પત્યજીવન, દિયર-ભોજાઈ, નણંદ-ભોજાઈ, સાસુ અને વહુ, સસરો અને જેઠ, લોકનારી અને પિયર જેવાં મથાળાં સાથે તેના ભાવ-અનુરૂપ સમાવીને તેની અભ્યાસપૂર્ણ ચર્ચા કરી છે. એકાદ ઉદાહરણ જોઈએ. સાસુ-વહુના સંબંધો સારા અને નરસા એમ બન્ને પ્રકારના હોય છે. અગરવચ્છા ચારણોમાં પ્રચલિત રાસડામાં સાસુને 'સમંદરલેર' કહી છે. જુઓ,

'ઊંડો કૂવો મધ સાંકડો જળ ભરવા દો. !
જળ જિંકોરા ખાય પાણીડાં ભરવા દો
મારી સાસુડી સમંદર લેર પાણીડાં ભરવા દો.' (પૃ. ૯૦)

પાંચમા પ્રકરણમાં વસ્ત્રાલંકારના રાસડા સંગ્રહિત કરવામાં આવ્યા છે. જેમાં સ્ત્રીનો વસ્ત્રાલંકારો પ્રત્યેનો લગાવ, પતિને આકર્ષિત કરવા પણ ઘણી વાર શણગાર સજવામાં આવતો હોય છે. આવા બધા ભાવો અને લગાવને વ્યક્ત કરવા રાસડા નારીના મનોભાવોને પણ પ્રગટ કરે છે. એકાદ ઉદાહરણ જોઈએ,

'એ...છેલ મારી નથ ઘડી દે સોનારા !
એ રે નથડીમાં શું રે બિરાજે સોનારા !
હીરા, મોતી, પરવાળા એ છેલ મારી નથ ઘડી દે સોનારા !
હાથ પરમાણે મુંને ગુજરી ઘડાવજો
એ ચૂડલાના ચમકારા... એ છેલ મારી નથ ઘડી દે સોનારા :
ડોક પરમાણે મુંને દાણિયું ઘડાવજો,
એ ઝરમરના ઝબકારા.. એ છેલ મારી નથ ઘડી દે સોનારા :'

(પૃ. ૧૦૯)

ભોપા રબારીમાં પ્રચલિત આ રાસડામાં ભોપી રબારણ કેવી ચતુરાઈથી પ્રારંભે નાની એવી નથની માંગણી કરીને ક્રમશઃ બીજા અલંકારો માટે પતિને સંમત કરી લે છે અને એવી રીતે સંમત કરે છે કે પતિને પણ એમ લાગે છે કે આટલું તો જુએ જ, આના વિના શોભા નહિ લાગે.. આ રીતે સુચેતાબહેને આવા વસ્ત્રાલંકારના રાસડા જે માલધારી જાતિમાં પ્રચલિત છે તે અહીં એકત્ર કરીને મૂકી આપ્યા તેની સરળ સમજૂતી સાથે.

છઠ્ઠા પ્રકરણમાં ઋતુગીતોના રાસડા સમાવ્યા છે. આ રાસડાઓને તેમણે વિષયવાર વિભાજિત કરતાં બે ભાગમાં વિભાજિત કરીને તેની સમજૂતી અને અભ્યાસ સાથે મૂક્યાં છે. પ્રારંભે ઋતુગીતોમાં વર્ષાઋતુના રાસડા છે. માલધારી જાતિઓમાં વર્ષાઋતુનું આગવું મૂલ્ય હોય છે. કારણ કે માલધારીઓ પોતાનાં ઢોર-માલના ચરિયાણ માટે ચોમાસા સિવાયના આઠ મહિના પોતાની ભૂમિ-વતનને છોડીને વાંઢ્યે જતા હોય છે અને વરસાદના સમાચાર મળતાં વતન પાછા ફરે છે. આ ઋતુને લગતાં ગીતો-રાસડા ગાઈને લાડ લડાવે છે, કારણ કે વરસાદ જ માલધારી પ્રજાની મુખ્ય જીવાદોરી છે.

આ પ્રજાનાં જે ઋતુગીતો છે તેમાં વિરહનો ભાવ પણ મુખ્યપણે રહેલો છે, કારણ કે પુરુષવર્ગ વાંઢ્યે જતો હોવાથી અહીં સ્ત્રી એકલી છે એટલે વિરહનું દર્દ પણ આ ગીતોમાં પ્રગટ્યું છે. આ વિરહગીતો બારમાસી પ્રકારનાં છે. એકાદ ઉદાહરણ જોઈએ,

‘બાયું કોઈ દેખાડો દીનોનાથ, વાલોજી મારો કીયા રે લોલ,
માહે મહિયર વિવાહ, પિયુ વિના શું ઉજમ રે લોલ’
ફાગણે ફરફરતી હોળી, પિયુ વિના કોણ રંગે હોળી.’

(પૃ. ૧૩૨)

આ પ્રકારના રાસડા તેના ભાવસંદર્ભ સાથે સુચેતાબહેને પ્રસ્તુત પ્રકરણમાં ખોલી આપ્યા છે.

સાતમા પ્રકરણમાં રાસડામાં ગવાતાં લોકગીતોની વિશિષ્ટતાઓ અભ્યાસપૂર્ણ રીતે ચીંધી આપી છે. જેમાં પેટા મુદ્દાઓ પાડ્યા છે. જેમ કે, લોકગીતોનું શિલ્પવિધાન, નાટકીય તત્ત્વોનો સમાવેશ, રૂઢિગત શૈલી, લોકગીતોની ભાષાકીય લક્ષણ જેવા મુદ્દાઓમાં માલધારી પ્રજાની બોલીગત લાક્ષણિકતાઓ સદૃષ્ટાંત ચર્ચી છે. ત્યારબાદ લોકગીતોમાં ઢાળો, લોકગીતોનું ભાવતથ્ય, લોકગીતોમાં રસ, લોકગીતોમાં પ્રકૃતિ-ચિત્રણ જેવા વિભાગોમાં સાધાર અને સઘન અભ્યાસ પ્રસ્તુત કર્યો છે. ઉત્સવો પ્રજા સાથે તંતોતંત જોડાયેલા છે. આપણે ત્યાં પ્રાચીન સમયથી

ઉત્સવો ઊજવાતા આવ્યા છે. ઉત્સવો સાથે ગીતો રચાતાં ગયાં. આ સંદર્ભે શ્રી પુષ્કર ચંદરવાકરનું વિધાન નોંધવા યોગ્ય છે : “લોકોત્સવ અસ્તિત્વમાં આવ્યા ત્યારે આર્યો પંચસિંધુને તીરે સ્થિર પણ થયા હતા. શાંતિ અને સ્થિરતાના કાળમાં ઉત્સવો અને તેમાં સંસ્કારિતાનું સિંચન થાય છે. આર્યોના ઉત્સવ શાંતિ અને સ્થિરતાનું ફળ છે, પેદાશ છે. તેમના મુખ્ય મુખ્ય ઉત્સવો, લોકમેળાઓ હતા. જુદાં જુદાં દેવ-દેવીઓની પૂજા અને અર્ચનાના પણ ઉત્સવો તે લોકો ઊજવતા. તેમની ભાવનામાં ગોઠવાઈ શકે તેવા વીર પુરુષની પાછળ પણ તેઓ ઉત્સવો ઊજવતા થયા હતા. સંસારની ઘરેડમાં પણ એવા અનેક પ્રસંગો આવતા કે તેઓ ઉત્સવો ઊજવતા થઈ ગયા હતા. લોકોએ તેમના આનંદ અને ઉલ્લાસના પણ ઉત્સવો ઊજવવા માંડ્યા હતા. તેમાંના કેટલાકનો નિર્દેશ જાતક-કથાઓમાં ઋગ્વેદ આદિમાં થયેલ છે. ઉપરાંત ઋતુવાર ઉત્સવો ઊજવાતા. આ ઉત્સવોએ તો પછી પરંપરાનું સ્થાન લીધું, લોકો તેને ઊજવતા જ. આ ઉત્સવોની જેમ સંસારના ઉત્સવો તેઓ ઊજવતા થયા હતાં અને તેનાં ગીતો પણ રચાવા લાગ્યાં હતાં.”

પુષ્કરભાઈ ચંદરવાકર સુચેતાબહેનના પીએચ.ડી. પદવી માટેના માર્ગદર્શક હતા અને તેમના માર્ગદર્શનથી સુચેતાબહેને ઉત્તમ કાર્ય કર્યું છે. પરિશિષ્ટોમાં સંદર્ભગ્રંથસૂચિ, થોડા ફોટોગ્રાફ અને માલધારી લોકબોલીનો શબ્દકોશ પણ આપ્યાં છે. સર્વાંગી રીતે તપાસતાં પ્રસ્તુત ગ્રંથ સુચેતાબહેનના દષ્ટિપૂત વર્ગીકરણ અને તર્કપૂત સ્વાધ્યાયનું તેજસ્વી દષ્ટાંત જણાય છે.

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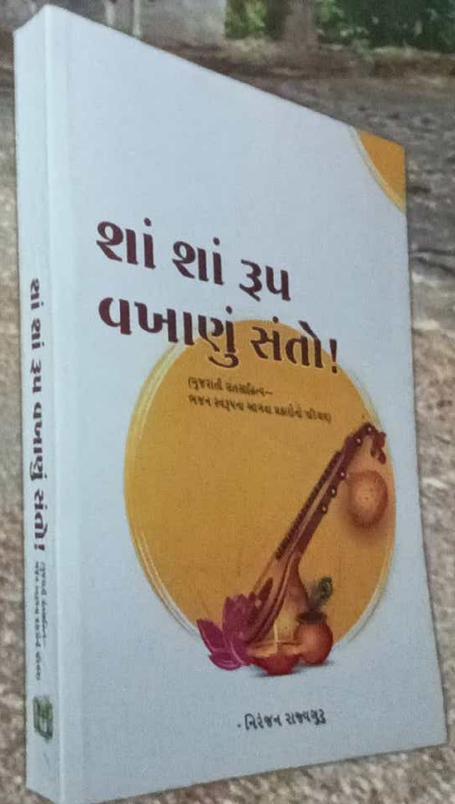
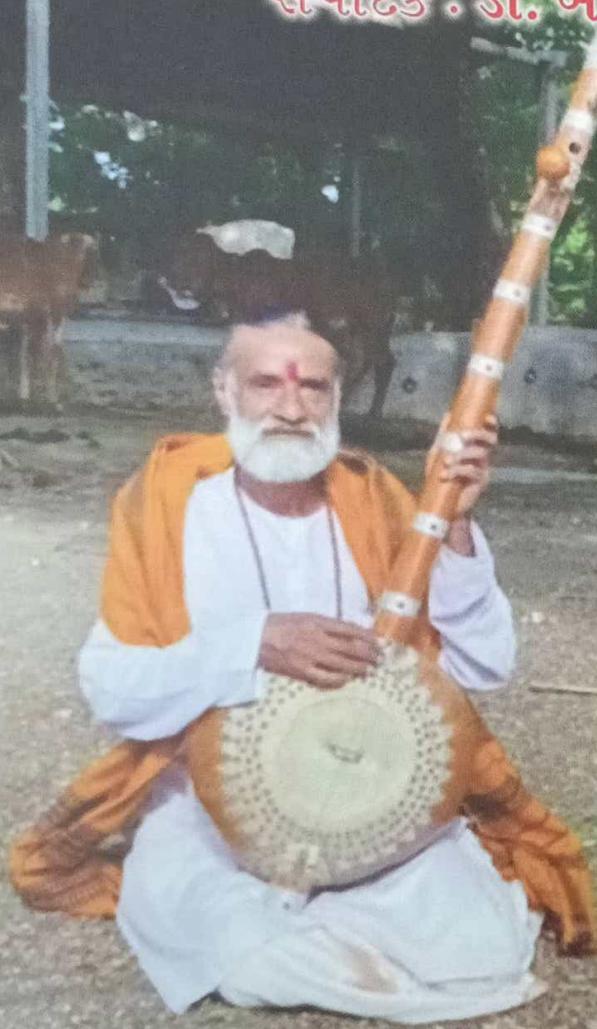
લોકગુર્જરી



સળંગ અંક : એકસઠ

(ત્રૈમાસિક : દસમું વર્ષ, બીજો અંક, સપ્ટેમ્બર-૨૦૨૧)

સંપાદક : ડૉ. બળવંત જાની



શ્રી ઝવેરચંદ મેઘાણી લોકસાહિત્ય કેન્દ્ર
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૧૨. લોકશિક્ષણ અને મનોરંજનનું માધ્યમ : 'લોકનાટ્ય ભવાઈ'	ડૉ. બળદેવ મજાપતિ	૧૩૩



માલધારી જાતિનાં સંસ્કારગીતો : અભ્યાસપૂત સંશોધન ડૉ. પ્રતિભા પંડ્યા

લોકસાહિત્યના પ્રખર અભ્યાસી શ્રી પુષ્કર ચંદરવાકરે લોકસાહિત્યક્ષેત્રે પીએચ.ડી.ના સંશોધન નિમિત્તે ખૂબ મહત્વનાં કામો કરાવ્યાં જેમાં મુખ્યત્વે પરશુરામ દેવમુરારી પાસે 'પાંચાળ પ્રદેશના માલધારીઓનું લોકસાહિત્ય', 'પી.ટી.પટેલ પાસે 'ઓખા મંડળના' વાઘેરોનું લોકસાહિત્ય' અને સૂચિતાબહેન ભાડલાવાળા પાસે 'હાલારના માલધારીઓનું લોકસાહિત્ય' વિશેનું કાર્ય નોંધપાત્ર છે. આમાંથી સૂચિતાબહેન ભાડલાવાળાએ પીએચ.ડી. નિમિત્તે કાર્ય કરતા સમયે બીજું જે કેટલુંક સંગ્રહેલું તેને બે પુસ્તક રૂપે પ્રકાશિત કર્યું છે. જેમાં 'માલધારી જાતિનાં સંસ્કારગીતો' અને 'હાલારની માલધારી જાતિના રાસડા'નો સમાવેશ થાય છે. આ સંદર્ભે સૂચિતાબહેન નોંધે છે : "લોકશાસ્ત્રના એક અંગ તરીકે માલધારીઓનું લોકસાહિત્ય અને તેમાંથી પ્રતિબિંબિત થતી તેમની લોકસંસ્કૃતિનું વૈજ્ઞાનિક પદ્ધતિએ અધ્યયન અને સંશોધન શરૂ કર્યું. ક્ષેત્રીય સંશોધનમાં હાલાર પ્રદેશને ખૂંટી વળી અને જે કંઈ લાધ્યું એ એટલું તો વિપુલ હતું કે એક મહાનિબંધમાં સમાવવું મુશ્કેલ બન્યું. તે સામગ્રીને મહાનિબંધનું સ્વરૂપ આપતી વખતે માહનિબંધમાં જે જે સામગ્રીનો સમાવેશ ન થઈ શક્યો તે અને મહાનિબંધ માંહેની સામગ્રીને અલગ પુસ્તક રૂપે પ્રસ્તુત કરવાને મનમાં એક વિચાર ઊઠ્યો જે અલગ એક પુસ્તક રૂપે અત્રે પ્રગટ કરું છું. ક્ષેત્રીય સંશોધનકાર્ય માટેના પ્રવાસ દ્વારા આ લોકજાતિનું લોકસાહિત્ય વિપુલ પ્રમાણમાં એકઠું થયું છે. તેમના ભિન્ન ભિન્ન સ્વરૂપના લોકસાહિત્યને

ક્રમશઃ સંપાદિત કરી પ્રગટ કરવાનું વિચાર્યું છે. અહીં તેમાંથી તેમનાં લોકગીતોનું વર્ગીકરણ કરીને, માત્ર એક જ પ્રકારનાં લોકગીતો - સંસ્કારગીત ગ્રંથસ્થ કરીએ છીએ.” (પૃ.૦૧ અને ૦૨) અને ‘માલધારી જાતિનાં સંસ્કારગીતો’ ગ્રંથ વિશે ચર્ચા કરવાનો ઉપક્રમ છે.

ગુજરાત રાજ્ય લોકસાહિત્ય સમિતિ દ્વારા ઈ.સ. ૧૯૭૯માં સુચેતાબહેન ભાડલાવાળાનો ગ્રંથ ‘માલધારી જાતિનાં સંસ્કારગીતો’ પ્રકાશિત થાય છે. પ્રસ્તુત ગ્રંથને પાંચ પ્રકરણો અને ચાર પરિશિષ્ટોમાં વિભાજિત કરવામાં આવ્યો છે. જેમાં અનુક્રમે માલધારી જાતિનાં સંસ્કારગીતોની વિશેષતા, ખોળાભરણાંનાં ગીતો અને હાલાર, માલધારી જાતિનાં લગ્નગીતો અને લગ્નસંસ્કારો, ઓળાપણાનાં ગીતો, ફટાણાનાં ગીતો અને ચાર પરિશિષ્ટોમાં અનુક્રમે માલધારી લોકબોલીનો શબ્દકોષ, સંદર્ભગ્રંથસૂચિ, ફોટા, ચિત્રો અને નોંધ, છેલ્લે જામનગર જિલ્લાનો નકશો મૂકવામાં આવ્યો છે.

‘મારા મલકજા માડુ મીઠડિયું બોલીયું નાં...’ શીર્ષકથી પ્રારંભે સુચેતાબહેને દીર્ઘ અભ્યાસલેખ આપ્યો છે, જેમાં ઐતિહાસિક પાર્શ્વભૂમાં હાલાર પ્રદેશની ઓળખ અને તે સંદર્ભે પ્રચલિત દુહો આપ્યો છે. હાલાજીના નામ ઉપરથી આ પ્રદેશને હાલાર નામ આપવામાં આવ્યું છે. આ સંદર્ભે કચ્છીબોલીમાં - હાલાર પ્રદેશમાં આ મુજબનો દુહો છે :

‘વાઢેર વાલો વટંદા, જેઠવા બરડે બાર;
વાલા વીણ વટંદા, હદ ચોવાઈ હાલાર.’ (પૃ.૦૪)

ત્યારબાદ હાલારની રસાળ ધરતી, ખનીજ સંપત્તિ, ઉદ્યોગો, હવામાનની ઝીણવટથી ચર્ચા કરે છે અને પછી હાલારની માલધારી જાતિની વાત માંડે છે. હાલારમાં માલધારીઓની મુખ્યત્વે ત્રણ જાતિઓ વસે છે :

૧. રબારી
૨. ભરવાડ
૩. અગરવચ્છા ચારણ

આ ત્રણેય માલધારી જાતિના લોકજીવનની અભ્યાસપૂર્ણ ચર્ચા કરી છે; જેમ કે, રબારી શબ્દની ઉન્નતિ ‘રાહબારી’ (અરબી શબ્દ) પરથી આવેલ હોવાનું માનવામાં આવે છે અને તેના સમર્થનમાં જામનગર ગેઝેટિયરમાં આવેલ એક કથા પણ નોંધે છે તેમજ રબારીઓ તેમની ઉન્નતિ મહાદેવથી થઈ હોવાનું માને છે તે સંદર્ભે દંતકથા છે કે મહાદેવે, પાર્વતીના ઊંટની રક્ષાર્થે એમને ઉત્પન્ન કર્યા ને

કૈલાસ એ એમનું આદિ નિવાસસ્થાન છે. સૌરાષ્ટ્રમાં રબારીઓની છ જાતિઓ વસે છે. તેમાંથી 'ભોપા' અને 'સોરઠિયા' રબારીઓ હાલારમાં વસે છે. આ બન્ને રબારીજાતિના લોકજીવન સંદર્ભે સૂચેતાબહેને અભ્યાસ પ્રસ્તુત કર્યો છે. જેમાં ભોપા રબારી અને સોરઠિયા રબારીની જાતિગત ભિન્નતા વસવાટ વગેરે વિશે ચર્ચા કરી છે. જેમ કે ભોપા રબારીઓ કચ્છમાંથી રાવળજામની સાથે આવેલા અને સોરઠિયા રબારી રાજપૂતમાંથી ઊતરી આવ્યા છે. તેમના મતે નૃવંશશાસ્ત્રની દૃષ્ટિએ આ જાતિનો અભ્યાસ કરવામાં આવે તો તેઓનું મૂળ હૂણ જાતિમાં હશે. ભરવાડોનાં બે કુળો છે (૧) નાનાભાઈ (૨) મોટાભાઈ. હાલારપ્રદેશમાં જે ભરવાડો વસે છે તે મોટાભાઈ છે. ભરવાડો વિશેની દંતકથા શ્રીકૃષ્ણ સાથે તેનો સંબંધ તેના પહેરવેશ, રીત-રિવાજોની ઊંડાણથી ચર્ચા કરવામાં આવી છે. ત્યારબાદ અગરવચ્છા ચારણ વિશે વાત કરતાં નોંધે છે કે તેઓ પણ જામરાવળ સાથે ઈ.સ. ૧૫૩૫માં હાલાર પ્રદેશમાં આવ્યા. આ જાતિના રહેઠાણ, માન્યતાઓ, વસ્ત્રાલંકારો, રીત-રિવાજોની રસપ્રદ ચર્ચા કરી છે. આ રીતે ચાળીસ પૃષ્ઠોમાં હાલાર પ્રદેશની ભૌગોલિક સ્થિતિથી લઈને ત્રણેય માલધારી જાતિઓના લોકજીવન સંદર્ભને ખોલી આપ્યો છે.

માલધારીજાતિનાં સંસ્કારગીતોની વિશેષતા : નામના પ્રકરણમાં પ્રારંભે લોકગીતની સમજ આપીને તેનું મહત્ત્વ સમજાવતામ નોંધે છે : 'લોકગીતોમાં એવી ઘણી ઐતિહાસિક સામગ્રી વેરાયેલી છે કે જેના સંગ્રહથી ભારતનો સાચો જીવતો જાગતો ઇતિહાસ પ્રસ્તુત કરી શકાય. લોકગીતોના આધારે ઘટનાઓની તારીખો ભલે નિશ્ચિત ન થઈ શકે, પણ એમાં સંદેહ નથી કે આ ગીતોથી ઘણી ઐતિહાસિક ઉલ્કનો અવશ્ય ઊકલી જાય છે. અને ઐતિહાસિક ઘટનાઓની પ્રમાણિકતા પણ આ ગીતોથી તપાસી શકાય છે. તેમજ વિભિન્ન જાતિઓના આચારવિચાર, ધર્મનીતિ તેમજ રીત-રિવાજો સંબંધી ઇતિહાસ પણ મળે છે.' (પૃ. ૪૫, ૪૬) આરીતે લોકગીતની મહત્તા સિદ્ધ કરીને લોકગીતના કલાવિધાનને તાકે છે, જેમાં સંસ્કારગીતોની શૈલી, ટેક પંક્તિની પુનરાવૃત્તિ, નાટકીય તત્ત્વોનો સમાવેશ, લોકગીતોમાં પ્રચલિત રૂઢિગતશૈલી, રૂઢિગત આંકડાઓ, અનંત સંયોજન, ભાષાકીય લઢણ, અલંકારવિધાન, લોકગીતોમાં પ્રયુક્ત વિશેષણો, લોકગીતમાં પ્રયુક્ત ઢાળો, ટેક અને લય, લોકગીતોમાં ભાવનિરૂપણ, લોકગીતોમાં પ્રતીકયોજના, લોકગીતોમાં પ્રકૃતિચિત્રણ અને પશુ-પક્ષીઓ, લોકગીતનાં વિશિષ્ટ પાત્રો, લોકગીતોમાંથી પ્રગટતું રંગસૌંદર્ય કે રંગવૈવિધ્ય અને અંતે માલધારીજાતિના લોકગીતોનું વર્ગીકરણ - આટલા મુદ્દાઓ સાથે સૂચેતાબહેને માલધારીજાતિનાં સંસ્કારગીતો વિશે સાધાર અભ્યાસ પ્રસ્તુત કર્યો છે. કેટલાંક ઉદાહરણો જોઈએ - રૂઢિગત આંકડાઓ :

માલધારી જાતિનાં સંસ્કારગીતોમાં રૂઢિગત આંકડાઓ જેવા કે સો, સોળસો, હજાર અને લાખની સંખ્યાનો ઉલ્લેખ ઠેર ઠેર જોવા મળે છે :

‘સો સો જાનડિયા સોંઢ્યસી રે.
સોળસે સાંઢ્યું ને હજાર હાથી માણારાજ
લાખે ઘોડે ને હજાર હાથીએ વીર રી જાન.’ (પૃ. ૫૩)

- બહુવચનનો પ્રયોગ

ઠેર ઠેર બહુવચન પ્રયોજે છે જેથી લય સધાય છે.

જેમ કે :

‘સો સો શરણાયાં સોંઢ્યસી રે
કે
સો સો બાંથીડા સોંઢ્યસી રે
કે
એવા હાથીડા દેખીને
કે
તારા ઘોરીડાને નીરું નાગરવેલ્ય’ (પૃ. ૬૧)

ડુંગરાઓ :

માલધારી મોટેભાગે અસ્થિર જીવન જીવતા હોવાથી અને ડુંગરાઓ પર નેસડા બાંધીને રહેતા હોવાથી તેમનાં ગીતોમાં ડુંગરાનો ઉલ્લેખ સહજ રીતે આપી જાય.

જેમ કે

‘ડુંગર ઉપર દેરડી વઢિયારાનો ઢોલા,
દેરડી ઉપર ડાયરો વઢિયારાના ઢોલા’ (પૃ. ૮૫)

— રંગસૌંદર્ય કે રંગવૈવિધ્ય :-

પ્રકૃતિના વિવિધ રંગોએ લોકમાનસને આકર્ષ્યું છે એમાં માલધારી જાતિમાં લાલ રંગ મુખ્ય રંગ છે. એમનાં વસ્ત્રોમાં મહદ્અંશે ગૂઢો લાલ રંગ જોવા મળે છે એટલે એમનાં લગ્નગીતોમાં લાલ રંગનો ઉલ્લેખ વારંવાર જોવા મળે છે :

‘રાતી ચણોઠડી ને રાતા મારા દાંત છે
કે
ભાઈ કસુંબી મોળિયા રંગી લાવ્ય’ (પૃ. ૯૬)

આપણે ત્યાં લોકસાહિત્યના અભ્યાસીઓએ લોકગીતોનું વર્ગીકરણ આપ્યું છે તેનો આધાર લઈને સૂચેતાબહેન માલધારી જાતિઓનાં જે લોકગીતો મળે છે તેને બે પ્રકારે વર્ગીકૃત કરે છે.

૧. સંસ્કારગીતો : જન્મ પહેલાંથી-ગર્ભસ્થ અવસ્થાથી લઈને મૃત્યુ સુધીના સંસ્કારોનાં લોકગીતો

૨. ઉત્સવગીતો : સામાજિક અને ધાર્મિક ઉત્સવનાં લોકગીતો

આ રીતે બાસઠ પૃષ્ઠોમાં માલધારીજાતિનાં સંસ્કારગીતોની વિશેષતા અભ્યાસપૂર્ણ રીતે આલેખી છે.

‘ખોળા ભરણાંનાં ગીતો અને હાલર’ નામના બીજા પ્રકરણમાં ખોળાભરણાંનાં ગીતો અને હાલરડાં વિશે સાધાર ચર્ચા કરવામાં આવી છે. માલધારી લોકજાતિમાં પ્રથમ ગર્ભાવસ્થા માટે ‘ખોળાભરણું’ શબ્દ પ્રયોજવામાં આવે છે તે સમયે જે ગીતો ગવાય છે તેને ‘ખોળાભરણાંનાં ગીતો’ કહેવામાં આવે છે. આ વિધિની સમજ આપતાં સૂચેતાબહેન નોંધે છે : ‘માલધારી લોકજાતિમાં સ્ત્રી ગર્ભધારણ કરે ત્યારથી પાંચમે મહિને અનુકૂળ સમયે અને દિવસે યોગડિયું જોઈને, રાખડી બાંધવામાં આવે છે. આ ક્રિયામાં નણંદ ભાભીના જમણા હાથે રાખડી બાંધે છે. રાખડીમાં લીલા રંગનું રેશમી કપડું લઈને, લોઢાની કડી, હનુમાનની મળી, ત્રણ શેરીની ધૂળ, અડદના દાણા અને સોનાનું બોરિયું તેમાં નાખી કોડી સાથે જમણા હાથને કાંડે બાંધવામાં આવે છે. વહુને લીલું કાપડું પહેરાવી કુળદેવીને પગે લગાડવામાં આવે છે. સાતમે મહિને ‘ખોળાભરણું’ આવે છે. પિયરપક્ષના સભ્યો કપડાની એક જોડ્ય મઢી અને નાળિયેર તથા મીઠાઈ કે ગોળ લઈને આવે છે. વહુને લીલું કાપડું પહેરાવી ખોળામાં નાળિયેર મૂકી ચાંદલો કરી કુળદેવીને સ્થાનકે પગે લગાડવામાં આવે છે તે વખતે લાપસી રંધાય છે અને કુટુંબીજનો ખોળાભરણાંનાં ગીતો ગાય છે. આ લોકજાતિમાં એવી એક માન્યતા છે કે લીલું કાપડું એ શુકન ગણાય છે. લીલો રંગ એ તો પ્રકૃતિનો રંગ છે. ધરતીની ફળદ્રુપતાનો રંગ છે તેથી લીલું કાપડું એ સ્ત્રીની કૂખ ધરતીની જેમ ફળદ્રુપ બને એ માટે પહેરાવાય છે.’ (પૃ. ૧૦૫, ૧૦૬) માલધારીજાતિના જીવનમાં શ્રીકૃષ્ણ એ પ્રકારે એકરૂપ થઈ ગયા છે કે એમનાં દરેક ગીતો શ્રીકૃષ્ણને સંબોધીને જ રચાયાં છે. જુઓ.

‘દેવકી જશોદા બે બેનડી, જીરે હરિ હાલરડું !

બે બહેનો પાણીડાની હાર, ગોવિંદજીનું હાલરડું !

દેવકીએ જશોદાને પૂછ્યું, જીરે હરિ હાલરડું !

બેની તારે કેટલા તે માસ, ગોવિંદજીનું હાલરડું !’ (પૃ. ૧૭)

આમ નવ મહિનાનું વર્ણન આવે. જશોદાના પ્રતીક દ્વારા ગર્ભવતી સ્ત્રીના હૃદયના ભાવો ચિત્રિત થયા છે. સુયેતાબહેન દરેક ગીતનું આસ્વાદલક્ષી અર્થઘટન આપતાં આપતાં તેનો લોકસંસ્કૃતિ સંદર્ભ પણ સહજ રીતે ખોલી આપે છે એ તેની અભ્યાસનિષ્ઠા છે.

‘હાલર’ એટલે કે ‘હાલરડા’ વિશે વાત કરતી વખતે મહાભારતકાલીન હાલરડાંની ચર્ચા કરી તેની પૌરાણિકતાનો નિર્દેશ કરે છે પછી માલધારી જાતિમાં ગવાતાં હાલરડાંની ઉદાહરણસમેત ચર્ચા માંડે છે. રબારી અને ભરવાડ જાતિનાં હાલરડાં અન્ય જાતિનાં હાલરડાં જેવાં કે તેમને મળતા આવે પણ અગરવચ્છા ચારણ જાતિમાં પ્રચલિત ‘હાલર’ સામાન્ય હાલરડાં કરતાં વિશિષ્ટ પ્રકારનું છે. કારણ કે આ જાતિ ભ્રમણશીલ જીવન જીવે છે. માલના ચરિયાણ માટે જન્મભૂમિ છોડીને ઠેર ઠેર ભટકે છે તેથી જન્મભૂમિમાં પાછા જવાની ઝંખના તેમના ‘હાલર’માં છે.

જુઓ :

‘હીંચકો વા’લો હીરની દોરીએ,
હાં...આં...આં હાં...લાં...આં...હાં...લાં...આં...!
અમારો નકો સુઈને મોટો થાહે,
હાં...આં...આં...હાં...લાં...આં...હાં...લાં...આં...!
એનું હાલર મુંને વા’લુ ને દેહડે દેહમાં જાસું !
હાં...આં...આં...હાં...લાં...આં...હાં...લાં...આં...!

(પૃ. ૧૧૬)

આ હાલરના ગીત દ્વારા ભ્રમણશીલ જીવન જીવનારી લોકજાતિની લોકસંસ્કૃતિ કઈ રીતે પ્રતિબિંબિત પામે છે તેવી ખૂબ જ માર્મિક ચર્ચા સુયેતાબહેને કરી છે.

‘માલધારીજાતિનાં લગ્નગીતો અને લગ્નસંસ્કારો’ નામે ત્રીજા પ્રકરણમાં પ્રારંભે આ જાતિઓનાં લગ્નના રિવાજો વિશે વાત કરે છે. જેમ કે ભોળા રબારીઓમાં સગાઈ કે લગ્ન વખતે ગોરનું મહત્ત્વ હોય છે. સગોત્રમાં લગ્ન નથી થતાં. સગાઈની વિધિને ‘દૂધપીણું’ પણ કહેવામાં આવે છે જ્યારે સોરઠિયા રબારીમાં સગપણ નાનપણથી કરી નાખવામાં આવે છે. આમાં પણ ગોરબાપા તો મધ્યસ્થી હોય જ છે. જ્યારે ભરવાડ માલધારીઓમાં સમૂહલગ્નની પ્રથા છે. એક માણેકસ્તંભ આગળ અનેક કન્યાઓનાં કન્યાદાન દેવાય છે. તેને ‘માંડવવિવાહ’ કહેવાય છે. ભરવાડજાતિના સમૂહલગ્નમાં જે માણેકસ્તંભ રોપાય છે તે પણ લોકસંસ્કૃતિનું એક

વિશિષ્ટ પ્રતીક છે. અન્ય જાતિઓમાં મોટેભાગે માણેકથંભ લાકડાના નાના ટુકડાનો હોય છે. જ્યારે આ જાતિનો માણેકથંભ ખીજડાના આખા વૃક્ષમાંથી તૈયાર કરવામાં આવે છે. અમરવચ્છા ચારણોમાં પહેલા બાળલગ્નનું પ્રમાણ હતું. બાળક ચાર-પાંચ વર્ષનું થાય કે લગ્ન કરી નાખતા. લગ્ન પછી કન્યા ઉંમરલાયક થાય ત્યારે આણું કરતાં. જોકે હવે બાળલગ્નો નથી કરતા પણ સગપણ તો બાળપણમાં જ કરી નાંખે છે. સગપણ કરે તેને 'બોલ બોલ્યા' કહેવામાં આવે છે. આ પ્રકારે લોકજીવન-રીત-રિવાજોના સંદર્ભો ખોલી આપીને લગ્નગીતોની ચર્ચા સાધાર કરે છે.

સુચેતાબહેન નોંધે છે : આ માલધારી જાતિઓના લગ્નસંસ્કારો હિન્દુ લગ્નસંસ્કારોથી સાવ જુદા છે. કન્યાવિક્રયની પ્રથા હોવાથી સ્ત્રીની તાબેદારી તરીકેની સ્થિતિ એમનાં લગ્નગીતોમાં જ્યાં ને ત્યાં ડોકિયાં કરે છે. લગ્નના સંસ્કારો લૌકિક હોવાથી માત્ર મુખ્ય મુખ્ય વિધિનાં જ લગ્નગીતો પ્રચલિત છે." (પૃ. ૧૩૬) આમ વરપક્ષે અને કન્યાપક્ષે ગવાતાં ગીતો આખ્યાં છે જેમાં માંડવાનાં તથા પીઠીનાં ગીતો, હથેવાળાનાં અને ચોરીનાં ગીતો અને ફટાણાં બન્ને પક્ષે ગવાય છે. વરપક્ષે જાન જતી વખતે ગવાતાં ગીતો છે. કન્યાપક્ષે સામૈયાનાં લગ્નગીતો. વરપક્ષે જાન પરણીને આવતાં ગવાતાં લગ્નગીતો તો કન્યાપક્ષે ઓળામણાંનાં લગ્નગીતો છે. અમરવચ્છા ચારણ જાતિમાં સાદી સરળ રીતે સમસ્યાગીત છે તેની થોડી પંક્તિ જુઓ :

‘એ રામને સીતા વાદ વદે, એ લાવજો એક લીલું પાંદ,
હૃદયકમળમાં રામ રમે.’

‘એ લીલા પોપટ પાંજરે રે, લીલી તે સૂડાની પાંખે
હૃદયકમળમાં રામ રમે’

(પૃ. ૧૩૭)

માલધારી ભરવાડોમાં હાથીદાંતનું બલોયું (ચૂડલો) કન્યાને જમણે હાથે પહેરાવવામાં આવે છે આ વિધિ વરપક્ષ તરફથી લગ્ન વખતે થાય છે. આ ગીત ભરવાડજાતિની લોકસંસ્કૃતિનું પરિચાયક બની રહે છે જુઓ :

‘ચમકે ચમકે ચાંદલિયો ચમકે સે
બોનના ચાંદા સૂરજ સરીખાં તેજ
ચાંદલિયો ચમકે સે.

બોનને કાંડે તે મીઠોળ સોહે સે
બોનને બલોયાની ઘણી ઘણી હોંસ
ચાંદલિયો ચમકે સે...' (પૃ. ૧૩૯, ૧૪૦)

સુચેતાબહેનને લગ્નની દરેક વિધિનાં ગીતો ક્ષેત્રીયકાર્ય કરી મેળવીને અહીં અર્થઘટન સાથે મૂક્યાં છે જે તેની સંશોધનનિષ્ઠા અને અભ્યાસદૃષ્ટિનો પરિચય કરાવે છે.

‘ઓળામણાંનાં ગીતો’માં વિદાયગીતો આપવામાં આવ્યાં છે ખાસ કરીને સૌરાષ્ટ્રમાં વિદાયને ‘ઓળાવવું’, વળાવવું કહેવામાં આવે છે. લગ્નવિધિ પૂર્ણ થયા બાદ કન્યાવિદાય કરવામાં આવે છે તે સમયે જે ગીતો ગાવામાં આવે છે તેને ‘ઓળામણાંનાં ગીતો’ કહેવામાં આવે છે. જોકે માલધારીજાતિઓમાં લગ્ન પછી તરત જ કન્યાને વિદાય અપાતી નથી પણ ઝાંપેથી ગાડામાંથી ઉતારી લેવામાં આવે છે. બાળલગ્નો થતાં ત્યારે આવું થતું પણ હવે વિદાય આપવામાં આવે છે. મોટાભાગે તો કન્યાવિદાયનાં ગીતો જાણીતાં છે. જુઓ—

‘સહીયેર સંકેલો ઢીંગલા પોતિયા...

સહીયેર આવો રૂડો દાદાજીનો દેહ મેલી

કડવીબાઈ હાલ્યા સાસરે...’ (પૃ. ૧૭૪)

અગરવચ્છા ચારણ જાતિનું કન્યાવિદાયનું ગીત જરા જુદું છે. જુઓ—

‘મોર તારા હૈયામાં જડિયલ હીરા !

કે આંખ્યે તારે આભલાં રે લોલ’ (પૃ. ૧૭૨)

અહીં પણ જે મળ્યાં છે તે થોડાં ઓળામણાંનાં ગીતો અર્થઘટન સાથે આપ્યાં છે જેથી ગીતોમાં સંગોપિત હૃદયભાવને આપણે પામી શકીએ.

પાંચમા પ્રકરણમાં ફટાણાંનાં ગીતો આપવામાં આવ્યાં છે. ફટાણાં એ લગ્નગીતોનો જ એક પ્રકાર છે, આ માલધારીજાતિમાં જોવા મળતાં ફટાણાંઓને સુચેતાબહેને ત્રણ વિભાગમાં વિભાજિત કર્યાં છે :

૧. કટાક્ષ અને પરિહાસ પ્રગટ કરતાં ફટાણાં

૨. અત્યંત નિર્મળ અને નિર્દોષ મર્મ અને સ્થૂળ વિનોદ પ્રગટ કરતાં ફટાણાં

૩. અશ્લીલતા પ્રગટ કરતાં ફટાણાં

થોડાં ઉદાહરણ જોઈએ.

— ભરવાડજાતિનું ફટાણું જે નિર્દોષ અને માર્મિક કટાક્ષ પ્રગટ કરે છે :

‘તમે ઘરચોળું લઈ આવ્યા નવી ફેશનનું,

તમે મોરજલીમાં કર્યો અતિલોભ

જાનૈયા સહુ સાંભળજો’ (પૃ. ૧૮૩)

— સોરઠિયા રબારીનું ફટાણું :

‘જોયું જોયું વેવાઈ તારું ખોટપણું રે,
તારા મોટપણાંમાં એટલું ખોટપણું રે’ (પૃ. ૧૮૪)

— ભોળા રબારીનું ફટાણું :

‘મારે માંડવે રે હીરરા દોર,
ચીરરા દોર કુમકિયાળાં ગોદડાં’. (પૃ. ૧૮૭)

— અગરવચ્છા ચારણનું ફટાણું :

‘કે, મારે આંગણ તલાવડી રે, છબછબિયાં પાણી રે,
કે, આવતા વેવાઈ લપસ્યો રે, એની કેડચ ધ્રસકાણી રે.’
(પૃ. ૧૮૮)

ફટાણાં પણ તેના અર્થઘટન સાથે આપ્યાં છે જેથી સમજવામાં સરળતા રહે.

પુસ્તકના અંતે પરિશિષ્ટોમાં માલધારી લોકબોલીનો શબ્દકોષ આપવામાં આવ્યો છે જેથી ગીતોને સમજવામાં સરળતા પડે છે. પુસ્તક તૈયાર કરવામાં ઉપયોગમાં લીધેલ સંદર્ભગ્રંથોની સૂચિ, વિધિ-વિધાનોના ફોટોગ્રાફ આપ્યા છે અને અંતે જામનગર જિલ્લાનો નકશો આપવામાં આવ્યો છે. આ રીતે ખરા અર્થમાં જેને શાસ્ત્રીય સંશોધન કહી શકાય તેવું કાર્ય સુચેતાબહેન ભાડલાવાળાએ કર્યું છે. આ માટે તેમને ખૂબ ખૂબ અભિનંદન સાથે વંદન.

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- ડૉ. પુલકેશી જાની, સરસ્વતી કોલેજ ઓફ સોશ્યલ વર્ક, મોરિયાણા, નેત્રંગ પાસે, (દ.ગુ.). મો. ૯૪૦૯૪૭૦૦૦૭
- ડૉ. કવિત પંડ્યા, ગુજરાતી વિભાગ, એસ. એન્ડ ડી.ટી. મહિલા યુનિવર્સિટી, ચર્ચ ગેટ, મુંબઈ
- ડૉ. વિનોદ જાડા, મદદનીશ અધ્યાપક, સરકારી વિનયન અને વાણિજ્ય કોલેજ, બાબરા (જિ. અમરેલી) મો : ૯૭૩૭૧ ૨૫૬૯૮
- ડૉ. બળદેવ પ્રજાપતિ, ૬૫૩/૧, કિસાનનગર, સેક્ટર-૨૬, ગાંધીનગર-૩૮૨૦૨૭. સંપર્ક : ૯૪૯૦૦૩૮૯૦૧

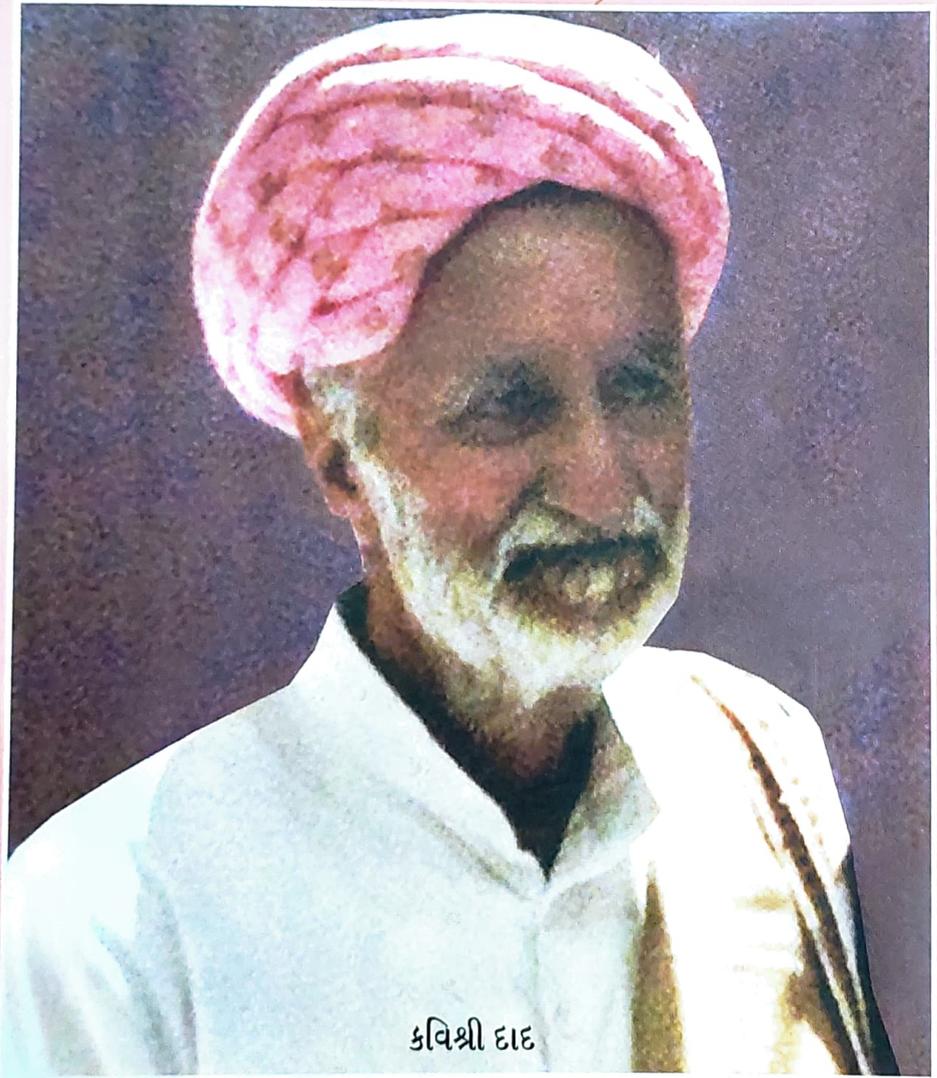
લોકગુર્જરી



સળંગ અંક : સાઠ

(ત્રૈમાસિક : દસમું વર્ષ, પહેલો અંક, જૂન-૨૦૨૧)

સંપાદક : ડૉ. બળવંત જાની



કવિશ્રી દાદ

શ્રી ઝવેરચંદ મેઘાણી લોકસાહિત્ય કેન્દ્ર

સૌરાષ્ટ્ર યુનિવર્સિટી, રાજકોટ-૫

અનુક્રમણિકા

વિભાગ - ૧ : લોકકથા સંશોધન

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| ૧. | ગુજરાતી લોકકથા : સંપાદનઅભિગમ
અને આલેખનપદ્ધતિ | ડૉ. બળવંત જાની | ૩ |
| ૨. | કાઠી-રાજપૂતવિષયક તથા
કાઠી-રાજપૂતઆલેખિત લોકકથાઓ | ડૉ. પુલકેશી જાની | ૩૬ |
| ૩. | પૂતળીબાઈનું સંપાદનકર્મ :
તપાસ અને તારતમ્ય | ડૉ. પ્રભુ આર. ચૌધરી | ૪૩ |

વિભાગ - ૨ : લોકસાહિત્ય સંશોધન

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| ૪. | લોકસાહિત્યસંશોધન :
અભિગમ અને અર્થઘટન | ડૉ. પિનાકિની પંડ્યા | ૫૩ |
| ૫. | સુચેતા ભાડલાવાળાનું
લોકસાહિત્યિક સંશોધન-સંપાદન :
ક્ષેત્રકાર્યકેન્દ્રી સૂઝ અને સમજ | ડૉ. પ્રતિભા પંડ્યા | ૬૮ |
| ૬. | લોકસાહિત્ય સમીક્ષા અને અભ્યાસ :
સામયિક લેખસૂચિ-૨૦૧૬ થી ૨૦૨૦ | ડૉ. કિશોર વ્યાસ | ૭૬ |
| ૭. | આપણું લોકસાહિત્ય :
વારસો અને વૈભવ | રાજુલ દવે | ૮૭ |
| ૮. | ‘અરજણિયો’ અને ‘જવાનડો’ :
લોકગીતોનો પાઠગત વિમર્શ | અરવિંદ બારોટ | ૯૨ |

વિભાગ - ૩ : સંતસાહિત્ય સંશોધન

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| ૯. | ‘સંત’ શબ્દ - લક્ષણ, પરિભાષા અને અર્થ | ડૉ. હસમુખ વ્યાસ | ૧૦૧ |
| ૧૦. | ‘સંતસાહિત્ય કી સમજ’ : સંતસાહિત્યની
તપાસનું પ્રભાવપૂર્ણ પગલું... | ડૉ. મનોજ રાવલ | ૧૧૧ |
| ૧૧. | જૈનસંત કવયિત્રી રતનબાઈ, રેંટિયો
અને ગાંધીજી | ડૉ. સંજય મકવાણા | ૧૧૭ |

વિભાગ - ૪ : ચારણીસાહિત્ય

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| ૧૨. | ‘લછનાયન’ : પ્રતિભાવંત સર્જકની
શબ્દકલાનું ઊંચું શિખર | ડૉ. વિનોદ જોશી | ૧૨૯ |
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સુચેતા ભાડલાવાળાનું લોકસાહિત્યિક સંપાદન-સંશોધન : ક્ષેમકાર્યકિન્દ્રી સૂઝ અને સમજ

ડૉ. પ્રતિભા પંડ્યા

લોકસાહિત્યના જાણીતા વિદ્વાન પુષ્કર ચંદરવાકર પાસે પીએચ.ડી. પદ માટે મહાનિબંધલેખન દરમ્યાન ક્ષેત્રકાર્ય દ્વારા લોકસાહિત્યિક સામગ્રીનું એકીકરણ થયત અને સંપાદકીય સૂઝસભર અભ્યાસથી આપણે ત્યાં બહુ વહેલા પોતાના અભ્યાસ દ્વારા પ્રદાન કરનારાં સુચેતાબહેન વિશે બહુ અભ્યાસ થયા નથી. આંછા જાણીતા, પણ પોતાની લોકસાહિત્યિક સમજથી પરિપક્વ પ્રદાન કરી શકેલા સુચેતાબહેનના સંશોધન-સંપાદનક્ષેત્રના કાર્યનો પરિચય કરાવવાનો ઉપાય આહી યોજ્યો છે.

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આતમી જુલાઈ ઈ.સ. ૧૯૪૨માં જામનગરમાં જન્મેલાં સુચેતાબહેન જામનગરમાં જ એ. કે. દોશી મહિલા કોલેજમાં નિવૃત્ત થયાં ત્યાં સુધી અધ્યાપકીય કાર્ય કરેલું. તે પહેલાં ઈ.સ. ૧૯૬૫થી ઈ.સ. ૧૯૭૧ સુધી જામનગરમાં આતમી કોલેજ ટાઈટેકુલમાં શિક્ષિકા તરીકે કરજ બજાવેલી. તેમણે ગુજરાત યુનિવર્સિટીમાંથી પુસ્તક એલએલ.બી.નો અભ્યાસ પણ કર્યો છે. તેમણે સૌરાષ્ટ્ર યુનિવર્સિટીમાંથી પુસ્તક ચંદરવાકરના માર્ગદર્શન હેઠળ 'હાલારના માલધારીઓનું લોકસાહિત્ય અને તેના લોકસંસ્કૃતિ સાથેનો સંબંધ' પર પીએચ.ડી.ની પદવી પ્રાપ્ત કરેલી. તેઓએ

પરિસંવાદોમાં ત્યાગ લઈને પોતાની સક્રિય ત્યુમિકા પણ ભજવી છે. તેમજ અનેક સંસ્થાઓ સાથે જોડાયેલાં સુચેતાબહેન એક સંનિષ્ઠ અધ્યાપક તરીકે વિદ્યાલયોમાં લોકપ્રિય હતાં. તેમની પાસેથી લોકસાહિત્ય સંપાદન-સંશોધનના બે ગ્રંથો પ્રાપ્ત થયા છે :

- (૧) માલધારી જાતિનાં સંસ્કારગીતો — ઈ.સ. ૧૯૭૯
 - (૨) હાલારની માલધારી જાતિના રાસશ — ઈ.સ. ૧૯૮૨
- આ બન્ને ગ્રંથોને આધારે સુચેતાબહેનના લોકસાહિત્ય-સંપાદન-સંશોધનક્ષેત્રે પ્રદાનને મૂલવવાનો ઉપક્રમ છે.

(૧) 'માલધારી જાતિનાં સંસ્કારગીતો' :

સુચેતાબહેન પાસેથી ઈ.સ. ૧૯૭૯માં પ્રસ્તુત ગ્રંથ પ્રાપ્ત થાય છે. જેમાં માલધારી જાતિનાં સંસ્કારગીતોની વિશેષતા, ખોળાભરણાનાં ગીતો અને હાલારના માલધારી જાતિનાં લગ્નગીતો, લગ્નસંસ્કારો, ખોળાપણાનાં ગીતો અને કટાણાંની સમાવેશ થાય છે. પ્રસ્તુત ગ્રંથને પાંચ પ્રકરણો અને ચાર પરિશિષ્ટોમાં વિભાજિત કરવામાં આવ્યાં છે. જેમાં અનુક્રમે માલધારી જાતિનાં સંસ્કારગીતોની વિશેષતા, ખોળાભરણાનાં ગીતો અને હાલાર, માલધારી જાતિનાં લગ્નગીતો અને લગ્નસંસ્કારો, ખોળાપણાનાં ગીતો, કટાણાંનાં ગીતો છે તો પરિશિષ્ટમાં અનુક્રમે માલધારી લોકબોલીનો શબ્દકોશ, સંદર્ભગ્રંથસૂચિ, ફોટો, ચિત્રો અને નોંધ અને બીજાં જામનગર જિલ્લાનો નકશો આપેલ છે. આ રીતે પુસ્તકનું સુનિયોજિત માણખું જ દર્શાવે છે કે આ શાસ્ત્રીય રીતે થયેલું સંશોધનકાર્ય છે.

સુચેતાબહેન પ્રારંભે જ 'કંઈક કહેવું છે'ના નામાભિધાન સાથે ધણુંબધું ઉપયોગી કહે છે. તેમની આ વાત ચાલીસ પૃષ્ઠો સુધી વિસ્તરે છે અને તેમાંથી જ ઊણાણાહારની માલધારી જાતિની વિશેષતા, તેના સામાજિક-સાંસ્કૃતિક સંદર્ભો સુપેરે આમી શકાય છે. પ્રારંભે તેમના શબ્દોમાં જ જોઈએ : "લોકશાસ્ત્રના એક અંગ તરીકે માલધારીએ લોકસાહિત્ય અને તેમાંથી પ્રતિબિંબિત થતી તેમની લોકસંસ્કૃતિનું સામાજિક પદ્ધતિએ અધ્યયન અને સંશોધન શરૂ કર્યું. ક્ષેત્રીય સંશોધનમાં હાલાર દેશને પૂંદી વળી અને જે કંઈ લાખું એ એટલું તો વિપુલ હવું કે એક મહાનિબંધમાં 'જે સામગ્રીનો સમાવેશ ન થઈ શક્યો તે અને મહાનિબંધ માંડેની સામગ્રીને લેખ પુસ્તક રૂપે અને પ્રગટ કરું છું. ક્ષેત્રીય સંશોધનકાર્ય માટેના પ્રવાસ દ્વારા આ લોકજાતિનું લોકસાહિત્ય વિપુલ પ્રમાણમાં એકઠું થયું છે. તેમના ભિન્ન ભિન્ન સંરેખના લોકસાહિત્યને ક્રમશઃ સંપાદિત કરી પ્રગટ કરવાનું વિચાર્યું છે. અહીં

તેમાંથી તેમનાં લોકગીતોનું વર્ગીકરણ કરીને, માત્ર એક જ પ્રકારનાં લોકગીતો સંસ્કારગીત ગ્રંથસ્થ કરીએ છીએ.” (પૃ. ૧, ૨)

આવાં ચાલીસ પૃષ્ઠોમાં વિસ્તરેલી રસપદ, સંશોધનપદ, ઐતિહાસિક પાર્શ્વભૂ, માલધારીઓની માત્મોમનાં રૂપદર્શન, ભૂમિપરિચય, મુખ્ય પેદાશ, ખનિજસંપત્તિ, ઉદ્યોગ, હવામાન, નિરાળી લોકજાતિ અને તેની નિર્ધારિત લોકસંસ્કૃતિ, રબારીજાતિ, ભોપારબારી, પહેરવેશ, અલંકારો, સોરઠિયા, રબારી પહેરવેશ, અલંકારો, ભરવાડ, વજ્રાલંકારો, અગારવચ્છા ચારણ, સૌરાષ્ટ્રનાં ચારણોનું આગમન, અગારવચ્છા કેમ કહેવાયા ? વજ્રાલંકાર-આટલા મુદ્દાઓમાં ચર્ચા-વિચારણા પ્રાપ્ત થાય છે. કેટલીક વિગતો તો ખૂબ જ રસપદ અને અવ્યાસીઓને આકર્ષે એવી છે. દા.ત. હાલાર નામ કેમ પડ્યું ? તેની વિગત આ રીતે મળે છે : “મમ રાવળે યુદ્ધયાતુર્યથી જુદા જુદા પ્રદેશ પર વિજય મેળવીને અને દેદા તથા ચાવડાઓને મચ્છુ નદી સુધી નસાડ્યા, વાઘેલાઓને ઓખાનું સ્થ આળંગાવ્યું અને આખા પ્રદેશને પોતાની સત્તા તળે લીધો ને પોતાના વંશ હાલાજના નામ પરથી આ પ્રદેશને હાલાર નામ આપ્યું. એ વિશે કચ્છી બોલીમાં અને હાલારી બોલીમાં લોકકૃત્તિઓ પ્રચલિત છે. જુઓ :

“વાઢેર વાલો વટંદા, જેઠવા બરડે બાર,
વાલા વીણ વટંદા, હદ ચોવાઈ હાલાર.” (પૃ. ૪)

તો હાલારની ભૌગોલિક સમૃદ્ધિથી આકર્ષાઈને ઘણી જાતિઓએ હાલારમાં સ્થળાંતર કર્યું છે. તે વિશે લોકબોલીમાં એક દુહો પ્રચલિત છે. જુઓ :

“અક દઈને તાદો બોલ્યો, આંધ વાવ્યું ઊગે,
હાલો જઈ જમને કંડોરણે, સરપેય ન સૂંઘે.” (પૃ. ૫)

ગોંડલ રાજ્યના વતની તાદા પટેલને રાજથી મનદુઃખ થયું. આથી તાદા પટેલે રાજને આ દુહો કહ્યો અને હાલારમાં આવી વસવાટ કર્યો. આ રીતે અનેક જાતિઓએ હાલારમાં આવીને વસવાટ કર્યો જેમાં પશુપાલક લોકજાતિમાંથી રબારી, ચારણ, ભરવાડ અને મતવાનો સમાવેશ થાય છે. આલેય અને ભરડાનાં જંગલોમાં નેસ બાંધીને રહેતા પછાતવર્ગના ભરવાડ, ચારણ અને રબારીઓને આ જિલ્લામાં અનુસૂચિત જનજાતિમાં ગણવામાં આવે છે.

હાલાર પ્રદેશમાં આ માલધારીઓ બરડા અને આલેયના ડુંગરાઓમાં નેસડા બાંધીને વસે છે. હાલારમાં માલધારીઓની મુખ્યત્વે ત્રણ જાતિઓ વસે છે : (૧) રબારી, (૨) ભરવાડ, (૩) અગારવચ્છા ચારણ.

રબારીઓ બરડામાં આવેલમાં અને બારાડી પ્રદેશમાં નેસડા બાંધીને વસે છે અને તેમનો મુખ્ય ધંધો પશુપાલનનો છે. તેથી માલના ચરિયાણ પાણી માટે પ્રસંગીયાત તેમને અસ્થિર જીવન ગાળવું પડે છે. સૌરાષ્ટ્રમાં રબારીઓની છ પ્રકારની વસે છે. તેઓ એકબીજા સાથે ખાનપાનનો વહેવાર રાખે છે. પણ જાતિઓ વસે છે. તેઓ (૧) ભોપા, (૨) સોરઠિયા, (૩) વિણીયા એ ત્રણ વનસંબંધ બાંધતા નથી. (૧) ભોપા, (૨) સોરઠિયા, (૩) વિણીયા એ ત્રણ મુખ્ય જાતિ છે. તેમાંથી ભોપા અને સોરઠિયા રબારીઓ હાલારમાં વસે છે. ભોપા રબારીઓ કચ્છમાંથી જામ રાવળની સાથે આવેલા. તેઓ નાના માલ એટલે કે ઘેટાં, બકરાં ઉછેરે છે. ઊંટ પણ રાખે છે તો બરડા અને આલેયના ડુંગરાઓમાં સોરઠિયા રબારીઓની મુખ્ય વસ્તી છે. તેઓ ભેંસો અને ઊંટો રાખે છે. આ રબારીઓ ગામની બહાર જ વસે છે. તેમના વિશે એક કહેવત છે,

‘ગામથી રબારી કેડો બાર...’ આ લોકજાતિમાં બાળલગ્ન, પુનઃલગ્ન અને દિયરવટાના રિવાજો છે. તેમની ધાર્મિક વિધિઓ લગભગ હિન્દુઓની વિધિને મળતી આવે છે. તેઓ પશુપાલન પર નિર્ભર છે. ભરવાડ જેઓ પોતાને નંદમોહનના વંશજ કહેવારાવે છે તેમ જ પોતાને શ્રીકૃષ્ણના ગોવાળિયા માને છે. તેઓ શ્રીકૃષ્ણ સાથે સ્થળાંતર કરીને નીકળ્યા હતા. પ્રારંભે કેટલાક ભરવાડો બનાસકાંઠા રોકાઈ ગયા. બનાસકાંઠે ભરવાડોનો જે સમૂહ રોકાઈ ગયો તેનાં બે કુળ થયાં : (૧) નાનાભાઈ (૨) મોટાભાઈ. નાનાભાઈ ભરવાડ, આલાવાડ ગોહિલવાડ જિલ્લામાં જોવા મળે છે. જ્યારે મોટાભાઈ હાલાર પ્રદેશમાં વસ્યા છે. હાલાર પ્રદેશમાં વસતા મોટાભાઈ ભરવાડો ગાડર-બકરાં રાખે છે અને ઊનનો ધંધો કરે છે. તેઓ સ્થિર જીવન જીવનારી જાતિ છે. ઊન વેચીને પોતાનું ગુજરાન ચલાવે છે. અગરવચ્છા ચારણ-ચારણો પોતાની ઉત્પત્તિ શિવથી ગણાવે છે. કહે છે કે શિવે ઢોર ચારવા માટે તેમને ઉત્પન્ન કરેલા છે. ચારણોના સાડાત્રણ પાડા છે : માટું, પરાજિયા, સોરઠિયા ને અડધો પાડો અગરવચ્છા ચારણોનો છે. તેઓ જામરાવલ સાથે ઈ.સ. ૧૫૩૫માં સગરકચ્છમાંથી મોટી બની થઈને હાલારપ્રદેશમાં આવ્યા અને હાલમાં તેઓ ઓખા અને બારાડીમાં વસે છે. અસ્થિર જીવન જીવનારી આ લોકજાતિ છે તેથી તેમનું નિવાસસ્થાન સ્થાયી હોવું નથી. તેઓ પાંચ-પંદરના જૂથમાં ઝૂંપડાં બાંધીને રહે છે. ઝૂંપડાને ‘ઝોડ’ અથવા ‘નેસ’ કહે છે. જ્યાં સુધી ઢોરને ચારવાનો ચારો આસપાસમાં મળી રહે ત્યાં સુધી તેઓ તેમના નેસમાં રહે છે, પરંતુ ધાસપાણીની તંગી થતાં નેસ છોડીને જ્યાં ધાસચારો હોય ત્યાં કામચલાઉ ઝૂંપડાં બાંધીને રહે છે. તેને ‘નેખમ’ કહે છે. વળી પોતાના નેસ આગળ ધાસચારો પૂરો થતાં તેઓ પાછા ચાલ્યા જાય છે. આ રીતે ધાસચારાની અનુકૂળતા લક્ષમાં રાખી પરકું જીવન ગાળે છે. હાલાર પ્રદેશમાં જોવાડની આસપાસ તેમજ જામનગર

લોકગુણસંહ્યા

તથા બારાડીના પ્રદેશમાં ઠેરઠેર આ લોકો વસે છે. તેઓ માલમાં મોટો માધ્યમ રાખે છે. સ્થળાંતર કરતી વખતે બળદ તથા ગધેડાંનો ઉપયોગ કરે છે. તેમના માલની સાથે બળદ તથા ગધેડાં પણ હોય છે. આ રીતે સુચેતાબહેન પ્રેમ કરતાં નોંધે છે : ‘સૌરાષ્ટ્રના બીજા પ્રદેશો કરતાં હાલારમાં હુંગરાઓ, મદિયા અને રસાળ જમીન હોવાથી માલધારીઓના માલને ચરિયાણ પાણીની સુવિધા પણ છે. તેથી આ પ્રદેશમાં વધુ ને વધુ માલધારીઓનો વસવાટ થયો છે. સાથે સાથે આ લોકજાતિની એક આગવી લોકસંસ્કૃતિનું મંડાણ પણ આ ધરતી પર થયું છે. આજેય નિરાળી લોકસંસ્કૃતિની શાખ પૂરતો બરડો સોરઠી રબારણો હલકામાં ગીતોથી ગાજે છે. તો બારાડી પ્રદેશ ત્રોપી રબારણોનાં દીપણ શિલ્પથી ઝળહળ જોગવડનો પ્રદેશ — અગરવચ્છળ ચારણોના દુહા, છંદ અને કવિતના પડછંદ છે. માલધારીઓનાં વિવિધ લોકસાહિત્ય અને લોકકલાઓથી શોભતો આ પ્રદેશ. સૌરાષ્ટ્ર-ગુજરાતમાં માલધારી લોકસંસ્કૃતિની એક નવી જ ભાત ઉપસાવીને ગુજરાતે માલધારી સમાજની ઉમ્મશશીલ જીવનશૈલીને પરિણામે એમની લોકસંસ્કૃતિની ભાતો પણ નિરાળી છે. આગળ આ ગ્રંથમાં હાલારના માલધારી સોરઠિયા રબારી માતાજીની જે ‘સરજુ’ ગાય છે, તેના સ્વરની વાત કરીને તેની સમજ આપી છે.

સુચેતાબહેન ખૂબ અભ્યાસપૂર્ણ રીતે આ સંસ્કારગીતોને રચનાલક્ષિત ખોલી આપે છે. ત્યારબાદ ખોળાભરણાનાં ગીતો અને હાલારમાં માલધારી લોકજાતિમાં પ્રથમ ગર્ભાવસ્થા માટે ‘ખોખાભરણું’ શબ્દ પ્રયોજાય છે. તેની ચર્ચા માંડીને ગીતોની ચર્ચા કરી છે. અંતે લગ્નગીતો, ફટાણાં સાધાર ચર્ચા આપે છે.

પુસ્તકના અંતે પરિશિષ્ટોમાં માલધારી લોકબોલીનો શબ્દકોશ આપ્યો છે. જેના કારણે આપણને ગીતોને સમજવામાં તકલીફ પડતી નથી અને અંતે સંદર્ભગ્રંથોની સૂચિ આપવામાં આવી છે. પુસ્તકમાં વિધિ-વિધાનના ફોટોગ્રાફ પણ આપવામાં આવ્યા છે, જેના કારણે સાચા અર્થમાં પામી શકાય છે. આ રીતે જોઈએ તો સુચેતાબહેન પાસેથી શાસ્ત્રીય અભ્યાસ પ્રાપ્ત થાય છે.

(૨) ‘હાલારની માલધારી જાતિના રાસડા’ :

સુચેતાબહેન પાસેથી ૧૯૮૨માં પ્રસ્તુત ગ્રંથ પ્રાપ્ત થાય છે. જેમાં માલધારી જાતિનાં લોકોત્સવો અને લોકનૃત્યો, કાનુકાનાં લોકગીતોના રાસડા, સામાજિક

સુચેતા આડકલાવાળનું લોકસાહિત્યિક મંથન-સંશોધન... લોકગીતોના રાસડા, પારિવારિક લોકગીતો-રાસડા, જાનુકીનીના રાસડા, રાસડામાં ગવાતાં લોકગીતોની પરિશિષ્ટોનાં સમાવેશ થાય છે.

સુચેતાબહેન આરંભે જ પ્રસ્તાવના રૂપે આ લોકગીતોના રાસડા શીર્ષક સાથે પોતાને આગળપુકાથી જ લોકગીતો અને રાસડા તે ક્રમશઃ કરી રીતે ધાર્યું તેની વાત કરે છે. તેમના અભ્યાસે જ્યાં સુધી ઉંચાર વાર્તા ન સંભળાવે, એકાદકે જમીન ન આવે !” આમ લોકસાહિત્યનો મને પરિચય થયો : ગિનના વ્યવસાયને લીધે માલધારી જાતિનાં જોડાણો પરિણામે ચિત્તમાં સૂક્ષ્મ રૂપે પડેલો લોકસાહિત્યનો સંસ્કાર જાતિઓના લોકસાહિત્ય અને લોકસંસ્કૃતિને નાશવા (પૃ. ૦૫). માલધારી જાતિનો મુખ્ય ઉત્સવ જન્મારજી રબારીઓ એ ઉત્સવને ‘કાનુકા’ કહે છે. પરંતુ આ ઉત્સવ છે. આ જન્મારજીની ઉત્સવ એ માલધારી જાતિનું દિવસથી આ ઉત્સવની તૈયારી થાય છે. સેનાના લોકનારીઓ રાસડાની રમકાટ ખોલાવવા લાગે છે. રાતો રાસડાની રમકાટ રૂપાળી અને સ્ત્રીલી મને પ્રસ્તુત ગ્રંથમાં સંપ્રાહિત કરવામાં આવ્યા છે.

પ્રકરણ એકમાં માલધારી જાતિના લોકો અભ્યાસમૂલક ચર્ચા કરવામાં આવી છે. જેમાં ઉત્સવો કરતાં નોંધે છે : ‘કૃત્રિ અને કૃત્રીઓના ઉત્સવ હતા. સાથે સાથે ઉલ્લાસ અને આનંદ મળવાવા ક્રિયાઓ આપોઆપ પ્રગટતાં આ જ વિચારથી તેમને આનંદ હતા અને તેમાં સમાજજીવનનું ચિત્ર મૂકીને આનંદ લોકજાતિઓના તકા પ્રકારના લોકોત્સવો ઉત્સવ પ્રકૃતિપૂર્ણના ઉત્સવો (૩) કૃત્રીઓના ઉત્સવો અલગ એક લોકનૃત્યની સમજ આપે છે. જેમ કે લોકોત્સવ લોકમેળાનો ઉદ્દેશ, તેમના લોકમેળા સંકર્ષે એક

‘સાત સાત સાંધેરની ઢોળો, જેલધારીનો મૂઓ, આરે જવે

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A study on the influence of metal (Fe, Bi, and Ag) doping on structural, optical, and antimicrobial activity of ZnO nanostructures

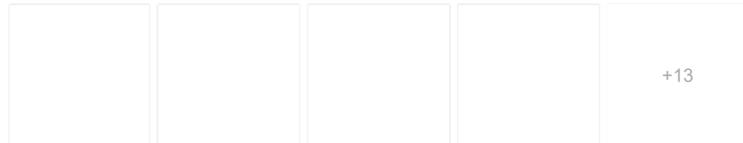
 SpringerDecember 2020 · [Advanced Composites and Hybrid Materials](#) 3(1)DOI: [10.1007/s42114-020-00174-0](https://doi.org/10.1007/s42114-020-00174-0)Project: [Nanocomposite materials for Wastewater Treatment](#)

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Abstract and Figures

In present work, the doping of ZnO nanoparticles has been achieved successfully by the addition of intentional impurity in the ZnO lattice by two transitions and one other metal namely iron, silver, and bismuth, respectively ($Zn_{1-x}O-Fe_x$, $Zn_{1-x}O-Bi_x$, and $Zn_{1-x}O-Ag_x$; $x = 10\%$). The aim of the work is to enhance the optical and antimicrobial efficiency of ZnO by doping with different metals and study the effect on structural, morphological, and optical properties. The as-synthesized pure and doped ZnO were characterized using advanced tools i.e. TEM, SEM, XRD, FT-IR, EDX, UV-DRS, BET, AFM, and Raman spectroscopy. The antimicrobial activity of pure and doped ZnO was tested on Gram-positive and-negative bacteria by agar disc diffusion assay. The significant change was observed in the optical and morphological properties of ZnO after doping clearly due to the influence of dopant ions. "The final publication is available at link.springer.com".



Flowchart of synthesis of... AFM of ZnO-Fe AFM of ZnO-Bi AFM of ZnO-Ag Raman spectra of pure and doped...

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*A study on the influence of metal (Fe, Bi,
and Ag) doping on structural, optical,
and antimicrobial activity of ZnO
nanostructures*

**Samreen Heena Khan, Bhawana Patha
& M H Fulekar**

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ISSN 2522-0128

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Citations (20)

References (81)

... The surface area of SnO₂ is in agreement to what is reported in literature (Mallikarjuna et al. 2020). Microporous materials are materials with pore sizes less than 2 nm, mesoporous materials have a pore size range of 2-50 nm, and macroporous materials have a pore size of 50-100 nm (Sameera et al. 2020). Mesoporous materials result in the accelerated transportation of the electrons which lead to more reactive sites and thereby achieving higher photoactivity (Chuanxin et al. 2021). ...

Cer-a-doped SnO₂ nanocubes for solar light-driven photocatalytic hydrogen production

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... This enhanced HER performance was attributed to the synergetic effects of Co, MoS₂, and rGO by high conductivity and interconnectivity, which formed abundant defects and accelerated the electron transfer. Several doping methods have been developed in recent years [44][45][46][47][48] [49]. Moreover, to overcome the poor charge transfer ability of transition-metal-doped MoS₂, carbonaceous materials (such as graphene, carbon nanotubes, rGO, etc.) have been widely applied in the fabrication of HER catalysts due to their large specific surface area, superior electronic conductivity, and good stability [33,[50][51][52]. ...

Direct growth of cobalt-doped molybdenum disulfide on graphene nanohybrids through microwave irradiation with enhanced electrocatalytic properties for hydrogen evolution reaction

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... Overall, the FESEM analysis indicates that the addition of Gly and oils to the BC/CMC composite membrane reduced the surface roughness. These results are in accordance with previous reports [44, 45]. ...

Development and characterization of plant oil-incorporated carboxymethyl cellulose/bacterial cellulose/glycerol-based antimicrobial edible films for food packaging applications

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... These observations are in accordance with previous reports of developing BC-based composites with Ag [40,56,57] or Ag-ZnO hybrid. [54, 58] The decreased intensity of cellulose peaks was further verified quantitatively by determining the crystallinity of pristine BC and BC/Ag nanocomposite. The relative crystallinities of pristine BC and BC/Ag nanocomposite films were found to be 69.51% and 52.77%, respectively. ...

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Groundwater for Sustainable Development

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Research paper

A hydrochemical and remote sensing approach to decrypt the groundwater salinization in the coastal district of Sabarmati basin, Gujarat

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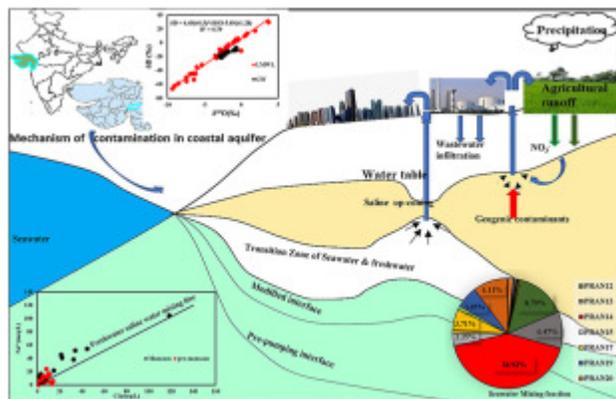
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Abstract

Coastal cities worldwide are witnessing large-scale salinity both inland as well as coastal salinity, especially in semi-arid regions. The economic aspiration and changes in land use associated with the excavation of unsustainable groundwater withdrawal is leading to saline up-coning as well as seawater intrusion in the coastal aquifers. The present study has been conducted in Anand district of Gujarat, India, to evaluate the genesis of salinity in the coastal aquifers. In the current study, both conservative elements and stable isotopes are used to explain the controlling factors of groundwater salinization and its hydrogeochemical processes. LISS-IV (Linear Imaging Self-scanning) was used to map land use land cover to understand the impact of land use practices on groundwater conditions and salinity mapping. From 2006 to 2018, the surveying and mapping of saline soil showed an increasing trend, which indicates high salinity in groundwater regime and soil characteristic. The chemical constituents coming from reverse ion exchange mineral dissolution as well as anthropogenic sources of saline water are demarcated using saturation index and ionic ratio. The origin of water salinity is described using $\delta^{18}\text{O}$ and δD , the relationship regression line below LMWL suggests significant evaporation of recharging water before infiltration.

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Introduction

Global land use/land cover change (LULC) has different natural hydrological imbalances. Land cover in any area affects evaporation as well as infiltration since the atmospheric precipitation passes through the soil media (Ford and William, 2013; Zhao et al., 2018). Various authors have reported the effect of LULC changes in hydrometeorological factors such as land surface temperature (Tan et al., 2017; Li et al., 2017), precipitation (Robertson and Sharp, 2015; Zhao et al., 2018), evapotranspiration (Bronstert et al., 2002; Raymond et al., 2008), recharge and runoff (Fletcher et al., 2013; Valle et al., 2014). This process is more intense in the semi-arid zone where evapotranspiration is more as compared to the humid zone which leads to negative water balance throughout the year (Gomez et al., 2017). Therefore, studying the water cycle pattern in these regions under different land uses is of great significance to address regional environmental issues related to hydrology (Marfia et al., 2004; Zhao et al., 2018). LU/LCC in Semi-arid alluvial plains is more significant due to productive land thus inland and coastal salinity both have a substantial effect on the aquifer. Several studies conducted in the coastal areas of India confirmed the salinity intrusion by using different geochemical, isotopic and numerical models like Maharashtra (Keesari et al., 2014; Omprakash and Gadikar, 2018), Odisha (Mohanty and Rao, 2019), West Bengal (Maity et al., 2017; Behera et al., 2019; Majumdar et al. 2014, 2016), Southern India (Thilagavathi et al., 2012; Singaraja et al., 2015; Satheeskumar et al., 2020; Sivaranjani et al., 2019), Gujarat (Chandrashankar et al., 1997; Singh et al., 2008; Desai and Desai, 2012; Rina et al., 2013; Maurya et al., 2019). There are multiple side effects of groundwater salinity on community health like increased cases of cardiovascular diseases, diarrhea, autoimmunity and abdominal pain are observed in coastal cities (Muller et al., 2019; Shammi et al., 2019).

The study area has been identified as the semi-arid coastal zone, so the coastal salinity is more noteworthy in groundwater (Smith and Compton, 2004; Duncan et al., 2005; Lorenzen et al., 2012;

Rina et al., 2013) along with inland salinity due to leaching of evaporites or the mineral weathering (Selle et al., 2010). In the coastal environment, aquifers are affected by marine evaporates (e.g. halite and gypsum), connate water (stored water in sedimentary rocks), or intrusion of seawater in freshwater aquifers (Weert et al., 2009; Rina et al., 2013) along with polluted seepage in the upper unconfined aquifer, or anthropogenic origin (e.g industrial activity and agricultural runoff) (Custodio, 2002; Calvache and Pulido-Bosh, 1997; Ghabayen et al., 2006; Trabelsi et al. 2007, 2012; Hchaichi et al., 2014; Carreira et al., 2014; Ouhamdouch et al., 2017). In density-dependent aquifers, natural discharge or abstraction wells may lead to up-coning of the saline water interface under low-pressure anomaly (Herzberg, 1901). The vertical up-coning and mixing of freshwater/saltwater by about 2–3% make it unsuitable for drinking and agriculture use (Bear et al., 1999; Gaaloul et al., 2012). The scenario of global groundwater overdraft indicates that exploitation of natural resource from shallow aquifers reduces the freshwater outflow towards the sea and create water table depression, causing seawater to migrate inland and rising towards well (Van Camp et al., 2014). The other responsible factors are lithology, coastal topography, groundwater recharge, and hydraulic gradient (Freeze and Cherry, 1979; Rina et al., 2013; Kazakis et al., 2016). Taken the view on account of groundwater abstraction through human activities, the groundwater is vulnerable to salinity in unconfined coastal aquifers (Werner et al., 2013).

In the past few decades, due to sporadic rainfall and high evapotranspiration, extensive research has been conducted on the salinity of arid and semi-arid coastal areas. The developmental activities are entirely dependent on groundwater in these regions. Therefore, the coastal water seems to be a complicated environment requiring multiple survey methods. The major chemical elements are the useful tracer at inland as well as coastal salinity (Vinson et al., 2013; Isawi et al., 2016; Li et al., 2016; Kumar, 2016; Najib et al., 2017). One such anion indicator is Br^- , which is widely used to distinguish the causes of salinity (Alcala and Custodio, 2008; Mollema et al., 2013; Li et al., 2016). The other combined applications of geophysical approach (Beaujean et al., 2014; Binley et al., 2015; Costall et al., 2018), numerical modeling (Dose et al., 2014; Surinaidu et al., 2015; Chang et al., 2018), geochemical indicators (Hernandez-Antonio et al., 2015), and isotopes are studied to characterize the brackish saline and freshwater origin and evolution (Guler and Thyne, 2004; Fernandez-Martinez et al., 2019; Kanagraj et al., 2018; Maurya et al., 2019; Bagheri et al., 2019). Multiple vulnerability assessment methods also provide encouraging results for the migration of pollutants in various types of aquifers (Motevalli et al., 2018). Among the available methods, the application of geochemistry and isotopes is an effective and simple way to quantify the origin of salinity in groundwater.

In the present study, LULC change detection intends to fill the gap between ongoing land-use effects on natural resources for both inland and coastal salinity using various hydro-chemical data in Anand district, Gujarat, India. Several problems of water quality had been identified like sea-level rise, declining water table and salinization makes it important to study (Rina et al., 2013; CGWB, 2014). LULC analysis shows that from 2006 to 2018, the population density doubled, thus the impacts are significantly important for the present study. This study also uses Cl/Br ratio and

stable water isotope to trace the origin of salinity of different water masses and various agriculture indices to see the impact of groundwater on soil salinity.

Section snippets

Study area

The present analysis is done in Anand district which is a part of the Sabarmati basin catchment area. Being a part of the outlet of the flow towards the coast, this area is having shallow aquifers near the coast and covers an area of 3092 sq km between north latitudes 22°06' & 22°43' and east longitude 72°20' & 73°12'. The lowest boundary is covered by the Gulf of Khambhat where river Mahi and Sabarmati are draining the district in east and west respectively. The region is known for its fertile ...

Material and methods

Groundwater samples (n = 33) were collected in Anand district for quality analysis from 33 operating wells in pre-monsoon dated May 13, 2018 and monsoon season on September 24, 2017 (Fig. 1a) in the vicinity of agriculture, coastal, and built-up areas. Since there are only a limited number of wells near the coast, tube wells and stable canal drainage are used to provide water for operational activities. The samples are collected in a clean polypropylene bottle, treated with 5% concentrated HNO₃...

Effect of changes in LULC on water regime

The changes in LULC have a significant effect on the water regime based on altered energy and hydrological balance (Quesada et al., 2017). The LULC change impact was assessed on the quality of groundwater. In the present study, eight LULC classes were demarcated during the year 2006–2018 as given in Table 1 in supplementary data and the map is given in Fig. 2. The accuracy statistics and kappa coefficient were computed which demonstrated a good correlation between the referenced and classified...

Conclusion

Inland and seawater salinity has led to the deterioration of groundwater quality in coastal cities which affects the availability of useable groundwater and has forced the closure of water supply at coastal areas. Hydrogeochemical and isotopic analyses along with the changes in LULC were applied to provide insights into regional hydrogeological processes and the degree of

groundwater salinity in the coastal aquifers of the Sabarmati River basin. The concentration of nitrate was found to cross...

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper....

Acknowledgment

The author Dr. Rina Kumari is thankful to the SERB, India for providing the ECR grant (ECR/2015/000202). JRF Tanushree is grateful for the fellowship to carry out the research. Dr. Rina expresses a gratitude to Dr. R.D. Deshpande, IWIN Laboratory, PRL, Ahmedabad for providing isotopes analysis facility....

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Assessment of secondary metabolite profile and quantification method development for Lupeol and Caffeic acid by HPTLC in *Avicennia marina* pneumatophore roots

Vinars Dawane  , Bhawana Pathak

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Abstract

It has been strongly endorsed that mangroves should be considered as a treasured source for phytochemical ingredients with potential medicinal, environmental, agricultural, biotechnological and nanobiotechnological applications. They are able to produce lots of natural structures, significant enough for widely acceptable in today's scenario, to generate various commercial and valuable products. The pneumatophore roots of *Avicennia marina* reported many botanical reference materials and bioactive compounds that found to display countless applications in pharmacognosy, pharmacy, biotechnology and nanobiotechnology. In this present investigation, different fingerprinting methods were designed and developed for the specific class of phytochemicals and their patterns were studied by using HPTLC to check and understand the phytochemistry, qualitatively and quantitatively. As Lupeol and Caffeic acid were previously well-known compounds having various pharmaceutical and nanobiotechnological applicability respectively, so validated HPTLC methods for quantification has also been discussed and developed.

Keywords

Avicennia marina pneumatophore roots; HPTLC fingerprinting; Secondary metabolites; Quantification; Lupeol and caffeic acid

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Geospatial approach to evaluate the morphometry of Sabarmati River Basin, India

Pooja Kumari¹ · Rina Kumari¹ · Deepak Kumar²

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Abstract

River basins play an essential role in ecosystem stability; they receive water through either snowmelt or precipitation which helps in groundwater resource development. Thereupon, land and water are ecologically linked which sustain the livelihood and food security. Since the river flows from higher to lower elevation, it carries sediment, nutrients or pollutants along with water flow to the outlet of the basin via channels. From the last few decades, the rapid change in land-use practices has led to water scarcity especially in the semi-arid region where there is an imbalance between precipitation and evapotranspiration. Therefore, there is a crucial requirement for water resource planning. The present morphometric analysis work has been performed on the Sabarmati River Basin, which falls in the semi-arid region of western India. Digital elevation model data, geostatistical tools and ArcGIS have been used to explore the hydrological and geomorphic processes in the basin. The morphometric variables like relief and areal and linear aspects were calculated to delineate the basin's drainage network. The area showed a dendritic drainage pattern with drainage density of 0.42 km^{-1} which infers the less permeable surface of the basin with thin vegetation and moderate to high elevation. The highest stream order of the basin is seventh order with an area of $31,921 \text{ km}^2$ and basin length of 481.75 km . The circulatory ratio of 0.18 and low-form factor of 0.14 infer the basin's elongated nature which increases the chances of water infiltration. This work will assist the decision-makers for allocation of artificial recharge points to reduce runoff in the basin for sustainable soil and water resource planning.

Keywords Drainage network · Sabarmati basin · Semi-arid region · Aster-DEM · GIS

Introduction

Semi-arid and arid regions cover around one-third of the total land surface area of the Earth (FAO 2019) and water shortage is a severe issue in such areas due to lower replenishment than consumption. India, especially western India (semi-arid regions), receives intense solar radiation which makes it more vulnerable to scarce and unreliable rainfall coupled with extreme temperatures, strong wind regions and high potential evaporation resulting in a low groundwater table and also influencing the phases of the hydrological cycle. Moreover, India has been observed vulnerable to water scarcity due to its

low gross per capita of water availability of $1800 \text{ m}^3/\text{year}$ in 2001, which is in the verge of shrinking to about $1100 \text{ m}^3/\text{year}$ in 2050 as per Gupta and Deshpande (2013). During the last few years, there has been a drastic variation in the rainfall pattern with fewer rainy days due to climate variability. Further, land-use and land-cover variation affects conventional food production and apparently water balance in the semi-arid tropics. Being an agro-centric country, India has the highest irrigated land area and it is expected to increase for agricultural purposes in future with the population outburst. Therefore, there is a requirement for sustainable water resource management plans to facilitate the water at a human timescale.

River basins are important fluvial landforms whose features are controlled by active tectonic activity. The extraction of the drainage network from a basin uncovers the linkage among surface activity and structural changes (Burbank and Anderson 2001). Some researchers have focused on proximity between stream orientation and tectonics (Beneduce et al. 2004; Hodgkinson et al. 2006). In a river basin, physiography

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and climate govern the hydrological processes. So, it is crucial to identify basins' features and their hydrological significance. Various hydrological processes are correlated to physiographic features like size, aspect, shape and slope of the drainage area (Gregory and Walling 1973; Magesh et al. 2012). The present work focuses on morphometry of the Sabarmati basin which is situated in the western semi-arid part of India having a total population of 3,69,08,052 (2011 census) with a per capita average annual water availability of 308 m³ (10,900 cu ft), which is significantly lower than the national average of 1545 m³ (54,600 cu ft) per capita (Central Water Commission 2016), due to scanty rainfall. The increasing demand by the growing population and the rapid industrialization have increased tremendous stress on the basin. So, there is an urgent need for water resource management for this water-scarce region.

Morphometry provides a quantitative assessment of the earth's surface characteristics and dimensions (Horton 1945; Strahler 1952a; Pareta and Pareta 2011). *A quantitative evaluation of the drainage network was proposed by Horton (1945) and revised by Strahler (1964) to evaluate the type, nature and empirical characteristics of the basin. The basin morphometry was governed by lithology, geology and tectonic activity as well as the climate of the study area* (Hack 1973; Cox 1994; Keller and Pinter 2002). *The morphometric characteristics play a prominent role in the dynamic nature of runoff and hydrologic phenomena like mass movement and flooding* (Eze and Efiog 2010) *which depends upon soil physical properties at the basin or watershed scale* (Dar and Bhat 2013). It also helps to realize the catchment behaviour and their hydrological implication, watershed prioritization and surface water potential (Astras and Soulankellis 1992). In the last few decades, many authors have described the role of drainage networks (Horton 1945; Leopold and Maddock 1953; Abrahams 1984) and various methods to quantify morphometric parameters (Schumm 1956; Nookaratnam et al. 2005). Earlier, conventional methods included topographic maps or field observation data for drainage extraction. However, with the advancement of geostatistical technique and remote sensing datasets, it is possible to reduce time and effort along with high accuracy (Verstappen 1983; Rinaldo et al. 1998; Bertolo 2000; Macka 2001; Ozdemir and Bird 2009). Consequently, digital elevation model (DEM) datasets are considered as a very exceptional and efficient method for catchment area delineation and drainage pattern extraction. Moreover, the integration of GIS with remote sensing helps in monitoring the morphometric parameters (Jain et al. 1995). Morphometric constraints like areal, linear, and relief features of the channel network have been estimated to assess the hydrological regimen in the basin (Nag and Chakraborty 2003). In some cases, morphometric evaluations of major streams like the Ganga and Godavari with their respective parameters are strenuous and complex. In such cases, the

evaluation was restricted to limited sub-basins of the river tributaries or sub-tributaries with a lithological variation. Prabu and Baskaran (2013) extracted the drainage network of the Western Ghats sub-basin whereas Bhatt and Ahmed (2014) assessed the flood using Cartosat DEM in the uppermost Krishna basin. Bajabaa and Masoud (2014) used ASTER DEM to spot flash flood-prone areas and recommended possible mitigation strategies. Thus, GIS-based studies have been done for the hydrological response, basin characterization, and watershed prioritization (Abdel-latif and Sherief 2012; Romshoo et al. 2012; Patel et al. 2013; Wakode and Dutta 2013; Yadav et al. 2014). However, studies highlighting the basin drainage are few confined to some small regions of the Sabarmati basin. Patel focused on the Hathmati watershed to identify an appropriate location for water restoration using multi-criterion-based decision and morphometric analyses to combat soil erosion during excessive rainfall. Sinha et al. (2012) computed the morphometry of the Wakal River basin using LISS III satellite data and SOI toposheets. Thakkar and Dhiman (2007) prioritized mini-watersheds of the Mohr River basin which are primary tributaries of the Sabarmati River and recommended control action for soil erosion. Pancholi et al. (2017) quantified Sabarmati-Cambay River basin drainage and geomorphic change to analyse tectonic activities for understanding the seismicity pattern in the region. As the present work was done in the semi-arid region of the western part of Gujarat, there is steady water scarcity in the Sabarmati River Basin, primarily in the upper part of the basin. The Sabarmati River is monsoon fed, so for the most part of the year it faces water scarcity, with little or no flows post-monsoon. The varying elevation in the basin makes it prone to runoff as the upper basin is at a higher elevation than the middle alluvial plains and the lower estuarine zone. The present research is an effort to delineate the drainage feature of the Sabarmati River by geospatial tools and techniques, which will further help to mark artificial recharge points for water harvesting. Hence, this morphometry study of the Sabarmati basin will help to comprehend the landform's impact on the watershed. Furthermore, these will aid to develop a better developmental plan for sustainable freshwater management plans and to identify check places to prevent erosion and runoff.

Material and methods

Study area: general physiography and climate

The research area of the Sabarmati basin lies in tropical and semi-arid regions of the western part of India and is located between 70° 58' and 73° 51' E longitudes and 22° 15' and 24° 47' N latitudes (as shown in Fig. 1). The river extends from Rajasthan to Gujarat states and runs through a south-westerly

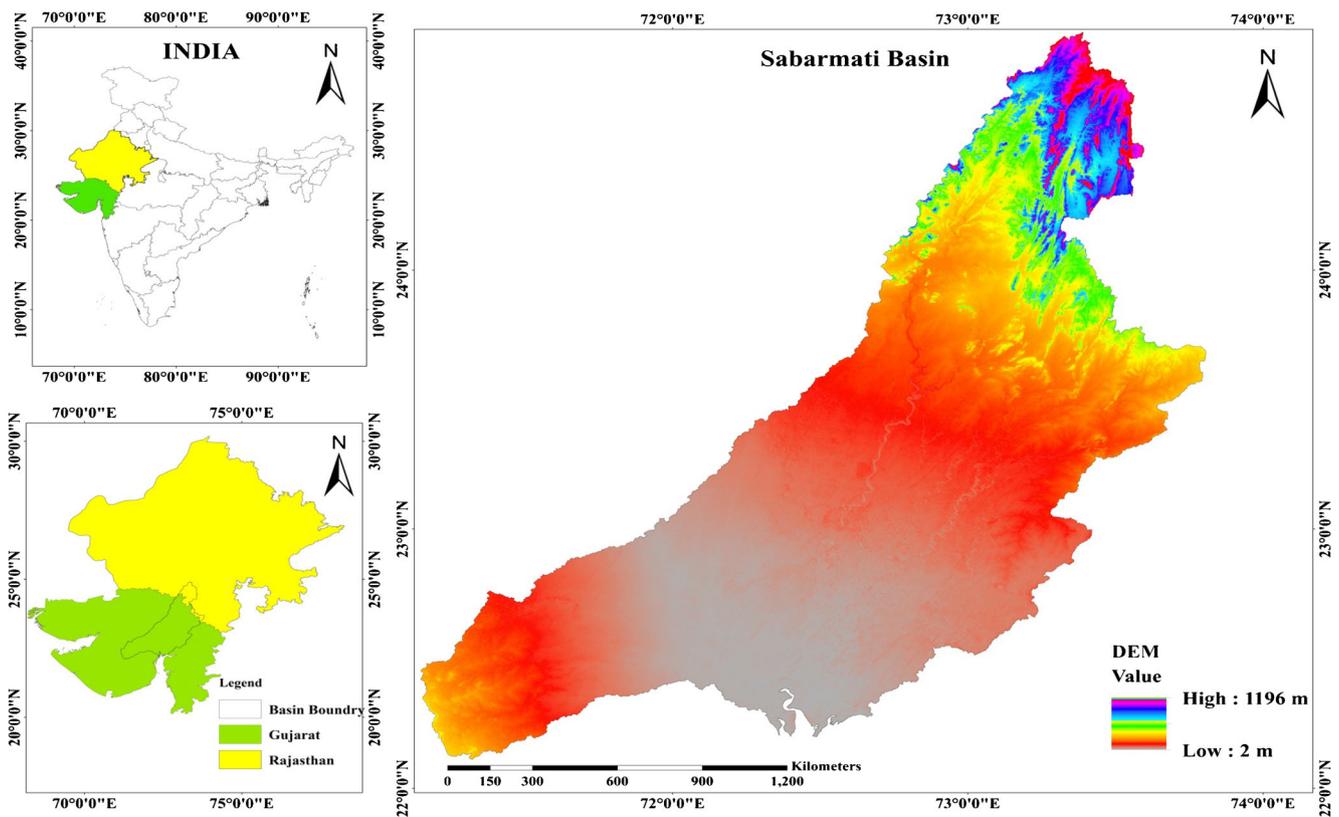


Fig. 1 Location map of the study area (Sabarmati basin)

direction with a total basin area of 21,674 km² and 371 km of total river length (Central Water Commission 2009). Sabarmati is a monsoon-fed river originating from Aravalli Hills with an altitude of 782 m above MSL (mean sea level) in the Udaipur district of Rajasthan state, and it traverses 48 km in Rajasthan and 323 km in Gujarat and finally meets in Bay of Khambhat of the Arabian Sea. The temperature ranges between 42 and 45 °C in summer during March to June, and in winter during October to February it has moderate temperature ranging from 6 to 12 °C. The magnitude of rainfall varies from region to region within sub-basins as low plains of the south-western parts receive low rainfall than the uplands. The average annual rainfall is approximately 750 mm (June to September); 80.7% and 19.03% area of the basin falls in Gujarat and Rajasthan, respectively. The basin outstretches in the Udaipur, Pali, Dungarpur and Sirohi districts of Rajasthan, as well as six districts of Gujarat, in Ahmedabad, Banaskantha, Gandhinagar, Kheda, Mehsana and Sabarkantha. The present basin has black, alluvial and sandy types of soil. The main tributaries are Wakal, Hathmati, Harnav, Vartak and Sei with their sub-tributaries in the basin. Guhai and Indrasi are two sub-tributaries of Hathmati. Shedhi, Khari, Mazam and Meshvo are sub-tributaries of Watrak. Sei and Wakal flow in the Rajasthan part of the basin in the south-west part parallel to the Sabarmati River up to their confluence with the river in

Gujarat (GOG 1996). Some reservoirs like Dharoi dam, Hathmati dam, Harnav dam, Guhai dam, Mazam dam and Watrak dam have been constructed to support major and minor irrigation projects, domestic purposes and industries (as per mowr.gov.in). The basin has hilly terrains in the early reaches (northern part) till Dharoi dam afterwards (southern part) where the river flows mostly in plains having a gentle slope. As per a study performed by Sareen, Tandon, and Bhola (1993), the Sabarmati basin exhibits a N-S to NNE-SSW topography at locations of alluvial plains with a regional slope of NE-SW topography in Gujarat due to non-tectonic action in the basin. The tectonic fault which originated during the cretaceous period is the East and West Margin Cambay, i.e. EMCBF and WM-CBF in the basin, respectively (Biswas 1987).

Data sets used, software and methodology

The data used for the current morphometric analysis of the Sabarmati basin includes ASTER DEM (downloaded from <https://earthexplorer.usgs.gov>.) with a 30-m spatial resolution and reference Survey of India (SoI) toposheet map at the 1: 250,000 scale with WGS UTM 1984, Zone 43N projection of the study area.

This work uses software like ERDAS Imagine 2014 for basic image processing and geoprocessing software like

ArcGIS 10.3 for basin demarcation and drainage network extraction with extensions like Spatial Analyst toolbox and Arc Hydro toolbox for related hydrological parameters of streams (Ahmed et al. 2010).

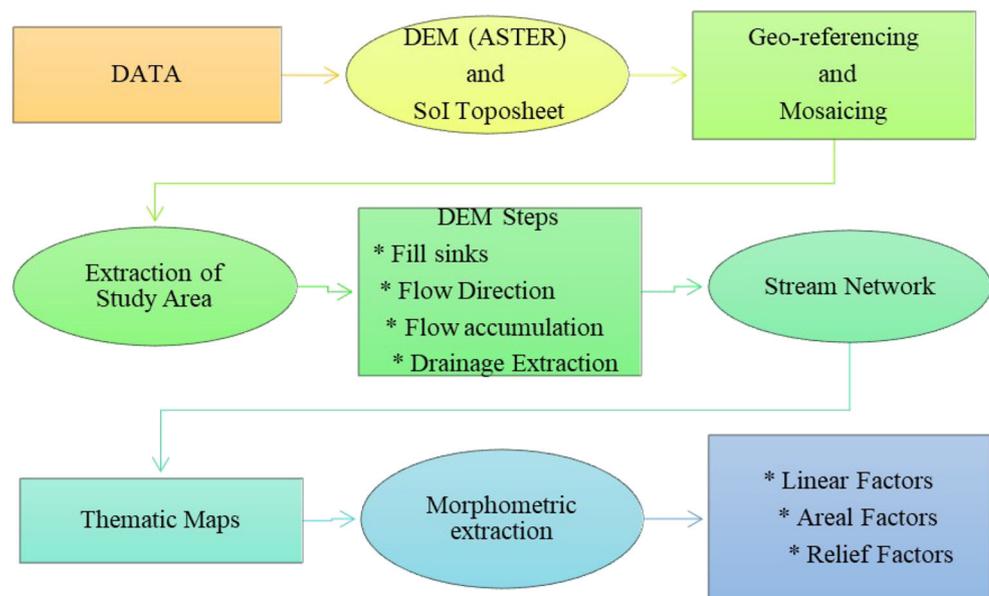
Different steps involved calculating drainage networks, and different aspects are shown in Fig. 2. It involves fill, flow accumulation, flow direction and stream order steps to generate a stream network. The automatic extraction of a drainage network is performed in ArcGIS software, and other related parameters are calculated manually. The Arc Hydro toolbox shows water current from one given cell into an adjoining cell, with the forth steepest slope assigned from 1 to 128 in various directions. The first step is filling the voids of DEM data using the hydrology toolset. The fill step is performed to remove or minimize unevenness or discontinuities in DEM elevation to estimate the proper stream network. Subsequently, the flow direction is estimated to discover stream flow for each cell in the landscape (Bhatt and Ahmed 2014). Then, flow accumulation is executed which displays the highest flow path of each cell on the terrain grid and the cells with flow accretion higher than the given threshold value were displayed in the stream networks (Thomas et al. 2014). The threshold value is required to maintain a minimum upstream drainage area, and in the present study threshold value the limit is 5000 (Gopinath et al. 2014). It is also a determining factor of total length and number of streams, as well as order of the basin (Quinn et al. 1995; Gandolfi and Bischetti 1997). Then, the stream network is extracted using a hydrology tool and different stream orders were assigned following Strahler's (1957) method. The total stream number and stream length are summarized for the different orders. Further, different parameters of each aspect are estimated and explained in 'Discussion'. Thus, basin geomorphic properties are regarded

as essential indices of surface processes. This helps in several studies related to surface-water hydrology and basin geomorphology (Jolly 1982; Ogunkoya et al. 1984; Breinlinger et al. 1993). The three key morphometric factors like linear, areal and relief aspects are analysed with the Hydrology toolbox of ArcGIS software, as suggested by various researchers like Strahler (1964), Schumm (1956) and Horton (1945). As per various researches performed by renowned scientists such as Horton (1932), Smith (1950). Hadley and Schumm (1961), Strahler (1964) and Mesa (2006), this study adopted different mathematical equations and formulae.

Results

The quantitative evaluation of the Sabarmati basin has been done by estimating various morphometric factors. These factors are classified into linear, areal and relief aspects. Linear aspects illustrate drainage channel patterns. The stream network properties are very significant; these comprise stream order, stream number, stream length, stream length ratio, bifurcation ratio, mean stream length and mean bifurcation ratio of the basin which have been determined for analysing basin features. Different areal morphometric parameters are area, perimeter, basin length, drainage density, stream frequency, form factor, drainage texture, infiltration number, length of overland flow and constant of channel maintenance. These areal parameters are computed by the formula suggested in different literature (Horton 1932; Smith 1950; Hadley and Schumm 1961; Strahler 1964; Mesa 2006). Different relief parameters basin relief (H), relief ratio (R_{hl}), relative relief (R_{hp}), absolute relief (R_a), ruggedness number (R_n) and slope have been derived. The estimated relief parameters were used

Fig. 2 Flow diagram showing the methodology followed for Sabarmati basin drainage extraction



to assess the denudation properties of the basin for predicting the degree of erosion. It impacts biodiversity as well as agricultural productivity of the area because received solar radiations rely on the aspect and reflection formed by topography.

Stream order (u)

For a hydrodynamic assessment of the drainage basin, a hierarchical arrangement of streams is fundamental. The stream hierarchy position in a basin is termed as stream order (Ali et al. 2016). Strahler (1964) proposed a well-versed method for stream ordering. The drainage pattern of the Sabarmati basin is a dendritic type as shown in Fig. 3a, with the highest order of seventh order as is shown in Table 1. Variation in stream order is mostly controlled by the physiographical, geomorphological and geological conditions of the basin (Rai et al. 2017; Mahala 2019). According to Ritter, Kochel, and Miller (1995), the prompt accumulator of rainwater is lower-order streams, which is also a better flood predictor. Thus, in the present basin, the stream-distributed area of the first order is maximum which can be used to allocate recharge points, and there are high chances of the overflow of water in higher-order streams which can be managed by forming small or large storage realms in such areas.

Drainage density (D_d)

As defined by Horton (1932), drainage density (D_d) is the total length of the stream per unit area which represents channel closeness or spacing. The drainage density map is generated using ArcGIS shown in Fig. 3b. Its value varies with relief, soil, vegetation and rock features, and the area with greater drainage density and stream frequency has more runoff. The semi-arid zone primarily has a finer drainage texture relative to humid regions. The obtained drainage density value of the basin is 0.42 km^{-1} as shown in Table 2. The values indicate effectual tectonic complexity, varying high reliefs and rocks of low-permeability properties of the basin which impact drainage density (Prasannakumar et al. 2013).

Slope

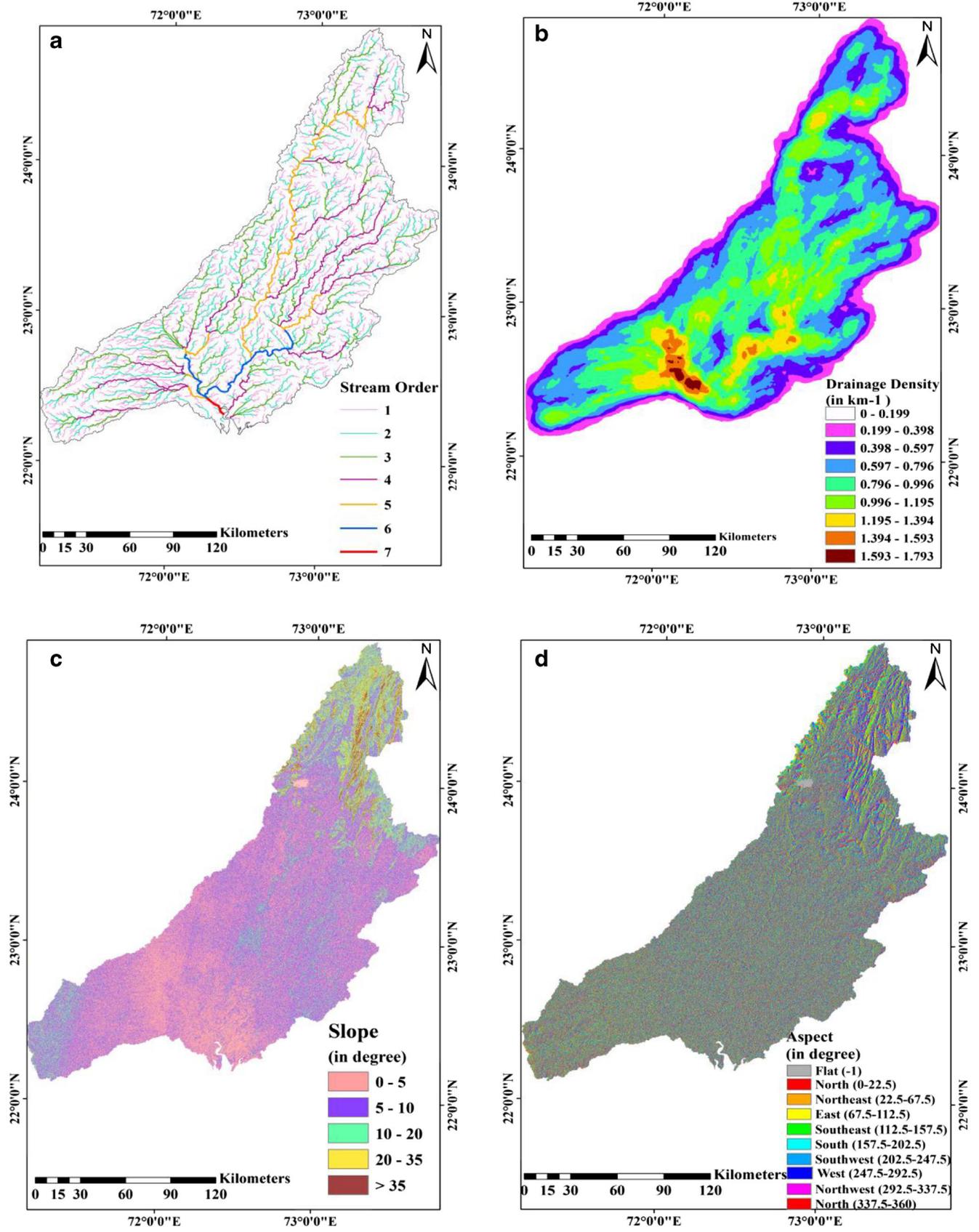
The slope plays a vital role as a significant parameter for geomorphic and hydrologic studies. The slope angle influences gravitational pull acting on a surface which produces changes in surface and subsurface flow (Strahler 1956) and impacts many earth processes (Wilson and Gallant 2000). It directly affects the permeation and runoff of any terrain. As per Wentworth (1930), the erodibility of a basin can be computed from its average elevation. Aster DEM data is being used to develop the aspect and slope map in the present analysis. Presently, the slope varies from 0° to 45° as shown in Fig. 3c. In the present work, the upper region of this basin has a steep slope than the middle and lower regions of the basin, and there will be high rates of erosion from the upper parts, causing siltation in the lower parts of the basin (Vittala et al. 2004; Samal et al. 2015). So, slope data can be used for planning settlements or structures by engineers, mechanization of agriculture and various conservation practices to combat the mass flow.

Aspects

Aspect applies to where the mountain slope faces. It is measured in degrees clockwise from the north and generally flat areas with an assigned aspect value of 1. It influences its local atmospheric condition as the sunlight remains in the west at the warmest period of daytime in the afternoon, and so in most cases, the area with the west-facing slope sensed warmer than the shaded east-facing slope (Magesh et al. 2011). It impacts biodiversity as well as agricultural productivity of the area because received solar radiations rely on the aspect and reflection formed by topography. The basin's aspect map was established using the ArcGIS Aspect tool. 0° is the true direction of the north, 90° is the dimension of the east, 180° in the south, etc. The basin aspect map created is shown in Fig. 3d.

Table 1 Generated Results obtained of linear aspects for the Sabarmati Basin

Stream order (u)	Stream number (N_u)	Stream length (L_u)	Stream length ratio (R_L)	Mean stream length (L_{sm})	Bifurcation ratio (R_b)	Mean bifurcation ratio (R_{bm})
1	3790	6419.38	0.52	1.69	2.04	--
2	1859	3322.71	0.58	1.79	1.69	--
3	1100	1929.07	0.54	1.75	2.22	--
4	496	1050.29	0.42	2.12	1.72	--
5	289	436.05	0.39	1.51	2.81	--
6	103	171.16	0.11	1.66	4.29	--
7	24	18.83	--	0.78	--	--
Total	7661	13347.48			14.76	2.46



◀ **Fig. 3** Generated map of the Sabarmati basin. **a** Drainage network with stream order of the basin. **b** Drainage density map of the basin. **c** Slope map of the Sabarmati basin. **d** Drainage density map of the Sabarmati basin

Stream number (N_u)

Stream number is composed of the total stream sum-up of individual stream orders (Horton 1945). Its formula is ($N_u = N_1 + N_2 + N_3 + \dots + N_n$). The stream number is negatively linked to the stream order as the stream number declines with the stream order increase which is shown in Fig. 4a. The stream numbers of different orders are calculated with various geospatial platforms. The Sabarmati basin has a total of 7661 streams, and out of it 3790 are of the 1st order, 1859 are of the 2nd order, 1100 are of the 3rd order, 496 are of the 4th order, 289 are of the 5th order, 103 are of the sixth order and 24 are of the seventh order, as shown in Table 1. It shows the maximum number of the first-order streams, and it decreases further with increasing order which will create more infiltrations in the stream of a lower order than successive orders in the basin by management.

Stream length (L_u)

Stream length is a cumulative sum of stream section lengths for each specific order ($L_u = L_1 + L_2 + \dots + L_n$) in a basin (Horton 1945). It is an expression of hydrological physiognomies of the bedrock. The total lengths of streams are 6419.38 km, 3322.71 km, 1929.07 km, 1050.29 km, 436.05 km, 171.16 km and 18.83 km respectively for the first-, second-, third-, fourth-, fifth-, sixth- and seventh-order streams as shown in Table 1. Usually, total stream length decreases with the increase in stream order as shown in Fig. 4b. It acknowledges surface run-off properties as smaller streams occur in high/steep slope areas with fine texture and longer streams found in low-lying/flatter areas. Thus, diverse lithologies and varying elevations across the basin create chances of more surface flow in irregular upper basin areas than in the lower areas.

Mean stream length (L_{sm})

As per (Strahler 1964), L_{sm} demonstrates the distinct structure of a drainage system and its respective stratum.

Its value is obtained by using formula $L_{sm} = L_u / N_u$ where L_u is the total stream length of order ‘u’ and N_u is the number of streams of particular stream order ‘u’. Its value varies due to slope, size and topography within the basin for different orders (Hajam et al. 2013). The values range from 0.78 to 2.12 which is shown in Table 1; this variation is probably due to changing topographic slope and elevation.

Stream length ratio (R_L)

Stream length ratio (R_L) is the ratio of the mean length of one order to the next lower order of the stream segment (Horton 1945). Its formula is $R_L = L_u / L_{u-1}$. The stream length ratio value varies inconsistently with elevation (Sreedevi, Subrahmanyam, and Ahmed 2005). In the current work, the R_L value varies from 0.11 to 0.58 as shown in Table 1. Its increasing value from lower-order to higher-order stream reflects the mature geomorphic development of the basin whereas the late youth stage of the geomorphic stage shows a decreasing trend. This study suggests the tectonically active nature of the terrain which affects the fluvial pattern of the Sabarmati River Basin which impacts discharge and sediment deposition.

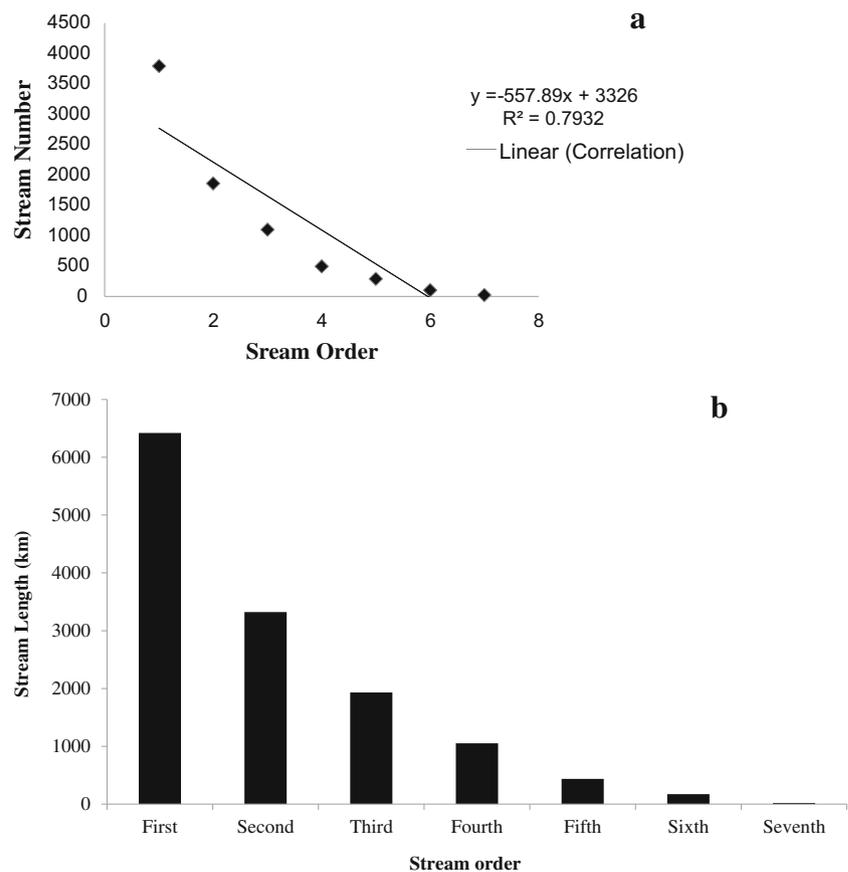
Bifurcation ratio (R_b)

As per Schumm (1956), R_b is a ratio of a given order’s stream number to the number of the next higher-order stream segments ($R_b = N_u / N_{u+1}$). The basin area with fewer structural disturbances has a lower bifurcation ratio values whereas a higher value indicates a major disparity between consecutive orders due to mature topography (Chandrasekar and Magesh 2014) and steep areas have a potential for flash flooding (Kumar et al. 2000). In this work, the value of the bifurcation ratio varies from 1.69 to 4.29 as shown in Table 1. The mean bifurcation ratio (R_{bm}) of the basin is 2.46, i.e. the average bifurcation ratios of all orders. The R_{bm} value suggests the need for structural control due to higher permeability and more geological complexity (Nag 1998), which can be done by more vegetation and check dam formation in the basin.

Table 2 Generated results of areal aspects for the Sabarmati Basin

Basin area (A) (km ²)	Basin perimeter (P) (km)	Drainage density (D_d) (km ⁻¹)	Stream frequency (F_s) (km ⁻²)	Drainage texture (T) (km ⁻¹)	Infiltration number (I_f) (km)	Constant of channel maintenance (C) (km)	Length of overland flow (L_g) (km)	Basin length (L_b) (km)	Form factor (F_f)	Circulatory ratio (R_c) (km)
31921	1503	0.42	0.24	8.88	0.10	2.39	1.20	481.75	0.14	0.18

Fig. 4 **a** Correlation plots of stream order vs. stream number. **b** Graphical representation of stream order vs. stream length



Stream frequency (F_s)

As per Horton (1945), it is the number of stream sections per unit area ($F_s = N_u/A$). It is also called ‘channel frequency’ or ‘drainage frequency’. It depends upon texture and lithological characteristics and mainly depends on rainfall (Veeranna et al. 2017). The present study area has 0.24 per km^2 of stream frequency which is shown in Table 2; therefore, there is a chance of recharge in more permeable sub-surface areas. The stream frequency and drainage density are rightly linked to each other, and their value increases with an increase in stream number.

Drainage texture (T)

Drainage texture (Horton 1945) is the ratio of total stream numbers of all orders and the perimeter (km) of the basin ($T = N_u/P$). It describes the comparative array of drainage lines to study geomorphology. It depends on the geology of terrains at different stages of development (Smith 1950). Very fine texture is present in unprotected vegetation and soft or weak-rock areas which give high erosion rates and lesser chances for groundwater recharge, whereas coarse texture is present in massive and resistant rocks

(Reddy et al. 2004). The drainage texture, as per Smith (1950), has been categorized into five distinct textures: very coarse (< 2), coarse (2 to 4), moderate (4 to 6), fine (6 to 8) and very fine (>8). Here, the basin’s drainage texture is 8.88 per unit area, as shown in Table 2, indicating that the basin has a finer texture and more chances of erosion but can be reduced by proper sustainable watershed management.

Length of overland flow (L_g)

It refers to the length of water channel flow over the surface prior to getting accumulated into the specific stream segment. Its value is ($L_g = 1/D_d * 2$) half reciprocal of drainage density (Horton 1945). Generally, streams and rivers receive water from rainfall or snowmelt. The low length of overland flow value shows moderate to abrupt slope and more surface runoff while higher (L_g) value indicates less structural disturbance with less runoff and gentle slope (Parveen and Kumar 2012). The estimated length of the overland flow value is 1196 km as shown in Table 2, suggesting a faster surface runoff because of the hilly terrain of the watershed and the runoff concentrate to form rills and gullies causing erosion.

Constant of channel maintenance (C)

The constant of channel maintenance (*C*) is the drainage density reciprocal (Schumm 1956). This represents the drainage area needed to sustain the channel unit length (Strahler 1957). It has the dimension of the length with the scale of the landform units. A low value is incorporated with weak resistance soils with mountainous terrain and sparse vegetation consequently where water discharges rapidly, while a high value indicates resistance soils with almost plain topography. Its value for the present study area is 2.39 exhibiting steep to very steep slopes with scarce vegetation cover as shown in Table 2. Through proper planning, vegetation cover can be increased in the basin which will help to restore biodiversity and water recharge.

Basin relief (H)

The basin relief is a salient feature in conceding denudation landforms which affects drainage patterns (Samal et al. 2015). It occurs as a consequence of active earth processes with varying geomorphic phenomena to form different landforms such as pediments and pediplains. Basin relief is the difference between the maximum and minimum elevations in the basin. In the present study, relief varies from 2 to 1196 m above MSL and the basin relief value is 1194 as shown in Table 3. It influences the flood pattern and sediment load in river flow (Hadley and Schumm 1961). The assessment will help in managing the landform, drainage and erosional extent in the basin.

Relief ratio (R_h)

As per Schumm (1963), relief ratio (*R_h*) is the ratio of basin relief to basin length ($R_h = H/L_b$). The basin area with high values of *R_h* refers to mountainous regions and low *R_h* values denote pediplains and valleys. It is a dimensionless attribute as it is a height-length ratio. The relief ratio value is 2.48 as shown in Table 3. This stands for the basin’s steepness and impacts peak discharge and runoff intensity.

Table 3 Generated results of relief aspects for the Sabarmati Basin

Minimum height of basin mouth (z) (m)	Maximum height of the basin (Z) (m)	Basin relief (H) (m)	Relief ratio (<i>R_h</i>)	Relative ratio (<i>R_{hp}</i>)	Ruggedness number (<i>R_n</i>)
2	1196	1194	2.48	0.79	0.50

Relative relief (R_{hp})

Relative relief (*R_{hp}*) is the ratio of maximum basin relief and the basin perimeter ($R_{hp} = H \times 100/P$) suggested by Melton (1957). The ‘*R_{hp}*’ value of this study as is shown in Table 3 is 0.79. It is also a dimensionless entity.

Ruggedness number (R_n)

Ruggedness number (*R_n*) is the product of basin relief (*H*) and drainage density (*D*), and it represents the structural complexity or roughness of the terrain and erosion possibilities of the surface (Strahler 1964 and Vijith and Satheesh 2006). Generally, ruggedness index data were employed by earth scientists to analyse the terrain morphology formed due to the complex geomorphic process (Bera et al. 2018). An extremely high ruggedness number value is observed until both parameters are large, i.e. *D* and *H*, and high elevation spread over a large area (Strahler 1956). In the present study, the value of *R_n* is 0.50 which denotes being more prone to erosion (Table 3).

Hypsometry curve

The hypsometric analysis was proposed by Langbein (1947). It illustrates the region above or below a specific elevation of the watershed (Strahler 1952b). It predicts the potential of watershed erosion and helps to prioritize for conservation. It has been mainly used to analyse small or large watersheds but is also possible to access the entire planet (Hurtrez et al. 1999). Hypsometry is described either as a hypsometric curve or as an integral called the ‘hypsometric integral’ just under the hypsometric curve (Schumm 1956). The hypsometric integral (HI) value is 0.18 as shown in Fig. 5. The shape of the hypsometric gives an idea of the evolving situation of watersheds. As per Strahler (1952b), the hypsometric curve of convex shape is characteristic of the youth stage of a watershed and

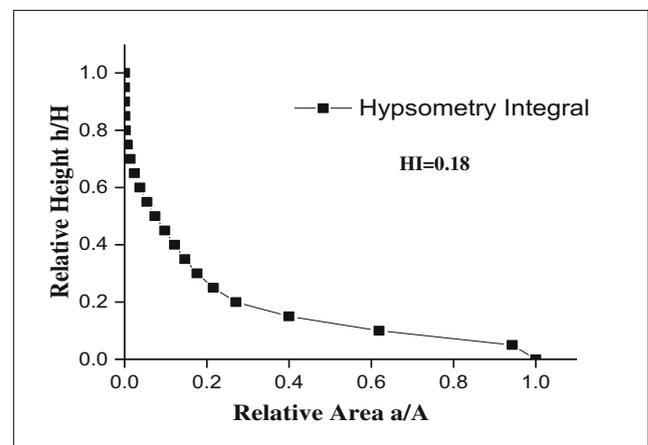


Fig. 5 The hypsometric curve of the Sabarmati Basin

an S-shaped curve forming a concave upwards at higher elevations and convex downwards formed at lower elevations represent the mature phase of watersheds, whereas a concave hypsometric curve indicates old or peneplain watersheds (Gajbhiye et al. 2014). The hypsometric curve of this basin is concave indicating a complex and mature landform with more denudation (Sharma and Mahajan 2020). The hypsometric curve forms a relation with basin hypsometry, and it will help to find erosion proneness due to flood phenomena to prevent soil abrasion.

Validation of obtained results

In the current study, an approach of drainage network extraction was performed by calculating the linear, areal and relief aspects. The results obtained were validated with the help of Google Earth Pro software. For this purpose, the extracted drainage network was converted into KML file and, later on, it was exported/overlaid on Google Earth Pro. The extracted drainage network was overlaid on corresponding basin areas with diverse order streams. Furthermore, junction points of stream order were extracted as point shape file with the help of the ArcCatalog GIS tool. The points were converted to KML file format for further overlay on drainage networks. A corresponding map was generated in Google Earth showing different stream orders, and points to represent validation of streams are shown in in Fig. 6.

Discussion

Morphometry provides a quantitative assessment of the earth surface characteristics and dimensions to evaluate the type, nature and empirical characteristics of the basin. The present study focuses on the morphometry of the Sabarmati basin which is situated in the western semi-arid part of India, where evapotranspiration exceeds precipitation due to scanty rainfall. This problem of water scarcity is aggravating with the growing population and industry, ultimately having increased stress on the basin. The present research is an effort to delineate the drainage feature of the Sabarmati River by geospatial tools and techniques for management in the water-scarce region of the basin. This will help to identify such area to increase the infiltration of water in the basin. The drainage pattern is of a dendritic type, with the highest order of seventh order in the present basin. As a matter of fact, the stream having no tributary is a first-order stream, then two first-order streams converge into a second-order stream and similarly two same-order streams join to form a higher-order stream, and so on.

In the present study, there is maximum area distribution of first-order streams which creates a chance to

allocate recharge points in such areas and there are high chances of overflow of water in higher-order streams which can be managed by forming small or large storage areas. The total number of streams estimated in the basin is 7661. The stream distribution for different orders in the basin is in following percentage, where 49% is of the 1st order, 24% is of the 2nd order, 14% is of the 3rd order, 6% is of the 4th order, 3% is of the 5th order, 1% is of the 6th order and 0.3% is of the 7th order. This shows that stream frequency is maximal for the first stream order but frequency declines with the stream order upswing. The low-frequency value of the stream typically shows porous sub-surface matter along with low relief, while higher-frequency values of streams indicate the resistant sub-surface matter and more elevation. The total lengths of streams are 6419.38 km, 3322.71 km, 1929.07 km, 1050.29 km, 436.05 km, 171.16 km and 18.83 km respectively for the first-, second-, third-, fourth-, fifth-, sixth- and seventh-order streams. Usually, total stream length decreases with the increase in stream order. It acknowledges surface run-off properties as smaller streams occur in high/steep slope areas with fine texture and longer streams found in low-lying/flatter areas.

The varying drainage density maps of the basin shown in Fig. 3b occur due to high relief and rock of low permeability property. The elevation directly affects the infiltration and runoff of any terrain. In the current work, the R_L value varies from 0.11 to 0.58 and R_b varies from 1.69 to 4.29, which suggests the tectonically active nature of the terrain which affects the fluvial pattern of the Sabarmati River Basin which impacts discharge and sediment deposition. This can be managed by more vegetation and check dam formation in the basin at specific locations prone to erosion.

Aster DEM data is being used to develop the aspect and slope map in the present analysis. Presently, the slope varies from 0° to 45° as shown in Fig. 3c. Thus, the upper region of this basin has a steep slope than the middle and lower regions of the basin; there will be the high rate of erosion from the upper parts, causing siltation in the lower parts of the basin. Further, slope data can be used for planning settlements or structures by engineers, mechanization of agriculture and various conservation practices to combat the mass flow. The obtained value of R_n is 0.5 and that of R_h is 2.48 which also denotes being more prone to erosion. Moreover, the hypsometric curve of this basin is concave indicating a complex and mature landform with more denudation. The hypsometric curve forms a relation with basin hypsometry, and it will help to find erosion proneness due to flood phenomena to prevent soil abrasion. Through proper planning, vegetation cover can be increased in the basin which will help to restore biodiversity and water recharge.

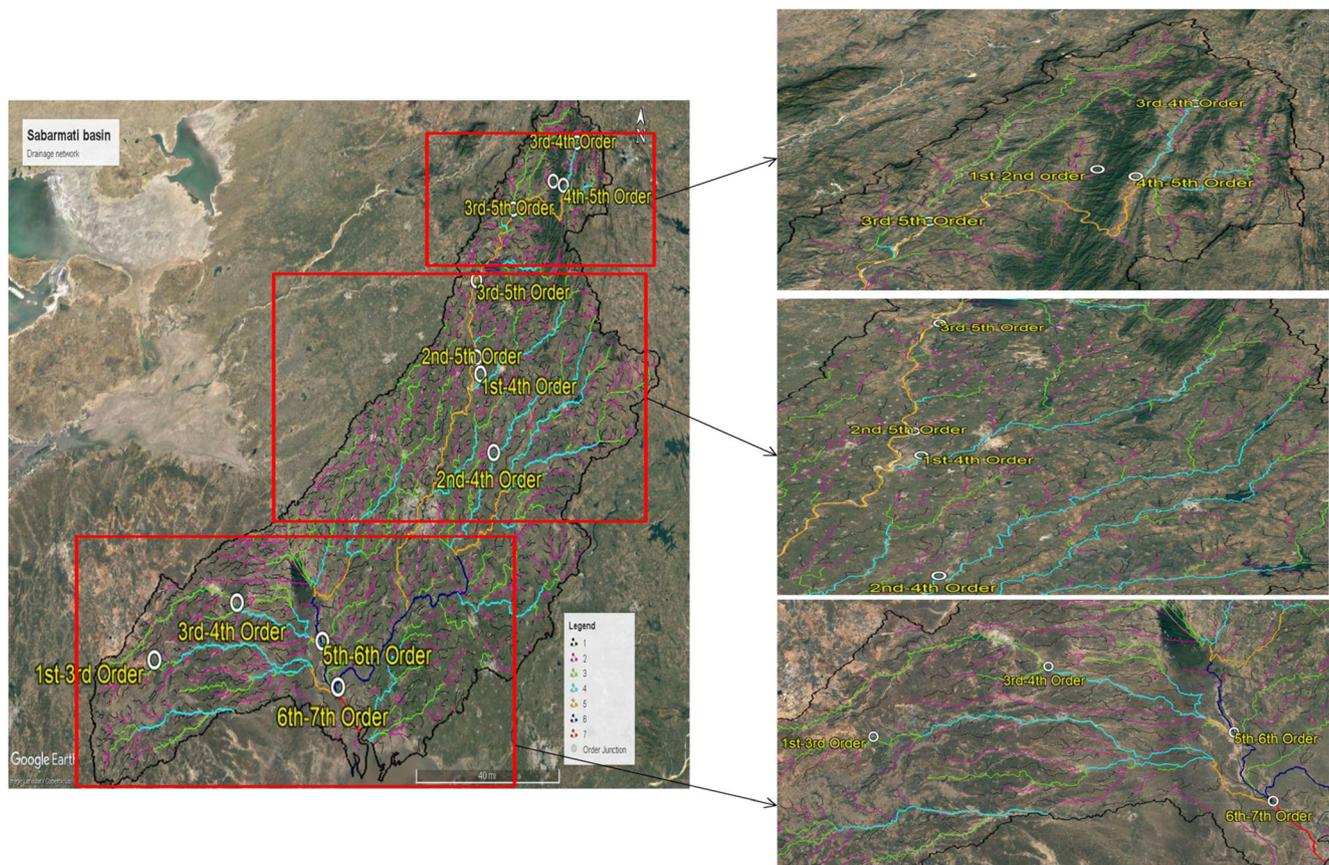


Fig. 6 Generated map of the basin in Google Earth showing different stream orders with junction points and a magnified view of the upper, middle and lower parts of the basin map

Conclusion

The current research work utilizes the geospatial approach in combination with remote sensing datasets to delineate morphometric variables of the basin by evaluating linear, areal and relief aspects. The present study considers the Sabarmati basin, which is an elongated basin with a dendritic drainage pattern. The elongated basin gives a longer duration for surface water flow which creates more chances of recharge. The elevation derived from the DEM varies from 2 to 1196 m above mean sea level. The basin has a seventh-order drainage pattern having area 31,921 km², perimeter 1503 km and basin length 481.75 km. The basin has a total of 7661 streams of which 49% is of the 1st order, 24% is of the 2nd order, 14% is of the 3rd order, 6% is of the 4th order, 3% is of the 5th order, 1% is of the sixth order and 0.3% is of the seventh-order stream. The basin has a maximum distribution of lower-order streams, and also the first order has a maximum total stream length which creates more chances of water potential recharge in the Sabarmati River Basin. The bifurcation ratio ranges from 1.69 to 4.29, and its higher value indicates that the basin is affected by geological structures. The value of the ruggedness number was estimated to be 0.50, and the region is more vulnerable to erosion. The basin drainage

density is 0.42 km⁻¹, suggesting scant vegetative area and moderating-to-high relief. The value of the circulatory ratio (0.18) and low form factor (0.14) represent the basin's elongated nature, which will help in water infiltration. The slope varies from 0° to 45° which influences the surface and subsurface flow of water or soil particles. Mostly, slope data is also used by engineers for planning settlements or structures and mechanization for agriculture and various conservation practices. The drainage network and structural details will help planners and decision-makers in sustainable watershed development and also allocate artificial recharge structures for resource management at the different regions of the basin.

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Spatiotemporal variation of the nutrients and heavy metals in mangroves using multivariate statistical analysis, Gulf of Kachchh (India)

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Abstract

The present study is an attempt to assess the spatial and seasonal variation of nutrients and heavy metals in mangroves water in the Gulf of Kachchh, India. The surface water samples were collected during pre- and post-monsoon to evaluate the hydrochemical processes occurring in the region. Seasonal changes and anthropogenic inputs have influenced the nutrients and metal concentrations. The results suggest high salinity (Pre-monsoon ± 45.59 PSU; post-monsoon ± 45.36 PSU) and chloride (Pre-monsoon ± 30251 mg/L; post-monsoon ± 29536 mg/L) concentration in both the seasons. Average values of Sulfate (SO_4^-), Nitrate (NO_3^-), Dissolved silica (DSi), and Phosphate (PO_4^{3-}) was 2503.69 mg/L, 10.47 mg/L, 35.41 mg/L and 0.85 mg/L in pre-monsoon, and 3474.50 mg/L, 13.66 mg/L, 31.54 mg/L and 0.64 mg/L in post-monsoon, respectively. Nutrient ratios indicate phosphorus limitation in both the seasons. Cluster and Principal Component analysis signifies that sampling locations in cluster 3 and factor 1 majorly impacted due to seawater mixing, high evaporation and discharge of brine from saltpans, while cluster 2, 3 and

They support the coastal food web, mitigate the nutrient pollution, sequester carbon, act as a buffer from catastrophic events such as tsunamis, cyclones, hurricanes, can dampen shoreline erosion, provides habitat for terrestrial and marine flora and fauna (Sappal et al., 2016). Further, mangroves exchange a significant amount of nutrients with the adjoining ocean, which maintains the nutrient dynamics (Maurya et al., 2021). Despite their importance, mangroves are being depleted and degraded rapidly due to increasing climate change and anthropogenic pressure. Globally 30–50% of mangrove has been lost in the last 50 years, and they are continue to be lost at a rate of 1–3% per year (Duke et al., 2007; Marchand et al., 2016). These losses have propelled by deforestation for coastal development, agriculture, aquaculture, upstream dams, dredging, and timber (Alongi, 2014; Jennerjahn et al., 2017). Further, natural losses of mangroves being caused due to pressure by extreme weather events, altered rainfalls and sea-level rise (Pendleton et al., 2012).

Water quality of mangroves becomes a major concern at present era because they are facing immense pressure due to the ever growing industrialization and urbanization. Coastal water has received plenty amount of waste from a variety of sources such as industrial effluents, sewage waste, saltpan brine, agricultural runoff, aquaculture effluents and discharge from the upstream river (Zhou et al., 2007), these situations resulting in degradation of water quality. As an outcome, the proportion of dissolved nutrients in mangrove water becomes altered which leads to changes in nutrient stoichiometric ratios (Si:N, N:P and Si:P), considerably influence the coastal food web structure and nutrient dynamics (Zhao et al., 2005; Manju et al., 2012). The periodic and consistent variations in hydro-metrological factors like temperature, salinity, oxygen and nutrients composition and climate synchronized with seasons and anthropogenic factors ultimately affect the environmental parameters, which in turn have a direct or indirect influence over flora and fauna. The seasonal distribution, abiotic and biotic processes also influence the nutrient cycling of the ecosystem (Saravanakumar et al., 2008). Mangroves act as a sink for pollutants (heavy metals, oil pollution, organic and inorganic nutrients) and having good phytoremediation potential. But due to human intervention, higher disposal rates of pollutants were observed, which is much higher than their uptake potential to remediate (Satheeshkumar and Khan, 2012).

For determining the health status of mangroves a thorough assessment of all the integrating factors at the ecosystem level is needed to select suitable indicators that can suitably reflect its instantaneous health status. Considering these environmental problems, the number of studies has been done on water quality monitoring in mangrove regions, especially to give a broader picture of the forces acting on the ecosystem. Indices for ocean health have been developed to assess several marine ecosystems (Tian et al., 2011; Marigomez et al., 2013). Nevertheless, indices to evaluate the status of mangrove ecosystem health are very limited. To assess the trophic status of marine water, Karydis and Tsirtsis (1996) suggested five useful phytoplankton variables (species number, abundance, Menhinick's index, Kothe's index, and evenness index). Beltrame et al. (2006) used four water quality variables (salinity, turbidity, pH and dissolved oxygen) for the hydrological

index. To construct an extensive assessment of the environmental status of coastal habitats, Berezina et al. (2017) utilized different variables such as water salinity, phosphorus, trace metals, polycyclic hydrocarbon, phytoplankton and benthic organisms. Samsudin et al. (2019) have employed spatial discriminant analysis for marine water quality index using 13 parameters (i.e. dissolved oxygen, total suspended solids, fecal coliform, nitrogen, nitrate, phosphate, oil and grease and heavy metals). Whereas in India, very few studies have been done related to the water quality of mangroves which are following: Bhitarkanika, Orissa (Chauhan and Ramanathan, 2008); Pondicherry coast (Satheeshkumar and Khan, 2012); Kerala (Manju et al., 2012); Mumbai (Pawar, 2013); Odisha (Behera et al., 2014); Sundarban Mangrove (Singh et al., 2016); Andhra Pradesh (Rao et al., 2018).

The current study has carried out in the Gulf of Kachchh, Gujarat. As this region is being an ecologically sensitive zone. Gujarat is the economic hub of the country and one of the fastest-growing economies among the Indian states and holds the 4th highest gross domestic product (GDP) in India (Maurya et al., 2019). Since it is the host of a large number of industries, it seems to be acting as sources of environmental stress from the last few decades. The coastal belt of the Gulf of Kachchh has increasing aggressive developmental activities, such as the fertilizer-manufacturing industry (IFFCO), vast number of salt manufacturing units, cement industries, aquaculture, thermal power plant, ship dismantling activities, and chemical manufacturing industries (like soda ash, sodium bicarbonate, caustic soda, petrochemicals) (Shirodkar et al., 2010; Chakraborty et al., 2014). Industries such as Reliance Industries Limited (petroleum terminal at Sikka), Indian Oil Corporation Limited and Bharat Oman Refineries Limited (Vadinar), Essar Oil Refinery (Vadinar), Shree Digvijay Cement Company Limited and many other industrial setups are situated in the study area, which may a potential threat to mangrove habitat (ICMAM, 2004; Prerna et al., 2015). Gulf has several ports such as Navlakhi, Bedi, Sikka, Vadinar, Okha, Kandla, Mundra, Mandvi, along with various jetties, pipelines, and single points harbors are associated with refineries. Incidental oil spilling from vessels during the unloading of large quantities of petroleum and its crude products is also a serious matter in the study area, which can cause a threat to marine flora and fauna (Vethamony et al., 2007). Thus, these multiple sources lead to high nutrient load causes eutrophication and also increases the heavy metal load in mangrove water. Maintenance of hydrochemical characteristics of mangroves is very important to its conservation, management, and sustainable development. So, it is requisite, to control and prevent water pollution, and enforce regular monitoring programs, which will help us to comprehend the spatial and temporal variation along with the present status of mangrove water quality. To our knowledge, this is the first study in the Gulf of Kachchh, which studied the entire gulf region. The present research work aims to investigate the water quality at different sites along the horizontal transect with respect to nutrients and heavy metals load in the mangrove water.

Section snippets

Regional setting

The present research work has been carried out between 23°22'25.44"N - 22°23'54.24"N latitude and between 68°35'33.05"E - 69° 3'20.57"E longitude in Gulf of Kachchh (GoK). The GoK is situated in the west coast of India, Gujarat which is covering an area approximately 7350 km² (Thivakaran et al., 2020). GoK is a semi-closed basin encircled by Kachchh mainland in the north, Saurashtra peninsula in the south and Arabian Sea to its west (Prizomwala et al., 2012). It is east-west oriented,...

Physical and chemical parameters

The seasonal variation of hydro-geochemical characteristics of the mangrove water at various locations has depicted in Table 1a, and a comparison of data with other studies has given in Table 1b.

The pH in GoK varied from 6.79 (S16) to 8.81 (S34) in pre-monsoon, while pH value in post-monsoon varied from 7.18 (S15) to 8.41 (S30). The pH in pre-monsoon observed acidic to alkaline, while in post-monsoon, alkaline pH was observed at all the locations. The alkaline pH can be attributed to the mixing ...

Heavy metals distribution and sources

Mean heavy metal concentration in pre-monsoon are in following order: Sr> Fe> Zn> Cu> Li> Mn> As> Ni> Pb> Ti> Tl> Mo. While, in post-monsoon mean concentration are as follows: Fe> Sr> Zn> Mn> Cu= Li> Ti> As = Pb> Ni> Tl= Mo. Heavy metal concentrations of both the seasons are depicted in Table 1a.

In both the seasons, the highest concentration of metals observed in pre-monsoon. At S21, the highest concentration of strontium (Sr) has observed in pre-monsoon, whereas in post-monsoon highest at...

Correlation

To evaluate the strength of relationship between the investigated parameters, pre and post-monsoon data sets were statistically analyzed with the correlation matrix is given in Table 2, Table 3. In pre-monsoon very high correlation observed between EC-TDS ($r^2=0.93$), EC-Salinity ($r^2=0.99$), EC- Cl⁻ ($r^2=0.80$), suggests the seawater mixing is a major contributing factor in the

studied region. Besides, this climatic condition (such as high temperature, low precipitation, and high wind velocity) also ...

Conclusion

The present study summarizes the spatial and seasonal variation in the water hydrochemistry at various locations of the Gulf of Kachchh. The nutrient balance in mangrove water in GoK was highly influenced due to high tidal amplitude, extensive saltpans, industrial, and seaport activities. The results suggested that there is a pronounced variation in most of the water quality parameters with variation in the seasons and geographical locations. The study area has experienced very high salinity...

Author contributions

PM and RK envisaged the idea. PM did fieldwork, laboratory analysis, statistical analyses, and wrote the first draft of the article. All contributed to the conclusive writing and editing of the manuscript...

Declaration of competing interest

The authors declare that they have no conflict of interest...

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Research Paper

Toxic metals distribution, seasonal variations and environmental risk assessment in surficial sediment and mangrove plants (*A. marina*), Gulf of Kachchh (India)

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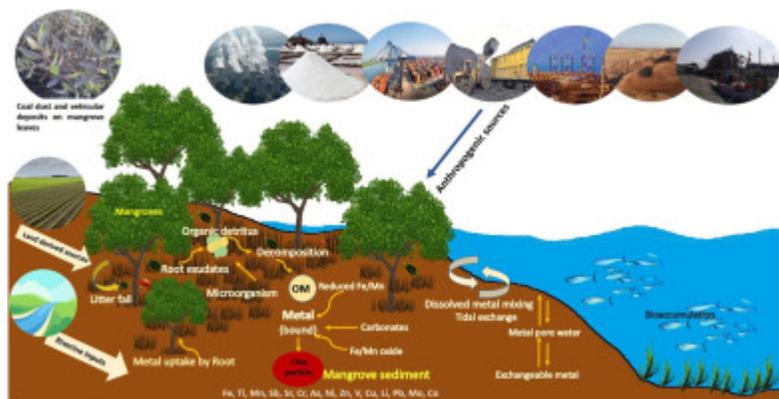
Highlights

- Seasonal variation of heavy metals in plants and sediment were assessed.
- Toxic metals in sediments were found in higher concentrations, Fe, Mn, Sb, Ti were higher among all the metals.
- Contamination indices demonstrate that sediments are extremely contaminated by metals, chiefly from Sb and As.
- Ecological risk assessment indicates about 72% of sites come under higher risk.
- *A. marina* efficiently translocates Cu, Pb, Fe, As, Zn, Ni, Sr, Mn and shows high bioaccumulation potential for Pb, Cu, Mo, Zn.

Abstract

Toxic metal pollution in the coastal ecosystem is becoming a serious problem, particularly in developing countries as a result of the industrial revolution. In recent years, mangroves are continuously contaminating with toxic metals and receiving global attention due to its toxicity, non-degradability, abundance, subsequent bioaccumulation, and biomagnification through successive trophic levels. This study aims to investigate the toxic metal content and pollution status in mangroves surface sediment and plants. Results showed that toxic metals in sediments were higher than natural background levels indicate anthropogenic sources. Fe, Mn, Sb, Ti found higher in concentration among all toxic metals, and site 9, 15, 18, 19, 21, 31 found the highest total metal load. Contamination indices like enrichment and contamination factor, geo-accumulation index, suggest minimal to extremely high level of contamination, and sediments have found extremely contaminated with Sb and As. Contamination degree and modified contamination degree suggest very high degree of contamination at all sites. Pollution load index indicates significant deterioration of sediment quality. Ecological risk and potential ecological risk index also indicate about 72% of sites come under higher ecological risk. Toxic metal in *Avicennia marina* was found higher in root than leaf. High bioconcentration factor has observed for Pb, Cu, Mo, Zn. Translocation factor for Cu and Zn at all sites, and As, Ni, Pb, Fe, Sr, Mn at some sites indicate high-efficiency in plants for toxic metal translocation.

Graphical Abstract



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Keywords

Mangroves; Sediments; *Avicennia marina*; Toxic metals; Contamination indices; Translocation and bioconcentration factors

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Impact of partially submersed iron scraps in simultaneously sulfate and nitrate removal using sulfate-reducing bacteria

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ABSTRACT

In this study, partially submerged iron scraps were allowed to react to the contaminants at the headspace air and liquid interface for pollutants removal in the serum bottle reactors. The experiments were conducted using Box–Behnken design of Response Surface Methodology in anaerobic batch reactors (120 ml) at different concentrations of sulfate (0.5, 1.75, 3 g/L), nitrate (0.25, 0.375, 0.5 g/L) and iron scraps (0, 0.125, 0.250 g/50 ml). The sulfate reducer enriched mixed culture was used for simultaneous removal of SO_4^{2-} and NO_3^- . The highest sulfate removal of 49.37 % was achieved at 0 g/50 ml ISs, 3 g/L SO_4^{2-} , and 0.375 g/L NO_3^- . Even at a higher concentration of both SO_4^{2-} (3 g/L) and NO_3^- (0.5 g/L), a significant SO_4^{2-} removal of 44.65% was achieved at an iron concentration of 125mg/50ml accompanied with NO_3^- conversion. Both the lower and higher concentrations of iron scraps were showing effective interaction at higher SO_4^{2-} and NO_3^- levels. The results of the confirmatory experiments with iron scraps of 0.039 g/50 ml with the desirability of 0.92 showed a maximum SO_4^{2-} removal of 50.62 % with complete conversion of nitrate.

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1. Introduction

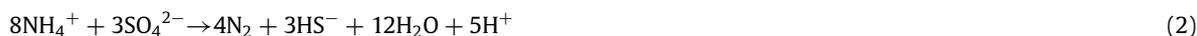
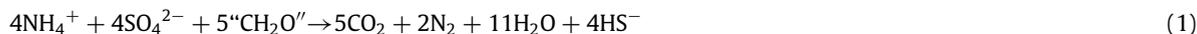
The discharge of untreated sulfate (SO_4^{2-}) and nitrate (NO_3^-) rich wastewater from industrial processes and other sources in large quantities may contribute to serious environmental pollution (Singh et al., 2011; Yamashita and Yamamoto-Ikemoto, 2014). The SO_4^{2-} rich wastewater discharged into surface water under anaerobic conditions results in the biological reduction of SO_4^{2-} to sulfide and increases the corrosion potential of receiving water bodies (Sarti et al., 2009). The sulfates can adversely affect human beings leading to several problems related to kidney dysfunction, liver damage, unconsciousness, brain disorders, lungs damage, breathlessness, endocrine disorders, heart problems, and sleep disorders (Duong et al., 2001; Hussain et al., 2016). Also, nitrates can infiltrate through the vadose zone due to their high solubility and eventually find their way to groundwater. In addition, nitrogen compounds are responsible to increase toxicity, bad odor, eutrophication, and other serious ecological threats to the receiving water bodies (Sun and Nemati, 2012; Xu et al., 2014). Additionally, it is also responsible for causing hemoglobin M disease and cyanosis in newborn babies. In some countries, groundwater resources have already been polluted with exceeded concentrations beyond the acceptable limits of NO_3^- (Show et al., 2013).

Instead of the expensive Physico-chemical methods which may generate toxic residuals, the biological treatment technologies are preferred to remove SO_4^{2-} and nitrogen contaminants (Midha et al., 2012). The treatment of contaminants by biological methods using microorganisms is a promising approach for wastewater treatment. Under anaerobic processes

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SO_4^{2-} is reduced to sulfide by Sulfate-reducing bacteria followed by its oxidation to elemental sulfur (S^0) at micro-aeration by sulfide oxidation bacteria (SOB) (Xu et al., 2014) and thus are helpful in the application for treatment of certain types of industrial wastewater (Lens et al., 1998; Muyzer and Stams, 2008). Simultaneous ammonium oxidation and sulfate reduction (80%) to dinitrogen gas and elemental sulfur respectively were observed in the treatment of Vinasse in an anaerobic fluidized-bed reactor (Fdz-Polanco et al., 2001). The approach towards SO_4^{2-} reducing, autotrophic denitrification, and nitrification integrated (SANI) process derive beneficial application of SRB for wastewater treatment (Van Den Brand et al., 2015). However, the mechanism of denitrifying sulfide removal (DSR) process which can remove nitrogen–sulfur–carbon (N–S–C) simultaneously is still unclear (Show et al., 2013). The DSR process having an unlimited supply of NO_3^- can partially oxidize sulfide (S^{2-}) to elemental sulfur (S^0) with the help of the autotrophic denitrifiers, whereas NO_3^- can also be converted into nitrogen in an anaerobic or anoxic environment (Reyes-Avila et al., 2004). Under the limited NO_3^- supply, fierce competition occurs among autotrophic and heterotrophic denitrifiers (Chen et al., 2008).

The multiple sulfate-driven ammonium oxidation (ANAMMOX) reactions performed by anaerobic ammonium oxidizing bacteria (AnOB) result in the formation of dinitrogen (Eqs. (1) and (2)) (Schrum et al., 2009).



Several studies have also reported the irreversible toxic effects of sulfide on the ANAMMOX process due to the denaturation of proteins of anammox bacteria (Zhu et al., 2018). Ferrous or ferric iron has been proven to remove sulfide toxicity by the formation of precipitates as ferrous sulfide (Pomeroy and Bowlus, 1946; Kiilerich et al., 2018). The iron sulfides also act as a possible electron source for autotrophic denitrification ((Hu et al., 2020; Tong et al., 2018). Iron under anoxic conditions can completely reduce nitrate producing ammonia as a major product (Hao et al., 2005). Ammoniacal nitrogen is also considered as one of the major pollutants of the leachate generated from the landfill site and has the potential to deteriorate the environment as well as health (Mor et al., 2019). The elevated ammonia levels (>1,800 mg/L) can also hinder the growth of methanogens that largely consume hydrogen and acetate for CH_4 production at low sulfate levels (Kratat et al., 2017; Raskin et al., 1996). The anammox bacteria can perform nitrate-dependent iron oxidation and thus oxidize ammonium with nitrite to produce nitrogen gas. Very limited research has been carried so far in the simultaneous removal of sulfate and nitrate in wastewater treatment. Also, the use of iron scraps for the simultaneous removal of both components further required more study. This study highlights the effect of iron scraps for the efficient removal of high sulfate and nitrate concentrations anaerobically using sulfate-reducing bacteria mixed culture. Moreover, the novelty of the work is also suggested by the fact that the iron scraps used for the study have been kept under partially submerged/floating conditions using porous plastic pouches. The submerged ISs were able to react with both the gases and contaminants lifted upwards during treatment as well as with the dissolved pollutants in the reactors. It also ensured better leachability of soluble iron and increased surface area for adhesion of culture on the ISs surface within the reactors to enhance pollutant removal rates.

2. Materials and methods

2.1. Inoculum source

The anaerobic sludge was collected from the Zydus Infrastructure Pvt. Ltd., Ahmadabad, Gujarat, India. The culture sample was collected from MBR tank of the treatment plant in a 5L can. The culture was kept for settling and the supernatant was removed from the culture. 2.5 L of this thick culture of 4000 mg L^{-1} (MLSS) was added to the modified Postgate growth media (Postgate, 1984) in a 5 L reactor and purged with nitrogen (99.999%) to ensure the anaerobic conditions. The culture in the reactor was maintained constantly by continuous feeding at week intervals at room temperature. The Postgate medium is as follow: KH_2PO_4 0.5 (g/L); Na_2SO_4 1.0 (g/L); NH_4Cl 2.0 (g/L); CaCl_2 0.06 (g/L); FeSO_4 0.005 (g/L); sodium citrate 0.3 (g/L); yeast extract 0.1 (g/L); glucose 5 (g/L).

2.2. SO_4^{2-} reduction study

The experiments were conducted in anaerobic batch reactors (Serum bottle 120 ml) at different concentrations of SO_4^{2-} (0.5, 1.75, 3 g/L), NO_3^- (0.25, 0.375, 0.5 g/L) and iron scraps (0, 0.125, 0.250 g/50 ml) by modifying the Postgate medium for simultaneous SO_4^{2-} and NO_3^- removal. 25 ml of the well-mixed culture was taken in the serum bottle and amended with the required amount of the supplements as suggested by the response surface methodology model. 25 ml of the Postgate medium was taken in each bottle and purged with nitrogen gas (99.99% purity) for 2–3 min to provide anaerobic conditions. The serum bottles were sealed with 20 mm rubber septa and aluminum crimp caps. The bottles were then incubated at 37 °C in the incubator under static conditions. The total gas produced was measured by withdrawing headspace samples at periodic intervals of 48 h, 96 h, and 120 h using the 20 ml glass syringe. The culture samples were harvested on the 5th day after incubation.

Table 1
Experimental factors and responses observed at various variables levels.

Std	Factors			Responses				
	ISs (mg/50 ml)	Sulfate (g/L)	Nitrate (g/L)	Sulfate (g/L)	COD (mg/L)	NH ₄ ⁺ (μg)	TG (ml)	H ₂ S (%)
1	0	0.5	0.375	65.63 ± 61.87	2426.67 ± 302.87	2445.71 ± 779.84	21.17 ± 3.15	5.57 ± 2.47
2	250	0.5	0.375	67.71 ± 135.68	2133.33 ± 394.63	2632.38 ± 734.62	28.17 ± 1.1	7.94 ± 4.35
3	0	3	0.375	1687.71 ± 51.91	1760 ± 60	3030 ± 30.30	24.87 ± 0.8	9.97 ± 0.44
4	250	3	0.375	1618.13 ± 25.37	1226.67 ± 777.002	1200.95 ± 589.59	23.83 ± 0.85	6.75 ± 3.73
5	0	1.75	0.25	660.63 ± 126.51	1893.33 ± 670.92	1245.71 ± 569.17	32.2 ± 2.55	10.14 ± 2.33
6	250	1.75	0.25	745.21 ± 35.71	1600 ± 138.6	1092.86 ± 167.69	35.93 ± 1.20	9.38 ± 0.30
7	0	1.75	0.5	868.75 ± 48.61	1200 ± 339.41	854.29 ± 16.16	34.97 ± 3.07	9.77 ± 5.16
8	250	1.75	0.5	741.88 ± 35.99	1786.67 ± 230.94	1731.43 ± 92.93	28.5 ± 2	6.13 ± 0.54
9	125	0.5	0.25	116.88 ± 34.03	2240 ± 922.6	3348.57 ± 1834.26	26.73 ± 1.9	7.59 ± 3.37
10	125	3	0.25	1494.79 ± 34.96	1386.67 ± 166.53	1253.33 ± 363.01	24.63 ± 1.65	5.92 ± 0.68
11	125	0.5	0.5	103.75 ± 2.65	1813.33 ± 725.9	2404.76 ± 752.58	27.57 ± 0.94	4.36 ± 3.00
12	125	3	0.5	1526.46 ± 46.85	1013.33 ± 323.32	2718.57 ± 345.47	25.73 ± 2.19	7.06 ± 1.84
13	125	1.75	0.375	755 ± 30.94	1840 ± 423.32	2448.57 ± 561.64	25.27 ± 2.2	5.81 ± 2.184
14	125	1.75	0.375	721.25 ± 99.9	1520 ± 835.22	2931.43 ± 864.69	26.43 ± 0.75	5.46 ± 0.300
15	125	1.75	0.375	765.21 ± 30.01	1520 ± 499.59	2321.43 ± 272.74	27.03 ± 0.98	6.22 ± 1.011
16	125	1.75	0.375	903.13 ± 37.12	1680 ± 240	2171.43 ± 965.71	24.3 ± 1.18	5.17 ± 0.482
17	125	1.75	0.375	786.14 ± 22.38	1640 ± 246.53	2446.43 ± 412.82	24.5 ± 0.72	5.66 ± 0.362

2.3. Analytical methods

The harvested samples were centrifuged at 5000 rpm at room temperature to prepare the cell-free supernatant for further analysis of different parameters SO₄²⁻, NO₃⁻, chemical oxygen demand and dissolved ammonical nitrogen. The instrument used for SO₄²⁻, NO₃⁻ and dissolved ammonical nitrogen measurement was Systronics Double Beam UV spectrophotometer 2203. The analysis of SO₄²⁻ (turbidity method), NO₃⁻ (phenol disulfonic acid method), dissolved ammonical nitrogen (nesslerization method) and COD (open reflux method) were carried accordingly using APHA (APHA, 2012). Lead acetate (10%) was used to measure the hydrogen sulfide (Kalia et al., 1992).

2.4. Box–Behnken design model

The response surface model of Box–Behnken design (Box and Behnken, 1960) based on Design-Expert software (Stat Ease version) was considered in the present study. In the present design, effect of individual variables, i.e. SO₄²⁻ (0.5, 1.75, 3 g/L Na₂SO₄), NO₃⁻ (0.25, 0.375 and 0.5 g/L KNO₃) and ISs (0,125, 250 mg/50 ml) were studied on responses, i.e. SO₄²⁻ and NO₃⁻ removal accompanied with its effects on dissolved ammonia, COD, H₂S and gas production. The study of the effect of these variables was carried after designing and running 17 experiments as suggested by the model. The data obtained from experiments were analyzed and the confirmatory conditions were obtained by considering the minimum residual sulfate and nitrate, highest gas production, and the lowest residual COD. The model variables were kept at different ranges to achieve the highest desirability. The following confirmatory experiments were run as suggested by the model with different concentrations of ISs (38.46, 37.15, 14.58, 10.8, and 4.06 mg/50 ml) added in the Postgate media having Na₂SO₄ and KNO₃ concentration as 3 and 0.5 g/L respectively. These experiments were performed to check the validity of the model's equation generated (Eq. (3)). The incubation for the confirmatory experiment was continued for 48 h after which no gas formation was observed during the optimization study.

3. Results and discussion

The effects of different concentrations of SO₄²⁻, NO₃⁻ and ISs on the removal of SO₄²⁻, NO₃⁻, COD, total gas and H₂S production is discussed as follows. A total of 17 sets (replicates) of experiments were conducted to understand the factor space using the central point (Table 1). The models were reduced for the stepwise regression with α to enter and to exit at 0.1. The common quadratic equation based stepwise regression on the experimental response was generated (Eq. (3))

$$\text{Response} = a_1 + a_2A + a_3B + a_4C + a_5A^2 + a_6B^2 + a_7C^2 + a_8AB + a_9AC + a_{10}BC \quad (3)$$

Where regression coefficients are, for the intercept (a_1), linear (a_2 – a_4), quadratic (a_5 – a_7) and interactive (a_8 – a_{10}) and A is ISs (mg/50 ml), B is sulfate concentrations (g/L) and C is nitrate (mg/L).

3.1. Effects of factors variables on responses

3.1.1. Effects of varying SO₄²⁻ and NO₃⁻ concentration

The varying concentrations of SO₄²⁻ and NO₃⁻ showed maximum SO₄²⁻ (49.37%) and COD removal at higher levels of NO₃⁻ (0.4 g/L) and SO₄²⁻ (2.96 g/L). The increase in COD removal was observed only at NO₃⁻ and SO₄²⁻ concentrations

exceeding 0.38 g/L and 0.85 g/L. The SO_4^{2-} removal efficiency of 51.6% in SRB system was improved to 76.3% for Fe^{2+} amended SRB system at SO_4^{2-} concentration of 3 g/L (Hu et al., 2018). The SO_4^{2-} removal was efficient by the addition of NO_3^- up to the highest concentration which might be possible due to the increased efficiency of SRBs which can outcompete methanogens in presence of NO_3^- . The addition of NO_3^- has already shown to inhibit the methane formation in various studies, may be due to the formation of intermediates during NO_3^- reduction leading to suppression of methanogenesis (Klüber and Conrad, 1998; Tugtas and Pavlostathis, 2007, 2008). The presence of NO_3^- has also been found to suppress SO_4^{2-} reduction activity by SRBs in situ (Jenneman et al., 1986; Davidova et al., 2001). Although it has also been observed in various studies that the SRBs were capable of showing possible resistance in the contaminated sites with high levels of NO_3^- (Gu et al., 2005; Bagwell et al., 2006). In a study, the nitrite stress has resulted in the up-regulation of Fur and PerR regulon as indicating oxidative stress response against nitrite toxicity (He et al., 2006). The maximum total gas was observed at SO_4^{2-} of about 1.68 g/L and NO_3^- of 0.25 and 0.5 g/L. The saddle point at which gas production was found to decrease was in the vicinity of SO_4^{2-} at 1.61 g/L and NO_3^- decreasing towards 0.37 g/L respectively as clearly observed in Fig. 3 A and 3B. The total gas production increased with increasing one of the components at a time. However, the H_2S production was least at highest NO_3^- (0.5 g/L) and lowest SO_4^{2-} (0.5 g/L) which increased by increasing SO_4^{2-} and decreasing NO_3^- towards the saddle point in the vicinity of 2.63 g/L of SO_4^{2-} and 0.38 g/L of NO_3^- . Sulfides as the reduction products of sulfate are highly toxic to microbes including SRB (Singh et al., 2011). It has been observed in various studies that the addition of NO_3^- has reduced the sulfide production with NO_3^- being the preferred electron acceptor as well as the production of nitrite inhibiting the SRBs population (Myhr et al., 2002; Hubert et al., 2003; Hubert, 2010). The ammonia production was least at the highest SO_4^{2-} of 3 g/L and lowest NO_3^- of 0.25 g/L which may be a result of denitrification. The decreasing SO_4^{2-} to the lowest concentration of 0.5 g/L at 0.32 g/L of NO_3^- and increasing SO_4^{2-} and NO_3^- towards highest concentrations of 2.96 g/L and 0.45 g/L respectively had increased ammonia production beyond the saddle point near 2.05 g/L of SO_4^{2-} and 0.42 g/L of NO_3^- (Fig. 2B). The H_2S generation or elemental sulfur production from sulfide due to SO_4^{2-} reduction might have possibly contributed to the dissimilatory reduction of NO_3^- resulting in ammonium formation (Brunet and Garcia-Gil, 1996). It has been observed that both NO_3^- and sulfide converted $38 \pm 1.6\%$ of NO_3^- to ammonium ($700 \pm 23 \mu\text{M}$) after 24 h in the anaerobic microcosm (Jones et al., 2017).

3.1.2. Effects of varying ISs and SO_4^{2-} concentration

The lowest and highest level of the iron showed maximum SO_4^{2-} and COD removal at the highest value of 3.0 g/L of SO_4^{2-} variable. Moreover, the highest gas production was also observed at the highest and lowest iron concentrations, keeping SO_4^{2-} levels to 1.5 g/L and 2 g/L respectively (Fig. 3 A). The saddle point was observed in the vicinity of 100 mg/50 ml of ISs and 1.83 g/L of SO_4^{2-} . The saddle point for less H_2S production within the vicinity of ISs of 135.65 mg/50 ml and 1.94 of SO_4^{2-} was observed, while moving towards the lowest iron concentrations with the highest SO_4^{2-} concentrations and vice versa (Fig. 4 A). The SRBs can reduce SO_4^{2-} provided the oxygen reduction potential (ORP) must be less than -200 mV which is also suitable for iron reduction (Cabrera et al., 2006). Thus, the reduction of ISs had possibly favored H_2S depletion by precipitating the ferrous (Fe^{+2}) state to FeS. A similar trend was observed in ammonia production with saddle point lying in the vicinity of 96 mg/50 ml of ISs and 1.93 g/L of SO_4^{2-} and its formation decreased keeping values of the variables away from saddle point (Fig. 2 A). The ammonia production decreased significantly at a higher value of SO_4^{2-} and ISs. The existence of SO_4^{2-} reducing ammonium oxidation was reported in sedimentary pore water (Schrum et al., 2009).

3.1.3. Effects of ISs and NO_3^- concentration

The maxima of SO_4^{2-} removal was observed at the lowest level of the ISs and the highest level of the NO_3^- , reversing the levels of the variables decrease its removal as inferred from the saddle point in the vicinity of 200.96 mg/50 ml of ISs and 0.37 mg/L of NO_3^- . The appearance of the saddle point in the vicinity of 200 mg/50 ml of ISs and 0.4 g/L of nitrate indicating either NO_3^- or ISs was necessary to be increased for efficient COD removal (Fig. 1B). The increasing concentrations of NO_3^- and ISs are individually responsible for decreasing the residual COD. The concentrations of both ISs and NO_3^- with either of the component at the highest or lowest concentration significantly decreased the total gas as well as H_2S production while moving towards the saddle point near to 110.55 and 144.03 mg/50 ml of ISs with NO_3^- nearly to 0.40 g/L (Figs. 3B and 4B). However, the reverse trend was observed in ammonia production which was decreasing with the concentrations moving away from the saddle point.

3.2. Confirmatory experiments

To confirm the validity of the statistical experiments, three replicates of batch experiments were performed under optimal conditions (Table 4). The confirmatory results were in reasonable agreement with the predicted responses with maximum desirability of 0.92 (Fig. 5). The iron optimized at 38.46 mg/50 ml was efficiently showing SO_4^{2-} removal of 50.63% even at higher concentrations of SO_4^{2-} and NO_3^- . Both hydrogenase and nitrogenase utilized Fe^{2+} as an essential cofactor for hydrogen production biologically (Liu et al., 2019), moreover, optima of hydrogenase activity and hydrogen production were observed at 300 mg/L and 400 mg/L Fe^{2+} concentrations respectively (Zhang et al., 2015). It was also observed that keeping the highest SO_4^{2-} (3 g/L) and NO_3^- (0.5 g/ml) and decreasing the iron concentration in the

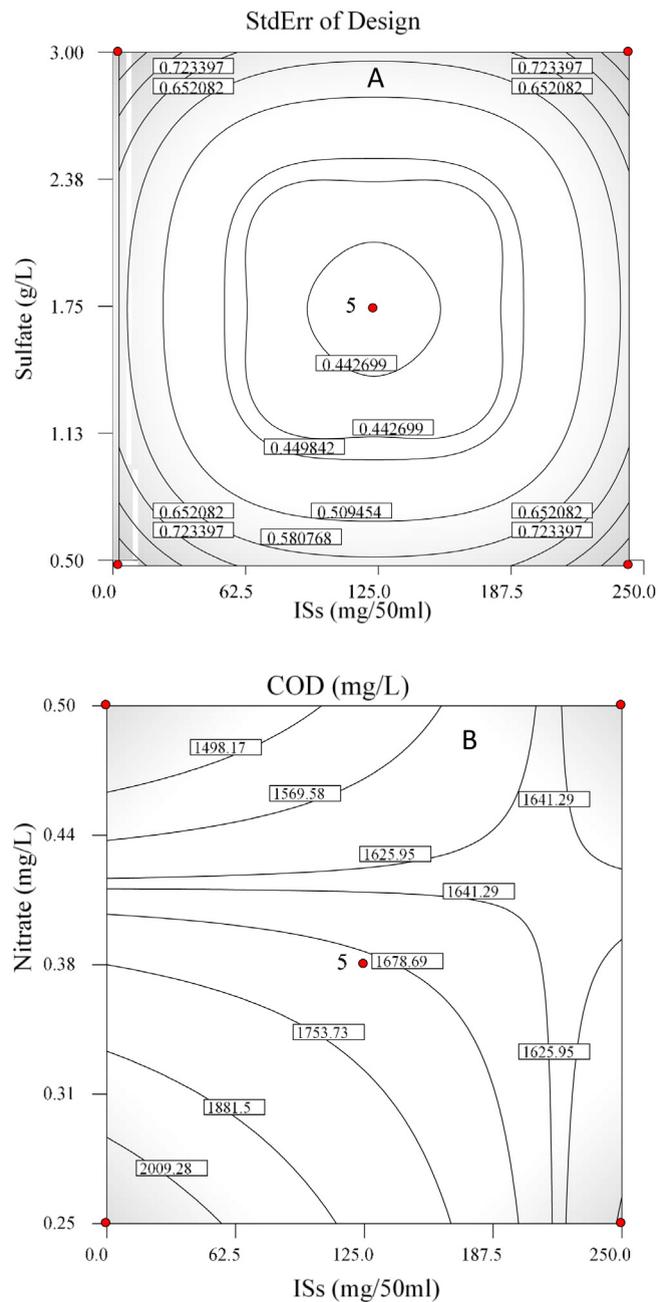


Fig. 1. (A) Standard error of the design considering the effects of ISs and sulfate concentration at the central point of the nitrate; (B) Interaction contour plots for the COD removal ISsVs Nitrate.

confirmatory experiment decreased the SO_4^{2-} removal efficiency accordingly with the maximum NO_3^- conversion. The COD removal and the gaseous production under the confirmatory conditions were also in good agreement as predicted by the model. Quite high level of the headspace H_2S and the presence of the NH_4^+ in very small concentration under the optimized conditions indicated the efficient denitrification and sulfate reduction simultaneously. The interaction of SO_4^{2-} and Fe^{2+} variables during the response optimization showed the differential H_2 production due to background sulfate levels with an elevated H_2 production at its lowest/highest levels during Phase-I. Whereas the optima was at 1300 mg/L (SO_4^{2-}) in Phase-II during optimization of the variables (Singh et al., 2021).

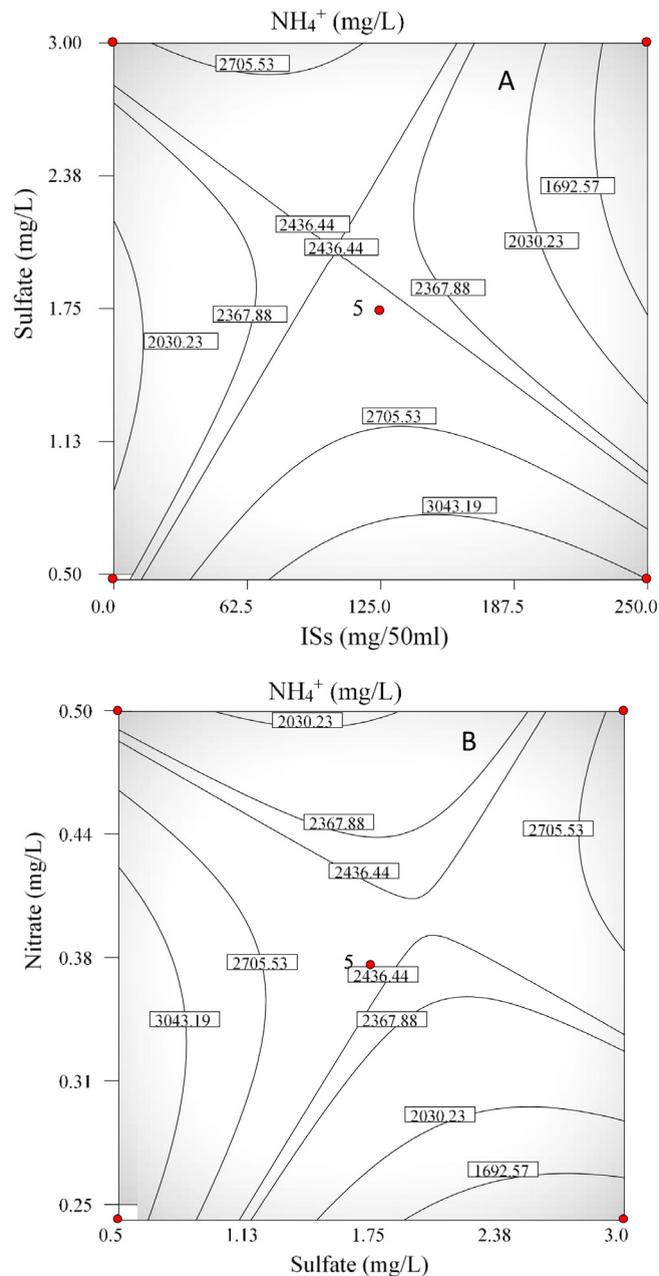


Fig. 2. Interaction contour plots for the NH_4^+ removal: (A) ISs Vs sulfate; (B) Sulfate Vs nitrate keeping respective third variable at central points.

3.3. Two-factor interaction plots

The positive (AC, +306) and negative (AC, -52.86) interactions for two-factor plots between ISs and NO_3^- for responses of COD and SO_4^{2-} removal can be achieved at higher levels of both the variables with increasing either of the variables respectively (Fig. 1B). This was also supported by the t value 2.24 and $\text{Prob} > |t| = 0.0428$ with R^2 of 0.774 (Table 3). The interaction coefficient values between ISs, SO_4^{2-} and NO_3^- for H_2S (AB = -1.40), (AC = -0.72), and (BC = 1.09) inferred its decreased production adding ISs simultaneously with either increased SO_4^{2-} or NO_3^- (Fig. 4 A&B). The decrease in H_2S may be also possible either due to the formation of FeS or the toxicity of added NO_3^- towards the SRB. However, the SO_4^{2-} and NO_3^- may also be effectively increasing H_2S production when increased simultaneously (Reg coeff = 1.09, $R^2 = 0.6468$). The ANOVA value (AB = -19.38) showed that there was a negative correlation between the interaction of ISs and SO_4^{2-} for ammonia production (Fig. 2 A) which was maximum with increasing either of the two variables as

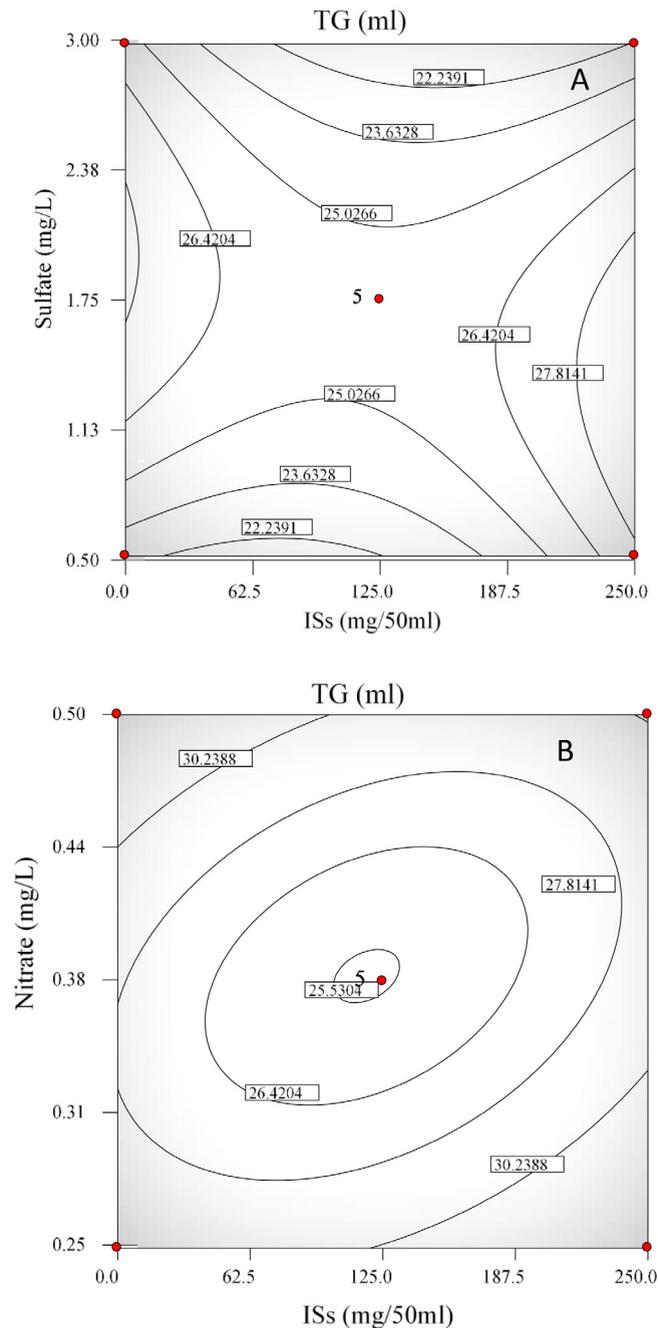


Fig. 3. Interaction contour plots: (A) Total gas production by varying ISs Vs sulfate; (B) Total gas production by varying ISs Vs Nitrate keeping respective third variable at central points.

supported by the regression coefficient value of -503.93 and R^2 of 0.7343 (Table 3). The ammonia production may be increased at a higher concentration of both ISs and NO_3^- ($AC = 9.90$). The higher concentration of SO_4^{2-} and NO_3^- ($BC = 23.16$) also increased ammonia production as also predicted by the regression coefficient of 602.26 and $\text{Prob} > F$ less than 0.05 (Table 3). This showed that the higher concentration of ISs was only responsible for efficient denitrification and less production of ammonia at constant NO_3^- when SO_4^{2-} concentration was low and vice versa. The presence of the ISs in the medium regulate the redox conditions, macro and micro patterns of redox factors create a very complex microbial community feasibly capable to promote several redox reactions (Bezbaruah and Zhang, 2004; Wiessner et al., 2005). The ferrous/ferric ions generated from the ISs may react with SO_4^{2-} to form various insoluble species of iron sulfate/sulfide,

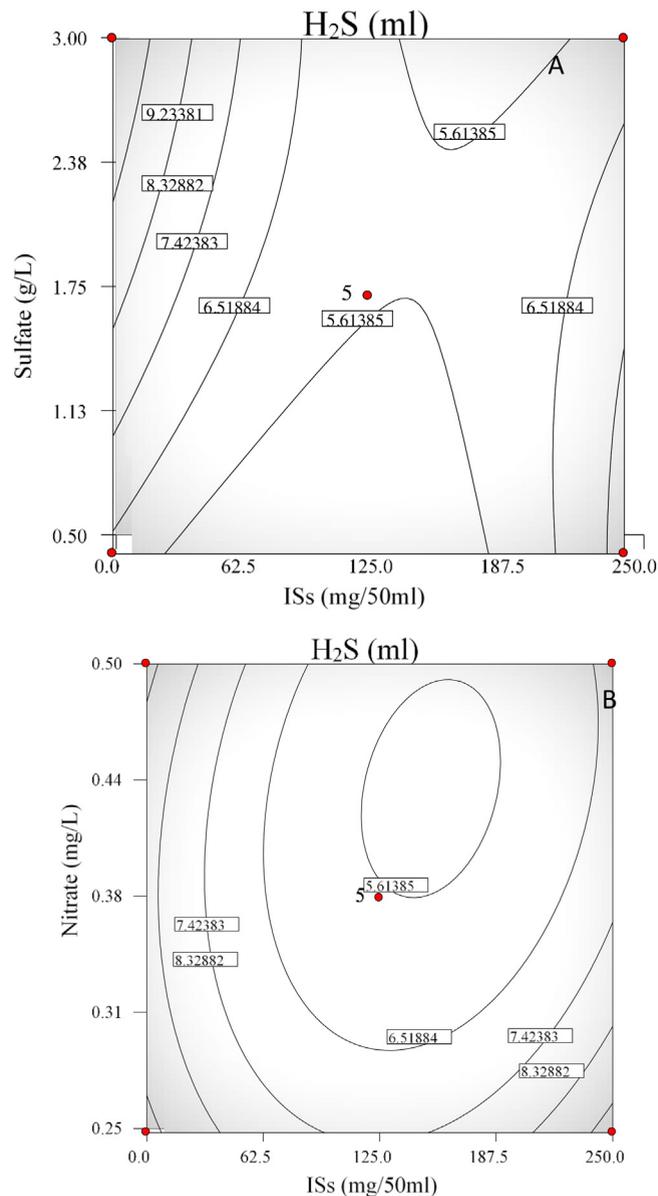


Fig. 4. Interaction contour plots for H₂S production: (A); ISs Vs sulfate; (B) ISs Vs Nitrate.

moreover, ISs can assist in the process of denitrification as electron donor to enhance nitrogen removal in CWs (Kumar and Singh, 2017).

3.3.1. RSM model development and validation of stepwise regression selection

The higher concentration of SO_4^{2-} of 3 g/L suggested by the model was found to be a responsible factor for its efficient removal with varying NO_3^- and ISs concentrations. The adjusted R^2 of 0.98 value was in reasonable agreement with the predicted R^2 value (0.984). The effect of a high concentration of SO_4^{2-} was highly significant as demonstrated by the ANOVA (Table 2) following stepwise regression with the Fisher's F^- test with a very low probability ($P < 0.01$) and non-significant lack of fit model ($P = 0.5364$). The SO_4^{2-} was responsible for positively increasing SO_4^{2-} removal showing a higher t value of 27.93 and lowest $\text{Prob} > |t|$ (< 0.0001). The addition of SO_4^{2-} alone might also be contributing to lowering the residual COD which was supported by the negative value of regression coefficient and t as -403.33 and -4.64 respectively and $\text{Prob} > |t| = 0.0003$. Although, the model suggested the Fisher's F -test with a very low probability ($P < 0.0001$) as the R^2 obtained for the model was 0.7898, yet the liability of SO_4^{2-} contribution for COD removal was not much significant with R^2 of 0.5894. The ammonia production was shown to decrease having a regression coefficient of

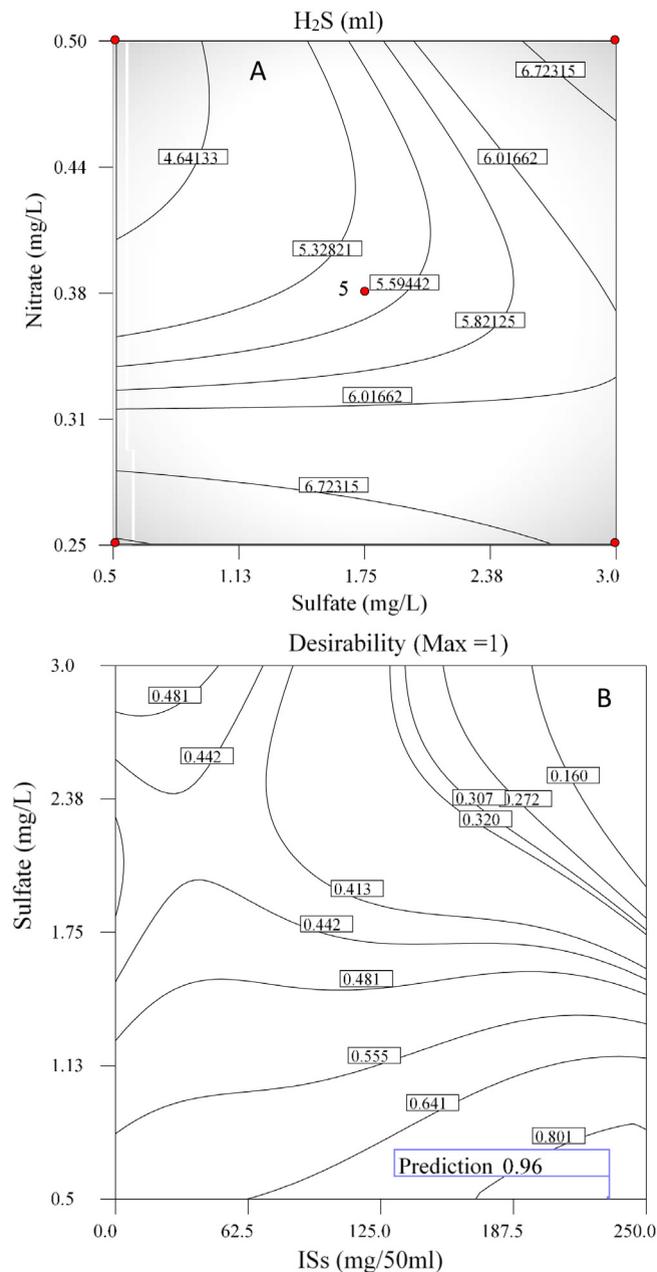


Fig. 5. Interaction contour plots for confirmatory experiment suggested by the model considering desirability as output by varying ISs Vs sulfate keeping nitrate at 0.26 (mg/L).

–328.57 and $\text{Prob} > |t|$ less than 0.05. However, increasing SO_4^{2-} to its quadratic value showed a lower performance of microbes in SO_4^{2-} removal. This was also understood by the fact that total gas production was decreasing effectively as supported by the regression and t values of –3.87 and –2.81 and $\text{Prob} > |t| = 0.0140$. The R^2 value of 0.5797 was not much significantly supporting total gas production by increased SO_4^{2-} concentration. The maximum removal of COD was achieved at NO_3^- of 0.5 g/L without ISs addition (Table 1). The nitrate individually was somehow responsible to decrease the residual COD as inferred from the negative relationship with the –166.33 regression coefficient at an increasing level. However, the t value, $\text{Prob} > |t|$ and R^2 were found as –2.08, 0.0568, and 0.6861 respectively which indicated the efficiency of COD removal to be slightly uncertain as a function of nitrate. The quadratic effect of nitrate was slight positively related with total gas production but with very low values of regression coefficient and R^2 of 4.53 and 0.3433 respectively and the significant value of $\text{Prob} > |t| (<0.05)$. This may be possible only due to the preference of nitrate consumption over SO_4^{2-} by microbial community further indicated from the NO_3^- removal in terms of dissolved ammonia conversion, as suggested

Table 2
Analysis of variance (ANOVA) output for the various responses obtained.

Source	Sum of Squares					F Value					Prob > F				
	SO ₄	COD	NH ₃	TG	H ₂ S	SO ₄	COD	NH ₃	TG	H ₂ S	SO ₄	COD	NH ₃	TG	H ₂ S
Model	4.477E+6	1.744E+6	7.818E+6	224.52	50.17	455.10	11.27	5.55	10.21	13.86	<0.001	0.0005	0.0129	0.0018	0.0006
A		35555.11	1.054E+5	1.31	3.43		0.92	0.60	0.48	7.59		0.3565	0.4615	0.5100	0.0249
B	4.460E+6	1.301E+6	8.637E+5	2.61	2.24	906.76	33.65	4.90	0.95	4.95	<0.0001	<0.0001	0.0577	0.3588	0.0567
C		2.134E+5	73837.76	0.93	4.10		5.52	0.42	0.34	9.07		0.0368	0.5354	0.5760	0.0168
A ²		–	1.881E+6	34.62	21.18		–	10.68	12.59	46.80		–	0.0114	0.0075	0.0001
B ²	16927.75	–	1.191E+6	62.93	–	3.44	–	6.76	22.88	–	0.0847	–	0.0316	0.0014	–
C ²	–	–	–	86.25	3.53	–	–	7.62	31.36	7.81	–	–	0.0247	0.0005	0.0234
AB	–	–	–	16.13	7.81	–	–	5.77	5.87	17.25	–	–	0.0431	0.0417	0.0032
AC	–	1.936E+5	–	26.00	2.07	–	5.01	–	9.46	4.58	–	0.0450	–	0.0152	0.0648
BC	–	–	–	–	4.77	–	–	8.24	–	10.54	–	–	0.0208	–	0.0118
Residual	68857.24	4.640E+5	1.409E+6	22.00	3.62	–	–	–	–	–	–	–	–	–	–
Lack of Fit	49553.20	3.936E+5	1.084E+6	16.29	3.01	1.03	2.80	3.34	2.85	4.91	0.5364	0.1679	0.1351	0.1674	0.0762
Pure Error	19304.04	70400.0	3.250E+5	5.72	0.61	–	–	–	–	–	–	–	–	–	–
Cor Total	4.546E+6	2.208E+6	9.226E+6	246.52	53.79	–	–	–	–	–	–	–	–	–	–
R ²	0.9849	0.7898	0.8473	0.9108	0.9327	–	–	–	–	–	–	–	–	–	–
Adj R ²	0.9827	0.7198	0.6946	0.8215	0.8654	–	–	–	–	–	–	–	–	–	–

Table 3
Regression coefficients for the models responses by modified process order and stepwise selection.

Coeff.	Regression Coeff estimated					t for H0 Coeff = 0					Prob > t					R ²					
	SO ₄	COD	NH ₃	TG	H ₂ S	SO ₄	COD	NH ₃	TG	H ₂ S	SO ₄	COD	NH ₃	TG	H ₂ S	SO ₄	COD	NH ₃	TG	H ₂ S	
a ₁	771.91	1687.06	2463.86	25.51	5.51	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
a ₂	–	–66.67	–114.76	0.40	–0.66	–	–	7.39	–	–2.08	–	–	–	–	0.0642	–	–	–	–	–	0.8525
a ₃	746.64	–403.33	–328.57	–0.57	0.53	27.93	–4.64	–2.33	–	1.88	<0.0001	0.0003	0.0419	–	0.0924	0.9811	0.5894	0.8279	–	–	0.8942
a ₄	–	–163.33	96.07	–0.34	–0.72	–	–2.08	–	–	–1.82	–	0.0568	–	–	0.0941	–	0.6861	–	–	–	0.7231
a ₅	–	–	–668.42	2.87	2.24	–	–	–1.97	2.55	3.25	–	–	0.0671	0.0240	0.0054	–	–	0.2062	0.7202	–	0.4130
a ₆	63.22	–	531.82	–3.87	–	1.86	–	2.03	–2.81	–	0.0847	–	0.0651	0.0140	–	0.9849	–	0.6242	0.5797	–	–
a ₇	–	–	–564.37	4.53	0.91	–	–	–1.84	2.80	1.85	–	–	0.0885	0.0134	0.0915	–	–	0.4951	0.3433	–	0.7887
a ₈	–	–	–503.93	–2.01	–1.40	–	–	–2.13	–2.57	–2.14	–	–	0.0561	0.0260	0.0501	–	–	0.7343	0.8911	–	0.5581
a ₉	–	220.0	–	–2.55	–0.72	–	2.24	–	–2.69	–2.14	–	0.0428	–	0.0195	0.0648	–	–	0.7737	–	–	0.8257
a ₁₀	–	–	602.26	1.09	–	–	–	1.86	–	1.81	–	–	0.0841	0.0940	–	–	–	0.3635	–	–	0.6468

a₁ is Intercept coefficient; a₂ is A; a₃ is B; a₄ is C, a₅ is A², a₆ is B², a₇ is C²; a₈ is A*B; a₉ is A*C, a₁₀ is B*C; COD: Chemical oxygen demand; TG: Total gas.

Table 4
The responses obtained under the confirmatory experiments.

Exp No.	ISs (mg/50 ml)	SO ₄ (g/l)	NO ₃ (g/l)	SO ₄ ²⁻ (mg/l)	NH ₃ (mg/l)	COD (mg/l)	TG (ml)	H ₂ S (ml)	NO ₃ ⁻ (mg/l)
1	38.46	3	0.5	1573.33 ± 118.02	139.10 ± 39.25	1386.67 ± 92.4	25.67 ± 0.6	11.63 ± 1.1	93.04 ± 7.41
2	37.15	3	0.5	1786.67 ± 83.18	198.17 ± 18.06	1333.33 ± 333.1	28.0 ± 0.9	13.83 ± 2.12	98.08 ± 10.24
3	14.58	3	0.5	1857.62 ± 107.71	181.23 ± 12.5	1573.33 ± 333.1	29.5 ± 0.5	13.52 ± 3.04	24.37 ± 27.65
4	10.8	3	0.5	1786.19 ± 29.74	173.90 ± 4.54	1440 ± 499.6	30.43 ± 0.9	10.87 ± 0.23	5.87 ± 8.12
5	4.06	3	0.5	1826.67 ± 13.5	186.36 ± 15.2	1786.67 ± 166.53	30.9 ± 0.4	5.63 ± 0.1	9.04 ± 3.66

by the very near values of 0.847 and 0.694 for the R² and the adj R² respectively. The ANOVA of the quadratic regression model for dissolved ammonia showed the lack of fit of 0.1351 as insignificant which is good for the model to be valid. In this case, the quadratic effect of iron scraps (A²), SO₄²⁻ (B²), and NO₃⁻ (C²) were also significant as 0.0114, 0.0316, and 0.0247 respectively. The quadratic effect of nitrate related to the decreasing total gas production was also evident from the fact where the negative regression coefficient and t value were observed as –2.01 and –2.57. The Prob > |t| required to be less than 0.05 was found 0.026 and a strong R² value of 0.891 was also obtained at constant nitrate. At constant sulfate, the interaction of ISs and nitrate for showing a negative relationship with total gas production was supported by t value and Prob > |t| as –2.69 and 0.0195 respectively. The quadratic effect of iron showed a positive impact on total gas and H₂S production having a t value of 2.55 and Prob > |t| = 0.024 with a supported R² of 0.7202 and Prob > F = 0.005. The increase in total gas and H₂S production might be possible due to the corrosion of iron scraps by the activity of SRB and thereby also inducing denitrification by reducing nitrate. The SRB needs a reducing environmental conditions with an ORP value lower than –100 mV for optimal performance (Postgate, 1984). The Fe ions not only promotes microbial growth but also the generation of Fe²⁺ from the ISs may participate in the denitrification and precipitation of soluble sulfide by reducing its toxicity. The microbial anaerobic growth performance achievement can be attributed by buffering the VFA generated and declining ORP of the bioreactor, besides, ZVI can also function as an electron donor to attenuate the competition of SRB with methanogens for substrate and reduce the toxicity of H₂S by improving the pH or precipitation (Zhang et al., 2011; Prajapati and Singh, 2018).

4. Conclusion

This study suggested that efficient removal of contaminants could be achieved simultaneously at higher concentrations of SO_4^{2-} and NO_3^- keeping ISs at optimized concentration. The highest concentration of iron and SO_4^{2-} showed efficient removal of SO_4^{2-} and COD with increased total gas production. The ISs in presence of SO_4^{2-} and NO_3^- also helped in declining H_2S and ammonia concentrations. However, at the highest iron and NO_3^- , the COD and SO_4^{2-} removal were less and increased ammonia at lower SO_4^{2-} levels following a dissimilatory nitrate reduction. The significant quadratic effect of nitrate was slight positively for total gas production at very low values of regression coefficient and R^2 of 4.53 and 0.3433 respectively. This might be possible only due to the preference of nitrate consumption over SO_4^{2-} by microbial community indicated in terms of dissolved ammonia conversion, as suggested by the very near values of 0.847 and 0.694 for the R^2 and the adj R^2 respectively. The confirmatory experiments with optimized iron showed the highest removal efficiency at the highest SO_4^{2-} and NO_3^- concentrations.

CRedit authorship contribution statement

Varsha Dhar: Investigation, Methodology, Data curation, Formal analysis, Writing – original draft. **Rajesh Singh:** Formal analysis, Methodology, Writing – review & editing, Validation, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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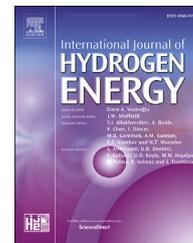
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Evaluation of pretreatment potential and hydrogen recovery from lignocellulosic biomass in an anoxic double-staged bioelectrochemical system

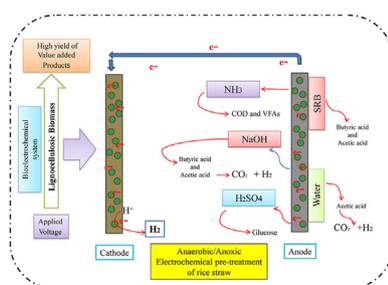
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HIGHLIGHTS

- ECP in presence of NaOH of RS favours the short carbon chain fatty acids synthesis.
- ECP by H₂O, NH₃ and H₂SO₄ favoured longer carbon chain synthesis.
- ECP in presence of NH₃ maximizes the solubilization of lignocellulosic biopolymer.
- Recovery of H₂ from ECP of RS with NaOH hydrolysate was found maximum using SRB mixed culture.

GRAPHICAL ABSTRACT



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ABSTRACT

The possible impact of bio and electrochemical reactions during pretreatment of rice straw using solvents, sulphuric acid (SA), ammonia (AM), sodium hydroxide (NA), and distilled water (W) was investigated. The total volatile fatty acids (tVFAs) of 163.48 ± 10.49 mM in the electrochemical pre-treatment in the presence of sodium hydroxide (ENA) followed by sulfate-reducing bacteria (EBA) (140.88 ± 0.07 mM) indicating the involvement of electrolytic breakdown of lignocellulosic biomass. The hydrogen production of 0.224 ± 0.05 and 0.218 ± 0.10 mM/g of the substrate was found in the ENA and anoxic sodium hydroxide pre-treatment (CNA) respectively. There was no detectable gasification in SA and AM electrochemical pre-treatments. The major advantage of the electrochemical process is the formation of acetic acid at a lower temperature, whereas processes like autohydrolysis form it at high temperatures during steam explosion pretreatment. The hydrogen production of 1.26 mM/g was found from anoxic hydrolysate pretreated (ECP) rice straw. The SEM and FTIR analysis show distortion of outer layers of biomass in the electrochemical pretreatment (ECP) system, which improved its accessibility towards enzymes for value-added product recovery.

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Introduction

The energy recovery from various wastes from treatment plants is a response to the double challenge of developing renewable energy sources while providing sustainable methods of waste management [1,2]. Expansion in the growth rate of the agricultural and agro-industrial sectors all over the world in the last few decades increases the significant quantity of waste generation [3]. Utilization of the lignocellulosic biomass such as agricultural crop residues to biofuel/value-added products is a sustainable approach over traditional fossil fuels to mitigate environmental pollution [4]. Rice straw, the lignocellulosic biomass is the most abundant agricultural waste with annual global quality production of 685 million tons [5]. In India, every year approximately 22,289 Gg of surplus rice straw is produced out of which 13,915 Gg is estimated to be burnt in the open fields causing serious environmental problems [6]. Apart from greenhouse gas emissions, the combustion process also produces toxic gasses such as carbon monoxide, carbon dioxide, nitrogen dioxide, sulfur dioxide, and hydrocarbons [7]. Lignocellulosic biomass, the rice straw consists of inorganic components (5–10%), lignin (5–24%), hemicellulose (19–27%), and cellulose (32–47%). The biohydrogen production economy depends on carbohydrates contents in the substrate and its biodegradation rate [8]. Theoretically, it can produce 41.0–43.4% of the glucose that can be utilized for the production of renewable hydrogen energy in anaerobic digestion (AD) [9].

Hydrogen production from waste lignocellulosic biomass as a sustainable process is a very interesting alternative. Besides, hydrogen (as well as biohydrogen) is one of the basic substrates for technologies like the synthesis of aniline from nitrobenzene, hexamethylenediamine synthesis, hydrocracking, synthetic gas generation (ICI process, Lurgi, gasification of coal), hydrogenation of hard coal, ammonia synthesis, methanol synthesis, hydrogenation of fats, oxo-processes [10].

However, for efficient hydrogen production, the lignocellulosic biomass should be processed using a suitable pretreatment method to consider the properties like crystallinity, adsorption/desorption of enzymes, and its enzymatic accessibility. The deconstruction of a highly recalcitrant complex lignocellulosic matrix into intermediates presents the principal hurdle to attain the economic viability essential to commercialize biomass-based technologies [11,12]. The enzymatic hydrolysis of hemicellulose and cellulose in untreated rice straw could not take place as these materials are backed by tough lignin dispersed between the cellulose layers. As lignin is among the major polymer in the cell wall of the rice straw, therefore it is necessary to consider all the components extracted in the hydrolysate for biofuel up-gradation. Therefore, pretreatment is a prior step to break lignin and expose hemicellulose and cellulose for enzymatic action [13]. To unlock these problems, several techniques like physical and chemical preprocessing have been developed to improve the efficiency of hydrolysis. The pretreatment technology requires a short

operation time without the use of any catalyst and no reduction in the size of lignocellulosic biomass [14] but each of these having some limitations. These advanced physico-chemical pretreatment technologies need expensive instruments [15], high energy costs with poor energy efficiency [16], low sugar yield [17], incomplete degradation of lignin and hemicellulose and produce high inhibitors [14]. Therefore, there is an urgent requirement for suitable pretreatment technology having minimum utilization of the chemical with maximum recovery of the soluble desired products/hydrolysate components.

The combination of electrical and chemical pre-treatment technology could be very effective for the recovery of value-added products from lignocellulosic biomass. This novel study can be used by applying small voltage for enhancing the biodegradability of rice straw to accelerate the hydrolysis step [18]. A significant amount of dead-end products such as sugars and VFAs in the hydrolysate are produced during the electrochemical treatment of the lignocellulosic biomass. This hydrolysate consisting of value-added products can be used in a second phase for hydrogen production to recover high energy from lignocellulosic biomass [19,20].

In this study, the electrochemical pretreatment of the rice straw under anoxic conditions offers the advantages of low-cost and stable technology for hydrogen and other value-added products production. Very limited studies concentrating on the monitoring of the gaseous emission as well as hydrolysate analysis to evaluate the feasibility of electrochemical pretreatments using various solvents are available. The objective of this study is to estimate the conversion potential of rice straw to gaseous components, volatile fatty acids, soluble sugar, and overall soluble chemical oxygen demand (sCOD) recovery during various electrochemical pretreatment. The hydrolysate produced was further evaluated for the biohydrogen production potential in a sulfate-reducing bacteria-based bioelectrochemical system.

Materials and methods

Rice straw collection

The rice straw was obtained from a rice field in Amarapur village, Gandhinagar District, Gujarat, India in the pre-winter season. The rice straw dried at room temperature was chopped to 2–4 mm. The chopped rice straw (moisture content <10%) was stored in plastic airtight zip lock bags. Further, the dried rice straw was milled to powder form with a mixer grinder and screened by a sieve (1 mm) before use.

Sulfate-reducing bacteria (SRB) inoculum preparation

The inoculum used in this study was prepared from the sludge sample collected from the anaerobic digester of Piranha Municipal Sewage Treatment Plant, Ahmedabad, Gujarat [21]. The collected sludge was stored at 4 °C for further use. The viscous sludge (1 kg) was added to a 5L aspirator bottle and fed with a 3L Postgate growth medium [22]. The reactor was maintained at

220 rpm at room temperature. The reactor culture was maintained by feeding the Postgate medium after regular intervals by decanting the 3L of the supernatant after settling the culture.

Rice straw pre-treatment experimental design

The present study was carried out in a 120 ml serum bottle equipped with two graphite electrodes procured from the local market (S.D. Industries), Ahmedabad (Gujarat). The graphite electrodes (Length 2.25 cm, Dia 0.6 mm) were separated 1 cm apart from each other using silicon tubing (Fig. 1). The electrodes were aligned vertically in the serum bottle using the copper wire, which was also used for the applied potential. The potential of 30 mV was applied and controlled through voltage controller 1V D.C and 5 V D.C. adaptor, procured from the local market Gandhinagar (Gujarat) India. Before use the electrodes were activated at 500 °C in the muffle

furnace (Model: Sonar) for about 30 min. The serum bottles reactors were constructed using the air distributor valve made of plastic as a sampling port [23]. The reactor was sealed with rubber septa and aluminium cap fitted with an airtight open lock device. One end of the open lock device was inserted into rubber septa and another end was fixed with a 1.5 cm silicon tube having an internal dia 0.6 mm. The septa were punctured with a needle for the narrow passage of the copper wires for voltage supply. The aluminium cap and open lock device were fixed with adhesive and m seal to prevent any leakage. Before the reactor operation, each reactor was dipped into a water bucket by over pressurizing with N₂ (99.999% purity) using a 60 ml syringe to avoid any leakage.

The quantity of rice straw was selected in such a way that it completely gets submerged in the 50 ml of the liquid content. The rice straw (7.5% w/v) was treated with 0.1 N of the respective solvents (sulphuric acid, nitric acid, ammonia,



Fig. 1 – Experimental system setup: A) Serum bottle reactor fabrication; B) Rice straw pretreatment using various solvents; C) Voltage converter system; D) Stainless steel capping of reactors; E) Electrochemical hydrolysate generation; F) Experiment set-up for H₂ recovery from hydrolysate.

sodium hydroxide), and bacterial consortium at room temperature for 15 days. The distilled water having rice straw of 7.5% w/v was kept as control. The microbial based hydrolysis of the rice straw was performed by mixing 25 ml of the culture (SRB) and 25 of the Postgate medium/distilled supplemented with 7.5% of rice straw as the substrate. Each serum bottle was purged with N₂ (99.99% purity) for 3 min and sealed with silicone rubber septa and an aluminium cap. The serum bottles were shaken manually twice a day to mix the rice straw properly. The following five sets of experiments were performed: Electrolysis with a combination of BA (Sulfate-reducing bacteria), NA (Sodium hydroxide), AM (Ammonia), SA (Sulphuric acid) and DW (Distilled water) supplied with 30 mV applied potential. Similar sets of experiments were also run parallel without electrolysis. The reactors conditions for the pretreatment of rice straw were coded with the following aberrations:

CBA: Rice straw pretreated in presence of SRB

EBA: Rice straw pretreated in presence of SRB at an applied voltage (30 ± 5.0 mV) CW: Rice straw pretreated in presence of water

EW: Rice straw pretreated in presence of water at an applied voltage (30 ± 5.0 mV)

CAM: Rice straw pretreated in presence of 0.1 N ammonia

EAM: Rice straw pretreated in presence of 0.1 N ammonia at an applied voltage (30 ± 5.0 mV)

CSA: Rice straw pretreated in presence of 0.1 N sulphuric acid

ESA: Rice straw pretreated in presence of 0.1 N sulphuric acid at an applied voltage (30 ± 5.0 mV)

CNA: Rice straw pretreated in presence of 0.1 N sodium hydroxide

ENA: Rice straw pretreated in presence of 0.1 N Sodium hydroxide at an applied voltage (30 ± 5.0 mV)

At the end of the experiments the whole reactor bottle was harvested and process for various parameters analysis.

Experimental design for H₂ recovery from hydrolysate

The hydrolysate was generated by treating the rice straw in the electrochemical system for 48h using the 0.1 N NaOH. Anoxic electrochemical pretreatment of rice straw was carried out using 7.5% w/v of dried grounded fine powder of rice straw fully submerged in 0.1 N NaOH solution. A 1.0 L reactor equipped with vertically placed two graphite electrodes (length 10 and dia 0.6 cm) at a 5 cm apart supported with a silicone tube was purged with N₂ (99.99% purity) (Fig. 1). The electrodes were continuously applied with 30 mV potential using a voltage converter. The reactor was at 240 rpm for 48 h for the generation of rice straw hydrolysate. The hydrolysate was separated from the reactor by settling the large particle and preserved in the refrigerator at 4 °C for later use.

Hydrogen recovery from hydrolysate was performed using 25 ml SRB culture with 25 ml pretreated NaOH hydrolysate in

120 ml serum bottles under anaerobic conditions in two phases of 72h duration. The SRB consortium was supplemented with Fe²⁺ (50 mg/L) and SO₄²⁻ (600 mg/L) in the Postgate media to maximize hydrogen production in Phase-I [21]. The second phase was proceeded by maintaining a pH of 6.5 with 6 N NaOH/HCl without the addition of Fe²⁺ and SO₄²⁻. The pre-treatment of SRB culture used in the study was done for 48h at 40 mV applied voltage in a 1 L aspirator bottle equipped with graphite electrodes (length 10 cm and dia 0.6). The electrodes were placed 5 cm apart [21]. All the experiments for the H₂ recovery from hydrolysate were performed at control conditions in a Meta-Lab incubator at 37 °C. Following reactors conditions were used for hydrogen production from the pre-treated rice straw hydrolysate:

- i. RS: Untreated culture with anoxic pre-treated rice straw
- ii. RSE: Untreated culture with anoxic electrochemical pre-treated rice straw
- iii. PTRS: Pre-treated culture at 40 ± 5.0 mV with anoxic pre-treated rice straw (Without electrochemical pretreated rice straw)
- iv. PTRSE: Pre-treated culture at 40 ± 5.0 mV with anoxic electrochemical pre-treated rice straw
- v. PTRS(WE): Maintain and pre-treated culture at 40 ± 5.0 mV with anoxic pre-treated rice straw (Without electrochemical pretreated rice straw)
- vi. PTRS + E (WE): Maintain and pre-treated culture at 40 ± 5.0 mV with anoxic electrochemical pre-treated rice straw.

Analytical methods

The physicochemical parameters considered in the manuscript were performed according to the standard method [24]. The pH and EC were measured using the Eutech Instrument (pH/CON 700). The Systronic Double Beam (2203) spectrophotometer was used for measuring the NH₃ and reducing sugars. The samples were digested in the SONAA COD digester unit for measuring the COD. Most of the parameters were analyzed within 48 h, whereas the samples were preserved in the refrigerator before analysis when required. Analysis of variance (ANOVA) was performed to test significant differences in the parameters between the various output data at $p < 0.05$ using Microsoft Excel 365.

Physicochemical parameters	Method	Reference
Chemical oxygen demand	Dichromate reflux method	[24]
Ammonia	Nesslerization method using Systronic double beam spectrophotometer 2203	[24]
Total Kjeldahl nitrogen	Reflux method	[24]
Reducing sugar	DNS method	[25]

Gas compositions and volatile fatty acids estimation

The volume of the gas generated was measured at 24 h intervals using a 20 ml glass syringe. The composition of the gas was analyzed using the gas chromatograph (PerkinElmer-680) configured with a thermal conductivity detector (maintained at 200 °C), using the Elite-plot Q column (30 m × 0.53 mm ID) [21]. Injector temperature was maintained to 150 °C. Initially, the oven temperature was kept to 60 °C for 3 min and it was further increased to 75 °C at the ramping rate of 25 °C/min, where it was held for 1.5 min. N₂ was used as carrier gas with a flow rate of 5.6 ml/min and the final makeup volume was 20 ml.

VFAs compositions were analyzed by gas chromatograph (PerkinElmer-680) equipped with a flame ionization detector (FID) using the Elite-wax column (30 m × 0.32 mm ID) [21]. The initial oven temperature was set at 95 °C for 5 min and subsequently increased at a rate of 10 °C/min to 140 °C, where it was held for 6 min. Further, the temperature was increased to 200 °C at a rate of 40 °C/min and hold for 5 min. The injector and detector temperatures were maintained at 250 °C and 240 °C, respectively. Helium was used as the carrier gas at a flow rate of 6.0 ml/min. Split injector mode was applied at 7:3 [21,26–28]. Before the analysis, the liquid samples were centrifuged at 5000 rpm for 15 min at room temperature. The supernatant collected was diluted 10 times with HPLC grade water and samples were acidified to pH 2–3 using Ortho-phosphoric acid. The samples were filtered using 0.2 µm membranes (Dia. 25 mm). 0.5 µl of the sample was injected using an auto-injector for analysis. A series of VFAs standards calibration curve was prepared from volatile free acid mix 10 mM (Sigma Aldrich) aqueous solution of acids: formic acid, acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, isocaproic acid, hexanoic acid, and n-heptanoic acid in the range of 0.5–5 mM. The following metrics were used for the investigations concerning the amounts and relative proportions of VFAs products [29].

- i. Alkyl groups (Methyl and Methylene groups) (mM) = $C_2 + (2 \cdot C_3) + (3 \cdot C_4) + (4 \cdot C_5) + (5 \cdot C_6)$, where C_x is VFA (mM) of the total carbon number x;
- ii. Average VFA chain length = (total mM alkyl groups/total mM VFA) + 1, where the 1 is non-energetic carbon (carboxyl group) in the VFA molecule.

Scanning electron microscopy and FTIR analysis

Scanning electron microscopy (SEM ZEISS EVO 18 Special Edition) with Smart SEM-[SYSTEM] software was used to analyse the surface morphology of the rice straw before and after treatment. Samples were prepared according to the method discussed [30,31]. Small dried pieces of rice straw samples were primarily fixed in 2.5% glutaraldehyde at 4 °C. After 3–6 h of fixation samples were washed 3 times at an interval of 15 min with 0.1 M phosphate buffer and then fixed in 1% osmium tetroxide for 2 h for post-fixation. The sample was again washed with 0.1 M phosphate buffer 3 times at an interval of 15 min at 4 °C. Thereafter dehydration of samples was done at 4 °C with increasing concentrations such as 30%, 50%, 70%, 90%, 95% acetone and 100% dry acetone for 30 min.

The rice straw was mounted on aluminium stubs with double-sided carbon tape sputter-coated with a palladium coater (Quorum SC7620 Mini sputter coater). After optimizing the image clarity, the magnification of rice straw was taken on a maximum 500× to 510 × level.

FTIR analysis was carried out using a JASCO FT-IR-4700 type A model equipped with a TGS detector with a resolution of 8 cm⁻¹ at scanning speed (2 mm/s). The spectral range of the peak was recorded 399.193 cm⁻¹ to 4001.57 cm⁻¹ at data interval 1.92847 cm⁻¹. The samples were dried in the hot air oven at 50 °C before analysis.

Results and discussion

Compositional analysis of liquid phase

Various pretreatment strategies are being used to enhance the accessibility towards enzymes by the modification of the recalcitrant structure of the lignocellulosic material (rice straw). [32], Tables 1 and 2 show the compositional analysis of the hydrolysates recovered during various pretreatment of the substrate. The decrease in pH of the hydrolysate was recorded for all the pretreatments except, SA, whereas a small increment to 2.13 ± 0.04 and 2.17 ± 0.02 was observed in the CSA and ESA respectively. However, the highest drops in the pH value of >5 were observed in the CNA and ENA treatments. The more drop in pH indicates a high accumulation of organic matter due to the higher hydrolysis process which results in the formation of simple sugar, fatty acids, and amino acids by conversion of carbohydrate, fats, and protein respectively [18].

The highest electrical conductivity observed in sulphuric acid electrolyzed treatment indicated the highest rate of mineralization of the rice straw and corresponds to a higher concentration of ions in hydrates after treatment. The electrical conductivity of hydrolysate depends on the concentration of minerals sulfate, chloride, nitrate, carbonate, and phosphates present in the biomass along with the nitrogen, sulfur, and alkaline metals which enhanced the electrical conductivity of hydrates [32]. Thus, the higher electrical conductivity in the acidic pH of sulphuric acid treatment was observed in comparison to other pre-treatments.

The significantly high level of the sCOD in all the treatments inferred the solubilization of the lignocellulose biomass. Though alkaline pre-treatment of the lignocellulosic biomass enables efficient removal of lignin, as well as the acetyl and uronic acids in hemicellulose [33], while hemicellulose and cellulose solubilization are minor [34]. Interestingly even higher sCOD of 11600 ± 842.85 mg/L in the EAM treatments associated with electrolysis inferring the higher solubilization of the lignocellulosic biomass as compared to CAM pretreatment. The absence of a CO₂ signature indicating no loss in the carbon components during the ammonia pre-treatment. This may be probably due to the formation of (NH₄)₂CO₃, which further can provide the alkalinity during the recovery of the energy (hydrogen/methane) at the second stage. Moreover, more drops in the pH in the EAM ammonia pre-treatment also confirm the formation of more VFAs and CO₂ in comparison to CAM pre-treatment. The electro-

Table 1 – Hydrogen production and compositional analysis of liquid samples.

Expt.	pH		Final	Residuals (mg/L)						Cumulative Gases		
	Initial	Final	EC (mS)	COD	NH ₄ ⁺	NO ₃ ⁻	TKN	Sulfate	Glucose	H ₂ (mM/g _s)	H ₂ S (μM/g _s)	CO ₂ (μM/g _s)
CBA	6.78	5.05 ± 0.04	17.3 ± 0.47	4160 ± 277.13	94.67 ± 26.23	8.14 ± 2.42	156.8 ± 11.2	N.D	723.12 ± 4.66	0.13 ± 0.04	9.85 ± 1.70	234.7 ± 78.31
EBA	6.78	5.13 ± 0.07	17.73 ± 0.32	5826.67 ± 976.80	44 ± 6.11	11.02 ± 2.23	186.67 ± 26.46	N.D	729.57 ± 9.45	0.06 ± 0.02	14.94 ± 3.70	337.88 ± 85.59
CW	6.85	5.06 ± 0.15	10.48 ± 0.38	4200 ± 848.53	27.3 ± 16.03	25.40 ± 3.79	522.67 ± 45.26	N.D	721.51 ± 2.46	0.093 ± 0.03	N.D	107.16 ± 38.56
EW	6.85	4.97 ± 0.10	9.83 ± 0.69	4720 ± 366.61	N.D	16.07 ± 11.79	444.27 ± 17.11	N.D	737.10 ± 5.82	0.021 ± 0.01	N.D	38.08 ± 16.77
CAM	9.67	9.13 ± 0.18	17.07 ± 2.10	5160 ± 206.17	122.67 ± 14.67	52.35 ± 6.44	3345.07 ± 233.15	N.D	797.31 ± 16.55	N.D	N.D	N.D.
EAM	9.67	8.96 ± 0.12	19.32 ± 0.76	11600 ± 842.85	186.22 ± 53.70	38.67 ± 1.76	3363.73 ± 161.66	N.D	965.59 ± 160.37	N.D	N.D	N.D.
CSA	1.9	2.13 ± 0.04	26.17 ± 1.16	3760 ± 366.61	27.33 ± 4.71	21.05 ± 2.78	210.93 ± 26.46	4661.90 ± 16.50	3006.45 ± 75.03	N.D	N.D	N.D.
ESA	1.9	2.17 ± 0.02	26.87 ± 1.14	4880 ± 603.99	40.0 ± 13.20	30.25 ± 12.03	235.33 ± 29.71	4980.95 ± 423.86	3050.54 ± 57.37	N.D	N.D	N.D.
CNA	11.4	6.14 ± 0.16	17.22 ± 0.46	4800 ± 831.38	51.56 ± 15.45	45.68 ± 6.04	397.6 ± 14.82	N.D	859.14 ± 74.62	0.218 ± 0.10	N.D	211.94 ± 89.73
ENA	11.4	6.02 ± 0.23	17.48 ± 0.23	6400 ± 733.21	61.33 ± 16.71	46.46 ± 8.85	468.53 ± 11.66	N.D	920.97 ± 57.55	0.224 ± 0.05	N.D	253.75 ± 91.54

The rice straw pretreated with; CBA: SRB; EBA: Electrolysis (30 ± 5.0 mV) in presence of SRB; CW: Water; EW: Electrolysis (30 ± 5.0 mV); CAM: 0.1 N ammonia; EAM: Electrolysis in presence of 0.1 N ammonia; CSA: 0.1 N sulphuric acid; ESA: Electrolysis in presence of 0.1 N sulphuric acid; CNA: 0.1 N Sodium hydroxide; ENA: Electrolysis in presence of 0.1 N sodium hydroxide; N.D: Not detected.

Table 2 – VFAs production during anoxic incubation in various pre-treatment.

Experiment Set-up	Volatile fatty acids (mM)									
	Acetic Acid	Formic Acid	Propionic Acid	Isobutyric Acid	Butyric Acid	Iso Valeric Acid	valeic Acid	Hexanoic Acid	n-Heptanoic Acid	Cumulative VFAs
CBA	16.69 ± 1.66	10.46 ± 1.87	0.20 ± 0.02	21.20 ± 2.93	49.50 ± 4.13	0.12 ± 0.01	1.34 ± 0.07	0.52 ± 0.08	0.14 ± 0.05	100.30 ± 6.23
EBA	20.80 ± 4.57	13.29 ± 1.60	0.35 ± 0.03	25.61 ± 2.92	70.46 ± 9.68	0.23 ± 0.03	2.01 ± 0.14	1.30 ± 0.40	1.23 ± 0.518	140.88 ± 0.07
CW	11.783 ± 6.602	1.28 ± 0.89	0.22 ± 0.01	10.38 ± 0.08	67.84 ± 2.75	0.17 ± 0.04	N.D	N.D	N.D	92.316 ± 4.837
EW	40.4692 ± 3.038	1.44 ± 0.33	0.44 ± 0.05	2.39 ± 0.16	58.64 ± 14.83	N.D	N.D	N.D	N.D	105.229 ± 11.493
CAM	7.562 ± 1.66	0.35 ± 0.02	0.18 ± 0.01	N.D	46.97 ± 12.06	N.D	N.D	N.D	N.D	46.15 ± 1.08
EAM	9.65 ± 3.29	0.55 ± 0.10	0.24 ± 0.06	0.24 ± 0.09	51.06 ± 13.23	0.09 ± 0.02	N.D	N.D	N.D	52.66 ± 4.40
CSA	2.39 ± 0.07	N.D	0.26 ± 0.03	N.D	55.51 ± 8.63	N.D	N.D	N.D	N.D	53.93 ± 6.21
ESA	2.69 ± 0.39	0.65 ± 0.12	0.32 ± 0.10	N.D	46.20 ± 4.78	N.D	N.D	N.D	N.D	54.52 ± 3.44
CNA	77.11 ± 16.3	11.34 ± 2.88	0.34 ± 0.05	6.40 ± 0.91	48.62 ± 0.05	0.12 ± 0.01	N.D	N.D	N.D	152.53 ± 14.08
ENA	80.14 ± 16.46	12.95 ± 1.87	0.34 ± 0.06	5.85 ± 0.45	66.45 ± 3.70	0.19 ± 0.06	N.D	N.D	N.D	163.48 ± 10.49

The rice straw pretreated with; CBA: SRB; EBA: Electrolysis (30 ± 5.0 mV) in presence of SRB; CW: Water; EW: Electrolysis (30 ± 5.0 mV); CAM: 0.1 N ammonia; EAM: Electrolysis in presence of 0.1 N ammonia; CSA: 0.1 N sulphuric acid; ESA: Electrolysis in presence of 0.1 N sulphuric acid; CNA: 0.1 N Sodium hydroxide; ENA: Electrolysis in presence of 0.1 N sodium hydroxide; N.D: Not detected.

hydrolysis pretreatment destructs the outer lignin layer to release intracellular and extracellular biopolymers in the soluble phase which increases the COD [35].

The nitrogenous components could play a significant role in the anaerobic process. The bacterial pre-treatment produces the lowest TKN of 186.67 ± 26.46 mg/L and 156.8 ± 11.2 mg/L in EBA and CBA respectively due to the consumption of nitrogen by bacteria for protein synthesis [36]. Moreover, the lowest ammonia in the electrochemical pretreatment (EBA, EW, and EAM) showing more ammonia availability for its redox reaction. Similarly, outcomes show that the $\text{NO}_3\text{-N}$ removal was always higher in the MEC integrated CW compared to the normal CW [37]. Whereas the electrochemical system operated in presence of the H_2SO_4 and NaOH solvents show a higher level of ammonia.

The highest glucose concentration of 3050.54 ± 57.37 mg/L and 3006.45 ± 75.03 mg/L was found in ESA and CSA respectively showing electrolytic pretreatment improves high sugar yield avoiding the use of enzymes in the saccharification stage [38]. This pretreatment operating strategy involves dilute acid for high glucose recovery by applying a small voltage at room temperature to produce value-added products. Interestingly the residual glucose in the hydrolysate was almost equal in the CBA, EBA, CB, and EW ranging from 721.51 ± 2.46 to 737.10 ± 5.82 mg/L. A slightly higher level of glucose to 729.57 ± 9.45 and 737.10 ± 5.82 mg/L in the EBA and EW showing the additional conversion of cellulose into glucose from the rice straw by the electrolysis of the polymer in presence of the respective solvents.

Effect of the solvent pretreatment of the gas production

Table 1 shows the cumulative gas and hydrogen production based on daily monitoring of gas of different pretreatment methods. The differences among the cumulative values of hydrogen production indicating NA pretreatment was more

effective for gas production. No gas production was observed in the pretreatment with SA, AM with and without electrolysis throughout the experiment.

Hydrogen production during pretreatment

Recovery of hydrogen production during various pretreatments was analyzed at regular intervals of time in bio-reactors and Bioelectrolysis reactors (BESs) with an electrode potential of 30 mV. Fig. 2 shows the hydrogen production profile in various pre-treatments for 15 days. As a result of various pre-treatments, an insignificant variance ($p = 0.056$) in the hydrogen production among the treatments having gas production was observed. Two-dimensional data matrix plots of hydrogen produced showing the major peaks during the initial 24h (5.38 ml) and 144h (5.69 ml) in the CBA and CNA respectively (Fig. 3A). With few samplings having no hydrogen production (white colour), all the pre-treatments showing the hydrogen production of <1.0 ml. The white region in the figure showing very lesser hydrogen production which may be below detection limits during the analysis. The direct and indirect electrochemical reactions between the reactant and the electrode surface directly (direct electrolysis), or by a solution-phase redox mediator (indirect electrolysis) generally involve outer-sphere electron transfer to the mediators and allow exploiting highly tailored molecular redox catalysts [39]. This change in the lignocellulosic material during the various physicochemical pretreatment, by various catalysts not only could remove lignin but could also effectively destroy the interaction of hydrogen bonds between celluloses as a result of the structural changes as revealed from the SEM and FTIR data. Moreover, the rice straw was not sterilized during the pretreatment, the microbes associated with biomass may also contribute to the emission of the gases under anaerobic conditions. Though the variation in the emission of the gases from the different treatments may be due to the different types of solvent used.

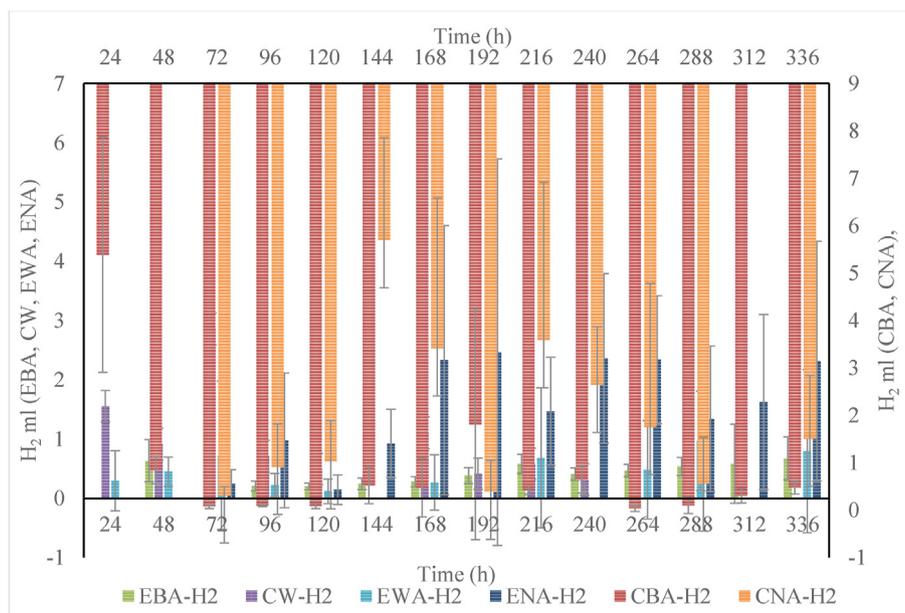


Fig. 2 – Hydrogen production profile as a result of various pre-treatments of rice straw.

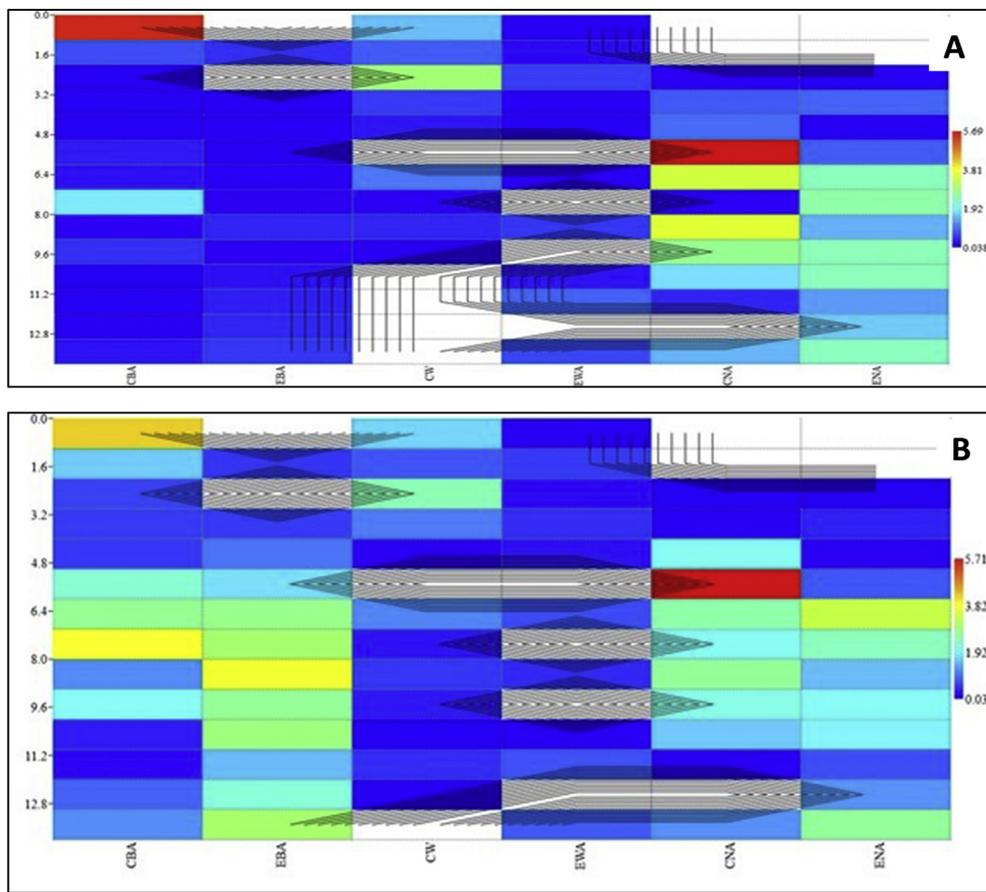


Fig. 3 – Two-dimensional data matrix plots of gas produced during various pre-treatment: A) H₂; B) CO₂. The colour scale provides the lowest to highest values of the respective gas. The white colour regions missing values showing the no gas production, red colour for highest gas production. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Despite the delayed appearance of the hydrogen production in the NaOH pre-treatment shown a comparatively much higher hydrogen production efficiency of 18.31 ml and 18.84 ml in the NA and ENA respectively. Whereas a little higher variance of 3.52% was observed in the NA in comparison to ENA pre-treatment (2.63%). It was observed that applied potential influences the higher percentage of hydrogen production by nearly 38%–59% after 216 h of pre-treatment. This occurs due to the combined effect of electrolysis due to applied voltage [18] and the corrosive nature of sodium hydroxide [40] which distracts the outermost lignin layer to form a high concentration of VFAs as soluble COD [35]. As a result, a large part of lignin is removed, cellulose is depolymerized and its digestibility is improved in the pretreatment performed with NaOH [41]. The detection of the hydrogen was ceased 168h onward of the incubation in CW, whereas hydrogen detection with few frequencies (4 times) was observed to 288 h of incubation in the EWA pre-treatment (Fig. 2). The disintegrated organic matter of rice straw was easily hydrolyzed during anoxic pre-treatment. Further, these simple monomers formed due to the combined effect of electrolytic and NaOH treatment are converted into H₂, CO₂, alcohol, and short-chain organic acids.

Hydrogen production during bacterial pre-treatment of rice straw has been accomplished by extensive hydrogenase activity of sulfate-reducing bacteria that produce H₂, CO₂ and acetate where sulfate is limited [42]. Interestingly SRB based BES integrated for pre-treatment comparatively show low H₂ yield due to high H₂S production during the electrolytic pre-treatment. Initially, a low percentage of hydrogen was found in 24 h due to the disturbance of the bacterial population by suddenly applied voltage in comparison to reactor operated without applying voltage. Researchers have reported that the applied potential of nearly 44.4 mV reduces the sulfate in the medium by electron flux for high H₂S gas production [21]. It has also been reported that H₂ production influence by sulfate addition in microbial growth medium that consumes H₂ to generate H₂S at lower redox potential [42,43].

Water pre-treatment shows a sudden increase in trend in H₂ production from 15% to 44% from 24 to 72 h, where it achieved maximum production. A further decline in trend till 144 h was seen and then its production increased by nearly 44% in 168 h (Fig. 2). However, beyond this incubation, no H₂ production was observed throughout the experimental period. Applied voltage triggers the hydrolysis steps in anaerobic digestion. Disruption of a complex

rice straw lignocellulose structure by electrophoresis, ohmic heating, and electro-osmosis results in fragmentation of polymer into ionized soluble monomer results in the release of 30% of H₂ production in electrolytic pre-treatment of water [18,44].

CO₂ production during pre-treatment

A significant variance in CO₂ production ($p = 0.003$) was observed during the various pre-treatments (Table 1). The CO₂ emissions of $143.96 \pm 109.37\%$, $35.54 \pm 43.49\%$, and $119.73 \pm 102.01\%$ times higher in EBA, EWA, and ENA respectively in the electrochemically pre-treatment of the rice straw showing the breakdown of the soluble components to CO₂ with the application of applied potential. Fig. 3 B shows the two-dimensional data matrix of CO₂ production in various pre-treatment. Initial monitoring of the bacterial treatment shows no CO₂ production due to sudden disturbance of the microbial population by the applied voltage on the electrodes. The CBA and CW show the CO₂ emission in 24h of incubation, whereas in the EBA and EWA the CO₂ was detected in 24 h of incubation followed by 72 h of incubation in the NaOH pre-treated sets. The CO₂ emission starts from 18% in 72 h and is enhanced to nearly 50% in 216 h of pre-treatment in the system in presence of NaOH. NaOH pre-treatment removes the lignin by opening the structure of lignocellulose material by breaking the ester bonds between lignin, hemicellulose and cellulose, as a result, it efficiently increases the accessibility of enzymes to the cellulose [45]. When the bacterial population was adapted to the applied voltage sudden enhancement of the CO₂ production occur after 48 h. The pre-treatment of rice straw coupled with the BES system was showing nearly 42–60% carbon-di-oxide emission from 96 h till the end of the cycle, which was nearly 1.12 times higher than pre-treatment in control. The highest cumulative CO₂ emission ($337.88 \pm 85.59 \mu\text{M/g}$) in the EBA might be due to the synergistic effect of the microbial activity and the potential applied.

The lowest CO₂ emission of 12.92 ± 1.04 and $6.90 \pm 1.77 \mu\text{M/g}$ in the CW and EWA might be due to the low accessibility of the lignocellulosic material for the breakdown in the water medium. The low hydrolysis of rice straw due to less solubilization of the lignin causes minimal delignification which results in the least damage to hemicellulose crosslinking across cellulose for CO₂ emission in comparison to NaOH and microbial pretreatment. Whereas in microbial and NaOH pre-treatment release enzymes and soluble components of the lignin respectively might be playing a significant role in the modification of the lignocellulosic biomass. The CO₂, CH₄, and H₂ concentrations in the biogas are directly influenced by the biodegradability of biomass that directly depends on the disintegration of (i.e., lignin, cellulose, and hemicellulose) [18].

Volatile fatty acids production during pre-treatment

Table 2 shows the VFAs composition analyzed under various pre-treatments. A significant variance in the VFAs produced under the various pretreatments was observed (Single-factor ANOVA $p < 0.05$), with the highest variance in the acetic acid (706.15%) followed by butyric acids (145.14%). Other VFAs except for isobutyric acid (58.04%) and formic acid (21.21%),

the variance observed was less than 0.4%. The electrolytic pretreatment operations' showing a significantly elevated level of VFAs production (Times higher: EBA = 207.13%, EWA = 137.84%, EAM = 53.86%, ESA = 52.77%, ENA = 96.67%) in comparison to respective without electrolysis treatment. This observation indicates that the electrolytic treatment enhances the degradation of organic matter by hydrolysis and conversion into value-added products. NaOH pretreatment was showing a high accumulation of VFAs production. In this treatment elevated levels of acetic acid and butyric acid were found. Bacterial pretreatment coupled with bioelectrolysis system produces butyric acid, isobutyric acid, acetic acid, and formic acid as the major constituent of the VFAs. The bioelectrolysis supports the production of these fatty acids which could be efficient in utilization of the continents in anaerobic digestion for bioenergy recovery. When acetate is the only reduced carbon byproduct, the H₂ yield is 4 mol mol⁻¹ of glucose, whereas, if acetate and butyrate are the byproducts, the maximum theoretical H₂ yield is further reduced to 3 mol mol⁻¹ of glucose [46]. The electrolytic treatment with ammonia shows a high accumulation of VFAs inferring applied voltage promotes its accumulation with major constituents of butyric acid, Iso-butyric acid, and acetic acid in EBA and EAM treatment. Studies reported that low pH influences the formation of acetic acid, propionic acid, and butyric acid [47].

Butyric acid production was found highest among all the fatty acids produced which is a vital metabolic product for H₂ evaluation and acetate production [48]. Researchers have found that undissociated butyric acid remains in the undissociated form at pH 4.9 [49]. Acetic acid production was found to be relatively higher in an alkaline pretreatment (NaOH) and relatively low accumulation was found high acidic medium of H₂SO₄. The highest concentration of acetic acid in the ENA, confirming the hydrolysis of the acetyl group for acetic acid formation. This is the major advantage of the electrochemical pre-treatment of the rice straw to form acetic acids, whereas another process of autohydrolysis of the acetyl group forming acetic acid occurs at high temperatures during steam explosion pretreatment [50]. The bacterial (SRB) pre-treatment shows a significant amount of acetic acid production due to the inhibition of methanogens by enriched SRBs.

The study illustrates the low detection of propionic acid was found in all the pre-treatment [51]. This attributes to the fact of low propionate yield inhibition occurs especially when the pH is below 6.0. Isovaleric and valeric acid synthesis was relatively found in minor concentrations in bacterial and sodium hydroxide treatments. The EAM and CW pre-treatment reactors were also showing their production in minor concentrations. Normally the higher level of these VFAs is observed in the degradation of the protein-rich substrates [52,53]. Hexanoic acid and heptanoic acid were also found in low concentrations and were only produced in bacterial treatment their formation was supported by applied voltage bacterial treatment. The shortest chain length in the VFAs was observed in the NaOH pretreated hydrolysate (ENA: 2.98, CNA:2.73), whereas the longest chain length was found in the sulphuric acid pretreated hydrolysate (ESA:3.85, SA:3.91). Interestingly except for EBA and ENA, the longer chain length was found without electrochemically pretreated hydrolysate.

Overoxidation and re-polymerization of monomers are some of the main problems found in lignin electrochemical oxidative depolymerization which can be overcome by removing the low-molecular-weight depolymerization products from the reaction medium as soon as they are formed [39].

Surface morphological change in rice straw raw solid residues after pretreatment

The surface morphological changes in rice straw were examined at the end of the experiments using SEM to reveal the structural changes in different solvents (Fig. 4). Significant surface morphological changes were observed in different solvents pre-treatments. The surface of untreated rice straw

(Fig. 4 UT) showed smooth and compact structure arranged fibers in bundle mainly composed of lignin and hemicellulose with some ridges and small dots indicating the presence of silica particles in the outer layers. The SA, AM, and NA treatments were found more severe. These treatments destroyed the lignin and hemicellulose fibers [54]. The removal of hemicellulose leads to increased porosity and improved enzymatic digestibility [55,56], when treated with weak acids. The highest damaged outer surface in the SEM image (Fig. 4 a.m.) of pre-treated with ammonia shows the possibility of the release of the polymer in form of COD. Thereby resulting in exposure of internal structure with clear pore formation. These changes will allow easier enzyme penetration and absorption to provide an increased susceptibility for enzymatic hydrolysis [57]. The

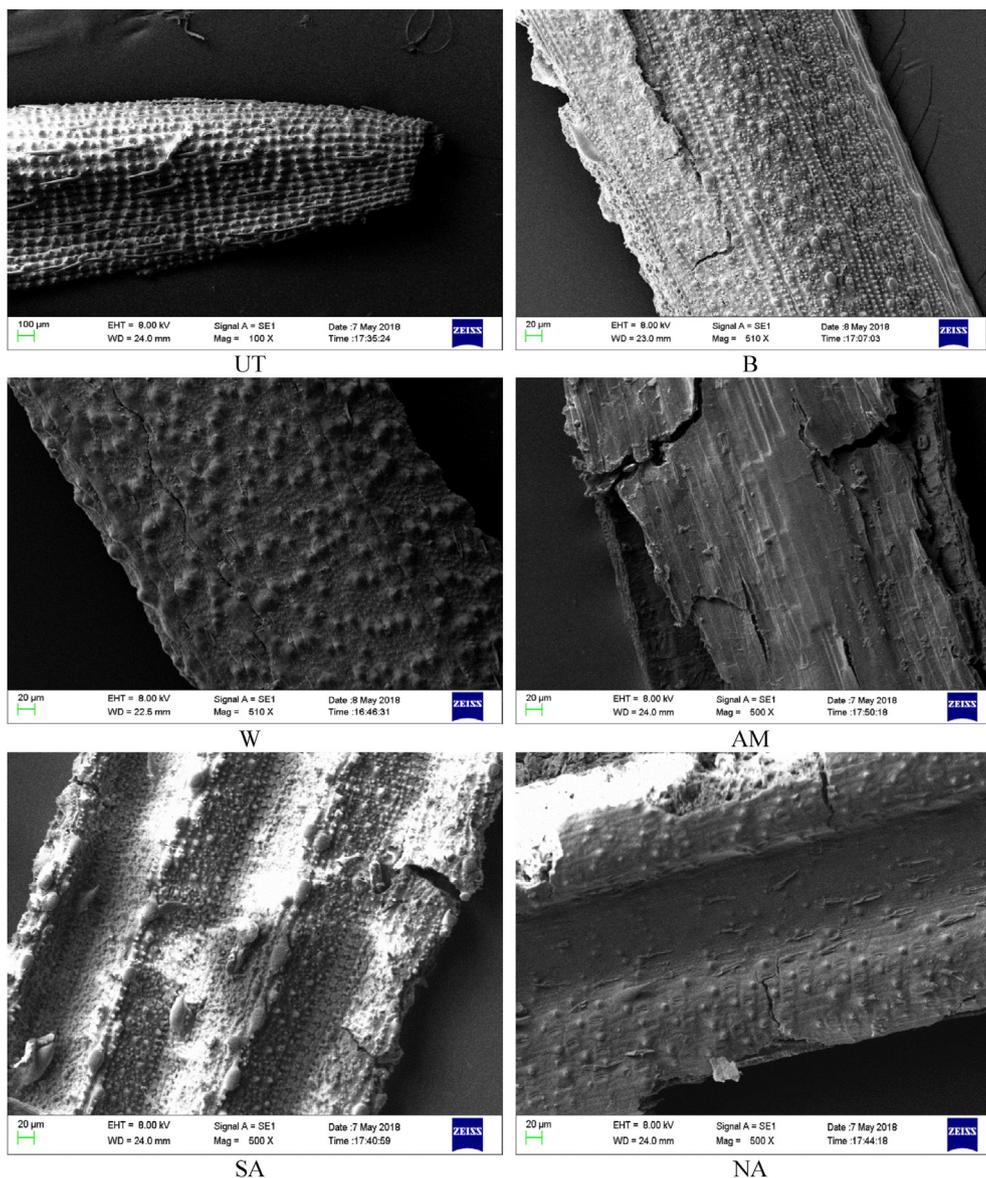


Fig. 4 – Scanning electron microscopy of the rice straw: UT) Untreated; B) Pre-treated with bacteria; W) Pre-treated with water; AM) Pre-treated with ammonia; SA) Pre-treated with sulphuric acid; NA) Pre-treated with sodium hydroxide.

bacterial and water treatment causes minimal changes in the surface morphology (Fig. 4 B and W). It shows the smooth layer with minimum destruction of rice straw layer as compared to sodium hydroxide, sulphuric acid and ammonia treatment (Fig. 4 NA, SA, AM) which is indicative of the inefficient removal of hemicellulose/lignin and low enzymatic digestibility of cellulose in rice straw. Fig. 4 (NA) showing NaOH treatment is quite effective in an increase in the internal surface area of cellulose, by the disruption of lignin structure.

The structural changes in the electrolytic pre-treated rice straw analyzed using FTIR spectroscopy are presented in Fig. 5. The spectra attribute the change in the various concentrations of lignin, hemicellulose, and cellulose in treated rice straw. Similar trends of the peak have been observed in electrolytic concerning without electrolytic treatment with the difference in the value of transmittance confirms the alternation of in chemical bonds [58]. Initial peak appearance in control and electrolytic treatment in the range of 880–890 cm^{-1} with nearly 1.7% in all the treatments which has been reduced to 0.004% in water treatment indicating the C–H deformation in cellulose [59,60]. Peak range between 1162 and 1157 cm^{-1} was found in higher transmittance in electrolytic ammonia treatment attribute to C–O–C vibrations in cellulose and hemicellulose [59,60]. A similar pattern of peak characteristic was noted in all the electrolytic pre-treatment with transmittance in a range near to 2.1% except in water treatment. Sulphuric acid treatment shows higher transmittance nearly 3.49% was found in the 1199–1200 cm^{-1} peak range due to symmetric stretching C–O–C glycoside [58]. The peak range between 1234 and 1244 cm^{-1} shows higher transmittance of 3.97% in sulphuric acid treatment due to C=O stretching; methyl, C–C, and C–O stretching vibrations vibration [59]. The peak range between 1319 and 1332 cm^{-1} nearly 4.1% transmittance was observed in all the treatments due to C–H vibration in cellulose and C–O vibration of S rings and peak range of 1363–1376 cm^{-1} having higher

transmittance of 3.28% was due to C–H deformation of cellulose and hemicellulose [59]. The peak range is between 1513 and 1400 cm^{-1} aromatic skeletal vibrations of lignin components in biomass [59]. The wavenumber of 1520–1590 cm^{-1} in all the treatments can be assigned as C=O stretching and aromatic vibrations of lignin [61]. The peak range between 500 and 1770 cm^{-1} is the lignin fingerprint region [54]. The decrease in the intensity of absorbance in electrolytic pre-treatment indicates the higher digestibility of lignin and hemicellulose [54]. The appearance of a distinct peak between 1640 and 1740 cm^{-1} may be due to C–O stretching vibrations and C–H stretching vibration (CH_3 , CH_2) was noted in the range of 2292–2296 cm^{-1} caused by lignin destruction [53,60].

Hydrogen recovery potential from hydrolysate in a BES

Hydrogen production from NaOH hydrolysate recovered under anoxic electrochemical pre-treatment to access its field implementation in various phases is presented in Table 3. Results obtained from ECP of rice straw hydrolysate using pre-treated anaerobic culture at 40 mV potential shows higher cumulative hydrogen production in both phases. Anoxic ECP of rice straw hydrolysate fermentative products were efficiently utilized for hydrogen production by SRBs. H_2 production was not detected under control conditions as methanogens were active which consumed H_2 and acetic acid for methane production [42]. Comparatively lower acetic acid production in the control conditions and elevated level of acetic acid in a reactor operated with ECP hydrolysate with a continuous supply of voltage confirms the inhibition of methanogens the common substrates for methanogens [21]. Simultaneously pretreatment of SRB culture at applied potential with the addition of an optimum level of Fe^{2+} and SO_4^{2-} support hydrogenase activity of the SRB culture in the bioelectrochemical system for higher H_2 production [21]. The drops in H_2 production in Phase-II might be due to the high accumulation of VFAs of 150.04 ± 22.52 mM in Phase-I which results in a drop in pH nearly 5 with lower H_2 production efficiency. The trace amount of H_2S production was found due to a low level of sulfate addition in media and a drop in pH below 6 inhibit the growth of SRB and sulfide production disappeared at pH 5.5 [62].

The VFAs monitored at the end of each phase are presented in Table 3 tVFA concentration was found maximum in ECP supplemented with the optimum levels of Fe^{2+} and SO_4^{2-} . Interestingly SRB using ECP rice straw hydrolysate showing in higher accumulation of acetic acid and lower butyric acid concentration showing effective growth of SRB and methanogens population inhibition [63]. The major concentration of acetic acid showed that hydrogen was produced by SRB through the acetic acid pathway from ECP rice straw hydrolysate [21]. The decrease in the concentration of formic acid in the reactor operated by ECP rice straw hydrolysate from Phase I to Phase II shows the successful existence of microbial electrolysis in these reactors. This drop might be due to polymerization of the chain length or H_2 conversion. The reactor PTRS + E (WE) showing the highest 26.55 ± 3.51 mM of Isobutyric acid and continuous supply of voltage to the ECP hydrolysate decrease its concentration by nearly half showing

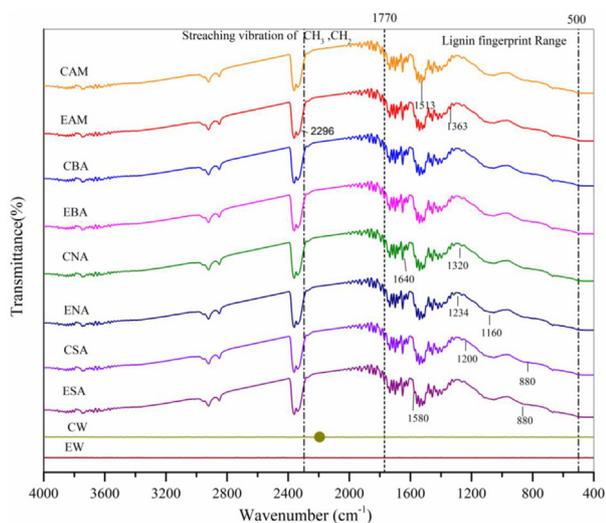


Fig. 5 – FTIR spectra of the structural changes of electrolytic pre-treatment of rice straw.

Table 3 – Gases and VFAs production from NaOH pretreated hydrolysate.

Parameters	Phase	Reactors					
		RS	RSE	PTRS	PTRS + E	PTRS(WE)	PTRS + E(WE)
pH	P-I	6.33 ± 0.06	6.27 ± 0.06	6.27 ± 0.15	5.93 ± 0.21	5.03 ± 0.06	4.90 ± 0.10
	P-II	6.60 ± 0.10	6.37 ± 0.06	6.23 ± 0.06	6.03 ± 0.06	5.33 ± 0.12	5.40 ± 0.10
H ₂ (mM/g _s)	P-I	N.D	N.D	0.253 ± 0.029	0.341 ± 0.031	1.049 ± 0.027	0.992 ± 0.141
	P-II	0.001 ± 0	0.032 ± 0.005	0.006 ± 0.001	0.039 ± 0.008	0.098 ± 0.028	0.265 ± 0.091
CH ₄ (mM/g _s)	P-I	0.008 ± 0.002	0.023 ± 0.005	0.012 ± 0.003	0.023 ± 0.002	N.D	0.005 ± 0
	P-II	0.062 ± 0.001	0.109 ± 0.017	0.061 ± 0.026	0.154 ± 0.028	0.008 ± 0.002	0.060 ± 0.018
H ₂ S (μmM/g _s)	P-I	N.D	N.D	2.167 ± 0.7324	0.89 ± 0.139	N.D	N.D
	P-II	N.D	N.D	35.31 ± 9.40	12.68 ± 1.08	N.D	N.D
Acetic acid (mM)	P-I	11.38 ± 0.80	12.92 ± 1.38	72.63 ± 5.13	79.77 ± 6.48	25.19 ± 9.86	91.47 ± 16.89
	P-II	11.27 ± 2.06	11.55 ± 2.07	26.87 ± 1.20	31.36 ± 1.41	12.63 ± 3.49	48.39 ± 6.03
Formic acid (mM)	P-I	1.04 ± 0.16	1.57 ± 0.10	6.24 ± 0.63	6.36 ± 0.52	0.88 ± 0.40	4.88 ± 0.53
	P-II	1.91 ± 0.40	9.56 ± 0.64	1.41 ± 0.20	4.75 ± 0.87	0.55 ± 0.16	2.17 ± 0.30
Propionic acid (mM)	P-I	0.21 ± 0.00	0.46 ± 0.05	0.73 ± 0.12	0.75 ± 0.07	0.24 ± 0.09	0.72 ± 0.21
	P-II	0.25 ± 0.08	0.75 ± 0.22	0.38 ± 0.14	0.56 ± 0.14	0.43 ± 0.21	0.59 ± 0.14
Isobutyric acid (mM)	P-I	0.52 ± 0.05	0.54 ± 0.12	7.54 ± 0.23	6.68 ± 0.84	9.96 ± 2.71	26.55 ± 3.51
	P-II	5.14 ± 0.41	14.68 ± 2.08	4.96 ± 2.48	9.77 ± 3.91	9.08 ± 2.06	13.32 ± 3.44
Iso-Valeric acid (mM)	P-I	27.11 ± 0.79	28.21 ± 0.43	28.10 ± 0.32	26.50 ± 0.36	28.83 ± 0.97	27.84 ± 1.79
	P-II	24.21 ± 0.17	26.05 ± 0.22	24.96 ± 0.73	26.90 ± 0.27	25.78 ± 0.83	26.07 ± 0.22
Iso-caproic acid (mM)	P-I	2.47 ± 0.03	2.57 ± 0.09	2.40 ± 0.03	2.54 ± 0.05	2.71 ± 0.01	2.76 ± 0.07
	P-II	2.17 ± 0.05	1.99 ± 0.10	2.17 ± 0.03	2.35 ± 0.03	2.40 ± 0.02	2.42 ± 0.02
Hexanoic acid (mM)	P-I	0.11 ± 0.01	0.16 ± 0	0.55 ± 0.16	0.25 ± 0.04	0.27 ± 0.03	0.90 ± 0.27
	P-II	0.12 ± 0	0.76 ± 0	0.20 ± 0.06	0.53 ± 0.09	0.50 ± 0.04	0.55 ± 0.13
Cumulative VFAs (mM)	P-I	47.15 ± 7.40	47.79 ± 1.90	123.35 ± 11.45	130.08 ± 18.40	68.26 ± 7.35	150.04 ± 22.52
	P-II	47.39 ± 3.89	66.24 ± 6.86	57.48 ± 3.75	66.58 ± 12.22	52.97 ± 5.24	93.52 ± 6.20

ND: Not detected.

that SRB efficiently utilizing it at applied voltage. Profiles of Iso-valeric acid show that are second-most dominant fatty acids with nearly 25 mM production was observed. The prominence of decreasing trend of Iso-caproic acid with the progression of the phase shows successful utilization of longer chain fatty acids by SRB [63]. A minor concentration of hexanoic acid less than 1 mM is found in the entire reactors.

Conclusion

The electrochemical pretreatment of the lignin-rich lignocellulosic biomass proved to be an especially useful method towards better understanding its recalcitrance nature in the production of value-added products and biofuels in an economical way. Additionally, the most suitable pH to 6.02 ± 0.23 and reactor study for hydrogen production at an optimized concentration of ferrous, sulfate with applied voltage in the ENA pretreated hydrolysate indicating its suitability for microbial biofuel production. Based on the soluble COD results, alkaline pretreatments are more effective, whereas cumulative VFAs data show SRB (EBA) and ENA pretreatments are more effective. The different constituents produced as a result of different pretreatment methods inferred that desired products are possible by controlling the pretreatment process parameters. Except for biological pretreatment of the rice straw in the Electrochemical system, all other conditions result in the emission of the lesser CO₂ gas. Therefore methods proposed for the pretreatment of the lignocellulosic biomass could be a good option for processing

the biomass for value-added products recovery at ambient temperature by applying very little voltage.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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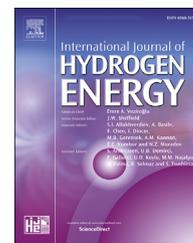
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Modeling and statistical analysis of heat-shocked sulfate-reducers and methanogens rich consortia for hydrogen and methane production in a bio-electrochemical cell

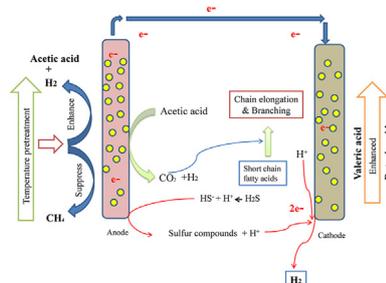
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HIGHLIGHTS

- SRB enriched culture in a Bio-electrochemical system (BES) show higher methane inhibition efficiency.
- Butyric acid was found the most dominant volatile fatty acid at all the temperature treatments.
- Optimum temperature stress for high H₂ yield was simulated by kinetic modeling to clarify computed data.

GRAPHICAL ABSTRACT



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ABSTRACT

This study was carried to investigate the effect of thermal pre-treatment at 55 °C, 65 °C, 75 °C, and 85 °C on sulfate-reducing bacteria (SRB) and methanogens to check their potential in a Bio-electrochemical system (BES) for hydrogen and methane production. The experimental results indicate that SRB culture pre-treated at 55 °C could be used efficiently in hydrogen production up to 2.814 ± 0.091 M/M of glucose. A significant drop in methane production from Phase-I to Phase-III with an increase in pre-treatment temperature was observed. Volatile fatty acids production was found highest in Phase-III, with butyric acid in major concentration at the pre-treatment temperature range of 55 °C–65 °C in SRB-based BES. The higher hydrogen production conditions were tested with various models i.e. the Gompertz model, Richard model, and Logistic model to confirm its validity and hydrogen production prediction. Richard's model was found best fitted for cumulative hydrogen production.

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Introduction

Global energy crises due to exhausting fossil fuel resources and the impact of energy recovery from this natural resource on the ecosystem, human health, and global climatic change promoting the researchers around the world to find out alternatives renewable resource to meet energy demand [1,2]. Having remarkable potential, hydrogen is considered an ideal substitute for meeting the energy demand. Hydrogen has the highest energy content of 120 MJ/kg for H₂, 44 MJ/kg per unit weight among the gaseous fuels [3]. Additionally, the oxidation products of hydrogen do not contribute to the emission of greenhouse gas, depletion of ozone, or acid rain [4]. Traditionally biohydrogen production is directed to deicy conditions with the emission of CO₂ which results in declining ecological balance [5]. Anaerobically hydrogen production using mixed culture has been focused particularly because it has advantages over methane production having no CO₂ emission and high energy density [6–8]. Compared to pure cultures, mixed culture potentially utilizes varieties of substrates, operates in non-sterile conditions, adapted to operational conditions in engineered bioreactors when cultured from natural ecosystems [9]. Sulfate-reducing bacteria (SRB) are a major group of important environmental anaerobes in the field of bioremediation and wastewater treatment technology playing a key role in the sulfur and carbon cycle [10–12]. In natural habitats, SRB has an extremely high hydrogenase activity. They produce fermentatively H₂, CO₂, and acetate in syntrophy with other organisms in sulfate-limited environments [12–14].

Under anaerobic conditions in the mixed cultures, SRB outcompetes with methanogens for substrates such as acetate and H₂, resulting in drops in the H₂ production rate [10,15]. The inhibition of the bacteria involved in the anaerobic digestion step of methanogenesis in the mixed bacterial culture is required for successful H₂ production [16]. Several studies prove that the COD/SO₄²⁻ ratio value of 1.7, dominates SRB [9,17]. Various methods are being used to enrich the hydrogen-producing bacteria by suppressing hydrogen consuming microbes include heat shock pretreatment [18,19], acid or alkaline pre-treatment [20,21], chemical inhibitors treatment [22–24], ultrasonication [25] and gamma irradiations pretreatment [19]. Chemical inhibitors are hazardous to the environment due to their presence in effluents, further acid and basic treatments need system monitoring and pH adjustment. So, these pretreatment methods having some limitations, including novel technologies irradiation, ultrasonic, and microwave are not given promising results and are not economically viable. The heat pre-treatment approached of microbial pretreatment has the most promising results by converting COD into biogas by effectively destroys volatile solids [26,27]. The heat-shock pretreatment (HST) is a useful method for the selection of the hydrogen-producing inocula for the utilization of the soluble substrates [28]. Repeated heating of anaerobic culture simultaneously with sulfate reduction would inhibit the H₂ consuming microorganisms to enhance hydrogen yield for long-term operation.

The Bioelectrolysis system an innovative promising green technology can be studied by integrating with

electrochemically active SRB hydrogenase-rich bacteria to produce hydrogen from organic matter without causing environmental pollution [29–31]. The application of the applied potential to a bioelectrochemical system (BES) enhanced hydrogen production by 1.7–5.2-fold (Approx) which further increases its production increase with an increase in applied potential [32]. Therefore, culture treatment and enhanced hydrogen recovery using the small applied voltage could play a significant role in economical energy recovery [33].

Kinetic models are beneficial tools in operation and optimization studies [34]. These mathematical models are also reported for providing and analyzing important aspects of policies for start-up inhibition pathways associated with the anaerobic digestion process [35]. The exponential and linear equations used for the production rate analysis of biogas showing a rising limb and a decreasing limb like the phase of bacterial growth [36,37]. The modified Gompertz, Logistic, and Richards models are used in various studies for simulation of methane and hydrogen production [38,39].

Although enhanced hydrogen productions in various pre-treatments are ambiguous as most of the studies are conducted using a specific microbial community and comparison of the simultaneous impact on different mixed anaerobic microbial communities are unavailable in the literature [22]. In the present study, we explore the thermal pre-treatment strategies for SRB, SRB coupled with bio-electrolysis and methanogens bioreactor system for hydrogen, methane, and H₂S production. The effectiveness of the combination of sulfate and heat treatment method in the first phase followed by only heat treatment in the latter two phases followed by the comparison of the impact of Bioelectrochemical system in gases production was evaluated. Our results demonstrate a very interesting effect of heat shock at various temperature treatment strategies which can be used in hydrogen production using SRB as new microorganisms for hydrogenase activity.

Materials and methods

Inoculum source and preparation

The inoculum A (methanogens enrich) used in this study was obtained from a 5 L capacity anaerobic bioreactor [40]. The reactor was decanted and fed twice a month with a 2 L slurry prepared by mixing 500 g cow dung in 2 L water. For sustaining the methanogen population the fresh cow dung was collected near the Central University of Gujarat, Sector-30, Gandhinagar, Gujarat (India) before feeding the reactor. The inoculum B (SRB enriched) used in this study was maintained in a 10 L capacity anaerobic bioreactor, prepared by mixing above collected cow dung with viscous sludge collected from anaerobic digester of Piranha Municipal Sewage Treatment Plant, Ahmedabad, Gujarat, (India) in 1:1 in 1 L water [33]. The inoculum B was further enriched in 5 L of Postgate media [41]. The reactor containing the master culture of inoculum B was maintained at pH 6.5 ± 0.5 using 6 N NaOH/HCl at room temperature. Every 5–6 days, the culture was kept for settling, and 3 L of the Postgate medium was added by replacing an

equal volume of supernatant from the reactor. The anaerobic condition in all the reactors was maintained by purging with nitrogen (99.999% purity) for 5 min. The presence of H₂S generation was detected by lead acetate [42], an indication of the blackening of the medium, and H₂S generation recorded as positive for SRB presence.

Experiment design and conditions

In the present study, Inoculum A and Inoculum B were used as a source for methanogens and sulfate-reducing bacteria (SRB) respectively. The heat-treatment or extreme pH eliminates the hydrogen consumers, the non-spore formers [43], whereas hydrogen producers, the spore-forming microbes are resistant to harsh conditions of high temperature, extreme acidity, and alkalinity [43,44]. Therefore, experiments were carried out in three phases of 72 h duration by heat pre-treating the cultures at various temperatures of 55 °C, 65 °C, 75 °C, 85 °C, and Control (37 °C) for half an hour in Electroquip hot air oven. Though the temperature and treatment duration during the heat shock treatment (HST) varies ranging from 65 °C to 121 °C [45,46] and from 20 min to 24 h, respectively [47,48]. The higher temperatures beyond the selected range are not suitable for both the SRB and the methanogens. The mixed culture heat-treated at a higher temperature of around 95–100 °C, the unfavorable conditions for hydrogen-producing bacteria, and eliminate methanogen which produces low hydrogen and methane [49]. The experiments were performed in the 120 ml serum bottles. 25 ml of pre-treated culture at different temperatures were mixed with 25 ml of the Postgate media followed by incubation at 37 °C. Phase-I was conducted in the Postgate medium; further Phase-II and Phase-III were conducted in the modified Postgate medium (without sulfate).

The SRB-based bioelectrolysis system was constructed using graphite electrodes procured from the local market,

Ahmedabad, Gujarat (Fig. 1). The electrodes of length 2.25 cm and dia 0.6 mm, were placed 1 cm apart from each other using the silicon tube. The potential of 40 mV was applied through voltage controller 1V D.C and 5 V D.C. adaptor, assembled from the local market, Gandhinagar, Gujarat. Each bottle was purged with nitrogen gas (99.999% purity) for 3 min and sealed with 20 mm silicone rubber septa and aluminium caps for control, whereas bio-electrolysis system was sealed with a specialized stainless-steel cap for the accommodation of the potential supply. During each phase, the gaseous sampling was carried at 24 h regular intervals for 72 h. The volume of the gas generated was measured using a 20 ml glass syringe. The liquid samples were taken at the end of each phase. All the phases were carried out in the same reactor while moving from one phase to the next phase, 25 ml of the liquid was drawn and replaced with 25 ml of the modified Postgate medium. All the reactors were heat-treated at respective temperatures and purged with nitrogen as mentioned earlier to ensure the anaerobic conditions while proceeding towards the next phase.

Gases measurement and composition analysis

The composition of headspace gas was analyzed using gas chromatography (PerkinElmer 680) configured with a thermal conductivity detector (200 °C), equipped with the Elite-plot Q column (30 m × 0.53 mm ID). Injector temperature was maintained at 150 °C. Initially, the oven temperature was kept at 40 °C for 3.5 min and then increased with a ramp rate of 40 °C/min to 65 °C, where it was held for 3 min. Nitrogen was used as the carrier gas with a flow rate of 2 ml/min. The concentrations of the individual gases in the mixture were calculated using calibration curves prepared from the H₂, CH₄, CO₂, and H₂S of high purity grades.

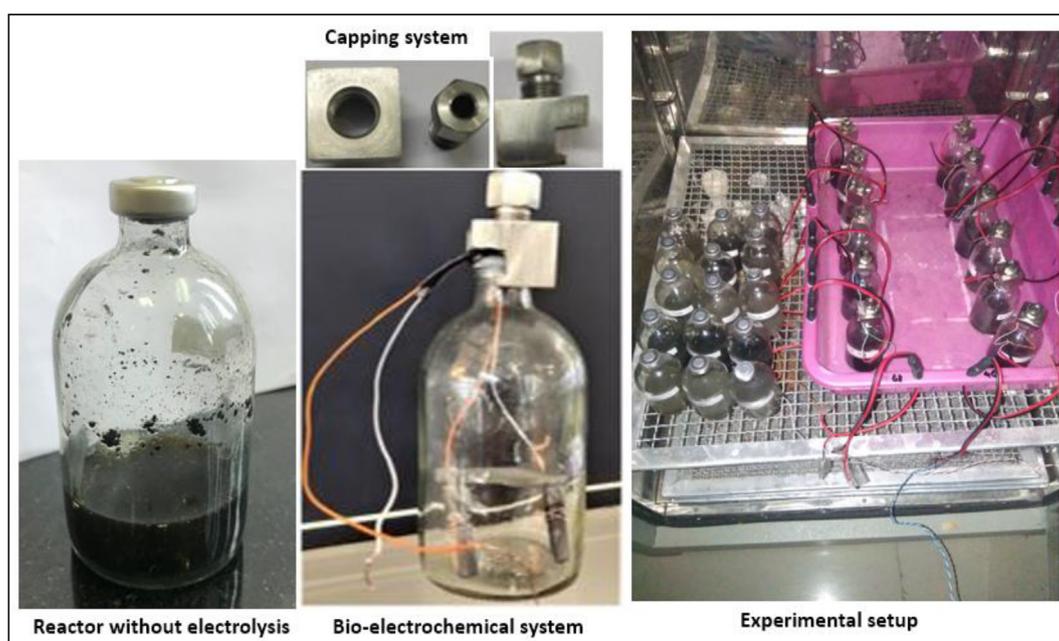


Fig. 1 – Bioreactors and Bioelectrochemical systems used in the study.

Liquid components analysis

Physicochemical characteristics of the liquid samples were analyzed at the end of each phase after harvesting 25 ml of the sample using the standard method [50]. The pH of the sample was measured immediately using (pH meter Eutech 700). The chemical oxygen demand (COD) and volatile fatty acids (VFAs) analysis were performed by centrifuging the samples at 5000 rpm for 20 min. For the analysis of VFAs, 2.5 ml of samples were acidified to 2–3 pH using Orthophosphoric acid [50] and were preserved in Eppendorf tubes at 4 °C for further analysis. Before analysis of VFAs samples were diluted to 10 times with HPLC grade water and filtered through 0.2 µm membranes (Dia. 25 mm) passing through the IR 120 regin filled column. The sample volume of 0.5 µl was injected using an autosampler in a gas chromatograph (PerkinElmer 680) equipped with a flame ionization detector (FID) using helium as a carrier gas in the Elite-wax column (30 m × 0.32 mm ID) [37]. The initial oven temperature was set at 95 °C for 5 min and subsequently increased at a rate of 10 °C/min until reached 140 °C, where it was held for 6 min. The further temperature was increased at a rate of 40 °C/min to 200 °C, where it was held for 5 min. The injector and detector temperatures were kept at 250 °C and 240 °C, respectively. The calibrations curve prepared (0.5–5 mM) using the volatile free

fatty acids mix of 10 mM (Sigma Aldrich) to calculate the concentration of formic acid, acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, isocaproic acid, hexanoic acid, and n-heptanoic acid.

Kinetic modeling

A number of the models are being used to assess the microbial growth curve, specific growth rate, and lag times in numbers of the disciplines. The kinetic and model analyses are widely applied in the design and scale-up of anaerobic digestion reactors [51]. The best results obtained for maximum hydrogen production from the bioreactors of different treatments were checked for the suitability of kinetic models. The non-linear models are mostly used for the interpretation of the gases produced during anaerobic fermentation. An adequate model can describe a curve, limits the number of interesting parameters, and is the one way to discriminate among models to compare them statistically [52]. The modified Richards, Gompertz, and Logistic models are widely used for the analysis of kinetics modeling of hydrogen production [37,38]. The Richards model has some problems sometimes, whereas the Gompertz and Logistic models never gave problems while fitting the non-linear curve [52]. The Non-linear fitting was assessed on each phase using Origin Pro Trial 2019b 64 bit software (Trial

Table 1 – Cumulative H₂, CH₄ and H₂S production in the various consortium.

Experiment		SRB			SRB + Electrolysis			Methanogens		
		H ₂	CH ₄	H ₂ S	H ₂	CH ₄	H ₂ S	H ₂	CH ₄	H ₂ S
					M/M of glucose					
Control	P-I	0.351 ±0.009	0.106 ±0.022	0.170 ±0.020	0.228 ±0.064	2.204 ±0.275	0.368 ±0.005	0.026 ±0.003	1.305 ±0.117	0.462 ±0.045
	P-II	1.926 ±0.136	N.D	0.289 ±0.043	0.879 ±0.012	0.194 ±0.034	0.188 ±0.044	0.155 ±0.006	1.009 ±0.126	0.199 ±0.037
	P-III	1.823 ±0.165	N.D	0.183 ±0.028	0.165 ±0.024	N.D	0.088 ±0.024	0.701 ±0.081	0.149 ±0.015	0.125 ±0.038
55 °C	P-I	0.058 ±0.010	2.298 ±0.137	0.441 ±0.065	0.311 ±0.084	1.603 ±0.252	0.631 ±0.083	0.019 ±0.00	1.369 ±0.069	0.421 ±0.022
	P-II	1.173 ±0.064	0.077 ±0.028	0.115 ±0.029	0.459 ±0.025	0.322 ±0.017	0.116 ±0.029	0.207 ±0.029	1.152 ±0.202	0.154 ±0.014
	P-III	2.814 ±0.091	N.D	0.159 ±0.016	1.692 ±0.272	N.D	0.072 ±0.017	0.814 ±0.004	0.101 ±0.004	0.088 ±0.008
65 °C	P-I	0.248 ±0.028	2.146 ±0.250	0.633 ±0.122	0.369 ±0.095	0.946 ±0.291	0.502 ±0.007	0.022 ±0.002	1.221 ±0.041	0.399 ±0.079
	P-II	0.784 ±0.275	0.174 ±0.041	0.099 ±0.019	1.341 ±0.078	0.023 ±0.007	0.229 ±0.005	0.391 ±0.071	1.556 ±0.229	0.129 ±0.024
	P-III	2.198 ±0.268	N.D	0.102 ±0.020	1.762 ±0.088	N.D	0.158 ±0.006	0.819 ±0.063	0.135 ±0.016	0.090 ±0.004
75 °C	P-I	0.332 ±0.068	0.773 ±0.237	0.348 ±0.047	0.426 ±0.072	0.116 ±0.003	0.451 ±0.099	0.010 ±0.001	0.989 ±0.018	0.372 ±0.0
	P-II	1.020 ±0.205	0.023 ±0.007	0.096 ±0.033	1.146 ±0.030	N.D	0.112 ±0.030	0.275 ±0.037	0.625 ±0.056	0.104 ±0.020
	P-III	1.778 ±0.152	N.D	0.086 ±0.015	0.243 ±0.075	N.D	0.035 ±0.005	0.885 ±0.026	ND	0.068 ±0.007
85 °C	P-I	0.199 ±0.27	0.101 ±0.013	0.230 ±0.037	0.527 ±0.014	0.018 ±0.001	0.414 ±0.097	0.042 ±0.014	0.653 ±0.012	0.281 ±0.095
	P-II	0.531 ±0.056	0.012 ±0.004	0.088 ±0.014	0.256 ±0.058	N.D	0.081 ±0.007	0.470 ±0.025	0.467 ±0.111	0.038 ±0.007
	P-III	2.075 ±0.178	N.D	0.058 ±0.005	0.033 ±0.007	N.D	0.045 ±0.006	0.840 ±0.035	ND	0.021 ±0.001

N.D: Not detected.

version). The cumulative hydrogen productions were simulated using the following equations [52,53].

Models Equations

$$\text{Logistic model } C = a/(1+b\exp(-kt)) \quad (1)$$

$$\text{Richard model } C = a * (1+(d-1)*\exp(-k*x-xc))/(1/(1-d)), \text{ for } d \neq 1(2)$$

$$\text{Gompertz model } C = a * \exp(-\exp(-k*(x-xc))) \quad (3)$$

where,

The Logistic and Richard models- C is cumulative gas production (ml); k is kinetic constant (h^{-1}); t is hydraulic retention time (h); a and b are the constants, xc is the center (=1).

The Gompertz model- C is cumulative gas production (ml); a is ultimate hydrogen production potential, exp is exponential (1); d and k are constants, t is hydraulic retention time (h); a and b are the constants, xc is the center (=1).

The statistical analysis or factorial analysis of two factors/single factor ANOVA of the gaseous and VFAs data was performed using the Microsoft Excel solver (Microsoft 365).

Results and discussion

Effect of heat pre-treatment on hydrogen and methane production

The effect of the heat pretreatment on SRB and methanogens culture for hydrogen and methane production in different

phases is presented in Table 1. The insignificant differences of the heat pretreatment within the subgroups were observed in SRB-Control culture ($p = 0.642$), SRB with electrolysis ($p = 0.146$), and methanogens ($p = 0.705$) in hydrogen production (Table 2). However, a significant difference in hydrogen production was observed between the groups of control cultures ($p = 0.001$) and cultures treated at 75°C ($p = 0.0273$). Whereas other treatments show insignificant differences in the hydrogen production between the groups ($p > 0.05$). The highest variance of 717.93% in the cumulative hydrogen production was observed in the SRB-control culture (preheated at 37°C), further increasing heating temperatures decrease the variance at 65°C (413.36%), beyond this pre-heating almost same variance of 341.675% and 348.84% was observed at 75°C and 85°C respectively with the progression of phases (Phase-I to Phase-III). This variance in hydrogen production decrease with increasing preheat treatment temperatures. The variance in hydrogen production was almost constant (35.86–52.08%) with increasing preheat treatment temperature with little increase in the variance (86.09%) at 85°C treatment of the methanogens. The different temperature heat treated culture, when applied with potential shows insignificant ($p = 0.146$) parabolic type of the variance, having maxima at 65°C , (variance, 236.09%; positive limb $R^2 = 0.996$) beyond this the sharp decreasing variance was recorded ($75^\circ\text{C} = 74.23\%$; $85^\circ\text{C} = 5.31\%$, negative limb $R^2 = 0.949$). The highest variance in the culture heat treated at 65°C which was applied with 40 mV potential showing the enrichment of the electrochemically active bacteria in the system. The increasing average hydrogen production ($R^2 = 0.7314$) and

Table 2 – Factorial ANOVA of hydrogen and VFAs produced under various treatment conditions.

Source of variations	Phase	p-value by		Variance				
		Treatment	Process	37°C	55°C	65°C	75°C	85°C
SRBC (H_2) ^a	Cum	0.642		717.929	569.373	413.355	341.675	348.835
SRBE (H_2) ^a	Cum	0.146		38.176	176.825	236.098	74.225	5.309
ME (H_2) ^a	Cum	0.705		35.858	40.413	52.081	50.944	86.093
Ace ^b	P-I	0.017	0.108	44.528	0.392	0.000	30.225	43.682
	P-II	0.646	<0.01	32.058	20.013	16.235	37.182	22.334
	P-III	0.974	0.049	32.810	37.541	4.222	6.469	3.402
For ^b	P-I	0.220	0.092	0.542	0.041	0.017	2.503	0.181
	P-II	0.627	<0.01	0.066	0.116	0.132	0.179	0.036
	P-III	0.986	0.026	0.184	0.123	0.026	0.025	0.026
Prop ^b	P-I	0.151	0.038	0.011	0.018	0.004	0.013	0.007
	P-II	0.287	0.135	0.001	0.009	0.005	0.004	0.003
	P-III	0.313	0.111	0.005	0.000	0.004	0.007	0.014
IsoBut ^b	P-I	0.606	0.541	4.398	0.191	0.100	0.035	1.335
	P-II	0.938	0.018	5.188	3.116	6.317	12.366	3.289
	P-III	0.897	0.031	7.859	26.398	1.520	1.420	3.351
But ^b	P-I	0.252	0.246	4.398	0.191	0.100	0.438	1.155
	P-II	0.076	<0.01	51.955	83.618	108.306	115.543	104.987
	P-III	0.874	0.021	70.449	34.088	21.266	1.589	4.310
Val ^b	P-I	0.121	<0.01	0.846	0.534	0.050	0.114	0.108
	P-II	0.056	<0.01	0.440	0.813	0.540	0.262	0.208
	P-III	0.078	0.090	0.000	0.096	0.000	0.039	0.003
Isocap ^b	P-I	0.058	<0.01	6.990	0.329	1.844	1.259	2.076
	P-II	0.350	<0.01	6.887	6.423	1.003	0.655	1.041
	P-III	0.027	<0.01	0.104	0.130	0.113	0.076	0.080

^a Single factor ANOVA.

^b Two factor ANOVA; Cum is cumulative hydrogen during all the phases.

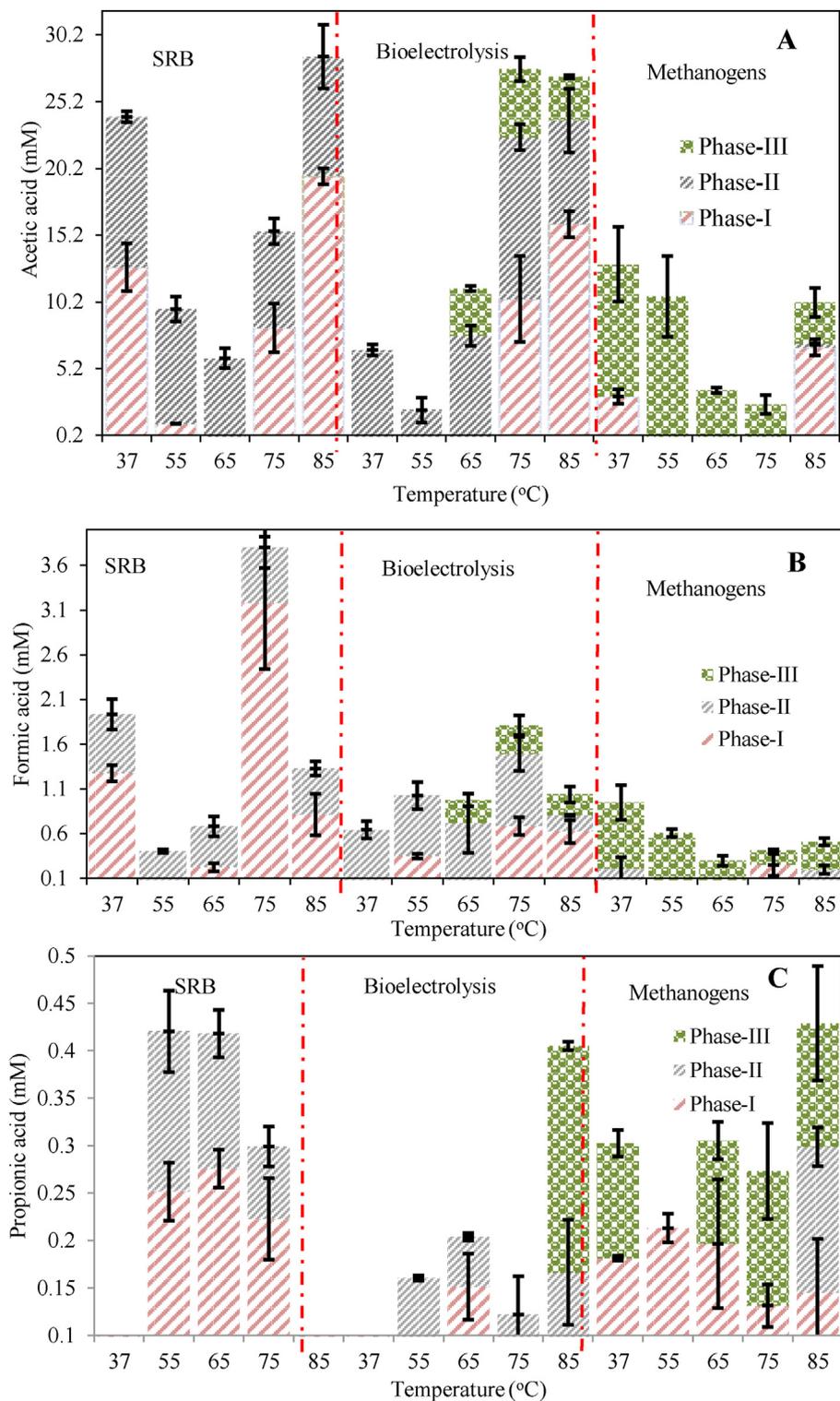


Fig. 2 – VFAs production profiling during heat treatment at different temperatures: A) Acetic acid; B) Formic acid; C) Propionic acid.

variance in hydrogen production ($R^2 = 0.7941$) with increasing heat pretreatment temperatures of 37 °C–85 °C effectively inhibiting the methanogens ($p = 0.705$). Similarly in the first stage of anaerobic digestion, no detectable CH_4 was observed in the reactors pre-treated either with waste frying oil (WFO)

or heat shock, indicating the efficiency of the pre-treatments on the inhibition of methanogens [24].

The maximum hydrogen and methane production of 2.814 ± 0.091 and 2.298 ± 0.137 M/M of glucose were observed in the SRB cultures pretreated at 55 °C during Phase-III and

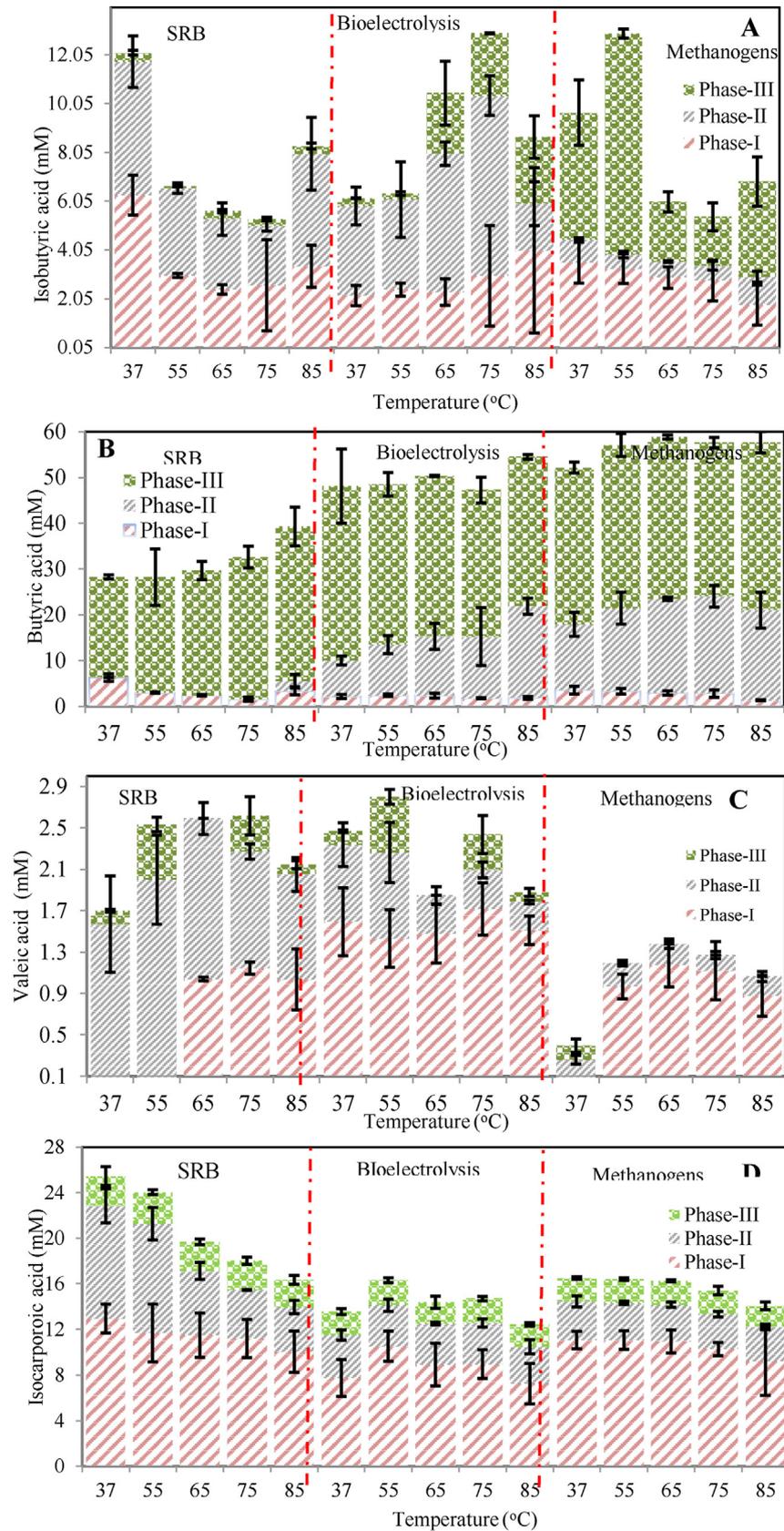


Fig. 3 – VFAs production profiling during heat treatment at different temperatures: A) Isobutyric acid; B) Butyric acid; C) Valeric acid; D) Isocaproic acid.

Phase-I respectively. This observation inferring that repeated heat shocking at 55 °C required to sufficiently inhibits the methanogens. Moreover, the lower hydrogen yield in the SRB heat pretreated culture at 55 °C indicated that the sensitivity of SRB culture to heat shocks. The hydrogen production potential to 70.35% of the SRB-based heat-treated mixed cultures is quite good considering the maximum theoretical hydrogen yield of 4.0 mol/mol of glucose. Moreover, this value seems to quite high based on the literature survey which reported values of 23.75 and 26.25% of theoretical hydrogen production by the heat and acid-treated mixed cultures [16]. Interestingly looking at the long-term consistency of the culture application (up to Phase-III), the present SRB-based heat pretreated culture showing higher hydrogen production potential (>2.5 mol/mol of glucose). This hydrogen production potential is quite high in comparison to the similar study of the researcher's Kan [54] and Yin et al. [55] who heat pretreated the sludge at 100 °C for 15 min and obtained hydrogen production of 0.9 and 2.15 mmol/mmol of consumed glucose, respectively using mixed culture and thermophilic conditions. The methane production drastically decreases from Phase-I to Phase-III with increasing heating pretreatment temperatures. This might be due to the combined effect of heat treatment and the presence of SO_4^{2-} in media which inhibits non spore-forming methane-producing bacteria (MPB) at higher temperatures [56,57]. Simultaneously change in pH nearly from pH 6.5 to 4.4 in the system during anaerobic digestion was due to the accumulation of VFAs which suppress most of the methanogens growing in a relatively narrow pH range of about 6.8–7.2 [58]. These results in favorable growth conditions for hydrogen-producing bacteria and enhanced hydrogen production from Phase-I to Phase III. Low hydrogen production in Phase-I obtained may be due to the addition of sulfate which gets converted into H_2S by consumption of hydrogen by SRB.

The SRB-based bioelectrolysis reactor operated at 40 mV and heat pretreated at 55 °C and 65 °C were showing the nearly similar hydrogen production of 1.692 ± 0.272 and 1.762 ± 0.088 M/M of glucose in Phase-III which was 1.7 times lower than reactor equipped without electrolysis. However cumulative hydrogen production in all phases was 1.4 times higher at a heat treatment temperature of 65 °C than 55 °C. This might be due to the low microbial diversity as a result of heat shock treatment which was unable to adapt to the bioanodes. Ziara et al. [59], investigated the effect of temperature on hydrogen production of anaerobic sludge and observed that higher temperature (45 °C) in dark fermentation could be attributed to an increase in the metabolic reactions responsible for the hydrogen production with enhanced biochemical reaction rate constant. Results also revealed that SRB bioelectrolysis reactor is more efficient to complete inhibition of methane production at higher temperatures of 55 °C–85 °C in Phase- II and Phase III. The study conducted on hydrogen and methane production in thermophilic conditions (57 ± 1 °C) using the bioelectrochemical system during anaerobic digestion inhibits methanogenesis at the applied voltage of 20 mV, 40 mV, and 80 mV, however, its production start at 120 mV [39].

Hydrogen production using methanogen-rich culture was found effectively low (<1 M/M of glucose) in comparison to other SRB bioreactor and SRB bioelectrolysis reactors. The highest cumulative methane production of 1.556 ± 0.229 M/M of glucose was found at a temperature of 65 °C in Phase-II and its production decrease to 0.135 ± 0.016 M/M of glucose in Phase-III. The thermophilic methanogen is more dominant at this temperature and an increase in the pre-treatment temperature from 65 °C to 85 °C inhibits methanogen in Phase-III. The low H_2 and CH_4 production were observed at a higher pretreatment temperature of around 95–100 °C, the

Table 3 – Kinetic modeling parameters of cumulative H_2 production.

Experiment	Model	Exp. (ml)	Pred (ml)	Constants				Statistics	
				A (ml)	xc	b/d	k	R ²	Adj. R ²
Cont. SRB	Richards	64.63	66.99	76.996	114.198	1.244	0.019	0.943	0.909
	Logistic	64.63	66.55	71.489		34.757	0.029	0.942	0.923
	Gompertz	64.63	67.21	80.379	109.604		0.016	0.943	0.924
SRB-55 °C	Richards	64.37	63.86	63.862	172.315	77.286	2.024	0.969	0.950
	Logistic	64.37	68.02	73.562		350.469	0.039	0.945	0.926
	Gompertz	64.37	69.11	95.869	148.178		0.017	0.934	0.912
Cont. SRB (40 mV)	Richards	17.30	16.50	16.504	96.566	3.579	0.078	0.959	0.934
	Logistic	17.30	16.93	16.982		56.355	0.046	0.954	0.939
	Gompertz	17.30	17.33	17.626	76.430		0.029	0.938	0.918
SRB-65 °C (40 mV)	Richards	46.53	46.57	104.063	185.679	0.986	0.007	0.978	0.964
	Logistic	46.53	46.24	62.894		20.795	0.019	0.976	0.968
	Gompertz	46.53	46.56	102.515	184.576		0.008	0.978	0.970
Cont. Meth	Richards	13.29	13.29	13.286	168.328	109.382	4.885	0.979	0.967
	Logistic	13.29	13.41	13.409		5.04E+10	0.163	0.961	0.948
	Gompertz	13.29	13.29	13.291	146.809		0.156	0.959	0.946
Meth –75 °C	Richards	17.82	17.82	17.825	170.752	103.397	3.001	0.958	0.933
	Logistic	17.82	18.99	20.119		607.288	0.043	0.927	0.903
	Gompertz	17.82	19.31	2.56E+01	145.119		0.0179	0.914	0.886

Cont. SRB: Untreated SRB culture; SRB-55 °C: SRB culture pre-treated at 55 °C; Cont.SRB-40mV: Control SRB with electrolysis; SRB 40 mV-65 °C: SRB with electrolysis pre-treated at 65 °C; Cont. Methanogens: Untreated Methanogen culture; Methanogens-75 °C: Methanogens pre-treated at 75 °C.

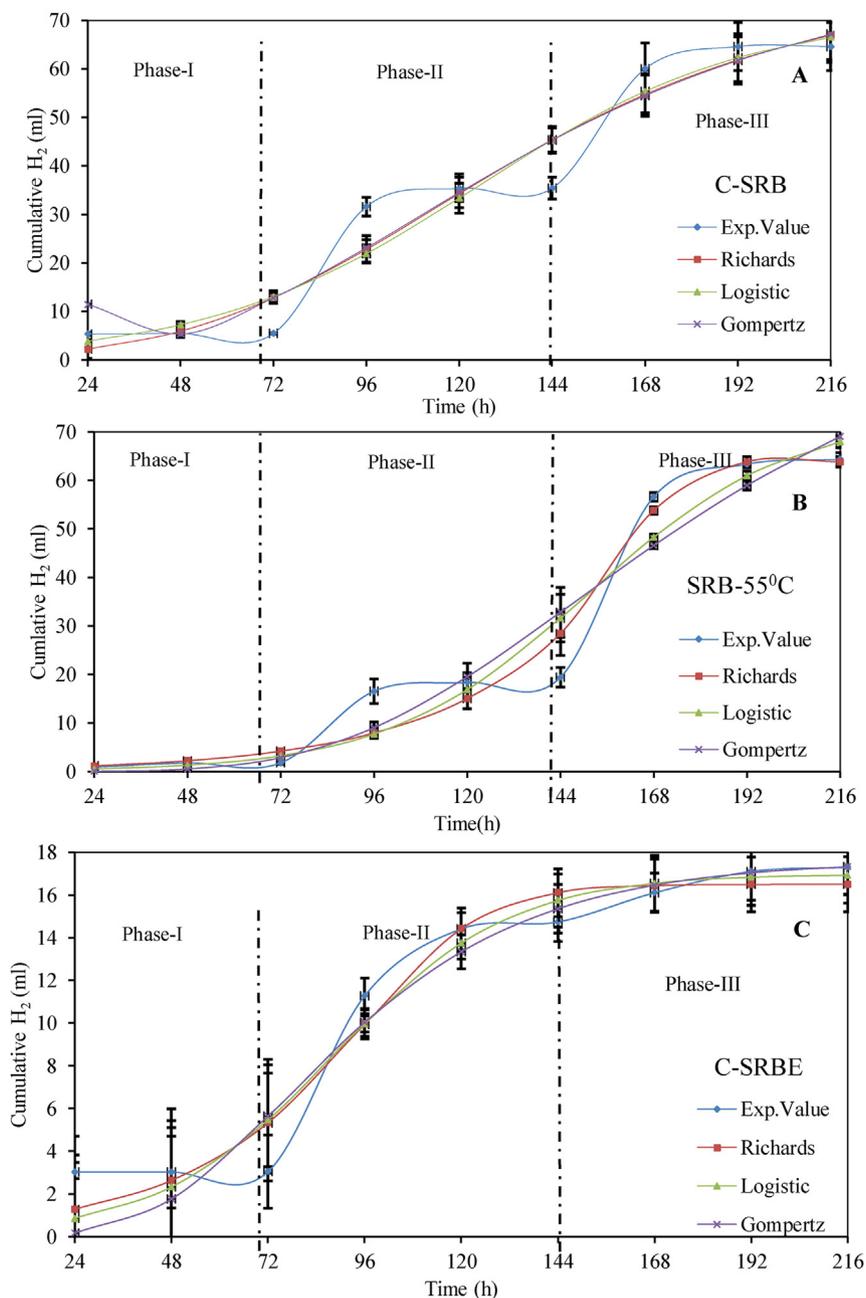


Fig. 4 – Kinetic modeling of cumulative H₂ production of experimental and predicted values: A) Control SRB; B) SRB heat pretreated at 55 °C; C) Control SRB coupled with bio electrolysis.

unfavorable condition for hydrogen-producing bacteria, which eliminate methanogens [49]. At a mesophilic temperature of 40 °C methanogen inhibition was found less compared to the thermophilic state [60]. Though considering the present findings the selection of the appropriate pretreatment method for the enrichment of the inoculum of interest might vary from other studies. Moreover, it is not necessary to use the inoculum prepared using the specific substrate that could be effectively applied to other substrates due to the strong effect of substrate biodegradability in achieving consistency [24].

Liquid phase components

The compositional analysis of the VFAs at different heat pretreatments in various bioreactors is presented in Figs. 2 and 3. The VFAs accumulated during each phase were also analyzed for factorial analysis of two factors ANOVA (Table 2). Except for acetic acid and isocaproic acid during Phase-I, and all VFAs in Phase-II and Phase-III (except for isocaproic acid) insignificant differences in the VFAs accumulation ($p > 0.05$) as a result of the heat pretreatment was observed. Whereas, acetic acid, formic acid, isobutyric acid, and butyric acid in Phase-I,

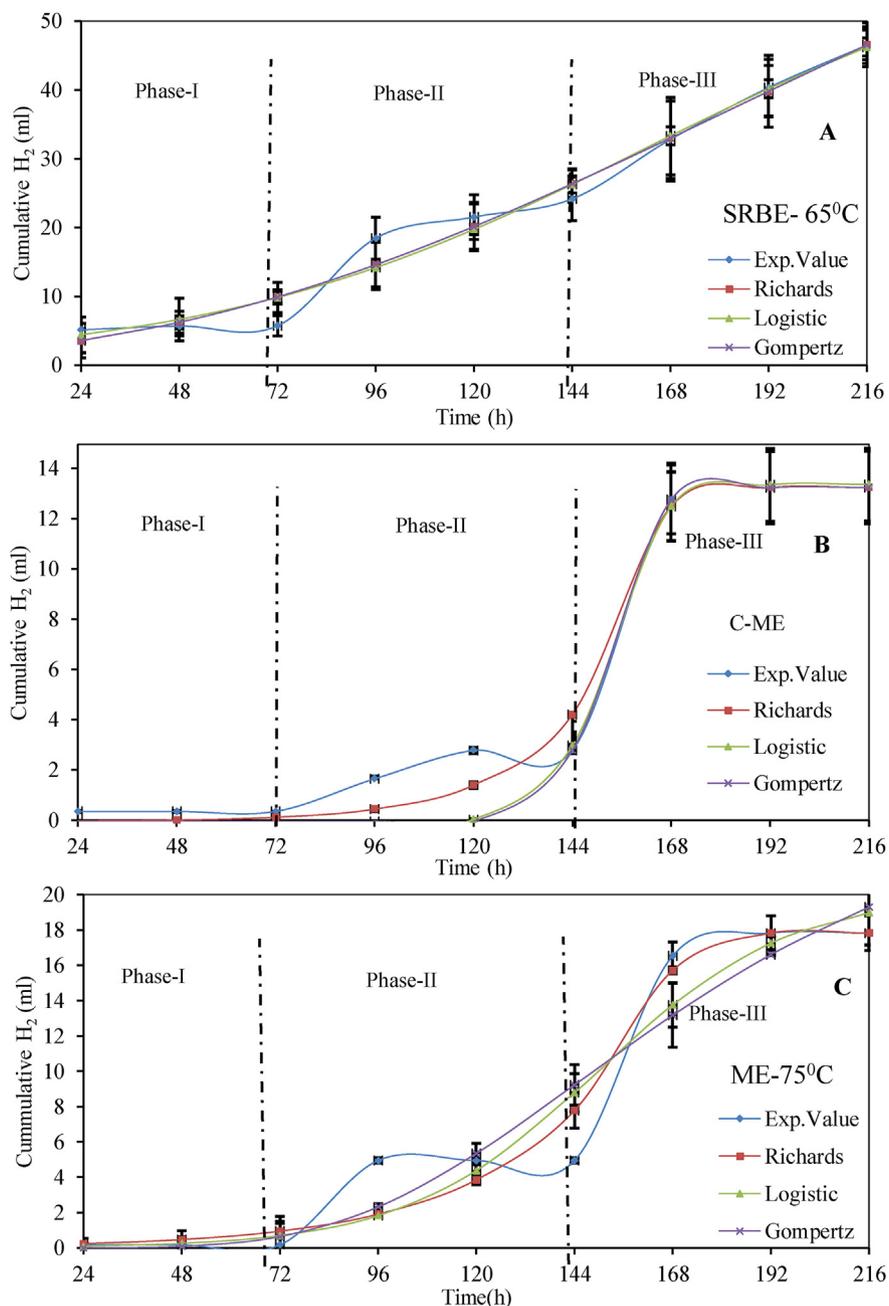


Fig. 5 – Kinetic modeling of cumulative H₂ production of experimental and predicted values: A) SRB coupled with bioelectrolysis pretreated at 65 °C; B) Control methanogens; C) Methanogen pretreated at 75 °C.

and propionic acids in Phase-II, propionic acid, and valeric acid in Phase-III, showed insignificant differences ($p > 0.05$). These observations indicate that despite different heat treatment as well as types of culture, these acids having insignificant differences are metabolically synthesized during the anaerobic fermentation in Phase-I. These findings inferring that electrolysis enhances the accumulation of VFAs in the culture heat-treated at a higher temperature. This might be due to the accumulation of toxic compounds etc. due to variation in pre-treatment temperature [61]. Acetic acid production was found low in methanogens anaerobic bioreactor in comparison to the SRB bioreactor (Fig. 2A). The highest

variations of 44.53% and 43.68% in acetic acid at the lowest (37 °C) and highest pre-treatment temperature (85 °C) respectively were observed, whereas reached to below detection level in the vicinity of 65 °C heat treatment. In the SRB-bioelectrolysis, acetic acid accumulation was observed in the heat-treated culture at 75 °C and 85 °C, whereas it was not accumulated at lower temperatures of heat treatment. However, this variation in the acetic acid as a result of heat treatment at different temperatures was insignificant ($p > 0.05$) in Phase-II and Phase-III. The suppression of the methanogens in the culture heat treated at 85 °C is also indicated by the presence of a higher level of acetic acid. The

methanogens largely consume 70% of acetate and 30% of H₂ for the production of CH₄ [62,63]. As a result of the pretreatment, an insignificant variation of the formic acids ($p > 0.05$) was observed. The formic acids and propionic acid accumulation were very low (<1 mM) in both methanogens and SRB-based electrolysis bioreactors (Fig. 2B and C). The highest butyric acid of 38.07 ± 8.09 mM was found in an SRB electrolysis reactor operated under control conditions (Fig. 3B). During temperature shock propionic acids produced in a relatively small fraction of VFAs at 37 °C [64], whereas butyric acid was found the second dominant after acetic acid at 37 °C, 55 °C, and 80 °C, however, our study shows the highest production of butyric acid followed by acetic acid.

The SRB bioelectrolysis triggers the production of valeric acid and butyric acid. Valeric acid concentration gradually decreases and butyric acid gradually increases from Phase-I to Phase-III (Fig. 3B and C). This might be due to a drop in pH, studies reported that a drop in pH in the range of 5–6 triggers the accumulation of butyric acid [65]. In the methanogen's bioreactor, a high accumulation of tVFAs was found in Phase-III at higher temperature heat pretreated cultures. Studies reported that an increase in temperature led to 10 times accumulation without pH adjusted due to the increase in hydrolysis rate compared to mesophilic conditions [66,67]. Isocarporoic acid significantly drops from Phase-I to Phase-III in both SRB and methanogen culture (Fig. 3D), however, the applied potential to the SRB culture was not showing this random drop in concentration.

Kinetic modeling of hydrogen production

The predicted values of cumulative H₂ and kinetic modeling constants (k) for reactors are presented in Table 3. Models are used to describe the behavior of microorganisms under different physical or chemical conditions such as temperature, pH, and water activity [52]. The Richards, Logistic, and Gompertz models suggested fit model values of hydrogen production found nearly equivalent to the experiment values (Figs. 4 and 5). The kinetic parameters obtained by the model equations showed improved H₂ production during the heat pretreatment in comparison to the control cultures. Hydrogen production plots of experimental and suggested fit model values for SRB with bioelectrolysis and without bioelectrolysis pre-treated at 55 °C show the enhanced hydrogen production in Phase-III, in comparison to without pre-treated culture. The delay in the hydrogen production (higher lag phase) for heat shock pretreated cultures might be due to the inhibition of some extent H₂ producing bacteria which required additional time to produce hydrogen. A significant drop in hydrogen production was found in the cultures treated beyond the temperature of 55 °C. A higher pre-treatment temperature of 85 °C was found effective in hydrogen production using methanogen culture, though production was found <1 M/M of glucose at the end of Phase-III. Highest R² (COD) and Adj. R² was found closer to 1 confirms the fitness of these models (Table 3).

The experimental data of cumulative H₂ simulated for the Richards model have R² values in the range of 0.910–0.970 and

Adj. R² of 0.885–0.970. In comparison to the Richards model slight variation in R² and Adj. R² values were found in the Logistic and Gompertz models. The rate constant (k) value was found highest in Richards models for control methanogens (4.885) and lowest in the Gompertz model in the SRB applied with 40 mV and heat pretreated at 55 °C (0.010). The lower rate constant (k) value of SRB culture may be due to the high conversion of glucose to H₂ and CH₄. Higher rate constant (k) value of methanogens might be due to low conversion of methane and high accumulation of organic matter. The Gompertz model equation could successfully describe the growth of microorganisms and estimate the hydrogen production in the anaerobic process based on kinetic parameters obtained [68].

Conclusion

This study investigated systematic evaluation of the effect of heat pretreatment for enhancement of H₂ production using anaerobic mixed culture enriched with SRB, SRB integrated with BES, and methanogens. The heat pre-treatment significantly enhances the H₂ production by suppressing the methanogen populations. Maximum H₂ production was observed at a heat pre-treatment temperature of 55 °C using SRB enriched culture. The thermophilic methanogens are more dominant at this temperature and an increase in the pre-treatment temperature from 65 °C to 85 °C inhibits methanogen in Phase-III. The highest cumulative methane production of 1.556 ± 0.229 M/M of glucose was found at 65 °C in Phase-II, which further decrease to 0.135 ± 0.016 M/M of glucose in Phase-III. The lesser H₂ and CH₄ production at higher pretreatment temperature showing the unfavorable conditions for hydrogen-producing bacteria, and eliminates the methanogens also. Acetic acid the most common product of the anaerobic shows the highest variations of 44.53% and 43.68% at temperatures of 37 °C, and 85 °C, whereas it reached the below detection limit level in the vicinity of 65 °C of heat treatment. The cultures heat-treated at a higher temperature and inoculated in the bioelectrochemical system enhanced the VFAs accumulation. The findings in this study, cost-effectively describe H₂ production using sulfate-reducing bacteria in a single-chamber MEC reactor.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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REVIEW ARTICLE

A review of disasters in Jammu and Kashmir, and Ladakh region in India

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Abstract: India has always been a disaster-prone country, with multiple states afflicted by different types of disasters. The impact of these disasters is exacerbated when an area is prone to multiple types of disasters. This study attempts to understand the impact of natural and man-made disasters on the people of Jammu and Kashmir (J&K) and Ladakh region in India as well as it also examines the resilience mechanisms adopted by the people, and identifies measures taken by the government in response to these disasters. To understand these disasters' dynamics, we conducted both offline and online desk reviews for this study. The review suggests that J&K and Ladakh region is afflicted not only by multiple natural disasters such as floods, earthquakes, avalanches, and landslides but also by the terrorism and violence, which has caused unparalleled death and destruction. These natural and man-made disasters have adversely affected most aspects of life and development in the region. To mitigate the risks, effective disaster risk reduction and management systems, early warning systems and infrastructure need to be strengthened. In addition, community engagement needs to be enhanced with the goal of addressing the grievances of the population and engaging them in the design and implementation of sustainable development programs.

Keywords: Natural disaster; Man-made disaster; Conflict; Terrorism; Violence; Jammu and Kashmir; Ladakh; India

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1. Background

Disasters are a global phenomenon. Natural and man-made disasters have had an impact on the development, economy, and health of both developing and developed nations and have put pressure on populations across the world. The United Nations International Strategy for Disaster Reduction (UNISDR) defines the term disaster as “a serious disruption of the functioning of a community or a society involving widespread human, material, economic, or environmental losses and impacts, which exceeds the ability of the affected community or society to cope using its own resources” (UNISDR, 2009). Disasters that are the result of natural hazards such as earthquakes, floods, cyclones, and droughts are called natural disasters. Those that are the result of anthropogenic activity such as industrialization, wars, global terrorism, political conflicts, and economic crises are defined as man-made disasters (Shaluf, 2007). A combination of natural and man-made disasters has affected most countries around the world and resulted in large-scale mortality and morbidity, destroyed livelihoods, creating millions of refugees, and migrants. Around 1.3 million people died due to natural disasters between 1996 and 2015, with low-income countries recording mortality rates that were 5 times higher than high-income countries (UNISDR and CRED, 2016). On the other hand, in the year 2016, global terrorism – a man-made disaster – caused 25,673 deaths and losses of \$84 billion to the global economy (Institute for Economics and Peace, 2017).

As a result of its geographical and climatic conditions, poor socio-economic profile, and numerous terrorisms-based violence, India is afflicted by multiple disasters. Over a period of 20 years, from 1996 to 2015, natural disasters killed 97,691 people in India. This represents the fifth highest mortality rate in the world (UNISDR and CRED, 2016). India has also been affected by violent man-made disasters in the form of wars, insurgency, and terrorism. The Bhopal gas tragedy in 1984 remains the country's worst industrial disaster thus far. The recently bifurcated union territories (UTs) of Jammu and Kashmir (J&K) and Ladakh (hence forth has been used as J&K and Ladakh region) in the northernmost part of India, is one of the most severely affected regions by both natural (Table 1) and man-made disasters. It is a region prone to multiple hazards on account of its topography and varying, extreme climate. The region has suffered massive floods, devastating earthquakes, and recurrent avalanches and landslides (SDMP, 2017). The earthquake in 2005, flash floods and landslides in 2010, and the massive floods of 2014 are among the major natural disasters the state has faced over the past 15 years (Kumar, Martha, and Roy, 2006; Gupta, Khanna, and Majumdar, 2012; SDMP, 2017). The state has also borne the brunt of disputes between India and Pakistan, witnessing regular cross-border infiltrations, state-sponsored terrorism, and violent attacks. The displacement of communities from their homelands, long-drawn-out protests leading to curfews, and regular clashes between terrorists and armed forces, have been some of the manifestations of the violence (Shekhawat, 2009; Behera, 2016).

This combination of natural and man-made disasters has had adverse effects on key facets of J&K and Ladakh region's economy, including key sources of revenues such as agriculture, horticulture, handicrafts, and tourism (Sharma, Sharma, and Waris, 2012). These disasters have stretched the administrative machinery of the region and left its population vulnerable. However, there are lack of studies which have focused on the aftermath of specific natural disasters and incidents of terrorism and violence as a form of disaster. There have been lack of studies which have explored the impact of natural and man-made disasters on people's lives and development in a comprehensive way. This article attempts to review the impact of different natural and man-made disasters (e.g., terrorism and violence) on the people of J&K and Ladakh region. It reviews the impact of disasters and the mechanisms of resilience adopted by the people of the region, and identify measures taken by the government in response to these disasters.

1.1. Geography and Weather

As per the recently formed UTs of J&K (as of October 31, 2019), UTs of J&K has 20 districts and UTs of Ladakh has two districts, both combined covering the geographical area of 222,236 km² (Census of India, 2011). The climatic conditions vary from tropical heat in Jammu, to temperate conditions of the Kashmir valley, to the arctic cold of Ladakh. There is large variation in temperature from the average maximum of 33°C (Jammu) in summers to the average minimum

Table 1. Deaths due to natural disasters in India and J&K and Ladakh region, 2005-2018.

Year	Deaths due to natural disasters	
	J&K (including Ladakh)	India
2005	1157	22,415
2006	345	21,502
2007	278	25,153
2008	307	23,993
2009	226	22,255
2010	575	25,066
2011	314	23,690
2012	321	22,960
2013	308	22,759
2014	518	20,201
2015	387	10,510
2016	280	8,684
2017	127	7143
2018	131	6891

Source: National Crime Records Bureau, Ministry of Home Affairs, Government of India.

of -14.4°C (Ladakh) in winters. The average annual rainfall for the region is 1028 mm with the months of July and August experiencing the maximum rainfall (IMD, 2014). Agriculture is the direct and indirect source of livelihood for the majority (75%) of the population while paddy and wheat are the two major crops. Sharecropping along with goat and sheep rearing is the sources of livelihood for the nomadic communities. Rain is the major source of irrigation followed by spring irrigation and nallah irrigation (SDMP, 2017).

2. Key Findings

2.1. Impacts of Earthquakes

Situated next to the Himalayas, J&K and Ladakh region falls in a mountain building geological zone and thus experiences recurring seismic activity (Hassan, 2014). It is an earthquake-prone area that falls under the most active seismic zones in India (Zones IV and V). It has endured several earthquakes over the years. Between 1889 and 1990, 170 earthquakes were recorded in the region (Hassan, 2014). The year 1885 witnessed one of the deadliest earthquakes to strike the region, the effects of which were felt from Srinagar to Gilgit and to Shimla in the neighboring state of Himachal Pradesh (Anees and Bhat, 2016).

In 2005, a major earthquake – with a magnitude of 7.6 on the Richter scale – struck the border region between India and Pakistan. The fallout affected both countries. More than 80,000 people – majority of them in Pakistan – lost their lives, resulting in an enormous humanitarian crisis (SDMP, 2017). The earthquake also resulted in injuries to approximately 100,000 people in Pakistan and about 6300 people in India (Ali, Mir, Jabeen, *et al.*, 2010). It is considered the deadliest earthquake in the recorded history of the Himalayan region (Anees and Bhat, 2016). Around 1300 of those killed were from Uri in Baramulla district of J&K. The township saw severe damage to most of its buildings, with 121 of them collapsing completely and many villages in the vicinity were severely affected by the earthquake (Kumar, Martha, and Roy, 2006). Kumar *et al.* (2006) also highlighted using remote sensing satellite data that the earthquake and the subsequent landslides resulted in collapse of 25% of the buildings in Uri and Poonch townships, collapse of bridges, and road blockage.

In the regions of J&K and Ladakh, earthquakes not only cause immediate destruction but also long-term damage to the socio-economic condition of the people in the region (Shah, Khwaja, Shah, *et al.*, 2018; Yousuf, Bukhari, Bhat, *et al.*, 2020). The collapsed houses left entire families homeless and the impact on hospitals and government buildings interrupted health and other essential services when they were most needed (Hamilton and Halvorson, 2007). A study on the morbidity patterns of victims of the 2005 earthquake reveals that a majority of the 6270 injured suffered upper and lower limb injuries, followed by spinal injuries (Ali, Mir, Jabeen, *et al.*, 2010). A study based on the mental health services provided in the region revealed that within 6 weeks of the earthquake, majority of the respondents had severe psychological impacts, with adjustment disorders (39.6%), depressive episodes (21.8%), anxiety (4.6%), and post-traumatic stress disorder (PTSD) (3.3%) being the most common (Chadda, Malhotra, Kaw, *et al.*, 2007). This study noted that a major concern reported by people was the lack of basic mental health services outside the city of Srinagar, which can be a journey of up to 8 h for people from the remote areas of the region.

With existing inequalities interacting with a natural hazard, the earthquakes also had a devastating impact on the women of J&K. Women faced an environment of increased personal insecurity and psychological stress due to a lack of sanitation facilities and immediate food insecurity due to their social responsibility as caretakers of the family. The disruption in health and reproductive facilities also had severe impacts on pregnant women (Hamilton and Halvorson, 2007).

2.2. Impacts of Landslides

Landslides are another geological hazard common in J&K and Ladakh region. The region is home to young mountain ranges, which have a fragile rock base that can trigger a flow of debris, mud, and rocks when the stability of the slope gets disturbed. Heavy rainfall, cloudbursts, and earthquakes can trigger landslides. Anthropogenic activities such as deforestation, road construction, and other unsustainable development activities have further increased the vulnerability of the area (Singh, Bhat, Sharma, *et al.*, 2012). Most of the areas in J&K are prone to landslides, with the districts of Bandipora, Kargil, Anantnag, Kishtwar, Pulwama, and Shopian being highly susceptible (SDMP, 2017). The environmentally fragile region of Ladakh has also been adversely affected by human activities such as encroachment of hill slopes, forest fires, terrace farming, and vibrations through heavy vehicular transportation, making it a highly vulnerable zone for landslides and mudslides (Barnard, Owen, Sharma, *et al.*, 2001).

In 2010, the Ladakh region witnessed one of its most destructive landslides as a result of a cloudburst. The extreme rainfall triggered multiple landslides and flash floods, leading to the deaths of 234 people with foreigners accounting for

about 10% of the deaths (Gupta, Khanna, and Majumdar, 2012). The flow of debris from the hills wreaked havoc on its path, destroying hospitals, houses, roads, bridges, farmland, and other infrastructure. The traditionally built houses of Leh and Ladakh were severely damaged, with over 1000 houses completely washed away by the flow of debris (Gupta, Khanna, and Majumdar, 2012). Roads were damaged and freshwater supply was interrupted due to the destruction of many canals. The destruction of storage facilities and difficulties in transportation due to heavy rainfall resulted in a temporary shortage of food supply. The destruction of hospitals and lack of sanitation facilities meant that public health was also severely affected. The remoteness of certain areas also meant that the availability of health services was further delayed (SDMP, 2017; Gupta, Khanna, and Majumdar, 2012).

Singh *et al.* (2012) argue that unplanned development in the form of construction of roads and dams is the major cause of landslides in the region. The landslide on the Batote-Doda road along National Highway 1B in 2009 was a case of slope failure resulting from the construction of the Baglihar hydro-power project. It washed away 150 m of the highway, killed one person, and affected the daily lives, livelihoods, and food security of 600,000 people for over a month (Singh, Bhat, Sharma, *et al.*, 2012). Mining sites in the region are also located in highly landslide-prone areas. Laborers work under conditions of constant risk and the mining endangers the fragile environment, creating a vicious cycle. The influx of tourists and the simultaneous infrastructural development – while positively affecting the J&K and Ladakh region's economy – has also made its environment more fragile and prone to natural hazards such as landslides (SDMP, 2017; Verma and Mushtaq, 2013).

The blocking of highways and other roadways, which leads to a disruption of normal life, is a common occurrence across the region during landslides. The Jammu-Srinagar Highway, a lifeline of the Kashmir valley, gets blocked every year due to landslides and results in hundreds of vehicles being stranded (Indian Express, 2018). This also prevents essential commodities from reaching the valley, creating shortages, and increasing prices. Cultural and religious activities such as the Amarnath yatra have also been interrupted from time to time due to landslides. Landslides cover agricultural land with debris and mud, damaging crops and making the land uncultivable for a long period of time (SDMP, 2017). This has an enormous impact on the livelihoods of farmers and of nomadic communities that live in hilly regions. The annual migration of the nomadic communities gets affected by the blocked roads. Landslides also cover large areas, preventing them from grazing their animals (Anees and Bhat, 2016). Like most other disasters, landslides have a severe impact on women due to existing social inequalities. There have been few studies on the psychological impacts of landslides in J&K. However, studies conducted in other parts of the world show that survivors of landslides are more likely than others to experience PTSD (Catapano, Malafrente, Lepre, *et al.*, 2001).

2.3. Impacts of Floods and Avalanches

Flooding is one of the most common and also one of the most devastating natural disasters across the world (CRED and UNISDR, 2015). It is generally a result of overflow of water due to rainfall, melting of snow, or other natural causes, which ends up submerging an area of land. Besides these natural causes, there are human activities such as deforestation, rapid and unplanned urbanization, construction of dams and bridges without proper research, and changing patterns of vegetation that make an area more vulnerable to flooding. The region is prone to floods, with major rivers such as Jhelum, Chenab, and Indus flowing through its populated areas (SDMP, 2017). Kashmir valley's bowl shape, with its vast variation in altitudes, makes the low-lying areas of the region specifically prone to floods. In the two-major urban centers of the region – Jammu and Srinagar – the number of wetlands such as lakes and ponds, which act as natural sponges, have come down severely, resulting in frequent urban flooding (Gupta, 2014).

In September 2014, extremely heavy rainfall led to one of the most severe and widespread instances of flooding in the region. The Jhelum, Chenab, and Tawi basins were overflowing as the amount of rainfall received in just few days was 2-6 times (depending on location) the monthly normal for September (SDMP, 2017). The floods severely affected ten out of 22 districts in the region, with districts in southern part of Kashmir being severely affected. About 30% of the urban area in the region was submerged and 2600 villages were affected with 400 being completely submerged (Vithalani and Bansal, 2017). The floods were followed by landslides that damaged roads and bridges, including one that washed away 50 people in a bus in Rajouri district (Gupta, 2014). More than 300 people lost their lives during the floods and lakhs of people were displaced as more than 80,000 *pucca* houses and about 21,000 *kachha* houses were completely damaged (Vithalani and Bansal, 2017). Multiple roadways across the region were blocked for days, including the Jammu–Srinagar highway, which remained closed for over 3 days, disrupting relief measures (Gupta, 2014). Farmers suffered huge losses as crops were destroyed, agricultural land was inundated, and thousands of animals reared for animal husbandry perished (SDMP, 2017; Shah, Khwaja, Shah, *et al.*, 2018). In the immediate aftermath of the floods, food security was a serious concern. A study claimed that 86% of respondents in Kashmir and 36% in Jammu reported a decrease in food consumption (Sphere

India, 2014). There was also a substantial decrease in the usage of piped water in affected villages as water resources were severely damaged (Gupta, 2014). The region's healthcare services were completely overwhelmed, with four out of five hospitals in Kashmir unable to function due to the floods (Vithalani and Bansal, 2017). The shortage of medical supplies affected the most vulnerable members of the society, such as the old and physically challenged and those suffering from chronic diseases such as diabetes and cancer. Disruption of electricity and damaged equipment in GB Pant hospital in Srinagar resulted in the deaths of 20 neonates (Venugopal and Yasir, 2017). There was an increase in mental illness cases in the hospitals of Srinagar, with people showing early symptoms of PTSD (Tabish and Nabil, 2015).

The floods have had a long-term impact on the people of J&K as they had not only lost their homes but also their livelihoods, livestock, and important documents. The Federation of Chamber of Commerce in Kashmir estimated the economic loss to the region till 2017 at around \$15 billion (Tabish and Nabil, 2015). The education of thousands of students across the region was affected as several government and private school buildings collapsed, leaving students without essential infrastructure (Venugopal and Yasir, 2017; Tabish and Nabil, 2015). As with all-natural disasters, women were affected even more than men because of existing gender inequalities and different social and cultural expectations. Their sanitation and reproductive health needs came under severe stress and their role as provider for the family led to considerable mental trauma. The lack of proper sanitation facilities in the relief camps and the lack of privacy for breastfeeding infants emerged as major issues for women in the aftermath of the floods (Gupta, 2014). Flooding of homes and the ensuing destruction caused severe psychological stress among people and aggravated existing mental health conditions. A study conducted 6 months after the year 2014 flood in Kashmir described that 60% of the population was suffering from severe PTSD (Fatima and Maqbool, 2017). The study also found that women and all elderly members of the society (above 60 years) were more affected by mental health issues such as depression and PTSD in the aftermath of floods.

Flash floods are extreme and sudden events that are usually triggered by a cloudburst or by the failure of dams. They trigger a high velocity current of water, submerging an area downstream within minutes or hours (SDMP, 2017). They usually occur in areas with steep slopes and, because of their sudden nature, can result in huge damage. Anthropogenic activities such as deforestation and unscientific road construction, which can trigger landslides, worsen the effects of flash floods. The cloudburst in the Ladakh region in 2010 resulted in the Indus river and its tributaries overflowing, triggering flash floods, and landslides. This led to the deaths of 234 people in the Ladakh region, left 800 injured, and caused many others to be washed away by the water and debris (Gupta, Khanna, and Majumdar, 2012). Transport was disrupted as many roads were washed away and the airport at Leh was flooded. Many buildings suffered severe damage with around 1000 houses completely collapsing. The damage was not limited to Ladakh region, with around 71 deaths being reported in 11 other districts (SDMP, 2017). Leh historically suffers a from lack of drinking water and sanitation facilities. These were further exaggerated by the flooding. Being a tourist destination, Ladakh was further hampered economically due to the destruction of tourist infrastructure. The district of Baramulla also suffered major losses of around \$8.7 million in the agricultural sector and \$8.3 million in the horticulture sector (SDMP, 2017).

Given the presence of the Himalayas in the region, J&K and Ladakh are also prone to avalanches. An avalanche is the flow of snow down a mountain slope and is very common in the high ranges of J&K (SDMP, 2017). Avalanches generally occur during episodes of heavy snowfall which can be attributed to the rising global temperature because of climate change (Rafiq and Mishra, 2018). Higher reaches of Kargil and Ladakh and the valleys of Kashmir and Gurez are the most avalanche-prone areas of the region (Hassan, 2014). Avalanches can also be highly difficult to predict and usually occur over a short period of time. The downward movement of snow can bring with it ice, soil, trees, and rocks, causing destruction to life and property (Ganju and Dimri, 2004). One of the most destructive avalanches struck the region in 1995, resulting in the deaths of 150 people and the closing of the Jawahar tunnel on the Jammu-Srinagar highway (Hassan, 2014).

Avalanches have also proven deadly for the Indian armed forces as they are normally posted in inhospitable locations of the region. An avalanche in Gurez sector in 2017 killed 20 army men and four civilians and caused destruction to a tourist place (Rafiq and Mishra, 2018). Avalanches also lead to the blocking of roadways, creating shortage of essential commodities. They essentially paralyze the life of the communities living in the high mountainous areas of the region, while also resulting in economic losses due to the impact on tourism. Agriculture is impacted for a longer term as large-scale movement of snow causes soil erosion making the soil unproductive (Ganju and Dimri, 2004). Historically, J&K and Ladakh has been a region with abundant water, but with rapid urbanization and increase in global temperatures, occasional droughts have become a reality for the majority of the region (Hassan, 2014).

2.4. Impacts of Man-made Disasters

Almost all-natural disasters in J&K and Ladakh region are, to an extent, exacerbated by human activities and their destructive potential is linked to human actions. Man-made disasters, on the other hand, are completely dependent on

human actions. The political conflict stretching back to India's independence has contributed to the most lethal, destructive, and continuous man-made disaster in the region. The region went through multiple crises in terms of violent insurgency and terrorism from the neighbor country in the Kashmir valley after India's independence (Bose, 2003). There were repeated instances of violence against the region's minority Pandit community, leading to a mass exodus of the Pandit community from the valley in 1990 (Shekhawat, 2009). In recent times, the region has witnessed large-scale protests and stone pelting as a form of collective civilian resistance, along with a new wave of militancy (Behera, 2016).

According to the South Asia Terrorism Portal (SATP), the militancy or terrorism in J&K and Ladakh region over the past three decades has led to 47,689 deaths between 1988 and July 30, 2020; among the casualties were 15,138 civilians, 6979 security personnel and 25,572 terrorists (SATP, 2020). The terrorism-based violence has also displaced lakhs of people – the majority among them being Kashmiri pandits. The people of the region continue to live in a heavily militarized zone under the constant threat of violence and terrorism. Normal life gets disrupted on a regular basis and a small incident can result in large-scale protests and violence (Behera, 2016). Violence has not only led to physical injuries and deaths but also resulted in the disruption of daily life. The family structure comes under stress, traditional and cultural events lose significance or get banned for security reasons, and overall community life gets affected (Amin and Khan, 2009). The violence has impacted the growth of children as they have been born and raised in a militarized zone that regularly witnesses violence. Growing up in a society ravaged by violence has not only hampered their mental growth but also their cultural growth (EFSAS, 2017).

All these factors lead to high prevalence of stress, trauma, and deep and lasting psychological impacts on all sections of the population. A study conducted in Kashmir highlights that about 55% of the population suffers from some level of depression and that the condition is much more prevalent in rural areas compared to urban areas as socio-economic factors add to factors like violence (Amin and Khan, 2009). Disability and violation of modesty (Violation of modesty is the local equivalent for sexual violence and includes inappropriate touching, in accordance with the WHO's definition of sexual violence) are the most common factors that cause psychological stress among men, whereas for women, the most common factors are exposure to violence and a sense of powerlessness (De Jong, Ford, Van de Kam, *et al.*, 2008). In the 1990s, the threat of sexual violence was used to terrorize the Pandit community (Shekhawat, 2009). Over the years, the terrorism-based violence in J&K has resulted in the deaths of many more men than women, leaving behind thousands of widows (Qayoom, 2014). A number of men have disappeared during the violence with no proof of death and their wives are referred to as half-widows (Qutab, 2012). The widows and half-widows, who were mainly dependent on their husbands economically and socially, have to struggle for their daily survival. Widows from poor backgrounds with no education have found difficulty in getting jobs other than manual labor (Qayoom, 2014). Half-widows not only suffer from economic deprivation but also stigma and psychological problems as they are unable to get closure due to the uncertainty that persists about their partners and their possibility of returning (Qutab, 2012).

The terrorism in J&K has had a major impact on the economic condition of the region. Due to the terrorism, in 1989, private investment in the region came to a complete halt and the economy of the region grew at a much slower pace than the rest of the country, resulting in high unemployment rates – especially in the private sector (Mahapatra and Shekhawat, 2008). Given the security concerns, the limited resources of the region are often diverted toward internal security and policing. The periods of violence have affected sources of livelihood—especially tourism, horticulture, and handicraft industries. The horticulture industry considered the backbone of Kashmir's rural economy, with numerous orchards producing apples, strawberries, almonds, walnuts, and saffron, was severely affected during the 1990s (Sharma, Sharma, and Waris, 2012). Kashmir was once India's favorite tourist destination, but as the terrorism raged on, the industry declined, affecting the livelihoods of thousands of people.

The number of tourists visiting J&K and Ladakh declined from 557,974 in 1989–8026 in 1993 (Sharma, Sharma, and Waris, 2012). The progress on increasing the footfall of tourists back to the previous levels has been marginal. This is reflected by the fact that only 27,358 tourists visited the region in 2002. Thus, during the most violent years of the terrorism, from 1989 to 2002, the valley lost an estimated 27 million tourists leading to tourism revenue loss of approximately \$3.6 billion (Sharma, Sharma, and Waris, 2012). Major tourist destinations and historical sites became militant hideouts. Terrorists attacked both Indian and foreign tourists, with tourist hotspots like Srinagar Airport being repeatedly attacked (EFSAS, 2017). It severely hampered the business of hotels and houseboats, while also crippling the handicraft industry that is dependent on purchases by tourists (EFSAS, 2017). In recent times, the number of tourists visiting the Jammu and Ladakh regions has grown faster compared to the Kashmir valley, which used to be the central attraction for tourists in the pre-insurgency days (Sharma, Sharma, and Waris, 2012). A study by Barbhuiya and Chatterjee (2020) also highlights that severe conflict or violence events affect domestic tourist arrival negatively, while natural disasters negatively impact international tourist arrival. Education also suffered during the conflict, with schools and colleges remaining shut during

militant attacks, protests, and indefinite strikes. Academic schedules have been disrupted and education has taken a back seat, with violence and anger taking their place, resulting in the loss of human talent and potential (EFSAS, 2017). The impact of violence on education and the economy has led to the reduction of job opportunities and this has created a generation of young people living in despair who can be potential recruits for militant activities.

2.5. Community Resilience and Government Measures on Disasters

In the aftermath of disasters, among the widespread death and destruction, individuals and communities have adopted various mechanisms to cope with the situation. This coping ability is directly linked to the vulnerability profile of the individual or the group. The vulnerability is determined by social and economic conditions such as age, gender, health, occupation, and other factors (SDMP, 2017). The ability of the population to cope with these natural disasters could also be linked to the socio-political history of the state, as noted in a study by Rakesh Chadda and others after the 2005 earthquake. The study argues that the years of violent conflict could have better equipped the people of the region to cope psychologically with natural disasters and to survive in tough conditions (Chadda, Malhotra, Kaw, *et al.*, 2007).

The social capital approach has been a key coping mechanism whereby people have gone out of their way to help each other, as was the case in the aftermath of the floods of 2014. Due to the lack of an early warning system, people were forced to evacuate in a hurry as the water level rose, which resulted in preventable losses. The Indian Army has been a key institution regarding the disaster relief in the regions of J&K and Ladakh. The army and the National Disaster Relief Force were brought into action and played a significant role in the rescue operations in 2014 (Venugopal and Yasir, 2017). The army also set up medical camps in flood-affected areas and a study shows that this played an important role in the aftermath of the disaster, but the low supply of certain drugs and the limited number of doctors available restricted the utility of these camps (Singh, Hasan, and Kasi, 2016). While the army and other administrative organizations did their best during the flood, it was the local youth networks that went out of their way to rescue people – including tourists – with the help of small boats and tyres (Venugopal and Yasir, 2017). There was also material help from other parts of the region in the form of food materials such as milk, vegetables, and rice, which helped people sustain themselves in the immediate aftermath of the floods (Bukhari, 2014). Organizations that are normally opposed to each other – such as the armed forces, non-government organizations (NGOs), separatists, and local youth – were all working toward the same goals during the natural disaster (Venugopal and Yasir, 2017). Women have played an important role in the event of disasters. In the aftermath of the earthquake in 2005, women organized relief efforts, helped in the building of temporary shelters, and prepared food in the aftermath of the earthquake (Hamilton and Halvorson, 2007).

The continuous presence of the armed forces in the rural and urban areas of J&K due to violence and terrorism has made the region a highly militarized zone. To avoid the constant patrolling by the armed forces, people have put up barriers made of rocks and pipes outside neighborhoods (Anjum and Varma, 2010). A study found that people living in J&K have reduced the number of times they leave their homes – especially during violent flare-ups (Khan, Ayoub, and Tahir, 2013). The age-old Kashmiri practice of storing dried foods and pickled vegetables due to the inaccessibility of the terrain during winters has re-emerged because of the conflict. People have been storing large quantities of food and grains at home as a way of coping with unforeseen circumstances such as prolonged protests and stringent curfews (Anjum and Varma, 2010). Displacement or migration is another coping mechanism. The biggest displacement from Kashmir was in the 1990s, when almost the entire Kashmiri Pandit community migrated to Jammu and other parts of India to escape the violence in the valley. Even after three decades, the community continues to live in exile (Shekhawat, 2009). There have also been other forms of displacement that is more cyclical in nature. People living in the border regions regularly migrate to more inner areas when increased tensions between India and Pakistan lead to cross-border shelling. In 2018, more than 1,000 people migrated from Uri district after heavy firing from across the border (SATP, 2020). A study conducted in the district of Srinagar found that people are open to migrating out of the conflict zone, but socio-economic conditions, and family and work commitments inhibit this migration (Khan, Ayoub, and Tahir, 2013).

During sudden and destructive disasters such as flash floods and landslides, it becomes imperative that the state machinery responds quickly and effectively to help people cope with the disaster. When flash floods and landslides wreaked havoc in Ladakh, the army, along with the civil administration and local people, launched massive search operations using army helicopters (Gupta, Khanna, and Majumdar, 2012). Due to concerns of contaminated water, purification units were installed to serve communities. Similarly, in the aftermath of the floods in 2014, Kashmiri student organizations from Delhi and other parts of India played an important role in helping people cope with the hazard (Venugopal and Yasir, 2017). The government has also followed a policy of providing compensation for the losses incurred by the people due to disasters. The central government provided \$720 million in multiple packages to the government of J&K (then state of J&K) for relief and rehabilitation after the floods of 2014 (SDMP, 2017). After the avalanche in 2018, the erstwhile

state government provided relief of \$5715 to the kin of those killed and \$172 to the injured in accordance with the policy of State Disaster Relief Fund (SDRF) (New Indian Express, 2018; SDRF, 2015). The government has also attempted to integrate Disaster Risk Reduction with schemes such as Mahatma Gandhi National Rural Employment Guarantee Act (MGNREGA) and Indira Awas Yojana to enhance the capacities of the most vulnerable sections of the population (SDMP, 2017). MGNREGA is integrated with disaster management by focusing on generating work related to water harvesting, management of irrigation canals, flood control in water-logged areas, tree plantation, and renovation of traditional water bodies. The government has also promoted crop diversification and implemented crop insurance schemes to create a level of security against disasters for the rural population (SDRF, 2015). The J&K government had aimed at a growth rate of 4% in the agricultural sector by giving access to quality inputs such as seeds and fertilizers and by giving better inputs on soil and water management to the farmers (GOJ&K, 2013). Self-help groups (SHGs) have also been initiated by the government and the NGOs, with a special focus toward women. Women's SHGs have promoted micro-credit schemes, generated self-employment by promoting activities such as carpet weaving and goat and sheep rearing (Irshad and Bhat, 2015). This has helped in building capacity of women in rural areas and made them active participants in the development of the state and increased its resilience to disasters.

The victims of terrorist attacks in the region have also been provided compensation. In 2017, the government of the erstwhile state of J&K announced compensation of \$8572 for the kin of the deceased after the attack on Amaranth pilgrims (Jameel, 2017). In 2015, the central government instituted measures to provide a monthly pension – apart from the one-time compensation – to widows of civilians killed in militant violence (GOI, 2008). A monthly cash relief of \$36 (with a maximum of \$145 per family) is also provided to Kashmiri migrants living in Jammu (GOI, 2015). The State Rehabilitation Council (SRC) has instituted schemes to rehabilitate thousands of women who have become widows over the many years of militancy. It also offers a one-time remittance of \$290 for widow remarriage (GOI, 2008). Himayat, a scheme sponsored by the Government of India, was launched in 2011 as a skill development and placement program with the goal of providing jobs for the youth of the region (GOI, 2011). It aims to provide jobs for 100,000 youth with socially backward youth and school and college dropouts being the priority. There is also a youth exchange program under the project *Watan Ko Jano*, run by the SRC of J&K and Ladakh region, that aims to positively influence children between the age of 14 and 24 – especially orphans of militant violence (GOJ&K, 2015).

3. Discussion

The previous sections of this article have clearly outlined the devastating effects of disasters on the everyday lives of the people. These disasters have caused death, physical and mental trauma, destroyed livelihoods, and affected the economy of J&K and Ladakh region. Landslides and avalanches have been the most common natural disasters in the region, while floods and earthquakes, though less common, have caused more death and destruction. The 2014 flood was the most widespread natural disaster in the region in recent times while the 2005 earthquake proved most fatal. This study has also underscored the importance of human activity in disaster management – the frequency of disasters and their destructive potential increases due to unscientific and unsustainable development. The study also shows that while natural disasters have caused great death and destruction, terrorism, and violence have been even more destructive in the region, causing deaths of more than 20,000 civilians and security personnel, and displacing lakhs of people. Disasters have affected every aspect of life in the region – from industries such as agriculture, handicrafts, and tourism to the physical and mental health of people.

This study effectively synchronizes other studies (Sharma, Sharma, and Waris, 2012; Vithalani and Bansal, 2017) that have assessed the impact of disasters in the region in a comprehensive manner. The decline of the tourism industry, a lifeline of the region's economy, has led to increased levels of unemployment, which has created further disillusionment – especially among the youth of the region. This lack of jobs, when combined with the ongoing violence and widespread feeling of injustice, can lead to more and more youth getting attracted to the terrorism and armed struggle. In fact, conflict, terrorism, and disaster have created a vicious cycle, where development is hampered due to the ongoing violence and the lack of development creates unemployed youth, who then become potential recruits for terrorist organizations seeking to further the violence.

Mental or psychological health is an important aspect of human life that is impacted during both natural and man-made disasters. Conventionally, it has not got the importance it deserves. This study also contrasts the incessant and relentless nature of the violence with the more sporadic nature of natural disasters. The physical and mental trauma caused by disasters has been highlighted throughout this study. A natural disaster or a violent attack immediately results in major challenges for the underequipped and overcrowded health sector of the region, as was evident after the floods in 2014

(Vithalani and Bansal, 2017). The widespread violence, repeated disruptions to daily life, and the militarized nature of the region have had an adverse psychological impact on the people. Numerous studies have underlined that the prevalence of stress, trauma, and depression is quite common among the people and is directly linked to their exposure to the violence in the Kashmir valley (De Jong, Ford, Van de Kam, et al., 2008; Amin and Khan, 2009; Housen, Lenglet, Shah, et al., 2019; Wani, Suhaff, Khan, et al., 2020). Another study after the earthquake in 2005 also highlighted the psychological impact of natural disasters, with depression, acute stress, and sleep disturbance being widely reported among the affected population (Chadda, Malhotra, Kaw, et al., 2007). The conflict in Kashmir has also left thousands of women as widows and half-widows in a volatile and patriarchal society where sexual violence against women is widely prevalent (Qayoom, 2014; Qutab, 2012). Further, in the aftermath of a natural disaster, women are confronted with an atmosphere of increased insecurity and stress and a lack of privacy, sanitation, and reproductive facilities (Hamilton and Halvorson, 2007; Kelman, Field, Suri, et al., 2018). In fact, natural disasters could potentially have a greater psychological impact on the people of the region than others due to the existing trauma arising from the violent conflict and due to the lack of resources to deal with mental health issues. The health facilities in the region require better infrastructure, more trained doctors, and mental health practitioners – especially in the rural and remote areas. Mental health needs to be a specific and ongoing focus. As the present study shows, psychological stress, trauma, and depression are widespread among the populace. The region also lags in health insurance coverage with only 4.2% of households having any insurance (GOI, 2017).

The study, while focusing on the impacts of natural and man-made disasters on the people of J&K and Ladakh region, also shows the linkages between the two types of disasters. First, the severity of natural disasters and their impact on people is largely determined by human activity. The impact of an earthquake is largely determined by the quality of the construction of buildings, whereas in the case of floods and landslides, human activities such as deforestation, urbanization, unscientific construction, and mining determine the extent of damage caused (Barnard, Owen, Sharma, et al., 2001; SDMP, 2017). Second, the extent of damage can be minimized by immediate and effective responses taken in the wake of the disaster. The response by the government after the flash floods in Ladakh in 2010 was considered mildly effective, whereas there was a widespread perception of government failure, inadequate preparation, and poor relief measures in the aftermath of the 2014 floods (Venugopal and Yasir, 2017; Gupta, Khanna, and Majumdar, 2012). The floods laid bare the lack of warning systems, the poor levels of preparation of the authorities, and the inadequate relief and rehabilitation measures (Venugopal and Yasir, 2017). Third, conflicts, while being rooted in their specific histories and the prevalent political and social condition, are also impacted by natural disasters. The destruction caused by natural disasters creates conditions of resource scarcity, aggravates pre-existing inequalities in the society, and can lead to a general sense of grievance among people, thus further aggravating the conflict. A study using the data from the second half of the 20th century concludes that the risk of violent conflict increases in the short- and medium-term after a rapid-onset disaster like a flood or an earthquake (Nel and Righarts, 2008).

The government has taken steps to mitigate the effects of disasters by integrating livelihood schemes with disaster management, by promoting crop diversification, crop insurance, and by providing compensation to people affected by disasters. It has also made efforts to implement an extensive disaster management plan. While the plan highlights detailed and effective measures against disasters, it is imperative that the policymakers go beyond and take into account the diversity of the conditions in the region. There is an urgent need to improve the response and rehabilitation measures in rural areas after natural disasters, as was evident after the earthquake in 2005 when remote villages were ignored, and most relief measures remained focused on towns and cities (Zahir-ud-Din, 2005). Early warning systems, which were not effective during the 2014 floods, need to be made functional for different types of disasters. The structural integrity of the existing infrastructure in the state needs to be improved with safety audit of existing buildings and strict adherence to earthquake resilience for construction of future infrastructure (Yousuf, Bukhari, Bhat et al., 2020). Social support among relatives, neighbors, and the community can also act as a source of resilience for people in the aftermath of disasters. A study done among adult survivors of 2014 floods in Kashmir region shows that high level of family and friends' support reduced the association between flood-exposure and symptoms of PTSD and depression to a great extent (Dar, Iqbal, Prakash, et al., 2018). There is also a need to engage with people and communities at the local level and formulate disaster management plans which makes use of the local and traditional knowledge systems. *Dhaji Diwari* is one such indigenous construction method which uses timber beams as means to reduce the impact of earthquakes on buildings. This method has been effective against earthquakes but is no longer widely practiced as people have moved toward more modern ways of construction using bricks and concrete which does not suit the unique landscape and climate of the region (Hassan, 2014; Yousuf, Bukhari, Bhat et al., 2020). Traditional and indigenous industries like handicraft need to be supported and private investment should be encouraged in industries such as biotechnology, mineral extraction, and leather goods (Mahapatra and Shekhawat, 2008).

In a disaster-affected area, while focusing on economic growth, it becomes imperative that the government pursues sustainable development which does not negatively affect the fragile natural environment of the region. Economic measures are important, but all economic activity such as investment, trade, and tourism depends, to an extent, on the security of the area. Over the years, the security situation in the region has improved substantially but remains fragile and prone to outbursts in the form of violent protests and terrorist attacks (Khan, 2017). There exists a trust deficit between the local population and the administration and this need to be bridged by more inclusive community engagement approaches. There is an urgent need to increase the social capital of the people of the region by making local communities' stakeholders in the functioning of the government. It can be done through decentralization of power and authority and through the implementation of developmental programs focused on the needs of the community, with their active participation. In addition, the recent bifurcation of the UTs and administrative policy change in the region has given government enough space to work on the safety, security, and economic development agenda of the region.

4. Conclusions

While our study has managed to present a comprehensive overview of the impacts of disasters on the lives of people in the region of J&K and Ladakh, it does have some limitations. The study is based entirely on available literature and no primary data were collected for it. In a dynamic situation like the one in J&K and Ladakh region, the study, while being true to the intrinsic nature of disasters, terrorism and violence, may fail to present the current situation. The study also leaves scope for in-depth research into the different impacts of the disasters highlighted here. Based on the findings, the study recommends the strengthening of effective disaster risk reduction and management systems, early warning systems and infrastructure – especially health facilities, schools and roads – in J&K and Ladakh region. In addition, emphasis must be laid on reducing the underlying vulnerabilities of the population through better community engagement approaches for both development initiatives and conflict resolution, with a special focus on the youth. Interventions made by the government to improve the resilience of communities should be implemented in a sustainable way, taking into account the risks posed by both natural disasters and terrorism. This study strongly recommends in-depth research and advocacy to ensure that resilience measures with regard to both natural and man-made disasters in the region are appropriately addressed.

Disclaimer

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Authors' Contributions

Conceived and designed: Sangram Kishor Patel. Review of literature: Sangram Kishor Patel, Ankit Nanda, Govind Singh and Sunita Patel. Contributed to tools/materials/data collection: Sangram Kishor Patel and Ankit Nanda. Drafted and wrote the manuscript: Sangram Kishor Patel, Ankit Nanda, Govind Singh and Sunita Patel.

Conflicts of Interest

No conflicts of interest were reported by the authors.

Ethical Approval

Not applicable.

Availability of Supporting Data

Open data sources.

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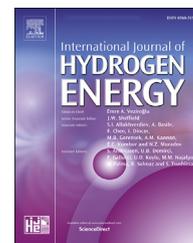
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A sequential approach to uncapping of theoretical hydrogen production in a sulfate-reducing bacteria-based bio-electrochemical system

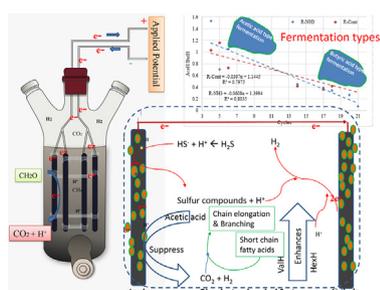
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HIGHLIGHTS

- Present study shows high feasibility of H₂ production using bio-electrochemical system.
- NH₃ pre-treated electrodes enriched with SRB's to increased H₂ production yield.
- CH₄ and H₂S production was found completely suppressed after 7th cycle.
- Kinetic modelling simulation is presented in residual to more clarify computed data.

GRAPHICAL ABSTRACT



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ABSTRACT

The presence of undesired methanogens with Sulfate-reducing bacteria (SRBs) is a serious challenge faced by the bioelectrochemical system (BES). In the present study, we investigate the impact of ammonia pre-treated electrodes on hydrogen production in a 600 ml anaerobic (BES) enriched with sulfate-reducing bacteria (SRBs) to inhibit the CH₄ production for achieving the theoretical H₂ production. The highest hydrogen production of 3.67 ± 0.31 M/M of glucose was recorded in the BES. The BES completely inhibited the growth of methanogens after the 7th cycle of operation. The higher hydrogen production efficiency of BES can be justified by assuming a higher hydrogen mass transfer from the electrode surface to the biofilm. In presence of sulfate, acetate acid type of the fermentation was dominating in hydrogen production, while limitation of SO₄²⁻ switch over to the dominance of butyric acid type fermentative hydrogen production. Despite the sign of change in the acetate to butyric acid type metabolism, the BES system was able to uncapped the theoretical hydrogen production. The notable change in vector orientation of H₂, butyric acid, and hexanoic acid inferring the significant differences in the microbial community adapted on the electrodes in the R-NH₃ and R-Cont. SEM image clearly showing ammonia-treated electrode harbour more microbial growth on the electrode surface. The ratio obtained for CH₃ and CH₂ for the R-NH₃ and R-CONT of 1.316 and 1.755 respectively by FTIR stretching vibrations showing the difference in the bacterial species

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adapted on the bioanodes. Cumulative hydrogen production data was computed to confirm its validity of the Gompertz model, Richard model, and Logistic model. The Richard model was found in the best-fitted models for cumulative hydrogen production.

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Introduction

The growing need for energy by human society and depletion of conventional energy sources demands renewable, safe, low-cost, and omnipresent energy [1]. Therefore, it is urgent to develop a cost-effective, sustainable, and eco-friendly technology for energy recovery, to mitigate the climate change impacts linked to the emission from fossil fuel consumption. Biological hydrogen production is an environmental process that consumes less energy than electrochemical and thermochemical processes [2]. The bio-electrohydrogenesis, namely the biohydrogen through microbial electrolysis cell (MEC) obtained the distinct advantage over traditional fermentation methods of reaching a higher hydrogen yield, and over traditional water electrolysis of running at greater energy efficiencies [3]. Among the widely employed microbial technologies, certain bacteria species are capable of generating electrons from their cell surface while decomposing organic matter and have been explored to generate bioelectricity or modified to produce hydrogen [4]. The SRB's having the ability to use lactate, ethanol, formate, and butyrate, have extensively high hydrogenase activity and produce H₂ in natural sulfate limited conditions [5]. Hydrogenase, an important electroactive enzyme of SRB, has been discovered to have the capacity to connect its activity to solid electrodes by catalyzing hydrogen evolution and oxidation [6].

Microbial fuel cells (MFCs) technology plays a crucial role in higher hydrogen production through electrochemically active biofilms from organics rich in waste [7,8]. Sulfate-reducing bacteria species *Desulfovibrio vulgaris*, *Desulfobulbus*, *Desulfomicrobium* have been documented as model organisms for hydrogen production in electrochemical systems [5,9]. Moreover, *Desulfobulbus propionicus*, a particular species of SRB, is known to conserve energy to support growth via electron transfer to insoluble electron acceptors, such as Fe (III) oxide and graphite electrodes [10].

Generally, due to higher conductivity graphite and copper electrodes are widely used for H₂ production in BES. The first pilot-scale (1000 L) biohydrogen producing MEC was also operated with the use of multiple electrode pairs in continuous mode for about 100 days, although the gas production could reach up to 0.19 m³m⁻¹d⁻¹, with the main component of methane (86%) suggesting that most of the cathodic hydrogen produced was consumed by the methanogens [11]. The major challenge in achieving higher hydrogen production is a mixture of acetate, ethanol, butyrate, and other products usually formed as end products yield <4 mol of H₂ per mole of glucose [12] further hydrogen production potential can be

reduced by hydrogen consumers methanogens [13] and propionate-forming bacteria [14]. Therefore, higher hydrogen yields can be possible by controlling the competition by inhibiting the methanogens to divert the electrons equivalent to assist the fermentation process using various strategies. Engineering, electrochemical and biological factors as well [15].

Therefore, to improve hydrogen yields in the BES, the inhibition of acetate and hydrogen consuming methanogens, sulfate reducers, and homoacetogens should be considered. Additionally, modifying materials that are essential for the increase in power performance can also be effective in increasing capacitance [16]. The represented example of this aspect is the nitrogen functionalization of carbon cloth anodes using heat ammonia treatment [17,18] or dimethylaniline [19] to produce protonated amine groups to be conducive to bacteria adhesion [16]. Therefore by combining the advantage of fermentation type and MECs, the co-cultured MECs reduced the metabolic inhibition and significantly improve the hydrogen production efficiency by regulating the distribution of electrons flow [20]. Prevailing literature suggests the methanogen population is selectively inhibited by ammonia toxicity and result in enhanced H₂ with a mixed culture [21]. Moreover the optimum level of SO₄²⁻ and applied potential also produce the suitable conditions for the SRB for higher performance efficiency for the long term operation for hydrogen production and sulfate reduction on the biocathode by overcoming a drop in pH problem, otherwise which fail BES [22,23]. Therefore, the ammonia pre-treated electrode would enrich the H₂ producing bacteria on the electrode by suppressing the methanogen population of anaerobic bioelectrolysis reactors [24]. The recent advances in BESs show that properly designed enriched anodic and cathodic biofilms hinder the need for adding mediators or chemical catalysts [25,26], as the rate-limiting steps and this limiting effect could be more significant for bioelectrodes when developed simultaneously in one system [27].

In the present study, we explored the potential of sulfate reducers (considered as hydrogen consumers) for hydrogen production in the BES system using ammonia pre-treated electrodes to suppress methanogens to facilitate the easy electron transfer to minimize the rate-limiting effects. Sulfate a preferred electrons acceptor by the SRB and the external applied potential strategies were employed to outcompete the methanogens from the mixed culture without using any specific inhibitor which is an innovative way. The kinetic modelling was applied to the hydrogen production for the analysis of best-suited conditions.

Materials and methods

SRB consortium source and enrichment

The inoculum used in the study was prepared by mixing a consortium of culture (A) collected from an anaerobic digester of the Pirana Municipal Sewage Treatment Plant, Ahmedabad, Gujarat, (India) with culture (B) cow dung in 1:1 ratio in a 1 L [23]. The culture was further fed with a 5 L of Postgate medium [28] in a 10 L capacity anaerobic bioreactor. The composition of the Postgate medium was (g/L): KH_2PO_4 0.5; Na_2SO_4 1.0; NH_4Cl 2.0; CaCl_2 0.06; FeSO_4 0.005; sodium citrate 0.3; yeast extract 0.1; glucose 5. The consortium was regularly fed with Postgate medium every 5–6 days under anaerobic condition by N_2 (99.99% purity) purging after decanting the 3.0 L supernatants. The reactor was kept at room temperature (35–40 °C) monitored using a thermometer and maintained at a pH of 6.5 ± 0.5 using 6 N HCl/NaOH. During the acclimation period and volume of gas was measured by the water displacement method.

Bioelectrochemical system design and operation

The present study was conducted in a 600 ml BES (working volume 400 ml) of diameter 6.5 cm and 25 cm height with two lateral openings on the sidewall and one at the bottom of length 4 cm having facilities of sealing with 20 mm septa and aluminium seal. The top central opening of 2.5 cm diameter equipped with cork was used to ease the wiring for the connection of the electrodes using the electric couple. Two graphite electrodes activated in a muffle furnace at 550 °C for 30 min and further pre-treated by dipping in 25% ammonia solution were inserted vertically and supported with a silicone tube. The electrodes (length 10 and dia 0.6 cm) were placed 5 cm apart. The reactor's fabrication and the electrode procurement were done from the local market, Ahmedabad (Gujarat). 200 ml of the active SRB culture from the master reactor maintained at room temperature was added to each reactor. The BES were operated at 400 ml working volume by feeding 200 ml of Postgate medium at 48 h hydraulic retention time (HRT) by applying 40 mV potential (pre-optimized potential). The initial pH of the reactors was maintained at 6.5 using 6 N NaOH/HCl. During Phase-I the reactors were optimized for constant volumetric gas production to ensure a steady-state. The headspace air was measured by dragging out air using a 60 ml plastic syringe. After obtaining the almost constant volumetric gas production from each reactor the compositional analysis of the gas was started and continued till Phase-II at a regular interval till the 6th cycle. The addition of sulfate in the medium was stopped and continue with gas composition analysis until the completion of the 17th cycle. After the 17th cycle, two additional electrodes, same in configuration and treatment were inserted in each reactor. The anaerobic condition in the reactors was generated by purging with N_2 (99.999% purity) for 5 min after feeding. Based on the observation of the gas production 60 ml–180 ml of headspace N_2 gas was drawn to accommodate the excess of the pressure generated in the reactors before incubation. The reactors set in triplicates were operated for the following phases:

- i. **Phase-I:** This phase was considered the stabilization phase. In this Phase, 200 ml of the well-mixed culture was added to each reactor. The ammonia-treated and untreated two graphite electrodes were inserted in the respective reactors through the top opening. The reactors were filled and decanted at 48 h HRT, by feeding the Postgate medium. The headspace gas generated was measured twice a day at 10:00 a.m. and 4:00 p.m. This phase was operated to insure an equal volume of gas production from each set of the reactors. The stabilization phase was carried for the completion of the three cycles. The stability of the reactors was assessed in terms of equal volumetric gas generation. Each reactor was producing 200 ± 10 ml of the gas. The qualitative analysis of the headspace gas (H_2 , CH_4 , CO_2 , and H_2S) was performed at the end of the last cycle.
- ii. **Phase-II:** During this Phase, 40 ± 5.0 mV potential was applied to each reactor. The reactors were continued till the completion of the three cycles by feeding with the Postgate medium at 48 h HRT. The headspace gas generated was measured both in terms of quantity as well as quality.
- iii. **Phase-III:** This phase was continued like Phase-II, except the Postgate medium fed was free of SO_4^{2-} . This phase was operated for the completion of the three cycles. At the start of this phase, the initial pH of the reactors was adjusted to 6–6.5. This phase was continued until the completion of 7 cycles.
- iv. **Phase-IV:** This phase was started with increasing the electrode's surface area by insertion of two more respective electrodes (NH_3 treated and Untreated) in the reactors. This phase continued to evaluate the effect of the electrode surface area on gas production.

Gas measurement and composition analysis

The composition of headspace gas was analyzed using gas chromatography (PerkinElmer-680) configured with a thermal conductivity detector (200 °C), equipped with the Elite-plot Q column (30 m \times 0.53 mm ID). The injector temperature was kept at 150 °C. Initially, the oven temperature was 50 °C for 7 min and increased with a ramp of 8 °C/min to 75 °C holding for 2.5 min. Nitrogen was used as carrier gas at a flow rate of 2 ml/min and makeup to 6 ml.

Liquid components analysis

The physicochemical analysis of the liquid sample was analyzed using the standard method [29]. 50 ml sample from each reactor was processed for further components analysis. pH was measured immediately after harvesting the samples using Eutech pH 700 m. The volatile fatty acids (VFAs) analysis was done at the initial, middle, and end cycles of each phase starting from Phase-II onwards. Before analysis, the VFAs samples were centrifuge at 5000 rpm for 20 min at room temperature. 2.5 ml of VFAs sample was acidified to 2–3 pH using Orthophosphoric acid and preserve at 4 °C for further analysis. Before analysis, the VFAs samples were diluted 10 times with HPLC grade water and filtered through 0.2 μm

membranes (Dia. 25 mm), and passed to IR 120 Regin. The gas chromatograph (PerkinElmer 680) equipped with a flame ionization detector (FID) was used for VFAs analysis by injecting 0.5 μl sample with autosampler in an elite-wax column (30 m \times 0.32 mm ID) [23]. The initial oven temperature was set at 95 $^{\circ}\text{C}$ for 5 min followed by an increase of 10 $^{\circ}\text{C}/\text{min}$ until the temperature reached 140 $^{\circ}\text{C}$, where it was kept for 6 min. Further, the temperature was increased at a rate of 40 $^{\circ}\text{C}/\text{min}$ to 200 $^{\circ}\text{C}$ holding for 5 min. The injector and detector temperatures were 250 $^{\circ}\text{C}$ and 240 $^{\circ}\text{C}$ respectively. The VFAs concentrations were calculated using a calibration curve prepared from volatile free acid mix 10 mM (Sigma Aldrich) of formic acid, acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, isocaproic acid, hexanoic acid, and *n*-heptanoic acid in the range of 0.5–5 mM. The following metrics were used for the investigations concerning the amounts and relative proportions of VFAs products [30].

- i. Alkyl groups (Methyl and Methylene groups) (mM) = $C_2 + (2 * C_3) + (3 * C_4) + (4 * C_5) + (5 * C_6)$, where C_x is VFA (mM) of the total carbon number x ;
- ii. Average VFA chain length = (total mM alkyl groups/total mM VFA) + 1, where the 1 is non-energetic carbon (carboxyl group) in the VFA molecule.

Kinetic and modelling

The data obtained for cumulative H_2 from the bio-electrochemical system were checked for the suitability of kinetic models. The Non-linear curve fitting was assessed on each phase using Origin Pro Trial 2019b 64bit software (Trial version). Cumulative H_2 production was stimulated by the following equations [31,32].

Models	Equations	
Logistic model	$C = a/(1+b\exp(-kt))$	(1)
Richard model	$C = a*(1+(d-1) * \exp(-k*x-xc)) / (1+(1-d))$, $d \neq 1$	(2)
Gompertz model	$C = a * \exp(-\exp(-k*(x-xc)))$	(3)

Where,

Logistic and Richard models- C is cumulative hydrogen production (ml); k is kinetic constant (h^{-1}); t is hydraulic retention time (h); a and b are the constants.

Gompertz model- C is cumulative hydrogen production (ml); a is ultimate hydrogen production potential, \exp is exponential (1); d and k are constants, t is hydraulic retention time (h); a and b are the constants.

The statistical analysis was carried out to evaluate the significant/insignificant variations between and within the treatment processes. The statistical evaluation was performed using Microsoft Excel 365 and the PAST software (version 3). The single factor ANOVA was used to test the variations in the various components. The principal components analysis was performed using the ordination PCA under multivariate analysis for the biplots preparation and further analysis.

Surface morphology and functionalities analysis of the electrodes

Scanning electron microscopy (SEM Zeiss Evo 18 Special Edition) with Smart SEM-[SYSTEM] software was used to analyze the surface morphology of the graphite electrodes before and after ammonia treatment, and electrodes collected from the R-NH₃ and R-Cont. After the completion of the experiments, the initially inserted electrodes were collected from the reactors and processed for the SEM analysis. The electrodes were dried under anoxic conditions, considering the air-drying as the only method that maintained the location of loosely attached bacteria on a surface [33]. A small piece of the electrode near the bottom (1 cm above) was cut for the analysis of the surface morphology of the electrodes. The pieces were cut in such a way so that surface having microbial growth remains intact. A small piece of the graphite electrode was mounted on aluminium stubs with double-sided carbon tape sputter-coated with a palladium coater (Quorum SC7620 Mini sputter coater). After optimizing the image clarity, the magnification of electrodes was taken on various levels.

FTIR analysis was carried out using a JASCO FT-IR-4700 type A model equipped with a TGS detector with a resolution of 8 cm^{-1} at scanning speed (2 mm/s). The spectral range of the peak was recorded 399.193 cm^{-1} to 4001.57 cm^{-1} at a data interval of 1.92847 cm^{-1} . The samples were dried in the hot air oven at 50 $^{\circ}\text{C}$ before FTIR analysis. The data was obtained in Excel format as well as FTIR spectrum (pdf file). It was difficult to compare the FTIR spectra obtained for R-NH₃, R-Cont, NH₃-Treated electrodes with a graphite electrode. The spectra were very well compared to the differences obtained in the spectra of the respective electrode from the spectra of the graphite electrode. This difference in % transmittance was plotted using excel graphs, which clearly showing even minute differences in the peaks and stretching in the FTIR spectra.

The graphite electrodes were further processed for the analysis of XRD. A small quantity of the powder was collected from the electrode surface for XRD analysis. The XRD analysis of the dried microbial film was performed to determine the change in surface compounds deposited on the electrodes during the hydrogen production. XRD analysis of dried fine powder of upper dried layer of electrodes was carried out using XRD (Bruker AXS D8 Focus P-XRD type model), with 2 θ ranging from 10 $^{\circ}$ to 80 $^{\circ}$ X-ray photoelectron spectroscopy.

Results and discussion

Start-up of the bioelectrochemical system

The present study illustrates the performance of the SRB-based BES systems equipped with ammonia pre-treated graphite electrodes operated at 48 h HRT. It is important to evaluate gaseous production efficiencies and stability in the BES for H_2 production. The BES acclimated with the microbial community in the R-NH₃ and R-Cont. reactors produce volumetrically and statistically indistinguishable variation in total gas ($p = 0.972$) during Phase-I, the stabilization period. Based on the stability during Phase-I, the BES systems were further

operated for hydrogen production potential. The insignificant variation in the volumetric gas production showing the merely NH_3 treatment of the electrodes is not affecting gas production. Interestingly application of the applied potential to the electrodes results in significant variations in the volumetric gas production ($p < 0.001$) in the R-NH₃ and R-Cont. The enhanced surface area as a result of ammonia treatment might be promoting good biofilm formation and increasing the electron transfer between the electrodes and the microorganisms. Though for most systems it is still not clear how microorganisms conduct the electron import, evidence was provided for some systems that hydrogenases can directly interact with the cathode and catalyze the production of molecular hydrogen [34]. At the start of the reactor, the gas production was 250.67 ± 62.21 and 254 ± 31.73 ml (mean \pm standard deviation) in the R-Cont. and R-NH₃ respectively. The respective reactors stabilized to 401 ± 12.17 and 399.5 ± 24.98 ml gas production in the R-Cont. and R-NH₃ respectively after achieving the maxima ~ 450 ml. The hydrogen yield analysis performance was started once the systems reached a stable condition as the reproducible cycles. The performance of the BES systems in terms of average H₂, CH₄, and H₂S production in M/M of glucose in different phases is presented in Table 1.

Hydrogen production potential

The H₂ recovery was tested under the ammonia-treated electrode and the untreated electrodes in the BESs. The hydrogen production on the biocathodes was analyzed for cathode potentials of 40 ± 5 mV for 38 days of Phase-I onwards in both the BESs (Fig. 1A and B). The much more hydrogen

production efficiency with statistically distinguishable variance ($p = 0.046$) by the type the R-NH₃ BES was observed in presence of the applied potential. Modifying the electrode with a positively charged ammoniacal compound promotes more bacterial adhesion, a faster start-up period, and an overall more efficient electron transfer during the MEC process [35]. The H₂ production profiles and gases (%) in various phases in the R-NH₃ and R-Cont. are presented in Fig. 1(A and B). The R-NH₃ produces higher hydrogen during all the phases. The amine functionalization of the carbon surface could be useful to improve microbial adhesion and power generation [19] by the heat and ammonia treatments that increased the nitrogen content and the positive surface charge (from 0.38 to 3.99 meq m⁻²) [17,18,36]. The higher hydrogen production in the R-NH₃ reactor showing the proper functionalization of the electrodes by treating with 25% ammonia solution for 30 min, otherwise, a highly functionalize surface can reduce the microbial adhesion [37]. The enriched biofilm on the ammonia-treated electrode was also observed in the SEM and FTIR (analysis of functional groups). At the onset of the applied potential of 40 ± 5 mV, the H₂ production improved with significant variations ($p < 0.001$) in both the BES in Phase-II. Though the improvement in the H₂ production was very less from 0.171 ± 0.062 M/M of glucose and 0.472 ± 0.115 M/M of glucose to 0.225 ± 0.085 M/M of glucose and 0.484 ± 0.08 M/M of glucose at the end of Phase-II in the R-Cont. and R-NH₃ respectively. The lesser hydrogen production during this phase might be due to the presence of SO_4^{2-} in the medium which acts as an alternative electron acceptor and will have a marked influence on its reduction to H₂S gas. This might be due to the presence of sulfate in media in Phase-II, where SRB consumed H₂ as it has a high affinity and a lower threshold

Table 1 – Gaseous compositions during the various phases in the R-NH₃ and R-Cont reactors.

Cycles		R-Cont			R-NH ₃		
		(M/M Glu)			(M/M Glu)		
		H ₂	CH ₄	H ₂ S	H ₂	CH ₄	H ₂ S
1	Phase-I**	250.67 \pm 62.21			254 \pm 31.73		
2		448.33 \pm 10.51			453.5 \pm 2.65		
3		401 \pm 12.17			399.5 \pm 24.98		
4	Phase-II	0.17 \pm 0.06	0.06 \pm 0.02	0.19 \pm 0.02	0.47 \pm 0.12	0.11 \pm 0.07	0.19 \pm 0.02
5		0.23 \pm 0.16	0.09 \pm 0.04	0.19 \pm 0.05	0.44 \pm 0.11	0.14 \pm 0.04	0.19 \pm 0.04
6		0.22 \pm 0.09	0.11 \pm 0.01	0.24 \pm 0.03	0.48 \pm 0.08	0.13 \pm 0.06	0.23 \pm 0.01
7	Phase-III	1.82 \pm 0.07	0.22 \pm 0.01	0.04 \pm 0.02	2.21 \pm 0.12	0.22 \pm 0.02	0.02 \pm 0.00
8		2.57 \pm 0.09	N.D	0.01 \pm 0.01	2.74 \pm 0.08	N.D	N.D
9		2.43 \pm 0.68	N.D	0.01 \pm 0.01	2.86 \pm 0.52	N.D	N.D
10		3.23 \pm 0.33	N.D	0.01 \pm 0.01	3.57 \pm 0.32	N.D	N.D
11		2.81 \pm 0.22	N.D	N.D	3.23 \pm 0.08	N.D	N.D
12		2.52 \pm 0.26	N.D	N.D	3.16 \pm 0.10	N.D	N.D
13		2.70 \pm 0.13	N.D	N.D	3.52 \pm 0.07	N.D	N.D
14		2.49 \pm 0.43	N.D	N.D	3.30 \pm 0.16	N.D	N.D
15		2.34 \pm 0.84	N.D	N.D	3.42 \pm 0.24	N.D	N.D
16		2.32 \pm 0.40	N.D	N.D	3.67 \pm 0.31	N.D	N.D
17	1.87 \pm 0.75	0.06 \pm 0.10	N.D	3.18 \pm 0.25	N.D	N.D	
18	Phase-IV	0.91 \pm 0.52	0.10 \pm 0.02	N.D	2.28 \pm 0.09	N.D	N.D
19		1.99 \pm 0.27	N.D	N.D	3.41 \pm 0.46	N.D	N.D
20		2.18 \pm 0.51	N.D	N.D	3.26 \pm 0.32	N.D	N.D
21		2.28 \pm 0.39	N.D	N.D	2.45 \pm 0.14	N.D	N.D

** Total volume of gas (ml); N.D: Not Detected.

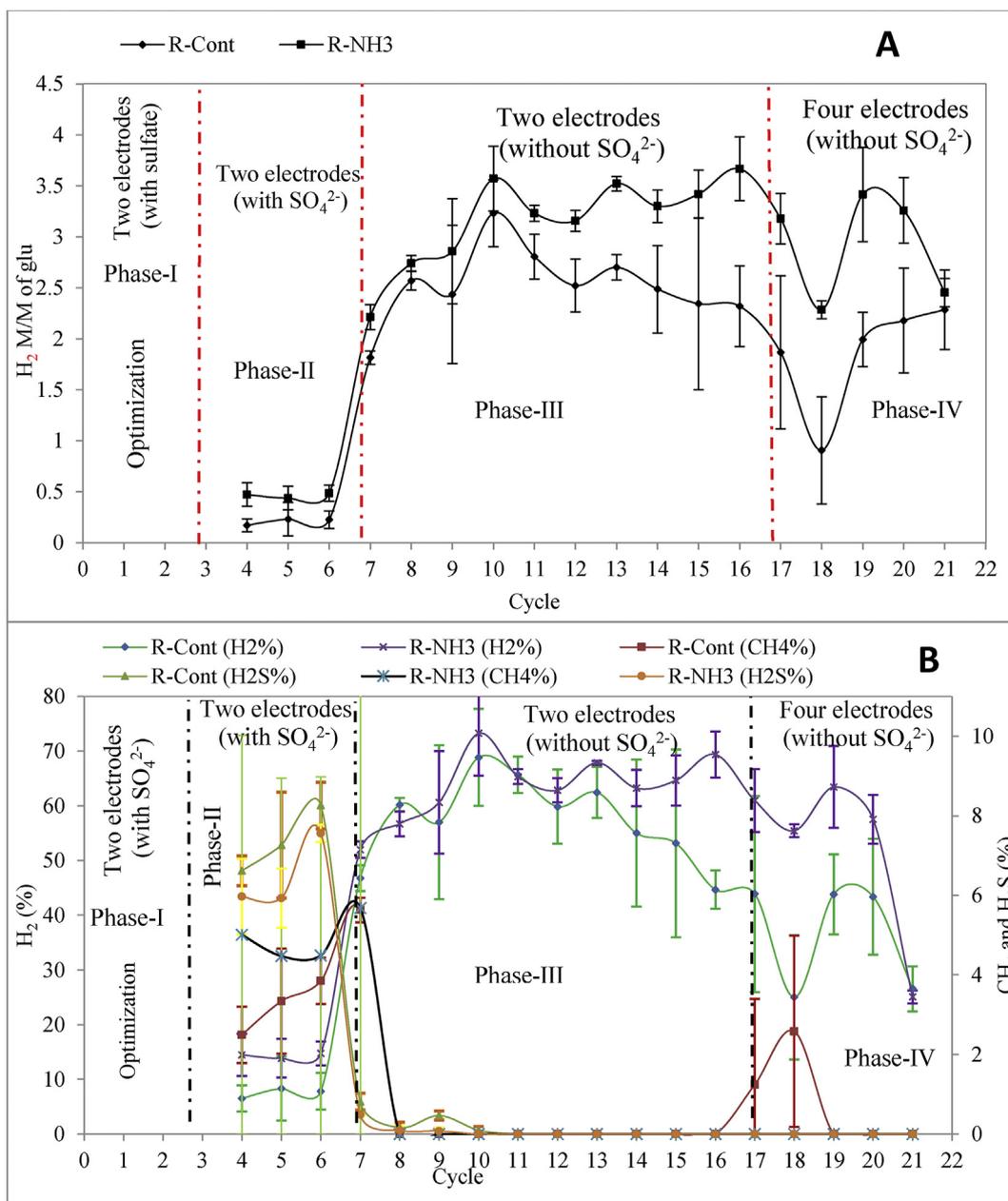


Fig. 1 – Gaseous component generated in various phase.

value for hydrogen. The lesser hydrogen production in the SRB-based biocathode might be due to the formation of metal sulfate precipitation as the result of high sulfate content in the cathode medium which inhibited the biofilm-electrode electron transfer [27]. Other researchers also reported hydrogenase activity of SRB enhanced with low sulfate addition during the enrichment phase of microbial growth [38]. During the subsequent Phase-III operation, the significant increase in 12%–63% hydrogen production coinciding with the decrease in the acetate concentration over time, when Postage media was not supplemented with sulfate (Fig. 2B). This was due to the SRB's extensive hydrogenase activity which fermentatively produces H₂, CO₂, and acetate in a limited sulfate environment with other organisms and functions as H₂

producers [5]. During Phase-III, hydrogen production was found in a range of 2.21 ± 0.12 to 3.67 ± 0.31 M/M of glucose. The results of our study reveal that the integration of HRT operation with ammonia-treated electrodes in the BES affects the overall performance of the reactor. Combining the advantage of fermentation type and MECs, the co-cultured MECs reduced the metabolic inhibition and further significantly improve the hydrogen production efficiency by regulating the distribution of electron flow [20]. Despite being mixed culture in MECs, hydrogen may also be lost due to the activity of hydrogenotrophic methanogens and acetogens, in the present BES system hydrogen recoveries of 56–73% was observed using graphite electrodes, whereas recoveries for platinum cathodes of 57–96% were achieved [35,39,40].

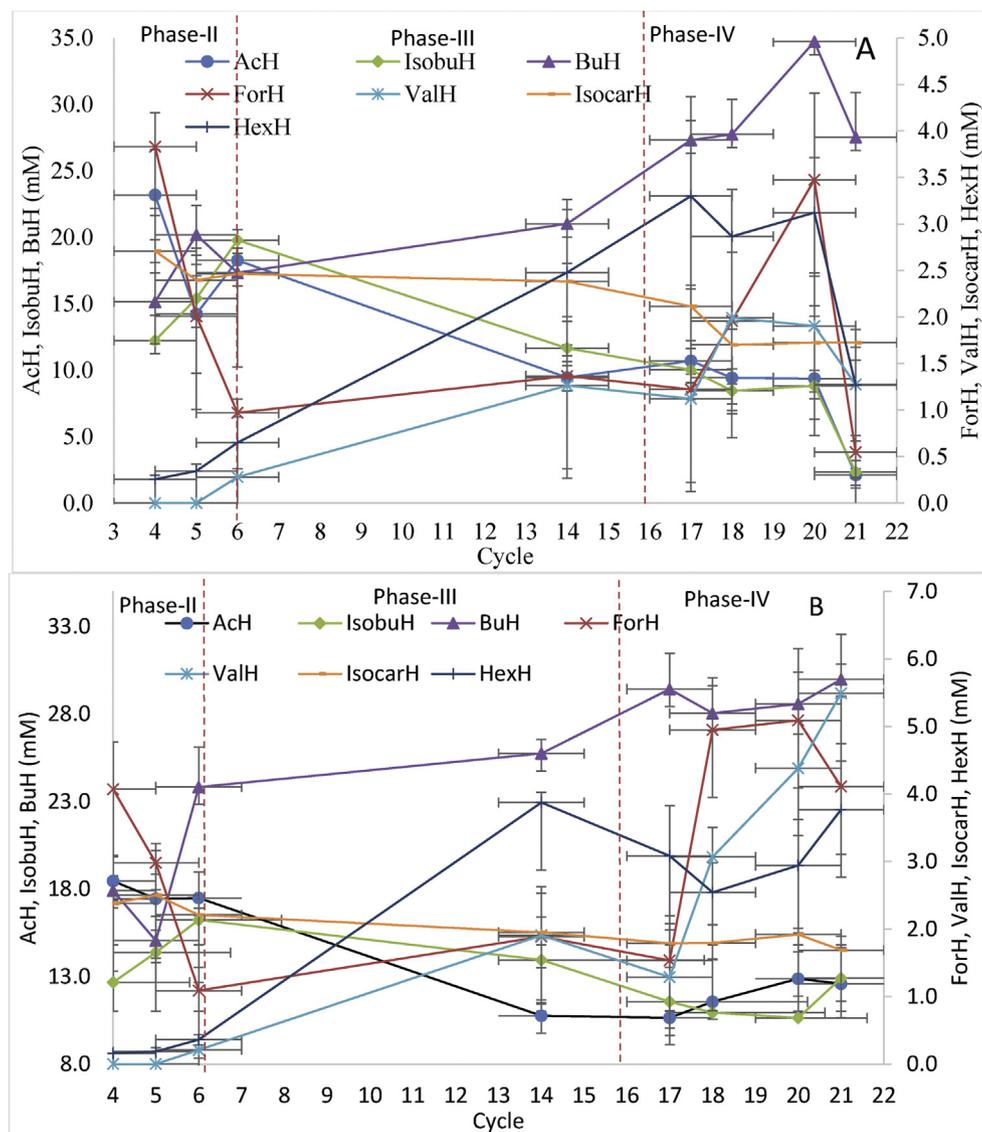


Fig. 2 – VFAs profile during various phases of operation in the reactors; A) R-NH₃, and B) R-Cont.

Ammonia pre-treated electrode enhances the hydrogen-producing bacteria population on the electrode by inhibiting the methanogen in anaerobic bio-electrolysis reactors [41–44]. In our case also the strong peak of the ammonia (FTIR data) was observed on the ammonia-treated electrode, which further disappears (diffuse from the surface) on the electrodes recovered from the BES. This finding inferred that the ammonia-treated electrodes inhibiting the methanogen bio-film formation and causing the toxicity by ammonia diffusion from the surface of the electrode during the initial acclimatization of the reactors. Studies have reported that ammonia causes toxicity to methanogens by diffusing free ammonia molecules into the cell through the cell membrane [45,46]. During Phase-IV significant drop in hydrogen production was found initially in the 18th cycle and its production tremendously increased to 3.41 ± 0.46 M/M of glucose in the 19th cycle. The sudden drops in hydrogen production initially in the 18th cycle might be due to the disturbance of the microbial population during the insertion of the additional electrodes.

The production tremendously increased to 3.41 ± 0.46 M/M of glucose in the 19th cycle, which might be due to more consumption of organic matter due to increase surface area for bio-electrolysis activity with additional electrodes. However, the systems were unable to maintain the continuous significant improvement in hydrogen production with the insertion of additional electrodes. This can be understood by the decrease in microbial electrode performance induced by increasing the electrode size (from 9 cm^2 to 50 cm^2) by looking at the current provided by the anodes at the same potential E_{measured} , the larger size the anode worked at potentials significantly far from applied value presented a broad distribution of local potential [47].

Further continuous drops in H₂ production was seen from 20th and 21st cycles might be due to the accumulation of toxic compound due to high organic degradation reduces the population of hydrogen-producing bacteria by continuous voltage supply in anaerobic bioreactors. Though despite a change in the fermentation types (based on the VFAs data), the present

SRB-based BES system has been successfully uncapping the theoretical hydrogen production potential as a result of the higher hydrogenase activities. Increasing butyrate production will cause the relative acetate to butyrate ratio to decrease while increasing the quantity of acetate via the homoacetogenesis will cause the ratio to increase [48]. Researchers have evaluated the efficiency of ammonia-treated platinum electrodes using carbonate/phosphate-buffered solutions in H₂ production of $1.7 \pm 0.4 \text{ m}^3\text{H}_2\text{-m}^{-3} \text{ d}^{-1}$ by inhibition of methane production less than 1% using acetate in a BES system [24]. So the SRB-based BES has higher potential as evidence is provided for some systems that hydrogenases can directly interact with cathodes and catalyze the production of molecular hydrogen [34].

Methane production

The treated electrodes show insignificant variations in methane production ($p = 0.477$) between the R-NH₃ and R-Cont. Though at lesser levels, CH₄ production was seen during Phase-II and up to the 7th cycle in Phase-III in both reactors. At the end of Phase-II in the 6th cycle, CH₄ production was found to nearly 4–5% in the BES reactors and further enhanced to nearly 11% in Phase-III in the 7th Cycle (Fig. 1B). The presence of the SO₄²⁻ in the medium is supposed to outcompete the methanogen by the SRB. The findings from the present study showing that the presence of sulfate in the medium successfully suppresses the methanogens, but unable to completely restrict their growth. The disappearance of the methane peaks/production on applying the potential ($40 \pm 5 \text{ mV}$) eliminated the methanogens 7th cycle onwards (Table 1 and Fig. 1B). In another study researchers completely inhibit the methane production using a two-chamber BES system fed-batch reactor feed with butyric acid-producing hydrogen nearly 80% using glucose [49]. So, the applied potential strategies for the suppression of the methanogens from the consortium are more prominent in comparison to the addition of only SO₄²⁻ in the medium. Similarly, the other researcher's study of the integration of bio-electrolysis at suitable low voltage with ammonia pre-treated electrode at lower HRT which inhibits methane production [50–52], due to more susceptibility towards ammonia toxicity by lowering down methane production in the anaerobic digesters [21,43,46,53].

H₂S production

The SRB utilizes sulfate under its elevated concentration as an electron acceptor by producing H₂ and H₂S using a suitable electron donor. At the end of Phase-I, both the reactors were producing average H₂S equally to $0.188 \pm 0.017 \text{ M/M}$ of glucose and $0.188 \pm 0.019 \text{ M/M}$ of glucose in the R-NH₃ and R-Cont. respectively. Though the H₂S production improved to $0.231 \pm 0.013 \text{ M/M}$ of glucose and $0.241 \pm 0.026 \text{ M/M}$ of glucose with insignificant variation ($p = 0.811$) between the R-NH₃ and R-Cont. respectively. Interestingly despite being a higher level of hydrogen production fed with an equal amount of sulfate, the lesser concentrations of the H₂S were seen in the R-NH₃. This finding inferring the reduction of the H₂S to elemental sulfur/other sulfur compounds on the treated electrode in presence of the applied potential. Otherwise in absence of

applied potential both the reactors were generating H₂S very equally. Similarly, another study reported that in SRB based bio-electrolysis reactor, H₂ is generated from biocathode which was used by SRB for sulfate reduction to hydrogen sulfide (H₂S) formation [54–56]. Moreover, the signature of H₂S generation was recorded up to the 7th cycle and 10th cycle in the R-NH₃ and R-Cont. respectively further indicating the more efficiency of the ammonia-treated electrodes. So, the residual SO₄²⁻ in the medium quickly vanished in the R-NH₃. A minor concentration of less than 1% of H₂S production was found in Phase-III till the end of the 10th cycle in R-Cont which might be due to residual sulfate in the media whereas comparatively, H₂S production was found till the 8th cycle in the R-NH₃.

Volatile fatty acids production

The VFAs profiles in the reactors during various phases are shown in Fig. 2(A and B). There was a significant difference in the distribution of various VFAs in various phases, which implied metabolic flux has been changed during various phases as well as in the type of reactor. The statistical analysis of the cumulative VFAs shows statistically indistinguishable data, however, the ANOVA value is very close to the significance level ($p = 0.056$). Moreover, the variations occurring to 63.829% and 32.544% in the R-NH₃ and R-Cont. respectively inferring the more dynamics (higher variance %) in R-NH₃ electrodes. At the end of Phase-I or start of Phase-II, the acetic acid ($23.176 \pm 3.417 \text{ mM}$), formic acid ($3.831 \pm 1.364 \text{ mM}$), and Isocaproic acids ($2.71 \pm 0.381 \text{ mM}$) were in the higher level in the R-NH₃. Whereas in the R-Cont., the acetic acid ($18.453 \pm 0.292 \text{ mM}$), formic acid ($4.069 \pm 0.702 \text{ mM}$), and butyric acid ($17.917 \pm 2.006 \text{ mM}$) were seen more in concentrations. Interestingly the concentration of the isobutyric acids seen was almost equal to $12.233 \pm 5.071 \text{ mM}$ and $12.668 \pm 1.785 \text{ mM}$ in the R-NH₃ and R-Cont. respectively. Moreover, the concentration of the isobutyric acid was statistically indistinguishable from the treatment factor of the electrode ($p = 0.364$). The higher variance in the R-NH₃ (26.54%) in comparison to the R-Cont. (3.61%) also support the finding of the polymerization of the VFAs in the earlier reactor.

A relative ratio of acetate and butyrate production greater than 1 indicates homoacetogenic activity as well as decreasing butyrate production [48]. The acetic acid/butyric acid can be a good indicator in the assessment of hydrogen yield. The Ace/But acids ratios of 1.5 and 1.02 in the R-NH₃ and R-Cont. respectively showing the favourable conditions for the SRB. This might be due to the presence of the SO₄²⁻ in the medium during the stabilization phase. In presence of SO₄²⁻ the SRB can outcompete methanogens and homoacetogens for substrate use. Further, the lesser concentration of the butyric acids in the R-NH₃, the preferred acid substrate by the SRB's is also a sign of their dominance. The hydrogen yield increased linearly with the increase of the acetic acid/butyric acid ratio. In sulfate-rich environments, the SRB outcompete the methanogens and homoacetogens, due to a higher affinity and lower threshold value for hydrogen. The regression analysis shows, the onset of the potential applied, the concentrations of the formate decrease from 3.831 mM to 0.969 mM and 4.069 mM–1.089 mM linearly in the R-NH₃

($R^2 = 0.975$) and R-Cont. ($R^2 = 0.976$) from the start to the end of Phase-II, respectively. Likewise, the impact of the onset of the applied potential was seen on the acetic acid also, where its concentrations drop to similar levels from 23.176 mM to 18.264 mM and 18.453 mM–17.487 mM in the R–NH₃ and R-Cont. at the start and end of Phase-II, respectively. The decrease in more acetate concentration in the R–NH₃ was due to its more oxidation. Even in the co-culture (*E. harbinense* and *G. sulfurreducens*) hydrogen production, it was recorded that the co-culture could mitigate the metabolic feedback of acetate [20]. So, the drops in the concentrations of formic acid and acetic acids suggest the existence of microbial electrolysis successfully in the reactors. The regression analysis showing more linearity in the consumption of formic acid in the R-Cont. ($R^2 = 0.710$) in comparison to R–NH₃ ($R^2 = 0.30$). This trend of the drops in formic acid concentration showing either its conversion to H₂ or polymerization of the chain length from 3.095 to 3.448 and 3.208 to 3.464 to long-chain fatty acids in the R–NH₃ and R-Cont. respectively. The previous studies also indicated the hydrogen production has a higher linear relationship with acetate concentration around a certain range and suggested a good correlation in pure substrate culture [57]. Moreover, the treatment of the electrodes with ammonia enhanced more polymerization of acids and the carbon chain length and goes on an increase to 4.038 and 3.701 in the R–NH₃ and R-Cont. respectively measured at the end of the operation. The concentration of the isobutyric acid increases from 12.233 ± 5.071 mM to 19.783 ± 0.792 mM and 12.668 ± 1.785 mM to 16.238 ± 1.931 mM in presence of SO₄²⁻, onset of the applied potential in the R–NH₃ and R-Cont. respectively. Interestingly onset of the potential (40 ± 5 mV) shows a statistically indistinguishable level ($p = 0.599$) of the isobutyric acids between the groups which changes to around statically distinguishable ($p = 0.059$) in the presence and absence of SO₄²⁻ respectively with higher variance in the R–NH₃. The SO₄²⁻ limitations in the medium continuously result in the decrease in the isobutyric acid concentrations in both the reactors and continue till the end of Phase-IV. However, the isobutyric acid concentration was more in the R-Cont. during Phase-II onwards. This might be due to the isomerization of butyric acid triggered by applied voltage [52]. Despite having fluctuations in the concentration of butyric acids its average phasic concentration increases to 17.545 ± 2.794 mM (Phase-II), 24.157 ± 3.059 mM (Phase-III), and 30.003 ± 3.802 mM (Phase-IV), and 18.932 ± 3.394 mM (Phase-II), 27.581 ± 2.921 mM (Phase-III) and 28.862 ± 1.572 mM (Phase-IV) in the R–NH₃ and R-Cont respectively. Very small differences in the butyric acid concentrations between the R–NH₃ and R-Cont. reactor shows that its level is not affected by the electrode's modification. The prominence of the butyric acid with increasing trends with the progression of the phases in both reactors might be due to the absence of the SO₄²⁻ in the medium. This might be due to increasing sulfate reduction during Phase-II is preferred by SRBs by butyric acid consumption and these microbes consumed lower chain fatty acid at a higher COD/SO₄²⁻ ratio [58], as was the case in Phase-III and Phase-IV in the present study. The concentrations of the isocaproic acid continuously decrease towards the end of the operation in both the reactors, however, a little higher concentration was seen in the R–NH₃ than the R-Cont.

Principal components analysis

The statistically indistinguishable cumulative VFAs having values very close to the significance level ($p = 0.056$) database was further assessed using the principal components analysis. The principal component analysis is a significant tool that helps in the understanding relationship between variables (correlation), similarities of individual data points (clustering), and relative importance of the observations to each independent variable using the biplots [59]. To correlate the H₂ production dataset with VFAs, the values of all the variables (VFAs and H₂) were considered from the same cycle. The variance in the dataset is presented in Fig. 3(A and B). The variance of about 83.70% and 87.22% in the R–NH₃ and R-Cont. reactor's data set respectively explain by the first 2 PCs. The cycles (4, 5, 6, 14, 17, 18, 20, and 21) in the biplots representing the scores of the respective component. The length and angle of the vectors showed variance and approximation of the correlation between the variable, respectively. The higher variance is seen from the longer length of the formic acid in the R–NH₃. The vectors H₂, ValH (<20°), ButH (20°), and HexH (<30°) directed in the same direction showing highly positively correlated variables with H₂ having correlation coefficients values of 0.847, 0.764, and 0.906, respectively. Vectors positioned in opposite directions indicate negatively correlated variables while orthogonal vectors indicate the absence of a correlation [60]. The IsoCapH vector angle was almost ~180° (actual 170°) to the H₂ vector angle showing the negative correlation (–1), while the vector ForH is found at 90° to H₂ vector inferring the smaller correlation between the vectors in the R–NH₃ reactor's VFAs profile. Whereas ButH and HexH vectors having correlation coefficients of 0.762 and 0.958 respectively followed by ValH (0.734) are very closely related to the H₂ vector in the R-Cont. Interestingly the vector angle of 180° between H₂ and AceH reflecting the negative correlation (–1) with a correlation coefficient of –0.846 in the R-Cont. However, the vector angle of interaction reduced to 130° depicting the consumption of the AceH on the NH₃ pre-treated electrodes in R–NH₃. The significant change in the orientation in H₂, ButH, and HexH inferring the significant differences in the microbial community adapted on the electrodes in the R–NH₃ and R-Cont. which is further confirmed by the surface characterization of the electrodes.

Electrodes (anode) surface characterization using SEM, FTIR and XRD

The scanning electron microscopy images of the electrodes used in the study are presented in Fig. 4(A–D). The number of microbes anchored on the anode surface affects the power output of MFCs to some extent [61]. The enhancement in the surface area as a result of the activation of the electrodes can be observed. It was found that nonuniform biofilm on the graphite electrodes from different reactors was covered with different morphologies (Fig. 4D). The SEM imaging of the anodes showed clear thick biofilm on the R–NH₃ electrode, whereas it seems thinner in the R-Cont electrode (Fig. 4C), indicating that the ammonia-treated electrode harbour more microbial growth on the surface. This electrode surface enriched with microbial growth could help in the direct

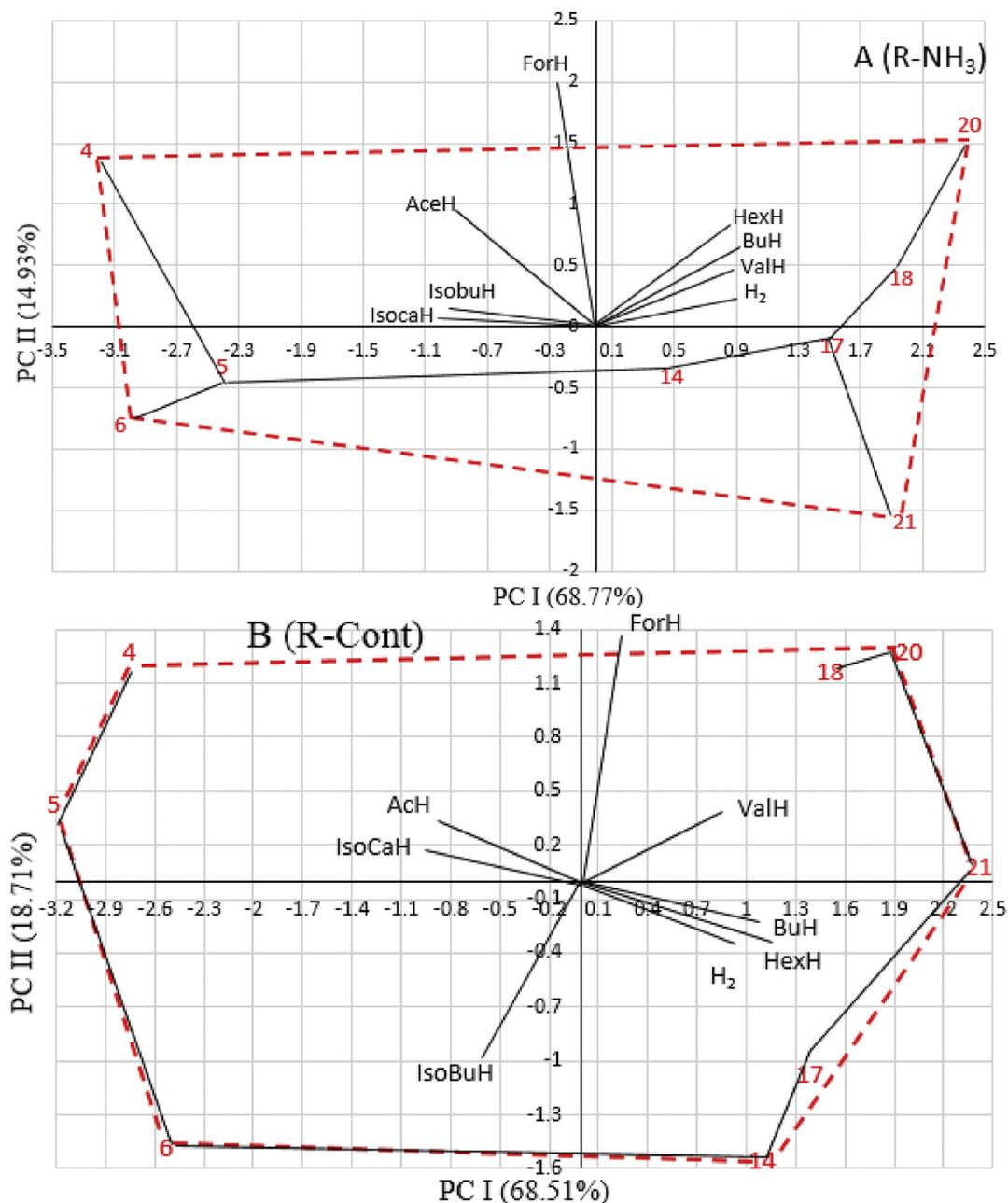


Fig. 3 – Principal Components analysis for the correlation in VFAs and H₂; A) R-NH₃, and B) R-Cont.

electron transfer without the mediator and results in efficient hydrogen production in the R-NH₃. SRB has been demonstrated as having the ability to connect their metabolic activities with the electron transfer on solid electrodes [6]. Further enhancing the image magnification, the additional interesting observation of pili-like appendages on the electrode surface (R-NH₃) facilitating the electron transfer and adhesion of the microbes, which did not appear in the R-Cont. electrode. These observations agree with a hypothesis in that the NH₃ treated electrode promotes the access of the bacteria to the electrode/electrolyte interface, enabling the electron transfer with graphite. Similarly, the best performance among the reported carbon-based ones for MFCs to date as a result of the biocompatibility and enhanced extracellular efficiency of

three-dimensional N-doped macropores carbon foam for the enrichment of *Geobacter* was observed [61]. Though extracellular electron transfer efficiency of the biofilm enriched anode is not only related to the morphological structure but also associated with the surface chemistry properties of the electrode.

The difference in FTIR spectra in R-NH₃, R-Cont, and NH₃-Treated electrodes were compared to the spectra obtained from the untreated graphite electrode to determine if differences are due to the interaction of the microbial growth and treatment of electrode with NH₃ as a result of functional groups happen if any (Fig. 5A). The difference in the spectra depicting the major changes in the functional group in the biofilm growth on the electrodes in the R-NH₃ reactor anode.

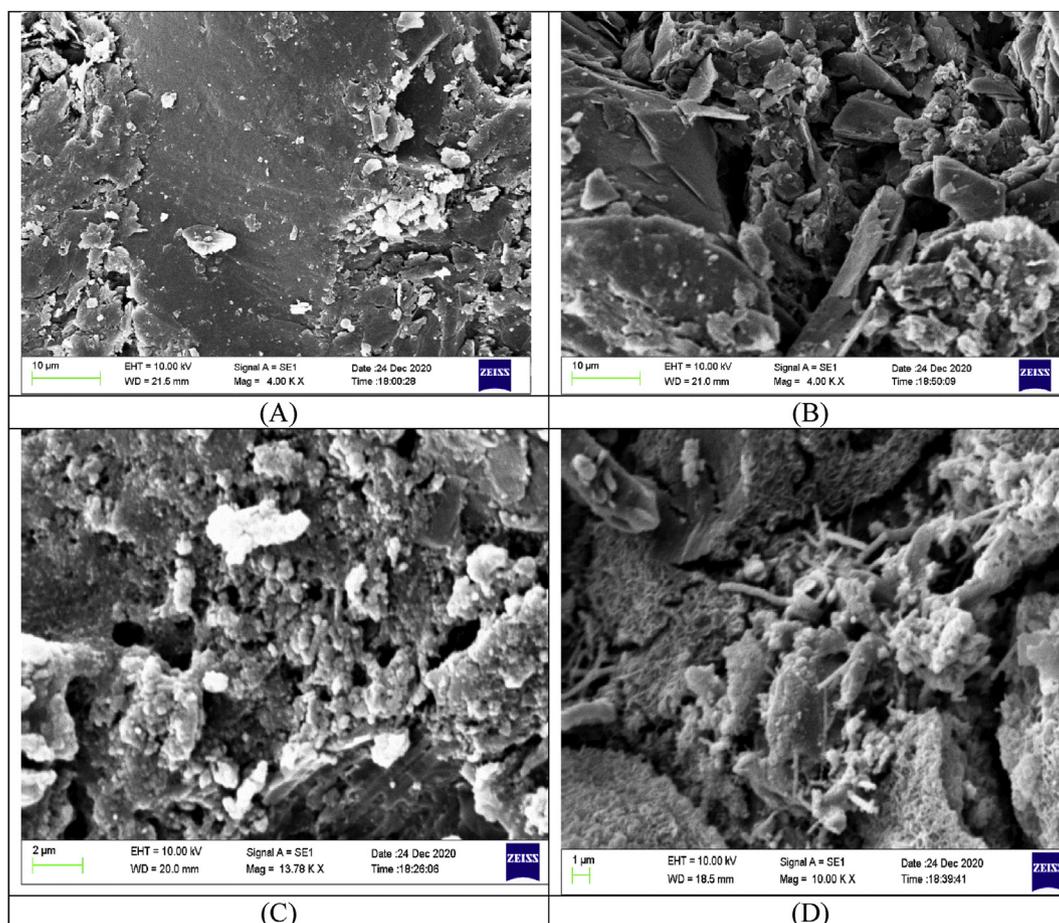


Fig. 4 – Scanning Electron Microscopy images: A) Unused electrode; B) NH₃ treated electrode; C) R-Control electrode; D) R–NH₃ electrode.

The comparative results show a high correlation of 0.9984 between the spectra of R–NH₃ and R–Cont, whereas it was 0.995 between the untreated and ammonia-treated electrodes. A broad peak at 3200–3600 cm⁻¹ indicates the presence of amino groups on the graphite electrode surface (unused) treated with NH₃. Interestingly this peak disappears in both R–NH₃ and R–Cont. reactor's anodes as a result of the microbial growth on the electrodes. The R–NH₃ showing strong bands in comparison to the R–Cont mainly due to phosphate stretching vibration in polysaccharides and nucleic acids in the range of 1200–1290 cm⁻¹ and 900–1147 cm⁻¹ spectral region corresponding to C–O–C and C–O–P due to healthy biofilm formation on the anode. These stretching vibrations imply oligo- and polysaccharides present in the bacteria [62]. The Naumann's IR region's [63] strong in the case of R–NH₃ whereas a little weak for R–Cont, are the absorbance peaks in the range of 2800–3000 cm⁻¹ related to the fatty acids region, further supporting more healthy biofilm on the electrodes in R–NH₃. The dominance of the peaks observed at 1500–1700 cm⁻¹ is associated with amide I and II of proteins and peptides in the R–NH₃ easily promoting the interface between the electrodes and the microbial cells. Similarly bending vibrations at 1200–500 cm⁻¹ attributed to a mixed region of fatty acids, proteins, and phosphate-carrying compounds [63]. The strong peaks at

877–1141 cm⁻¹ on the anode recovered from the R–NH₃ indicate absorption bands of the carbohydrates in microbial cell walls also inferring the healthier biofilm formation in comparison to the R–Cont. The ratio obtained for 2960 cm⁻¹ (CH₃) and 2923 cm⁻¹ (CH₂) asymmetrical stretching vibrations for the R–NH₃ and R–Cont of 1.316 and 1.755 respectively showing the difference in the bacterial species [63] adapted in the anodes, whereas it was 1.0 for the NH₃ treated electrode (Unused).

The XRD patterns of all the graphite electrodes showed the characteristic peaks of carbon at 26.5° [64]. The unmodified pattern XRD pattern (Fig. 5B) confirming the insignificant structural modification in the anodes recovered even after activation in the furnace. The only change in the intensity values was observed in the R–NH₃ and R–Cont.

Kinetics modelling of hydrogen production

The modified Richards, Gompertz, and Logistic models are widely used for the analysis of kinetics modelling of hydrogen production [52,65]. The experimental data of cumulative hydrogen production was used to confirm the performance of BES as a function of time. The experimental and predicted values of models are presented in Table 2. The experimental and predicted values of hydrogen production decided by

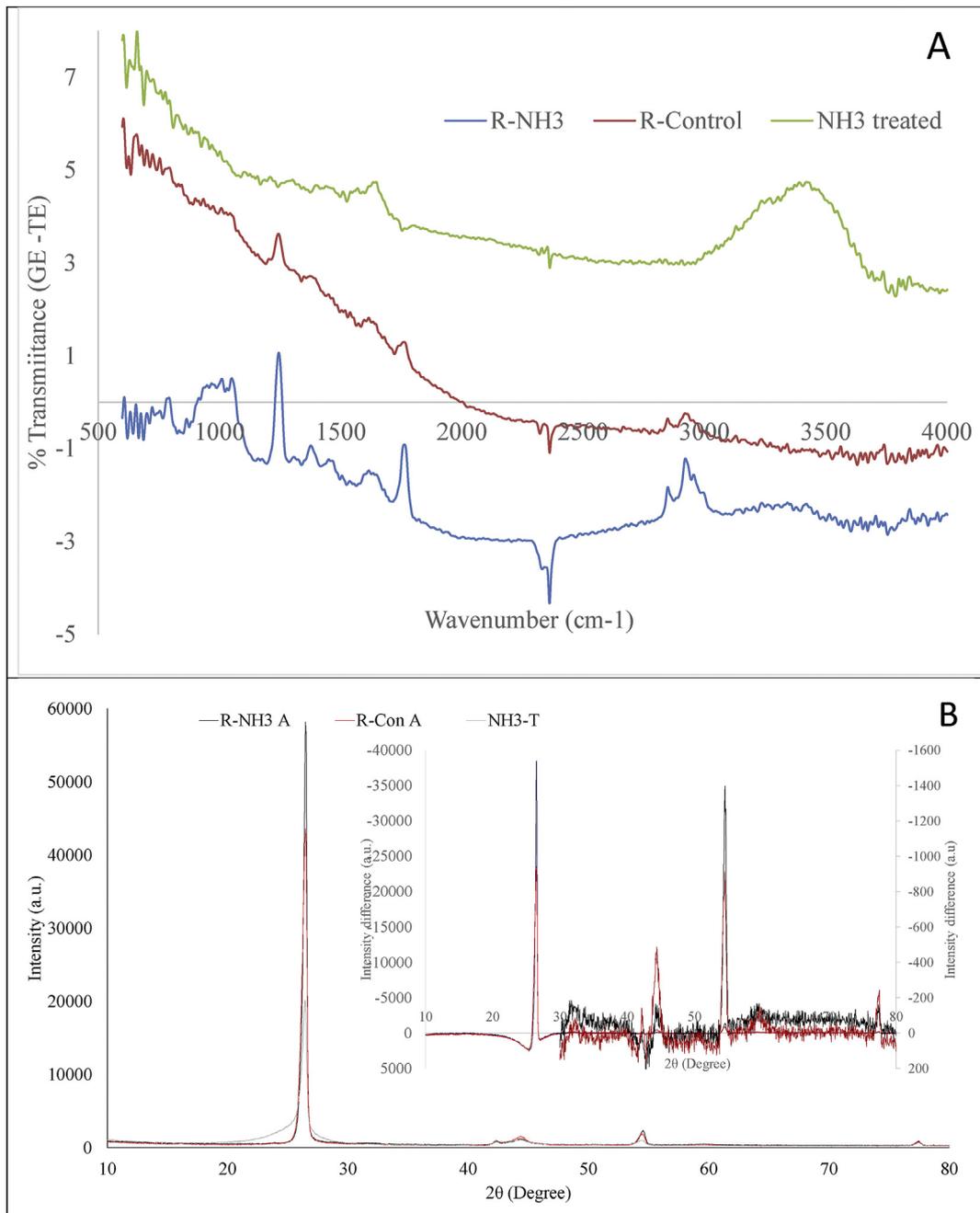


Fig. 5 – A) FTIR spectra obtained from the differences in the transmittance of the respective samples by subtracting from the spectra obtained of the graphite electrode; B) XRD spectra obtained for various electrodes (The small window image showing the intensity obtained at the respective values by subtracting the intensity of the respective electrodes from the untreated graphite electrode).

Table 2 – Kinetic modelling values and constants of cumulative hydrogen production.

Expt	Models	Exp. value (ml)	Predicted value (ml)	Constants				Statistics	
				a	xc	b/d	k	R ²	Adj. R ²
R-Cont	Richards	4321.14	4141.91	4880.719	11.153	0.847	0.185	0.998	0.998
	logistic	4321.14	4032.41	4207.421		112.204	0.374	0.992	0.991
	Gompertz	4321.14	4141.60	4779.232	11.508		0.205	0.998	0.997
R-NH ₃	Richards	5870.01	5796.68	8242.122	12.729	0.784	0.131	0.999	0.999
	logistic	5870.01	5649.35	6171.357		92.836	0.329	0.995	0.994
	Gompertz	5870.01	5780.35	7592.940	13.044		0.163	0.999	0.999

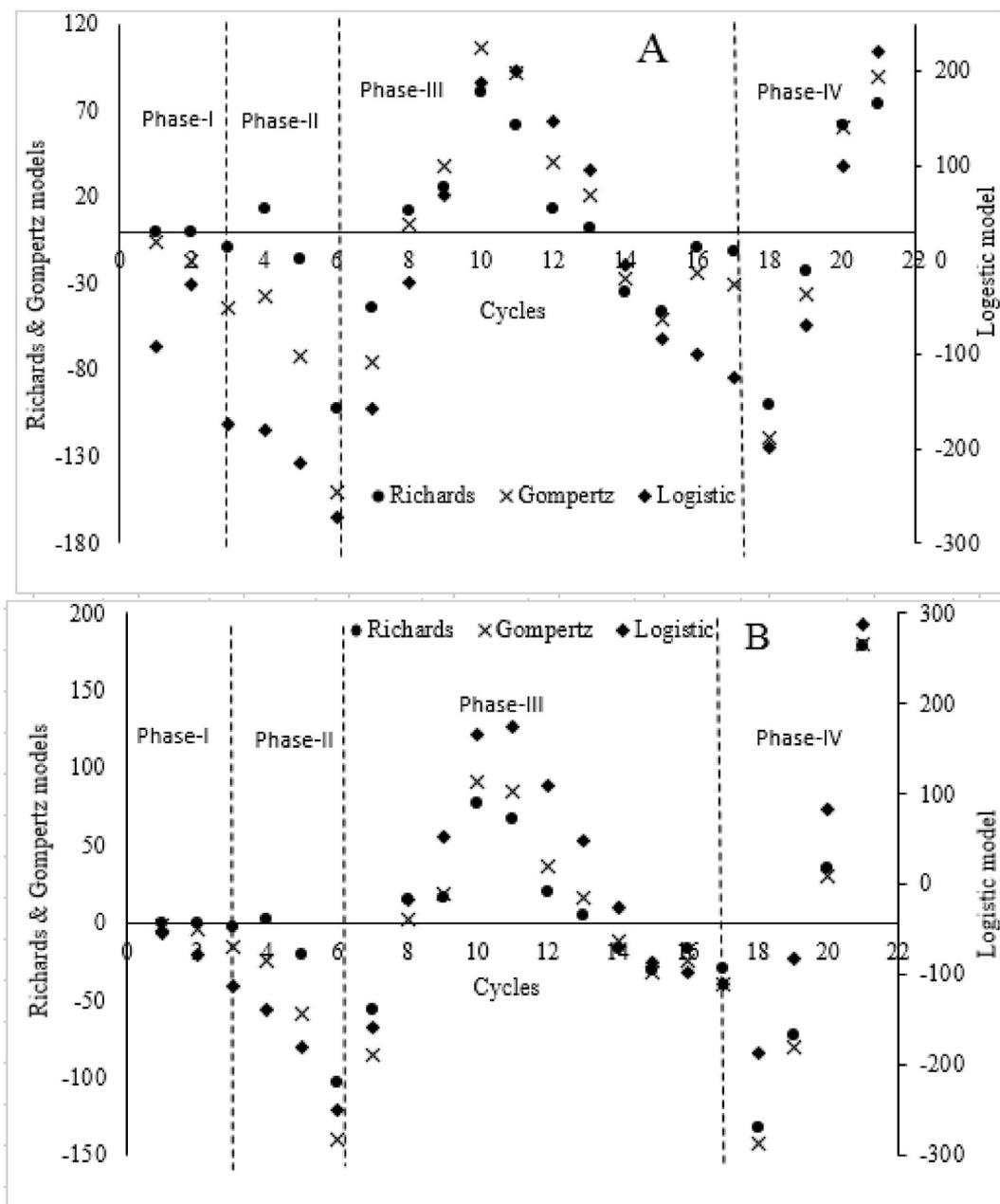


Fig. 6 – Distribution of the residuals of the experimental and models predicted values; A) R-NH₃ and B) R-Cont.

models with time in different phases are very close to each other. Therefore, it would be very difficult to interpret the values by plotting the predicted and experimental values over time. So, in the present study, the data was very clearly differentiated based on the residuals of the experimental and predicted values for all the models (Fig. 6A and B). The residual values are very scattered around the central lines during various phases. The highest predicted residuals values in the H₂ production were seen for the Logistic model in the mid of Phase-III (174.086 ml) and at the end of Phase-IV (288.72 ml) in the R-Cont. While the residual values of hydrogen production were lesser in the mid of Phase-III (200.44 ml) and at the end of Phase-IV (220.66 ml) in the R-NH₃. Similarly, overpredicted

values were also high for the Logistic model inferred from the lowest values of R² of 0.991 and 0.994 for the R-Cont. and R-NH₃, respectively. Among the tested models, the Richard model was found best fitted in the present study for R-Cont. (R² = 0.998) and R-NH₃ (R² = 0.999). When data was stimulated with the Gompertz model, the higher value of *a* and *xc* constants was obtained with higher R² (Table 2) showing higher hydrogen production during experimental conditions. Zhen et al. reported that pre-treatment methods, operating conditions, substrate property, and inoculum types influence the accuracy of the model [66]. In terms of bio-hydrogen production potential (*a*), the reactor with ammonia pre-treatment electrodes had a higher production potential predicted by all the models.

Conclusion

This study shows sulfate-reducing bacteria-based BES can be used as an efficient system for hydrogen production. The BES coupled with ammonia pre-treated electrode is a simple and efficient approach for higher hydrogen production using hydrogenase-rich sulfate-reducing bacteria by inhibiting the growth of methanogens. The VFAs profiling shows acetic acid and butyric acid were in dominant concentrations. Among tested models, the Richard model was found best fitted. The residuals values in the hydrogen production predicted were highest and lowest for the R-Cont and R-NH₃ respectively in mid of Phase-III and end of Phase-IV for the Logistic model. Similarly, overpredicted values were also high for the Logistic model as inferred from the lowest values of R² of 0.991 and 0.994 for the R-Cont. and R-NH₃, respectively. The finding proved the efficient suppression of the methanogen population cost-effectively in the BES system. This technology can be implemented sustainably for the recovery of energy cost-effectively from organic waste having limited sulfate using the single-chambered BES system. Further research on the mechanism of the acetate/butyric acid metabolism in the sulfate-reducing bacteria in the BES is necessary.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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A Statistical Approach to Study the Evolution of Groundwater of Vishwamitri River Basin (VRB), Gujarat

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ABSTRACT

Groundwater is the most vulnerable, exploited natural resource which is a key source of freshwater in the world. Its regular decline in both quality and quantity are an important concern of research. The focus of the present study is to understand the important hydrogeochemical processes involved in the groundwater evolution of Vishwamitri River Basin (VRB), Gujarat, India, using Principal Component Analysis (PCA) technique. To achieve a symmetrical data distribution, log-transformation was applied in this study. This statistical approach decreases the data outlier. A Shapiro-Wilk statistic was employed to test the normal distribution of the data set. The results revealed that ten elements (TDS, bicarbonate, chloride, sulfate, sodium, calcium, magnesium, fluoride, iron and strontium) showed normal distribution. The PCA of 60 groundwater samples using 10 normally distributed chemical parameters indicates that the dominant geochemical process is rock-water interaction through dissolution and cation exchange reactions within the VRB aquifers. This study also reveals that, due to over use of chemical and fertilizers, the sulfate loading is high in groundwater. Therefore, the combined process of natural rock-water interaction, weathering and anthropogenic activities play a significant role in controlling the chemical composition of groundwater of VRB.

INTRODUCTION

Overgrowing population, industrialization and environmental pollution are the most critical issues. Groundwater is extremely vulnerable and overexploited natural resources in the earth. It is the only primary source of drinking water (Clark et al., 1997; Leduc et al., 2017). Therefore, it is very challenging to meet increased requirements and afford adequate quantity and wholesome quality groundwater to the population (Stevanovic, 2010). Generally, inorganic pollutants like heavy metals, organic pollutants like detergents, fertilizers, pesticides, pharmaceuticals, microorganisms, wastewater, domestic or industrial effluents are the major sources of contaminants in groundwater (Foppen, 2002; Trojan et al., 2003; Hrudey and Hrudey, 2007; Hao et al., 2012; Verma et al., 2018). Hydrochemical composition of groundwater within the aquifer system is usually controlled by several factors like lithology, solubilization of rock materials, weathering process, dissolution and ion exchange (Meng et al., 2016; Ma et al., 2017; Funes et al., 2018).

Hydrogeochemical study and groundwater characterization are an indispensable element of management of this resource in order to scrutinize the groundwater quality in an aquifer system (Ballukraya and Ravi, 1999). Multivariate statistical approaches like Principal Component Analysis (PCA), has been carried out to facilitate understanding of complex groundwater system easier (Kshetrimayum and Hegeu, 2016; Khanoranga and Khalid, 2018; Nnorom et al., 2019).

India has scarcities of groundwater due to the over-growing

population and industrialization (Central Water Commission, 2014). Among 29 states of India, Gujarat and Rajasthan are harshly affected by the shortage of groundwater. More than 77% of its requirement for irrigation is fulfilled by exploiting groundwater. While considering the groundwater quality, Gujarat is one of the most affected states of the country. The main aim of the present study is to understand the hydrogeochemical evolution of groundwater using principal component analysis.

MATERIALS AND METHODS

Study Area

In the present study, Vishwamitri River Basin (VRB), Gujarat is selected (Fig.1). The VRB includes two districts of Gujarat state namely, Vadodara and a small part of the Panchmahal which lies between 22°00' and 22°30' N latitudes and 73°10' and 73°38' E longitudes. The major part of the study area is in Vadodara city, which is located on the banks of the Vishwamitri river. This river originates in the Pavagadh hills of Panchmahal district. It is a seasonal river and flows from east to west in the middle of the Mahi and Narmada rivers.

This area is most significant both in terms of ecology and industrial activity. The major industries of this area include agrochemical, fertilizer, food, cement, textile, pulp and paper, chemical and rubber-based, mineral and metal-based industries, etc. The Vishwamitri river has a special importance with regard to ecology as it provides habitat for a unique species of crocodile called mugger (*Crocodylus palustris*). This species has been enumerated by IUCN as one of the threatened species under the "Red" list. Even with enormous pollution and massive human interferences, the survival of the crocodile species in a vast number specifies the special ecological conditions of this river. As per the census 2011, the approximate population of this region is 4.2 million and major part of this population is usually dependent on groundwater.

Groundwater Sampling and Analysis

Sixty groundwater samples were collected from different locations of the study area. Teflon bottles were used to collect these samples. All these bottles were washed with low sulfate liquid detergents, then purified several times with double distilled water and dipped into 10% concentrated nitric acid. Then set aside overnight and again washed with double distilled water. Two separate groups of bottles were taken to collect the samples of groundwater. One group of these bottles was reserved for the analysis of anions such as nitrate, sulphate, chloride, bicarbonate, phosphate and fluoride and other physicochemical parameters like pH, EC, TDS, total hardness, total alkalinity. The second group of sample bottles were slightly acidified with 3 drops of concentrated nitric acid, and were reserved for the analysis of various trace heavy metals like iron, zinc, manganese, molybdenum, lithium, strontium, arsenic, selenium, thallium and vanadium and cations such

देश-प्रेम, जन-हित और लोक-शिक्षा के कथाकार प्रेमचंद

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प्रेमचंद शिक्षक थे तथा अनेक रूपों में शिक्षा विभाग से जुड़े रहे। उनका लेखन मनोरंजन एवं मनबहलाव का साधन मात्र नहीं था। दरअसल वे अपनी कहानियों के माध्यम से जन शिक्षा का महत्वपूर्ण कार्य कर रहे थे। यह शिक्षा केवल स्कूली कक्षाओं और पाठ्यक्रमों तक सीमित नहीं थी, अपितु उनके लिए भी थी, जो कक्षाओं और पाठ्यक्रमों से बाहर रह गए थे। जीवन ने उन्हें औपचारिक पाठ्यक्रमों की परिधि में आने का अवसर ही नहीं दिया था। ध्यान रहे प्रेमचंद भारत के नवसाक्षरों के लिए लिख रहे थे। उस पाठक वर्ग के लिए लिख रहे थे जो पहली पीढ़ी का साक्षर था। उसकी सीमित समझ को सरलता से विकसित करने, समाज और देश के प्रति जिम्मेदारी के भाव को जागृत करने, उनके नैतिक उन्नयन के लिए जैसे कथानक, चरित्र, भाषा और शिल्प की जरूरत थी, प्रेमचंद ने उसी को अपनाया। प्रेमचंद न अपने समकालीन विद्वान आलोचकों की आंखों का सूरमा बनने के लिए लिख रहे थे न आज के कथित विमर्शवादियों के लिए, जो प्रेमचंद के लेखन के खिलाफ एक सुनियोजित निंदा अभियान में लगे हुए हैं।

प्रेमचंद ने कुल 280 कहानियाँ लिखी हैं, जिनमें से 194 मूलतः उर्दू में लिखी गई हैं। हिन्दी और उर्दू में प्रेमचंद के कथा लेखन की अवधि लगभग तीस वर्ष उहरती है। औसतन उन्होंने एक कहानी प्रतिमाह लिखी है। इधर के कुछ शोधकों ने खोज निकाला है कि उन्होंने इतनी अधिक मात्रा में कहानियाँ पारिश्रमिक की लालच में लिखीं। जिसने सरकारी नौकरी को टोकर मारी हो, वह थोड़े से पारिश्रमिक की लालच में कहानियों का अंबार लगाता जाएगा, ऐसा मानना कुचेष्टा ही कही जाएगी। गोरतलब है कि जब वे गोरखपुर में कार्यरत थे, तभी वहाँ 8 फरवरी, 1921 को गांधीजी का पदार्पण हुआ था। गांधी जी के विचारों से प्रभावित होकर नौकरी से त्यागपत्र देने का निर्णय करने में उन्होंने बस एक सप्ताह का वक्त लगाया। 16 फरवरी 1921 को उन्होंने अपनी बीस साल पुरानी नौकरी से त्याग पत्र दे दिया था।

प्रेमचंद और उनका युग' पुस्तक में आलोचक रामविलास शर्मा, कहानीकार प्रेमचंद और उपन्यासकार प्रेमचंद की गैरजरूरी तुलना करते हुए लिखते हैं कि "यदि कहानीकार प्रेमचंद और उपन्यासकार प्रेमचंद में एक को ही हिन्दी साहित्य में जगह देने की बात हो तो शायद उपन्यासकार प्रेमचंद को ही उस जगह के लिए चुना जाएगा। "उनके अच्छे से अच्छे और घटिया से घटिया उपन्यासों के बीच इतना बड़ा फासला न मिलेगा जितना उनकी बढ़िया और घटिया कहानियों के बीच मिलता है।" इस तरह वे प्रेमचंद की लगभग पचास कहानियों को 'बढ़िया' की श्रेणी में रखते हैं। किसी भी रचनाकार की सभी रचनाएँ कथित श्रेष्ठता के मापदंड पर खरी नहीं उतर सकतीं। पर एक शीर्षस्थ कहानीकार की कतिपय कमजोर कहानियों को घटिया जैसे खाते में नहीं डाला जा सकता। वह भी तब जब वह हिन्दी कथा रचना की नयी राह निकाल रहा हो।

प्रेमचंद के हिन्दी-उर्दू कथा क्षेत्र में आगमन के पूर्व अरब की रातें, सिंदबाद का जहाजी, बेताल पचीसी, सिंहासन बत्तीसी, गुल बकावली, तोता-मैना, तिलिस्म-ए-होशरूबा आदि की धूम थी। ऐसे ही माहौल को लक्ष्य कर के आधुनिक हिन्दी साहित्य के उन्नायक भारतेंदु हरिश्चंद्र ने 1884 में आह्वान किया था कि "उन्हीं किताबों को पढ़िए जो आपके नैतिक उत्थान में योग देती हैं। विदेशी वस्तुओं और विदेशी भाषा पर भरोसा न कीजिए, अपनी भाषा में प्रगति कीजिए... अपने बच्चों के हाथ में मीर हसन की 'मसनवी' और 'इंदर सभा' पकड़ा कर उन्हें बर्बाद न कीजिए।... उसे अपनाइये जो देश और राष्ट्र के हित में है।" बंग

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भंग के खिलाफ आंदोलन से लेकर प्रगतिशील लेखक संघ के पहले अधिवेशन तक के तीन दशकों के भारतीय जीवन की धड़कन, उसका संघर्ष, उसकी पराजय, उसके दुख-दर्द अपनी समस्त गहराई और व्यापकता के साथ अगर किसी एक भारतीय लेखक में मिल सकता है, तो वह लेखक कोई और नहीं प्रेमचंद ही हैं।

उर्दू में प्रकाशित अपने पहले कथा संग्रह 'सोजे वतन' की भूमिका में उन्होंने लिखा "हरेक कौम का इल्म और अदब अपने जमाने की सच्ची तस्वीर होता है। जो खयालात कौम के दिमागों को मतहरिक (सक्रिय) करते हैं और जो जज्बात कौम के दिलों में गूंजते हैं वो नज्म-ओ-नस्त (गद्य-पद्य) के सफों में ऐसी सफाई से नजर आते हैं जैसे आइने में सूरत। "कौम के नये और पुराने खयालात में जिंदगी और मौत की लड़ाई शुरू हुई और इस्लाहेतमदुन (सांस्कृतिक सुधार) की तजवीजें सोची जाने लगी। इस जमाने के कसम-व-हिकायत (किस्से तथा कहानी) ज्यादातर इसलाह(सुधार) और तज्दीद (नवीनता) ही का पहलू लिये हुए हैं। अब हिंदुस्तान के कौमी खयाल ने बलोगीयत (बालिगपन, बुद्धिमत्ता) के जीने पर एक कदम और बढ़ाया है और हुब्बे-वतन के जज्बात लोगों के दिलों में उभरने लगे हैं। क्यूंकर मुमकिन था कि इसका असर अदब पर न पड़ता? "ये चंद कहानियाँ इसी असर का आगाज हैं और यकीन है कि ज्यों-ज्यों हमारे खयाल वसीह (विस्तृत) होते जाएंगे इसी रंग के लिटरेचर को रोज-अफजों(प्रतिदिन बढ़ना) फरोग (उन्नत) होता जाएगा। हमारे मुल्क को ऐसी किताबों की अशद (सख्त) जरूरत है जो नयी नस्ल के जिगर पर हुब्बे वतन (देश प्रेम) की अजमत (महिमा) का नक्शा जमाये।" हिन्दी साहित्य की प्रतिष्ठित पत्रिका सरस्वती में अपने कहानी संग्रह की समीक्षा के प्रकाशन हेतु तत्कालीन यशस्वी संपादक आचार्य महावीर प्रसाद द्विवेदी को लिखे पत्र में उन्होंने दावा किया कि "यह किताब नफाए-आम (जनहित) में लिखी गई है। इस लिहाज से कीमत भी कम रखी गई है। जाती (निजी) नफा मकशूद(उद्देश्य) नहीं। बर बख्ते रिव्यू किताब मिलने का पता जो नीचे दर्ज है जरूर नोट फर्मा दीजिएगा, नवाजिश होगी।"

जनहित के उद्देश्य और नयी नस्ल के हृदय में देश प्रेम का नक्शा जमाने के लिए उन्हें राजद्रोह के आरोप झेलने पड़े। अपने पहले शाहकार को, अपनी ही आँखों के सामने जब्ती और जलाए जाने का दृश्य देखना आसान नहीं रहा होगा। एक नवोदित कथाकार के लिए यह कितनी बड़ी कुर्बानी थी, अंदाज लगाना मुश्किल नहीं। प्रेमचंद के लिए देश प्रेम मात्र भौगोलिक विस्तार तक सीमित नहीं था। उनका देश प्रेम जनता को सभी स्तर भेदों से परे धकेल कर मनुष्यता की सामान्य आधार भूमि पर प्रतिष्ठित करना था। आलोचक मधुरेश ने अपनी पुस्तक 'हिन्दी कहानी : अस्मिता की तलाश' में प्रेमचंद पर राजद्रोह का आरोप लगाने वाली ब्रिटिश हुकूमत की नीयत का विश्लेषण करते हुए लिखा है "भारतीय कथा साहित्य में प्रेमचंद कदाचित पहले लेखक हैं, जिन्होंने स्वाधीनता आन्दोलन के सन्दर्भ में इस जातीय सद्भाव (जो सोजे वतन की पांच कहानियों में अभिव्यक्त हुआ है) के महत्व को समझा और भारी जोखिम उठा कर उसके विकास का रास्ता तैयार किया।" जातीय सद्भाव और सांप्रदायिक सौहार्द का जैसा निरूपण प्रेमचंद की कहानियों में हुआ है, वह उनके समकालीन कहानीकारों में विरल है। आज के समय में प्रेमचंद के देश प्रेम को समझने की जरूरत और बढ़ गई है। देश प्रेम को संकुचित नवराष्ट्रवाद के कदमों तले रौंदे जाने से बचाना होगा। कथित राष्ट्रवाद को देश प्रेम का पर्याय मान लिये जाने के खतरे सामने आने लगे हैं।

प्रेमचंद भारतीय स्वाधीनता आंदोलन में जन को भागीदारी के लिए प्रेरित, प्रभावित और प्रतिबिंबित करने वाले कथाकार थे। वे देश की सामाजिक-सांस्कृतिक आजादी के स्वप्नद्रष्टा थे। वे भारतीय नवजागरण की सुदीर्घ परियोजना के महत्वपूर्ण कार्यकर्ता थे। वे सामाजिक सुधार के लिए व्यक्ति के अंतर और बाह्य को परिष्कृत करने की जरूरत पर बल देते थे। भारतीय स्वाधीनता आंदोलन की जितनी आलोचना उनके कथा साहित्य में मिलती है, वह उनके समकालीनों में दुर्लभ है। प्रेमचंद के लिए देश की स्वाधीनता का अर्थ राजनीतिक सत्ता का हस्तांतरण भर नहीं था। उनके लिए आजादी का मतलब सामाजिक संरचना के सबसे निचले पायदान पर रह रहे दलित-पीड़ित किसान, मजदूर, ग्रामीण स्त्री-पुरुष में सहज मानवीय जीवन की आकांक्षाओं का बिरवा रोपना था। उनकी कलम का उद्देश्य हाशिए पर जी रहे लोगों में आत्मबल भरना और

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राजस्थान के आदिवासी समुदायों में

गरासिया समुदाय में अधिक जनसँख्या सिर्फ मीणा और भील समुदाय की है। जनजातीय जनसँख्या की दृष्टि से राजस्थान में तीसरी बड़ी जनजाति गरासिया है। सन् 2011 की जनगणना में इनकी जनसँख्या 3,14,194 व्यक्ति परिगणित की गई। गरासिया जनजाति राजस्थान के सिरोही (152575), उदयपुर (103847) और पाली (49465) जिलों में मुख्य रूप से निवास करती है। अन्य जिलों में इसकी जनसँख्या बहुत कम है और वहाँ के ये संभवतः अस्थायी निवासी ही हैं। राजस्थान के सीमावर्ती गुजरात के बनासकांठा और साबरकांठा क्षेत्र में भी यह समुदाय निवास करता है, लेकिन गुजरात में यह अनुसूचित जनजाति की श्रेणी में नहीं है। राजस्थान में गरासिया जनजाति का लिंगानुपात 978 तथा साक्षरता दर 33.1 प्रतिशत है, जो राजस्थान में सबसे कम साक्षरता दर वाला आदिवासी समुदाय है। साक्षरता दर से उनमें शिक्षा की स्थिति को देखा जा सकता है। इनकी भाषा मेवाड़ी और गुजराती से प्रभावित भीली भाषा है। आर्थिक और शैक्षणिक दृष्टि से पीछे रहने के बावजूद गरासियों का सामाजिक संगठन

सुदृढ़ है। आज के दौर में भी गरासियों में सामाजिक-आर्थिक और सांस्कृतिक सहकार जबरदस्त पाया जाता है।

'गमना' गरासिया आदिवासी समुदाय पर केन्द्रित संज्ञान में आने वाला संभवतः पहला और एकमात्र उपन्यास है। 'गमना' उपन्यास का परिवेश प्रकाशन वर्ष के आसपास का ही है और क्षेत्र पाली तथा उदयपुर जिले का प्रतीत होता है। राजस्थान के पाली-उदयपुर जिलों के कुछ स्थलों के नामोल्लेख भी उपन्यास में हैं। उपन्यास के माध्यम से गरासिया समुदाय के सन्दर्भों को देखा-समझा जा सकता है। इस उपन्यास में सभी गरासियों के जिक्र नहीं हैं। कहा जा सकता है कि उपन्यास में पूरे गरासिया गाँव के विविध सन्दर्भ नहीं हैं। यह इस उपन्यास की सीमा प्रतीत होती है। यहाँ गून्दा गरासिया का परिवार प्रमुखता से चित्रित हुआ है। गून्दा का बेटा गमना, उसका भाई जीवा, गमना की संभवित पत्नी सतवंती प्रमुख आदिवासी पात्र के रूप में उभर कर सामने आते हैं। गमना के माध्यम से गरासियों की स्थिति को समझा जा सकता है। गमना बिना वजह कई बार चौधरी करणसिंह और रेवतसिंह के हाथों पीटता है। "बस करो बापसी। गलती हुई। माफ़ी दो।" बूढ़ा गून्दा चीथड़े-सी मैली धोती में बेटे के बचाव में रेवतसिंह के पैरों पड़ गया।

'कुछ पता भी है इसने क्या गलती की हैं?' हाथ रोककर हांफते हुए रेवतसिंह ने गुस्सेल आँखों से वृद्ध की ओर देखा।

'हमें तो इतना पता है कि हमारा इस धरती पर होना ही गलती है बापजी।'¹ इससे गरासियों की स्थिति को समझा जा सकता है। उसका यह कहना कि उनका इस धरती पर होना ही गुनाह है। गुनाह भी कुछ खास नहीं है। गरासियों के सन्दर्भ में रेवतसिंह चौधरी के विचार देखने योग्य हैं- "ये तो

मासिक

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Review

Anticancer nano-delivery systems based on bovine serum albumin nanoparticles: A critical review

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ABSTRACT

Among the health-promotional protein-based vehicles, bovine serum albumin nanoparticles (BSA NPs) are particularly interesting. Meeting requirements e. g., non-toxicity, non-immunogenicity, biodegradability, biocompatibility, and high drug-binding capacity, has introduced BSA NPs as a promising candidate for efficient anti-cancer drug delivery and its application is now a rapidly-growing strategy to promote cancer therapy. Nevertheless, the leverage of such carriers requires an in-depth understanding of structural/physicochemical features of the BSA molecule and its derived nanovehicles, together with the utilized nano-formulation approaches, effective variables in delivery mechanism, specific shortfalls, and recent nanoencapsulation progresses. The current review highlights the novel advances in the application of BSA NPs to engineer drug vehicles for delivering anti-cancer agents. The factors influencing the efficiency of the therapeutics in such nano-delivery systems, alongside their advantaged and limitations are also discussed.

1. Introduction

Despite the tremendous progress in the development of novel therapeutics over the past few decades, cancer is still among the most common causes of death worldwide due to enhanced prevalence, morbidity, as well as mortality induced by complications [1,2]. Cancer is created via the uncontrolled growth and spread of unusual cells, essentially affected by external agents (*i.e.*, unhealthy diet, infectious organism, *etc.*) and internal factors (*i.e.*, hormones, immune conditions, and inherited genetic mutations) [3,4].

Conventional strategies in cancer therapy, like chemotherapy and radiotherapy, can destroy cancer cells, yet cause high damages to healthy tissues. Besides, the overall therapeutic efficiency of an anti-cancer agent is not just relevant to its *in vitro* capability. Upon physiological circumstances, bioactives/drugs confront biological obstacles, *e. g.*, aggregation, degradation, undesired immunogenicity, poor solubility, non-specific tissue distribution, low tissue penetration, inefficient cellular internalization, as well as off-target toxicities [5–7]. A drug's effectiveness can also be seriously jeopardized by environmental variations (*e.g.*, pH, pressure, temperature, humidity, *etc.*), which can occur during storage, administration, and even systemic circulation [8,9].

Strategies for tackling such limitations typically relates to the

application of controlled drug delivery systems of high potency, especially nature-inspired nanoparticles (NPs)-based vehicles, which can preserve anticancers from prompt release and attain a preferential accumulation of the bioactive/drug within solid tumors [10,11]. Taking benefits of these properties, several natural-based nanoparticulate structures have been utilized as carrier systems of various anti-cancer agents to promote their efficacy and tolerability while decreasing their off-target toxicity [11,12]. These systems may preserve the drug from environmental stresses, modify pharmacokinetic and drug tissue distribution profile, increase drug biological stability, promote intracellular penetration/distribution, and improve drug absorption *via* enabling diffusion through epithelium [13].

In this framework, proteins, as a valuable gift by nature, can be applied in the form of drug vehicles for cancer treatment, in part, due to their exceptional characteristics such as bioactivity, biocompatibility/biodegradability, exquisite tunability, metabolizability, non-immunogenicity, high drug-binding capacity, scalability, as well as acceptable (bio)stability *in vitro/in vivo* [14,15]. Protein NPs with unique functional attributes (including emulsibility, foaming, gelling capacity, and water-binding property) have the possibility of surface modification, facilitating the binding of protein molecules onto many therapeutic agents [16,17].

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comprises two tryptophan residues (namely Trp-212 and Trp-134), as fluorophores able to quench fluorescence light [22]. Moreover, the presence of some functional groups like amino and carboxylic units on the surface of BSA molecule enables the covalent attachment of cell-targeting agents (e.g., transferrin, apolipoproteins, surfactants, folate, monoclonal antibodies, etc.) and thus promotes the selectivity of these structures [32].

2.2. Beneficial applications

BSA is a small, stable, and moderately non-reactive protein molecule, which possess a myriad of biochemical applications in ELISAs (Enzyme-Linked Immunosorbent Assay), immunoblots, as well as immunohistochemistry. By virtue of its unique physiological functions, BSA is utilized extensively as a representative short peptide in drug delivery studies. A vast variety of endogenous/exogenous components (i.e., bioactives, drugs, hormones, and xenobiotics), once entering the bloodstream, are delivered/disposed through their complexation with BSA and other serum albumin proteins. Besides its contribution to the colloid blood osmotic pressure and the preservation of blood pH, this protein is capable of binding to various compounds in a reversible manner, facilitating the transport of the BSA NPs within the body and release of the drug/bioactive within the therapeutic site of interest. Serum albumin proteins' family can enhance the apparent solubility of liposoluble therapeutics within plasma and affect the circulation, metabolism, and efficacy of bioactives/drugs [33].

Upon immunohistochemistry (the process of using antibodies to recognize antigens in cells), tissue section is typically incubated with BSA blockers to bind non-specific binding sites, increasing the specificity and sensitivity of the antibody-antigen binding. BSA is an interesting bioactive protein in cell and microbial cultures. It has been widely exploited to stabilize enzymes, determine the quantity of proteins, and synthesize nanocarriers for drug delivery. The BSA nanocarriers are metabolizable to naturally occurring components, as the hydrolysis of the proteins lattice *via* digestive enzymes leads to the generation of bioactive peptides, exerting a myriad of physiological properties *in vivo*.

2.3. Interactions of BSA with anticancer drugs

Investigation of BSA-drug interactions is crucial in delivery studies as it plays a pivotal role in bioavailability, (bio) distribution, release properties, and absorption of therapeutics *in vitro/in vivo* [34]. As stated before, subdomains of IIA and IIIA are two main binding sites on BSA structure, which also known as Sudlow's site I and II, respectively [35]. Sudlow's site I, a large and flexible multi-chamber cavity located within the core of subdomain IIA, binds bulky heterocyclic and negatively charged compounds mainly through hydrophobic interactions [36]. While at Sudlow's site II, the negatively charged bioactives/drugs typically bind to the BSA structure *via* hydrophobic linkages, electrostatic interactions, and H₂ bonding [36].

Essentially, the drug loading within BSA NPs can be executed by two main mechanisms; covalent and non-covalent conjugation (vis. physical entrapment through electrostatic/hydrophobic interaction or H₂ binding) [33]. Compared to the covalent conjugates, the non-covalent ones need less chemical synthetic procedures; however, the reversible BSA-drug interactions may jeopardize both stability and reproducibility of the protein-drug systems [37].

To provide a proper, efficient interaction between the BSA NPs and anti-cancer molecules, the protein vehicles should be engineered in a way that balance the attractive and repulsive forces within the BSA configuration, increase the protein network unfolding, and decrease its intra-molecular hydrophobic interactions [38]. During drug encapsulation, the protein architecture undergoes conformational changes depending on its molecular concentration, cross-linking agent, and conditions like pH, ionic strength or solvent used.

3. Different preparation approaches for BSA NPs

BSA NPs can be easily engineered upon soft circumstances using different nanoparticulation strategies such as [22,39]:

3.1. Controlled desolvation (coacervation)

It is a convenient approach and no specialized equipment or complex operating circumstances are needed to fabricate NPs [40,41]. The desolvation technique is based on the continuous addition of a dissolving agent (e.g., ethanol or acetone) into an aqueous solution of BSA upon stirring [39,42]. The anticancer drug is previously added to the protein solution or antisolvent medium relying on its solubility. During the anti-solvation process, BSA solubility is gradually diminished, and the produced unstable protein aggregates are hardened *via* chemical cross-linking to avert re-dissolution phenomenon [17]. The concentration of desolvating agent, pH, and temperature of the system are key parameters affecting the particle size and zeta potential of the final NPs [43].

3.2. Emulsification

This strategy lies on the drop-wise incorporation of a non-aqueous phase (vis. an organic phase contained of an organic solvent or lipid) to aqueous BSA medium (upon stirring) followed by homogenization (i.e., high or low-energy homogenization approaches) [44]. Through the emulsification process, the water-soluble therapeutics can be incorporated into BSA aqueous solution and the hydrophobic ones are added into the organic phase [45]. Following the application of high pressure homogenization techniques, the shear forces provoke the creation of new disulfide bridges *via* oxidation and crosslinking of sulfhydryl units in the protein structure [46]. In this approach, both thermal and chemical treatments are employed to stabilize BSA NPs. The main parameters affecting the particle size of developed NPs are protein concentration, aqueous phase: organic phase ratio, homogenization rate, process time, and the number of cycles [47,48].

3.3. Nano-spray drying

It is an emerging, simple, scalable, and reproducible nanofabrication method and plays a critical role in producing BSA NPs with adjustable size/shape [49]. Nano-spray drying is generally relying on four main steps, including: (i) atomization of the specimen (comprising BSA, drug, and/or surfactant); (ii) spray air contact (following the entering a drying gas into the system) for the evaporation of solvent used; (iii) drying of spray (following the solvent evaporation, the spray dried NPs are formed); and finally (iv) collection of the product *via* an electrostatic particle collector comprising of grounded star electrode and cylindrical collecting electrode. Variable parameters e.g., inlet temperature, system pressure, gas flow rate, as well as mesh size reckon as effective factors in drug loading using spray dried BSA NPs [50,51].

3.4. Thermal gelation

It includes the heat-induced unfolding followed by protein-protein interactions (e.g., H₂ linkages, electrostatic attraction, hydrophobic bonding, or disulfide-sulfhydryl linkages) [39,44,52].

3.5. Self-assembly

In this technique, the incorporation of hydrophobic anticancer drugs into the aqueous BSA solution enhances the protein hydrophobicity, which causes the cleaving disulfide bonds and eliminating primary amines [39,44,53].

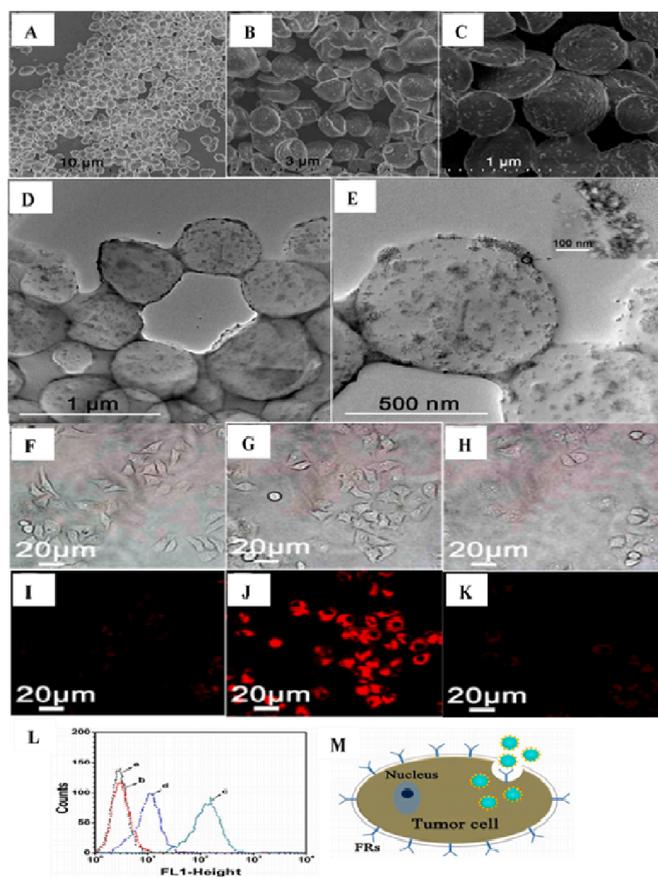


Fig. 4. SEM (A, B, and C) and TEM (D and E) images of hydrophilic drug-loaded multifunctional BSA NPs; CLSM images of tumor cells incubated in the serum-free medium (SFM) with RhB-encapsulated multifunctional BSA NPs: different cells lines (F,I,G,J) and Hela cells following the pretreatment of folic acid (H and K) (images F, G, and H were in the bright field, while images I, J, and K were in the dark field); flow cytometry of Hela cells incubated in diverse nutrient media (pure SFM) (a), pure SFM following the pretreatment of folic acid (b), SFM with RhB-loaded BSA NPs (c), and SFM with RhB-loaded BSA NPs following the pretreatment of folic acid (d); schematic of cellular uptake of hydrophilic drug-loaded BSA NPs [56].

long-circulating half-life, BSA NPs exhibit active targeting. Because of these beneficiary applications, recently, several albumin-based formulations have been approved by FDA such as Abraxane, Levimir and Victoza.

In passive targeting, BSA NPs mediated drugs within a size range of 20 to 200 nm can accumulate at the tumor target site because of the EPR effect [69]. The vasculature in an active tumor is different from the vessels present in normal tissue. Angiogenesis leads to high vascular density in tumors, large gaps exist between endothelial cells in tumor blood vessels, and therefore tumor tissues show selective extravasation and retention of macromolecules and NPs > 10 nm in a tumor. Below this size, drugs can easily diffuse in or out from the blood vessels without accumulating. However, NPs within the size range of 20 to 200 nm can accumulate at the tumor target site without returning to the bloodstream, because of their large size (Fig. 3b). So in conclusion, for the EPR effect, size of the nano system is an important characteristic and therefore it is important for passive targeting too. Tumor microenvironments passively facilitate the EPR effect that brings the drug-loaded BSA NPs into tumor sites from circulation and enhance tumor target ability [70].

Furthermore, the surface properties of BSA NPs play a vital role in the cellular uptake by the cancer cells. A neutrally charged surface does not show a tendency to interact with cell membranes, while charged

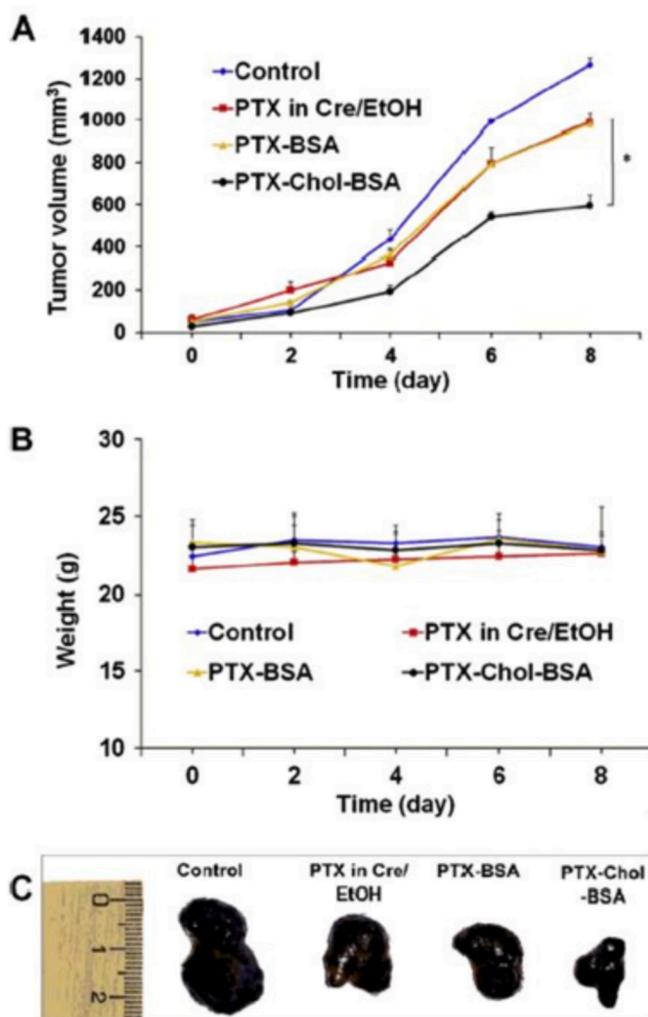


Fig. 5. *In vivo* anti-tumor efficacy of PTX-Chol-BSA NPs [85].

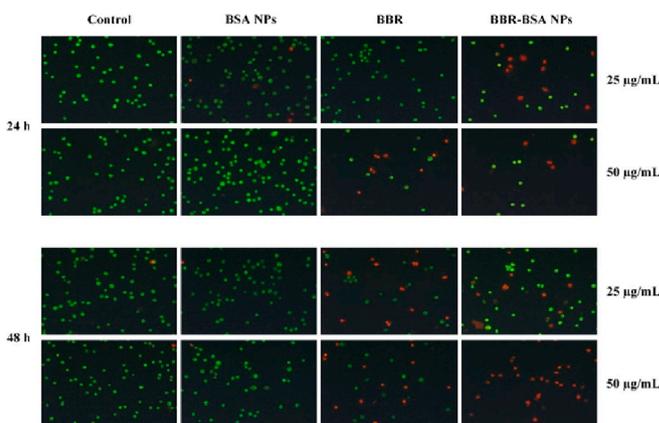


Fig. 6. Apoptosis study by Acridine orange/Ethidium bromide (AO/EtBr) staining. Effect of BBR-BSA NPs on MDA-MB-231 breast cancer cell line for 24 h and 48 followed by fluorescence microscopy [86].

groups present on the surface of BSA NPs are actively involved in NPs - cell interaction. If we talk about charged surface NPs, then reported studies suggest that positively charged surface NPs have greater binding affinity to cell membranes than negatively charged surface NPs because cationic NPs can bind negatively charged functional groups such as sialic

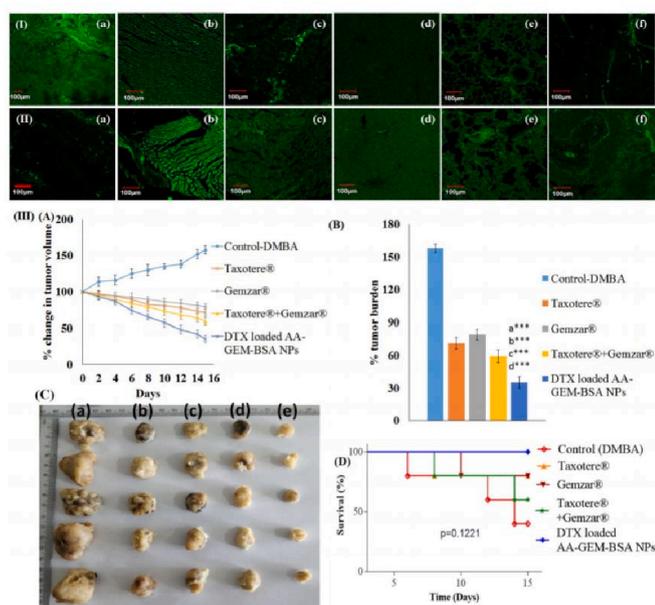


Fig. 7. Fluorescence intensity of (I) C-6 loaded AA-GEM-BSA NPs, (II) Free C-6 in different organs and tumors. (A) Comparison of % change in tumor volume and (B) % tumor burden of different formulations. (C) Representative photographs of excised tumors from different treatment groups and (D) Kaplan–Meier survival curve [88].

acid, present on the cancer cell surface and initiate translocation [71]. Along with this, cationic NPs help masking the negative DNA charge and thereby facilitate entrance into cells and enhanced transfection efficiency. Cationic NPs can form nanosized complexes with DNA and small interfering RNA (siRNA) then can deliver encapsulated drug alone or with DNA simultaneously for enhanced gene therapy (BSA NPs mediated gene therapy discussed in next paragraph) [72]. Moreover, BSA possesses amine groups that are protonable in acidic conditions. Hence, when entering into endosomes, they accept protons, which results in resisting the drop in pH; so they can destabilize the encapsulated DNA or oligonucleotide, negatively affecting gene transfection. Therefore, these unique properties of cationic surface charged NPs provide advantages over anionic or neutral charged surface NPs. A wide variety of polymers such as chitosan, polyethyleneimine (PEI), poly-L-lysine (PLL) and polymethacrylates forming dendrimers can be used for the coating of NPs with a positive charge. Besides the advantages for cellular uptake, unique properties of cationic NPs also cause some unwanted effects such as aggregation, instability, toxicity and rapid clearance of the particles by the Mononuclear Phagocyte System (MPS) [72].

Gene therapy involving plasmid DNA, siRNA, miRNA and antisense oligonucleotides have been showing immense potential in cancer therapy. However, there are limitations associated with nucleic acid delivery including enzymatic degradation by nuclease, rapid clearance, triggering the immune system, biological barriers, accessing deeply seated tumor sites and effects in non-targeted genes [73]. In addition, negative charge, large size and hydrophilicity are challenges for naked nucleic acid to enter cancer cells. Considering properties like biodegradability, biocompatibility, tumor target specificity and non-immunogenicity of BSA NPs, they can be utilized for the delivery of nucleic acid. Various studies suggest that to ensure the targeted delivery of DNA and RNA complexes to the cancers and enhanced therapeutic potential of these complexes, they can be protected from enzymatic degradation, physiological pH and protein binding in circulation when encapsulated into BSA NPs or in the form of nanocomplexes [74–76].

Another major advantage of BSA NPs is their high binding capacity towards water soluble/insoluble drugs because of different binding sites available in the BSA molecule [77]. The solubility enhancement of

poorly soluble drugs is a remarkable application of BSA NPs [78]. Almost 60 to 70% of drugs developing as therapeutics are poorly water soluble which is a main drawback in drug development because it leads to slow absorption and poor bioavailability limiting its desirable clinical applications. Solubility of drugs, enhanced by BSA NPs through forming reversible binding complexes with ligands, allows the bound molecules to move in the blood at higher concentration compared to their initial solubility [79]. Also, as discussed in the section of structural properties of BSA, it has two main sites where ligands can be bound *via* hydrophobic or electrostatic interactions. Hydrophobic molecules, anionic molecules and poorly water soluble drugs have a high affinity to bind with BSA *via* these interactions. Furthermore, BSA has numerous accessible free carboxyl and amine groups where acid or basic drugs can bind and produce soluble salts. Additionally, the buffer capacity of BSA may help in enhancing the solubility of drugs that are ionisable in the pH range of BSA. Khoder et al. [79] investigated the use of BSA as a drug solubility enhancer for a hydrophobic drug (Indomethacin) and demonstrated solubility studies by preparing drug-BSA solid dispersions by spray drying and freeze-drying techniques. The study suggested that solubility of drug was enhanced by formulating in BSA solid dispersions significantly up to 1,00,000 and 71,000 folds through freeze-drying and spray drying techniques, respectively.

To utilize the advantages of NPs for targeted drug delivery, it is essential to understand their physicochemical properties such as size, shape, flexibility, water solubility, surface charges, etc. The unique properties of BSA NPs mentioned above, including long half-life, the ability of cellular receptor-mediated transcytosis, surface properties, enhanced drug solubility, improved bioavailability and low toxicity make them an ideal nanocarrier for the delivery of anticancer therapeutics in biomedical applications.

5. Nanoencapsulation of anti-cancer drugs/bioactives within BSA NPs (*in vitro/in vivo* studies)

A wide range of bioactive molecules and anti-cancer agents (*i.e.*, drugs, curcuminoids, carotenoids, proteins, peptides, etc.) suffer severe shortcomings resulted from their short-time release *in vivo* via liver and kidney [80]. This can be suppressed by leveraging the exceptionally long half-life of BSA NPs-based carriers and their cancer homing attributes (in both preclinical models or clinical trials) along with their intrinsic characteristic to accumulate at tumor sites and inflamed tissues, while preserving anticancer therapeutics until the vehicle is ruptured at the intended locus [81]. In the following parts, we will underscore *in vitro/in vivo* results related to the capability of BSA NPs to promote the pharmacokinetic features of anti-cancer therapeutics/bioactives *i.e.*, (bio) distribution, (bio)stability, efficiency, and tumor targeting.

In a study performed by Zhao et al. [82], mannosylated BSA NPs (formulated *via* 4-isothiocyanatophenyl- α -mannopyranoside) were designed for drug-resistant cancer therapy to target cancer cells and M2 Macrophages. The protein structure was denatured by urea/BH4, and then the subsequent incorporation of liposoluble therapeutics (*i.e.*, disulfiram/copper complex and regorafenib) and altering the salt concentration led to the self-assembly into drug-encapsulated BSA NPs. The co-encapsulation strategy using BSA nanocarriers efficiently averted the growth of drug-resistant colon tumor and promoted the treatment mechanism (apoptosis, anti-angiogenesis, upregulation of intracellular ROS, as well as tumor-associated macrophage re-education) in the tumor-bearing animal model.

In another attempt, folate (vitamin B₉)-decorated BSA NPs were engineered *via* an anti-solvation approach and utilized for the *in vitro* targeted delivery of anti-cancer drug, paclitaxel [83]. The technique exploits the overexpression of folate receptors, occurring on a vast diversity of tumor cells. The resultant receptor-targeted BSA NPs (–30 mV) could effectively target a human prostate cancer cell line. The formulated drug-NPs were ~210 nm in particle size and attained drug loading efficiency and entrapment efficiency of ~27% and 95%,

Table 1
An overview of natural anticancer drug-loaded BSA nanoparticles.

Anticancer drugs	Other conjugates/ drugs/NPs	Particle size (nm)	Entrapment efficiency (EE) (%)	Loading capacity (LC)	Cancer type	Cell line/animal model	Brief findings (compared with native drug)	Reference
Berberine	–	166	85.65	7.78%	Breast	MDA-MB-231	<i>In vitro</i> anticancer activity ↑	[89]
	D – glucose	394.9	55.45	168.04 mg/ g	Liver fibrosis	LX-2, Male Balb/C mice	Anticancer activity ↑	[90]
Curcumin	Galactosylated	116.24	55.47	14.00%	Hepatocellular carcinoma	HepG2	Cell apoptosis ↑ Cell proliferation and migration ↓ Clinical use*	[84]
	Genipin	153–184.4	72.54	14.508 µg/ mg	–	–	–	[91]
	–	225.1–228.7	74.76–91.01	–	Breast	MDA-MB-231	Clinical use*	[92]
	–	92.59	78.12	2.61%	Breast	MCF-7, HFF-2	Cytotoxicity ↑	[93]
	Dextran	115	73.5	2.8%	Colon	Caco-2	Antioxidant activity ↑	[94]
	N-Acetyl-L- cysteine	251.6	85.79	10.9%	Colon	Caco-2, Adult male SD rat, Male ICR mice	Synthesized/optimized NPs Excellent permeation and absorption rate, Oral bioavailability ↑	[95]
	Gold nanoclusters	177.54	–	–	Neuroblastoma	SH-SY-5Y	Inhibition of tumor cell growth ↑ Cell apoptosis ↑	[96]
	Galactosylated	–	–	–	Colon	Caco-2, Male Sprague-Dawley rats	Intestinal absorption capacity ↑ Oral bioavailability ↑	[97]
	Poly-D-lysine	212 ± 11	46.7 to 60	3.1 to 22 µg/mg	–	–	Clinical use*	[98]
	Doxorubicin	80–100	Cur:95.46 Dox:86.69	Cur:9.3 Dox:13.27	Breast	MCF-7	<i>In vitro</i> anticancer activity ↑	[99]
Oridonin	Galactosylated	130.9 to 160.2	58.2 to 72.4	2.8 to 4.8%	Liver	–	Clinical use*	[100]
	Galactosylated	172.0	–	–	Liver	Wistar rats, Kunming strain mice	The prolonged blood circulation of drug Drug concentration and relative distribution percentage in liver ↑	[101]
Chrysin	Folic acid	97.5	–	2.09%	Breast	MCF-7 HFF-2	Suppression of cell growth ↑	[61]
	–	13 to 28	56 to 70	–	Breast	MDA MB 231, MCF-7	<i>In vitro</i> antitumor activity ↑	[27]
	–	13 to 28	44 to 84	–	–	–	Clinical use*	[103]
Quercetin	–	130	~85	–	–	–	Antioxidant activity ↑	[104]
	2-MI, zinc nitrate hexahydrate, folic acid	45.1	–	24%	Skin	B16F10, Male BALB/C mice	<i>In vitro</i> and <i>in vivo</i> antitumor activity ↑	[105]
Ginsenoside	Folic acid modified Ginsenosid-Rg5	201.4	73.59	12.64%	Breast	MCF-7, L929	<i>In vitro</i> and <i>in vivo</i> anticancer activity ↑ NPs exhibited tumor accumulation ability	[60]
	Ginsenoside Rh2	175.8	–	–	Lung, colon, skin	A549, HT29, HaCaT, RAW264.7	<i>In vitro</i> anticancer activity ↑	[106]
	PEGylation, Ginsenoside Rg3	149.5	76.56	17.65%	Liver, lung	L929, HepG2, A549, tumor- bearing mice	<i>In vitro</i> anticancer activity and apoptosis ↑ <i>In vivo</i> anticancer activity ↑	[107]
Glycyrrhizin	10-Hydroxy camptothecin	157.5	93.7	10.9%	Liver	SMMC7721, White rabbits	<i>In vitro</i> anticancer activity ↑ IC ₅₀ ↓	[108]
	Calcein	79	–	5.93 µg/mg	Liver	Male Wistar Rats	Uptake of NPs by hepatocytes increased 4.43 fold	[109]
	–	50 to 100	75.0	30.2%	Liver	Kunming mice	Serum drug concentration ↑ for a prolonged period and enhanced liver uptake	[110]
Rutin	–	316	32	–	–	–	Antioxidant activity ↑	[111]
Capsaicin	–	205	98.3	–	–	Male Wistar rats	Antioxidant activity of NPs was concentration and time-dependent	[112]
Catechin	–	45	60.5	–	Lung	A549	Scavenging activity ↑	[113]
Epicatechin	Poly-3-lysine, Chitosan	48	54.5	–	Colon	Caco-2	Anticancer efficacy ↑ Clinical use*	[114]

(continued on next page)

Table 1 (continued)

Anticancer drugs	Other conjugates/ drugs/NPs	Particle size (nm)	Entrapment efficiency (EE %)	Loading capacity (LC)	Cancer type	Cell line/animal model	Brief findings (compared with native drug)	Reference
Epigallo catechin gallate (EGCG)		BEN: 186 PBEN: 259 CBEN: 300	32.3 35.4 32.7	18.9% 17.0% 16.0%				
Myricetin	Folic acid	78	88.24	14.56%	Breast	MCF-7	<i>In vitro</i> anticancer activity ↑ ROS production ↑	[59]
Resveratrol	–	–	27.13 to 57.47	–	Ovarian	SKOV3, Balb/c (nu/nu) female nude mice	Drug concentration in liver ↑ <i>In vivo</i> tumor inhibition ↑	[115]
	Zein NPs, caffeic acid	217.2	86.5	7.3%	Colon	Caco-2	Antioxidant activity ↑	[116]

Note: ↑ - Enhanced, ↓ - Reduced, Clinical use* - Synthesized and characterized the anticancer drug-loaded BSA NPs but there is a need to do biological studies to prove clinical use. In the brief finding results column, results showed activity of anticancer drug-loaded BSA NPs compared to native drug.

respectively.

A convenient technique using sonochemical method was rationally applied to develop multifunctional BSA NPs encapsulating hydrophilic anti-cancer agents. The drug-vehicle system possessed a satisfying particle diameter (Fig. 4) with an exceptional magnetic responsive capacity. The fabricated protein NPs displayed unique controlled release for hydrophobic drugs *via* their redox responsiveness and possessed access to the cellular uptake by the folate-mediated endocytosis [56].

Huang et al. [84] developed galactosylated (Gal) BSA NPs for improving the bioavailability of anticancer drug (curcumin, Cur). They performed the *in vitro* cellular uptake assay to assess the cellular internalization and cell targeting evaluation of Gal-BSA-Cur NPs by fluorescence microscopy. The results showed that green fluorescent intensity of Gal-BSA-Cur NPs was stronger in HepG2 cells compared to alone Cur indicating that galactose residues displayed on the surface of Gal-BSA-Cur NPs improved the cell uptake ability through ASGPR mediated endocytosis. Moreover, they suggested that Gal-BSA-Cur NPs have a high efficiency of anticancer activity than only Cur and Cur-loaded BSA NPs. PTX was used as a model drug by Battogtokh et al. and they prepared PTX-loaded cholesteryl (Chol) BSA NPs by emulsion method [85]. Chol BSA NPs showed sustained drug release and circulated in the bloodstream for a longer time. As shown in Fig. 5, the tumor volume in PTX-Chol-BSA NPs and PTX in Cre/EtOH treated mice were 78.23% and 46.94%, respectively. Results indicated that tumor volume in PTX-Chol-BSA NPs treated mice was significantly smaller in comparison to PTX in Cre/EtOH treated mice. So, greater anti-tumor efficacy was observed through PTX-loaded Chol BSA NPs.

Solanki et al. [86] synthesized berberine-loaded BSA NPs by desolvation method. They performed an apoptotic study to determine the changes at the cellular level after treatment with drug-loaded BSA NPs and only drug. The results suggested that berberine-loaded BSA NPs trigger higher apoptosis in a time and dose-dependent manner in the cancerous cells compare to only berberine (Fig. 6).

For the incensement of the efficacy of BSA-based nanomedicine, different drugs and contrast agents could be co-loaded for combinational therapy and integrated theranostics, respectively. Zhao et al. developed paclitaxel/resveratrol (PTX/RES) co-loaded BSA NPs for synergistic combination cancer therapy [87]. They showed that PTX/RES NPs possessed enhanced *in vitro* cytotoxicity towards cancer cells. *In vivo* anti-tumor efficacy studies also showed promising enhanced tumor growth suppression. Concerning *in vivo* studies, the anticancer drug-loaded BSA NPs showed high anticancer activity than alone drug.

Varun Kushwah et al. [88] prepared anacardic acid-modified self-assembled BSA NPs for the co-delivery of docetaxel and gemcitabine. They well characterized NPs through FTIR, NMR, MALDI-TOF, Raman and CD spectroscopy, DSC, TGA, SEM and AFM. *In vitro* study explored on MDA-MB-231 and MCF-7 cell lines then explored *in vivo* study on tumor bearing rats.

Fig. 7(a), depicts the biodistribution of only C-6 and C-6 loaded NPs

in various organs and tumors and suggested that prepared NPs are nontoxic for organs. Fig. 7(b) suggested that prepared NPs treated animal model demonstrated significant suppression of tumor growth as compared to the control group. Fig. 7(d) showed that tumor bearing rats treated with NPs survived more compared to DMBA control and other groups. Overall data suggested that AA-GEM-BSA NPs are safer, low or nontoxic, responsible for enhancing apoptosis, and have promising potential as an anticancer agent.

Different ligands, drugs and conjugates used for the modification or combinational therapy are listed in Tables 1 and 2. Apart from PTX and berberine, varieties of drugs such as doxorubicin, docetaxel, curcumin, and quercetin are loaded into BSA nanocarriers and have been evaluated through *in vitro* or *in vivo* studies. Detailed information regarding anticancer drugs loaded into BSA NPs, their size distribution, entrapment efficiency (EE%), loading capacity (LC%), types of cancer, cell lines or animal model used for evaluation are represented in Tables 1 and 2.

In general, reported studies related to drug-loaded BSA NPs, showed an average size distribution between 100 and 200 nm, which is generally an ideal size range and acceptable for systemic drug delivery systems.

6. Drawbacks and challenges

Till now, several drug-loaded BSA NPs have been developed by different methods and it is clear that many advancements and modifications have been done. But there are some drawbacks, which should be minimized as much as possible. Controlling the particles size, shape, stability and distribution-controlled production of BSA NPs are the major challenges. Also, the composition and surface properties of nanocarriers need to be precisely controlled because these are the factors affecting the toxicity of BSA NPs. As discussed, solvents, anti-solvents, surfactants, and cross-linkers are used for the formation of BSA NPs; the use of a certain organic solvent such as chloroform and ethyl-acetate, cross-linkers such as glutaraldehyde, as well as surfactants such as polysorbate-80 and poly (vinyl alcohol) is undesirable because they can alter the biological activity of therapeutics. Batch to batch variation of BSA NPs formation is another drawback, which may hinder the scaling-up process for industrial applications. An interesting strategy to overcome this drawback is recombinant protein technology [39]. The investigation of BSA NPs is limited to *in vitro* studies in most of the publications; so *in vivo* studies should be done to understand the biological efficacy of BSA NPs used as versatile nanocarriers for anticancer drugs. However, compared to HSA, BSA may induce unfavorable immunogenic reactions.

7. Conclusion and future perspective

The development of drug nano delivery systems is a novel approach, to provide safe and effective cancer treatments. Whereas anticancer drug-loaded BSA NPs hold promising applications in cancer as they

Table 2

A summary of synthetic anticancer drug-loaded BSA nanoparticles.

Anticancer drugs	Other conjugates/drugs/NPs	Particle size (nm)	Entrapment efficiency (EE%)	Loading capacity (LC)	Cancer type	Cell line/animal model	Brief findings (compared with native drug)	Reference
Paclitaxel	Cholesteryl	147.6	94.8	37.9%	Breast	B16F10, MCF-7, male C57B16 mice	<i>In vivo</i> and <i>in vitro</i> antitumor activity ↑	[85]
	Octyl	123.3–152.8	90.5	33.1%	Liver	Hepg2	<i>In vitro</i> antitumor activity ↑	[117]
	Folic acid	217.0	95.3	27.2%	Prostate	PC3	Targeted delivery	[63]
	Biotin	163	86	14.7%	Breast, skin	MCF-7, B16, PBMC	Cytotoxicity towards cancer cells ↑	[62]
	Folate, difluorinated curcumin analogue (CDF)	PTX: 194.4 CDF: 197.8	PTX: 77.37 CDF: 78.42	PTX: 33.71% CDF: 37.5%	Ovarian, Cervical	SKOV-3, HeLa	Synergistic anticancer activity ↑	[118]
	Phenethyl isothiocyanate	133	~ 75	~9.5%	Breast, cervical	MCF-7, BCap37, 4T1, ICR mice, Nude mice bearing BCap37	Anti-tumor activity ↑ Prolonged blood circulation Tumor accumulation ↑	[119]
	Resveratrol	150	PTX: 99.93 RES: 98.24	PTX: 4.00% RES: 1.54%	Breast, lung	A549, A549/PTX, 4T1, Female BALB/c mice	<i>In vivo</i> and <i>in vivo</i> antitumor activity ↑	[87]
TPGS	170–340	75.3–78.8	1.85–13.62%	Breast	MCF-7, MCF-ADR, Kunming Mice	<i>In vitro</i> cytotoxicity and apoptosis ↑	[10]	
Doxorubicin	Gold nanocluster	186	83.05	–	Cervical	HeLa	<i>In vivo</i> antitumor efficacy ↑	[120]
	–	50 to 150	85	88%	Lymphoblastic leukemia, Uterine sarcoma	MOLT-4, MES-SA/DX-5	<i>In vitro</i> antitumor activity ↑ Cytotoxicity towards cancer cells ↑	[121]
	Cyclopamine	151	–	DOX: 1.2% CYS: 9.1%	Breast	MDA-MB-231, MCF-7, BALB/c female nude mice	Anti-breast cancer efficacy <i>in vitro</i> and <i>in vivo</i> ↑	[122]
	Gambogic acid	DNP: 249.3 GNP: 217.7	34.8 56.0	3.8% 4.2%	Liver	HepG2, Female nude mice	Synergistic antitumor efficacy ↑	[123]
	Dextran–folic acid	90	90	14%	Liver	H22 tumor-bearing mice	Tumor inhibition rate ↑ Average half-life ↑ Toxicity of native drug ↓	[124]
	Vanillin	92.7	88.60	9.09%	–	BGC-823, ICR mice, Heps Tumor xenograft mice	Cytotoxicity towards cancer cells ↑ Tumor suppression ↑ Tumor Targeting capacity ↑ Cardiac toxicity of native drug ↓	[125]
	Fe ₃ O ₄ , folic acid, modified dextran	55	88.7	14.2%	–	KB, ICR mice, Male SPF BALB/c, nude mice	Tumor inhibition rate ↑	[126]
	Galactosamine	187 to 194	52.3 to 61.2	1.76 to 2.03%	Liver	Hepg2	Nanocarriers are nontoxic Anticancer activity ↑	[127]
	Folic acid, magnetic iron oxide	180	~80	~4%	Nasopharyngeal carcinoma	KB, BALB/C nude mice	Two-fold incensement in cytotoxicity Inhibition of tumor <i>in vitro</i> and <i>in vivo</i> ↑	[128]
	Haloperidol	218	89	–	Lung	–	Clinical Use*	[129]
Dextran	182	90	30%	Liver	Kunming mice	Life time survivability ↑	[130]	
PLGA	–	~ 80	–	Liver	HepG2	Cell viability ↓	[131]	
Chlorin e6, Folic acid	103.5	–	–	Cervical	HeLa, zebrafish larvae	<i>In vitro</i> anticancer activity ↑ <i>In vivo</i> anticancer activity in Zebra fish larvae	[132]	
Lactoferrin, mPEG2000	–	98.5	4.3%	Glioma	BCECs, C6, Healthy SD rats	Circulation time ↑ Cytotoxicity towards cancer cells ↑ Accumulation of drug in the brain ↑	[133]	
–	166.9–84.8	47.9–71.8	15.6–25.2%	Ovarian, lung	NIH 3T3, A549, A2780 and NCL-H460	<i>In vitro</i> cytotoxicity ↑	[19]	
Docetaxel	Egg yolk lecithin	110.1	89.85	4.65%	Lung, glioma	A549, U87, HUVEC, Raw264.7, BMECs, bEnd.3, Male and female BALB/c mice, BALB/c nude mice	Cytotoxicity and apoptosis ↑ Drug accumulation within	[134]

(continued on next page)

Table 2 (continued)

Anticancer drugs	Other conjugates/drugs/NPs	Particle size (nm)	Entrapment efficiency (EE%)	Loading capacity (LC)	Cancer type	Cell line/animal model	Brief findings (compared with native drug)	Reference
	Quercetin	209.26	DTX: ~75 QT: ~68	–	Breast	MDA-MB-231, MCF-7, Female Sprague Dawley rats	glioma tissue ↑ No organic toxicity observed Bioavailability ↑ <i>In vitro</i> cytotoxicity and apoptosis ↑ <i>In vivo</i> toxicity ↑ Anti-proliferative effect ↑	[135]
	Biotin	159.0–84.8	–	–	Breast, gastric, colon, lung	MCF-7, SGC7901, LS-174T, A549		[136]
Metformin	–	97 to 120	92	–	Pancreatic	MiaPaCa-2	<i>In vitro</i> anticancer activity ↑ ROS production ↑	[137]
Salicylic acid	–	182.20	57.5	–	–	–	Clinical use*	[18]
Scutellarin	–	283.4	64.46	6.73%	Liver	HepG2, Male Sprague-Dawley rats	Bioavailability ↑ Elimination half-life ↓	[138]
5-methylmellein	–	154.95	73.26	7.09%	Prostate, colon, breast	PC-3 HCT-116 MCF-7	<i>In vitro</i> anticancer activity ↑ Cellular apoptosis ↑ ROS production ↑	[139]
Gemcitabine	–	147.2	–	3.23%	Pancreatic	MIA PaCa-2, PANC1	<i>In vitro</i> cytotoxicity and apoptosis ↑	[140]
	–	110, 406	82.92, 92.56	11.25%, 13.40%	Pancreatic	BXPC-3	<i>In vitro</i> anticancer activity ↑	[141]
	Anacardic acid, docetaxel	163	–	DTX: 9.1% GEM: 3.7%	Breast	MDA-MB-231, MCF-7, Female Sprague Dawley	<i>In vitro</i> cytotoxicity and apoptosis ↑ IC ₅₀ ↓ Tumor Growth ↓ No organic toxicity observed	[88]
Gefitinib	Folic acid, carboxy-methyl-β-cyclodextrin Chitosan	90.2	89.2	–	Cervical	HeLa	<i>In vitro</i> cytotoxicity and apoptosis ↑	[142]
	–	167	85.4	9.3%	–	–	<i>In vitro</i> cytotoxicity and apoptosis ↑	[143]
Cisplatin	–	55	98	–	Pancreatic	–	<i>In vitro</i> anticancer activity ↑	[144]
	–	172	75.02	–	Breast	MCF-7	<i>In vitro</i> anticancer activity ↑	[145]
5-Fluorouracil	–	210	27.5–30.0	1.1 and 1.2 mg/200 mg BSA	–	–	Clinical use*	[146]
Vinblastine sulfate	Folic acid	156.6	84.83	42.37%	–	–	Clinical use*	[147]
Atorvastatin	–	97–125	71.45–73.33	–	Pancreatic	MiaPaCa-2	<i>In vitro</i> anticancer activity ↑	[148]
Bexarotene	Folic acid	195.3	65.45	1.73%	Lung, breast	A549, MCF-7 c	<i>In vitro</i> cytotoxicity and apoptosis ↑ <i>In vivo</i> plasma concentrations ↑	[149]
Sorafenib	Folic acid	158.00nm	77.25	7.73%	Liver	LO2, SMMC-7721, female SD rats	<i>In vitro</i> anticancer activity ↑ Tumor targeting ↑	[150]
Oxaliplatin	–	187.9	53.3	1.5%	Breast	HFFF2, MCF7	<i>In vitro</i> anticancer activity ↑	[151]
Bufalin	–	125.1	76.02	12.62%	Liver	SMMC-7721, Wistar rats	<i>In vitro</i> antitumor activity ↑ Systemic circulation time and drug concentration ↑	[152]
Tamoxifen	–	155.13–199.167	–	12.07%	Breast	4T1, HFF-2	<i>In vivo</i> tumor inhibition ↑ <i>In vitro</i> cytotoxicity and apoptosis ↑	[153]

Note: ↑ - Enhanced, ↓ - Reduced, Clinical Use* - Synthesized and characterized the anticancer drug-loaded BSA NPs but there is a need to do biological studies to prove clinical use. In the brief finding results column, results showed activity of anticancer drug-loaded BSA NPs compared to native drug.

improve the anticancer efficiency of drugs. To conquer the limitations of conventional anti-cancer therapeutic agents, drug delivery systems based on BSA NPs rise as a victor for cancer patients, which have various advantages and applications in cancer therapy. The future of BSA NPs with no doubt could yield innovative platforms for cancer therapy, and this review may improve general consideration of cancer treatment with anticancer drug-loaded BSA NPs.

Declaration of competing interest

The authors declare no conflict of interest.

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मुख्यपृष्ठ > 37

शोध आलेख : औपनिवेशिक काल में झारखण्ड के आदिवासियों का शोषण एवं संघर्ष / प्रो. संजीव कुमार दुबे, सियाराम मीणा

📄 सम्पादक, अपनी माटी 📅 सोमवार, नवंबर 01, 2021



शोध आलेख : औपनिवेशिक काल में झारखण्ड के आदिवासियों का शोषण एवं संघर्ष (हिन्दी उपन्यासों के विशेष संदर्भ में)

प्रो. संजीव कुमार दुबे, सियाराम मीणा

शोध सार : साम्राज्यवादी, उपनिवेशवादी और पूंजीवादी ताकतों के खिलाफ आदिवासियों का संघर्ष श्रृंखलाबद्ध आन्दोलन के रूप में अनवरत चल रहा है। स्वतंत्रता पश्चात् इस संघर्ष के स्वरूप में बदलाव आया है। औपनिवेशिक काल से लेकर वर्तमान अस्तित्व और जल, जंगल, जमीन के साथ साथ परंपरागत अधिकारों, स्वशासन को सुरक्षित एवं संरक्षित करने के लिए उपन्यासकारों ने अपनी रचनाओं में प्रमुखता से स्थान दिया है। इसी परम्परा में राकेश कुमार सिंह ने 'हुल पहाड़िया' और 'जंगल आदिवासी शोषण व संघर्ष को सामने लाने का सफल प्रयास किया है। मधुकर सिंह ने 'बाजत अनहद डोल' उपन्यास में आदिवासियों को ऐतिहासिक ग्रन्थ तो नहीं कहा जा सकता है लेकिन उपन्यासकारों ने घटनाओं व इन उपन्यासों में आदिवासियों के सामूहिक संघर्ष का स्वर उभर कर आता है। यह सामूहिक संघर्ष उनकी अस्मिता व हथियार भी है। औपनिवेशिक काल में आदिवासी संघर्ष चेतना की परम्परा रमना पहाड़िया से तिलका मांझी, सिदो, कानो देती है।

बीज शब्द : उपनिवेशवाद, पूंजीवाद, आदिवासी आन्दोलन, जल, जंगल, जमीन, स्वायत्त स्वशासन।

अपनी माटी



पटिचय > सम्पादकीय > विमर्श > विधाएं > विविधा > विशेषांक > नवीनतम > ताजा अंक विभिन्न अंक

सुख्यपृष्ठ > 37

कुछ कविताएं कुछ बात : कविता में 'होना' / प्रमोद कुमार तिवारी

सम्पादक, अपनी माटी ७ सीमावाट, नवंबर 01, 2021

पृ 2



कुछ कविताएं कुछ बात : कविता में 'होना' / प्रमोद कुमार तिवारी

सबसे अच्छी स्थिति तो यही होती है कि कवि नहीं, उसकी कविता बोले पट कई बात कवि का बोलना भी जरूरी होता है। दुनिया भर के टचनाकार इस > पट एकमत हैं कि कविता कम बोलने वाली साहित्यिक विधा है; दूसरे शब्दों में कविता जिताना बताती है उससे कहीं ज्यादा छुपाती है और इस छुपाने से ही बहुस्तरीयता और



अपनी माटी

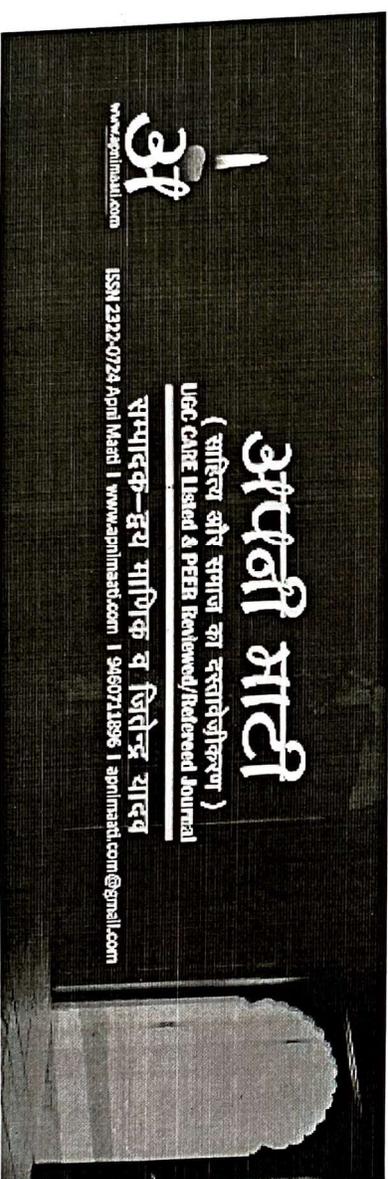


सूचनापत्र > 37

शोध आलेख : आदिवासी साहित्य के समक्ष चुनौतियां / डॉ. राजेन्द्र कुमार मीणा

ॐ सम्पादक: अपनी माटी ॐ सोमवार, नवंबर 01, 2021

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शोध आलेख : आदिवासी साहित्य के समक्ष चुनौतियां / डॉ. राजेन्द्र कुमार मीणा

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Area-based speciation kinetic analysis of multipollutant removal in constructed wetlands to enhance the treatment efficiency

Manoj Kumar and Rajesh Singh *

In the present study, area-based pollutant removal kinetic analysis was considered using the zero-order, first-order decay and efficiency loss (EL) models in the constructed wetlands (CWs) for municipal wastewater treatment. Except for SO_4^{2-} elimination, overall higher average pollutant removal performance was found in the CWs with gravel media only (P-CWs). The lowest value of 6.47 ± 4.72 of the efficiency loss co-efficient indicated the highest performance with a minimum discharge concentration of $0.54 \pm 0.24 \text{ mg L}^{-1}$ of TN (84.70% removal) in the CWs integrated with iron scraps and granular activated carbon (ISs + GAC-CWs). The lowest level to 0.903 ± 0.89 of the model's predicted value (C_t -ISs) also confirmed the relatively higher average removal efficiency of 63.37% of nitrate with narrower discharge distribution in the ISs-GAC-CWs. The factors affecting pollutant removal in the CWs and trends of the reactions of the multipollutants were interpreted by using the kinetic modeling analysis. The results confirmed that VF-CWs, an ecotechnology, should be considered as an alternative to the conventional treatment methods of municipal wastewater for small communities, which is a sustainable solution.

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Water impact

The main objective of this paper is to study the feasibility of the integrated constructed wetland zero-valent iron and activated carbon micro-electrolysis system for sewage water treatment. Though studies on iron-carbon micro-electrolysis system integration have been reported by scientists, in the present study, we tried to integrate CWs with iron-carbon micro-electrolysis, and we have compared the design of multiple-layer iron-carbon beds in CWs. We have also found a microbial strain (*Bacillus stratosphericus*) in CWs, and this microbial strain will be used for microbial fuel cell and organic and inorganic pollutant degradation. The data presented in the manuscript are very useful for the further designing of the iron-carbon micro-electrolysis system for sewage water treatment without electricity supply, which can be applied in domestic household wastewater treatment sustainably.

1. Introduction

Freshwater is one of the most valuable commodities on earth but is expected to become scarce in the next decades. Contaminants released by wastewater into the environment are harmful to humans and the ecological environment.¹ To keep water supplies free from eutrophication, nitrogen removal from wastewater is necessary.² While denitrification with exogenous carbon addition and chemical precipitation to extract phosphate (P) are well-established methods for reaching the limits of nutrient discharge, facilities are searching for more efficient and cost-effective methods to meet their permits.³ Therefore, there is an urgent need to address the problem of wastewater contamination with alternative treatment technologies. The high removal efficacy

of biological effects as a measure of total effect-scaled concentrations of chemicals provides further support to the use of intensified wetlands for wastewater treatment.⁴ The CWs significantly affect the overall cost of nutrient removal, allowing savings of up to 86% and 42% for biochemical oxygen demand and phosphorus removal, respectively, particularly for low concentrations and flow rate.⁵

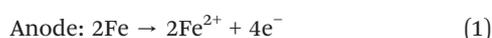
The combination of electrodes and microorganisms has led to several methods to remove or recover nitrogen from wastewater *via* oxidation reactions, reduction reactions, and/or transport across an ion exchange membrane.⁶ A microbial electrolysis cell (MEC) integrated into CWs has helped to investigate the probability of denitrification under low carbon conditions.⁷ During the past three decades, CWs have been used to treat acid mine drainage, industrial wastewater, agricultural and urban storm runoff, and effluent from livestock operations.⁸

As one of the commonly used wastewater treatment technologies in advanced oxidation processes (AOPs), iron-

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carbon micro-electrolysis has proved to be a high-efficiency low-cost treatment system for specific wastewater treatment.⁹ Fe(II) can easily be oxidized to Fe(III) under aerobic conditions, while Fe(III) can easily revert to Fe(II) under reductive conditions.¹⁰ The use of iron in CWs is an appropriate option to increase the removal efficiency of contaminants.¹

Substrate materials in CWs could provide a large surface area for microorganisms to bind and serve as either a filtration and/or a pollutant adsorption medium.¹¹ The tremendous automatic potency and good porosity upon use might be the reason for their use as the filter medium as well as a resource of H₂ in CWs.¹² A combination of granular activated carbon (GAC) and iron scraps interacts with wastewater (electrolyte solution) to form galvanic cells between the elements of Fe and C (ref. 13) as represented in the following half-cell reactions. Anodic half-cell reactions:



Cathode half-cell reactions: acidic conditions/neutral to alkaline conditions



At microscopic scales, these half-cell reactions occur; however, if these reactions happen at the same time on the surfaces of a large number of iron chips and GAC particles, the device will experience substantial electron flows.¹³

The objectives of this research are to evaluate the pollutant removal efficiency of CWs and characterize the adapted indigenous microbial consortiums during wastewater treatment. Through this analysis, the influence of iron scraps and granular activated carbon in the CWs is evaluated based on the fate of nutrients/contaminants and organic matter. Based on the iron scraps and granular activated carbon-CWs domain, comparisons are made between CWs that contribute to the output of CWs.

2. Materials and methods

2.1 Constructed wetland configuration and operation

The CWs experimental installation consisted of the integration of iron scraps and granular activated carbon.

Each of the CWs was multilayer with various substrate sizes (pebbles, ISSs and GAC) and was planted with *Eichhornia crassipes*. Three types of VF-CWs in duplicates with sampling ports at 5 cm and 15 cm from bottom to top were constructed. A detailed description of the various types of wetlands is presented in Table 1. The CWs were covered with aluminum foil to guard against the growth of periphyton (periphytic algae). The fresh iron scraps (ISSs), granular activated carbon (GAC), and gravels were purchased from the local market, Gandhinagar, Gujarat, India. The gravels were washed several times with tap water before integrating into the CWs. The gravels, GAC, and ISSs occupied 2.83 L volume out of the 8.5 L total capacity of the CWs. Thus, 4.53 L of the volume was available as working volume (Fig. 1).

All the CWs were maintained by filling and decanting treated domestic sewage wastewater at regular intervals of time for nearly 72 days for the acclimation of native bacteria. The actual treatment of wastewater was carried out for seven weeks with the addition of plants at 48 h HRT under semi-continues modes. Wastewater collection and serving, plant collection and adaptation, HRT, and pollutant removal performance were considered according to the study by Kumar and Singh.¹⁴

2.2 Analytical methods

Eutech Multimeter Instruments (Eutech Instruments Pte Ltd Blk 55, Ayer Rajah Crescent # 04-16/24, Singapore 139949) was used to measure the physicochemical parameters of pH (Eutech pH 700), electrical conductivity (Eutech CON 700), and total dissolved solids (Eutech CON 700). The NH₄⁺-N, NO₂⁻-N, NO₃⁻-N, SO₄²⁻, PO₄³⁻, dissolved oxygen (DO) and chemical oxygen demand (COD) were measured using standard methods.¹⁵ The total nitrogen concentration was calculated by the sum of NH₄⁺-N, NO₂⁻-N, and NO₃⁻-N measured individually using standard methods.

Microbial isolation and 16S rDNA characterization. The microbial community analysis was performed at 10-day intervals during the stabilization period and end of the cycles during the treatment phase. The characterization of the isolated microbial strains was performed using 16S rDNA-based molecular methods. The 16S rDNA gene fragment was amplified by 27F and 1492R primers, from Sagar Sequencing Department Eurofins Genomics Bangalore-560048 (India). A single isolated PCR amplicon band of 1500 base pairs was

Table 1 Type and construction dimensions of the wetlands

Layers	P-CWs				GAC-CWs				ISSs-GAC-CWs			
	Material	Thickness (cm)	Size (mm)	Weight (kg)	Material	Thickness (cm)	Size (mm)	Weight (kg)	Material	Thickness (cm)	Size (mm)	Weight (kg)
1st	Gravels	6	15	3	GAC	1	2-4	0.3	Gravels	4	15	2.0
2nd	Gravels	6	10	3	Gravels	4	15	2	ISSs	3		0.25
3rd	Gravels	5	5	2.5	Gravels	4	10	2	Gravels	4	10	2.0
4th					GAC	1	2-4	0.3	GAC	1	2-4	0.3
5th					Gravels	6	5	2.5	Gravels	4	5	0.2
6th					GAC	1	2-4	0.3	GAC	1	2-4	0.3

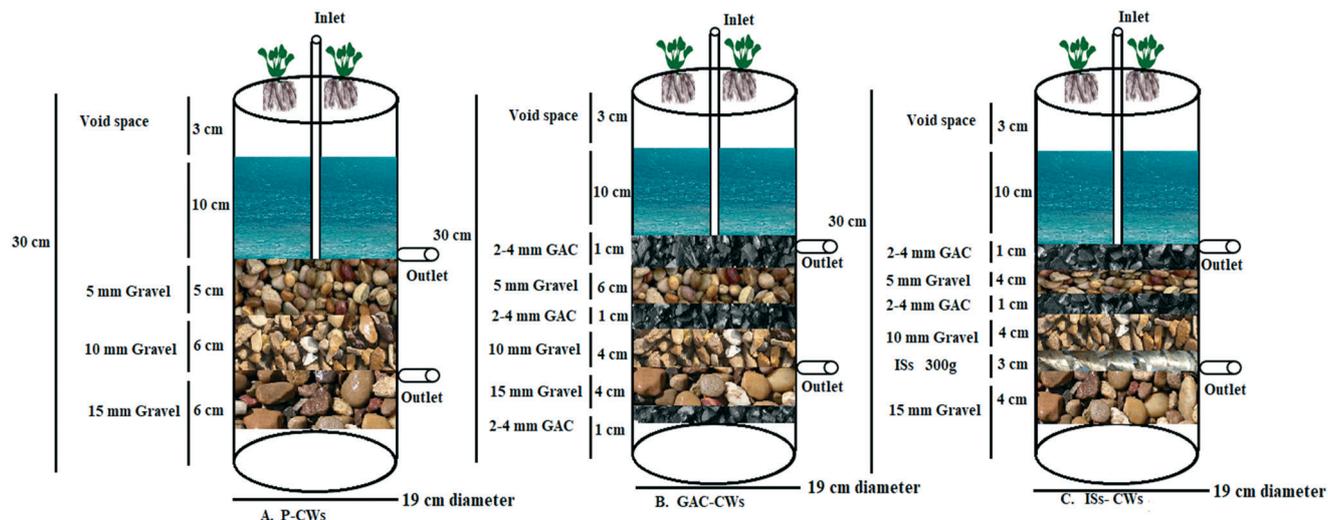


Fig. 1 Constructed wetlands: A) P-CWs, B) GAC-CWs and C) ISs + GAC-CWs.

noticed when it was resolved on an agarose gel. The DNA sequencing reaction of PCR amplicon was carried out with forward and reverse primers using a BDT v3.1 sequence kit on ABI 3730xl Genetic Analyzer.¹⁶ The phylogenetic tree construction was performed using MEGA 7.¹⁷ The microbial strain presented showed high likeness with the strain founded on nucleotide homology.¹⁸

2.3 Statistical analysis

The Past 3.0 (Version 3.12) software was used for Pearson's correlational analysis and employed to identify the linear relationship between the pollutant removal performance and variable parameters in the CWs. One-way ANCOVA test was performed with Past 3.0 (Version 3.12) software to demonstrate the differences in pollutant (ammonia, nitrite, nitrate, total nitrogen, total phosphate, sulphate, and COD) removal performances. All data calculations including averages and standard deviations were performed in three replicates by Microsoft office excel 2016 (Microsoft, USA). The Box plot graphs were prepared using Origin 9.60, 2019 software at 75th percentile and 25th percentile interquartile scale. For the processing of the plots, the 5th percentile minimum value and the 95th percentile maximum value were chosen. The horizontal median line indicates the average pollutant concentration.

2.4 Kinetic modeling

The pollutant removal affecting factors in the CWs were interpreted by using the kinetic modeling analysis. The speciation kinetic analysis of the multipollutants helps in finding the trends of the reactions. In the present study, the following area-based pollutant removal kinetics analysis was considered.

Area-based pollutant removal rates (J_{NN} , $\text{mg m}^{-2} \text{d}^{-1}$) were determined using eqn (4).¹⁹

$$J_{NN} = \frac{(C_i - C_t)}{A \times t} \quad (4)$$

where C_i is the initial pollutant load in the CWs (mg), C_t is the pollutant load in the CW effluent at sampling time (mg L^{-1}), A is the surface area of the CWs (m^2), and t is the time or mean residence time of wastewater held within the CWs.

The Zero-order model was considered to assume that contaminant reduction is independent of pollutant concentration.²⁰

$$J_{ZO} = \frac{(C_1 - C_F)xD}{t_{F-1}} \quad (5)$$

where C_1 is the initial pollutant concentration in the CWs added (mg L^{-1}), t_{F-1} is the influent mean residence time in the CWs after the influent is well mixed (48 h), C_F is the pollutant final concentration at the end of residence time (mg L^{-1}), and D is the average depth (m) of the CWs. After the estimation of the J_{ZO} , the C_t was estimated using eqn (6).¹⁹

$$C_t = C_{\text{Applied}} - \frac{(J_{ZO}xt)}{D} \quad (6)$$

where C_{Applied} is the pollutant concentration applied initially in the CWs at the time of feeding the influent.

Further, the second model, the first-order decay model (FO), assumes that the pollutant reduction rates are directly proportional to its final concentration.²¹ The ρ_{FO} , the mass transfer coefficient (cm h^{-1}), accounts for the intrinsic ability of the matrix to retain pollutants with depth, which is often ignored by using other removal rate constants (k (h^{-1})) in first-order decay model evaluations. The model can be expressed mathematically as eqn (7).²²

$$\rho_{FO} = - \frac{\left(Dx \ln \frac{C_F}{C_1} \right)}{t_{F-1}} \quad (7)$$

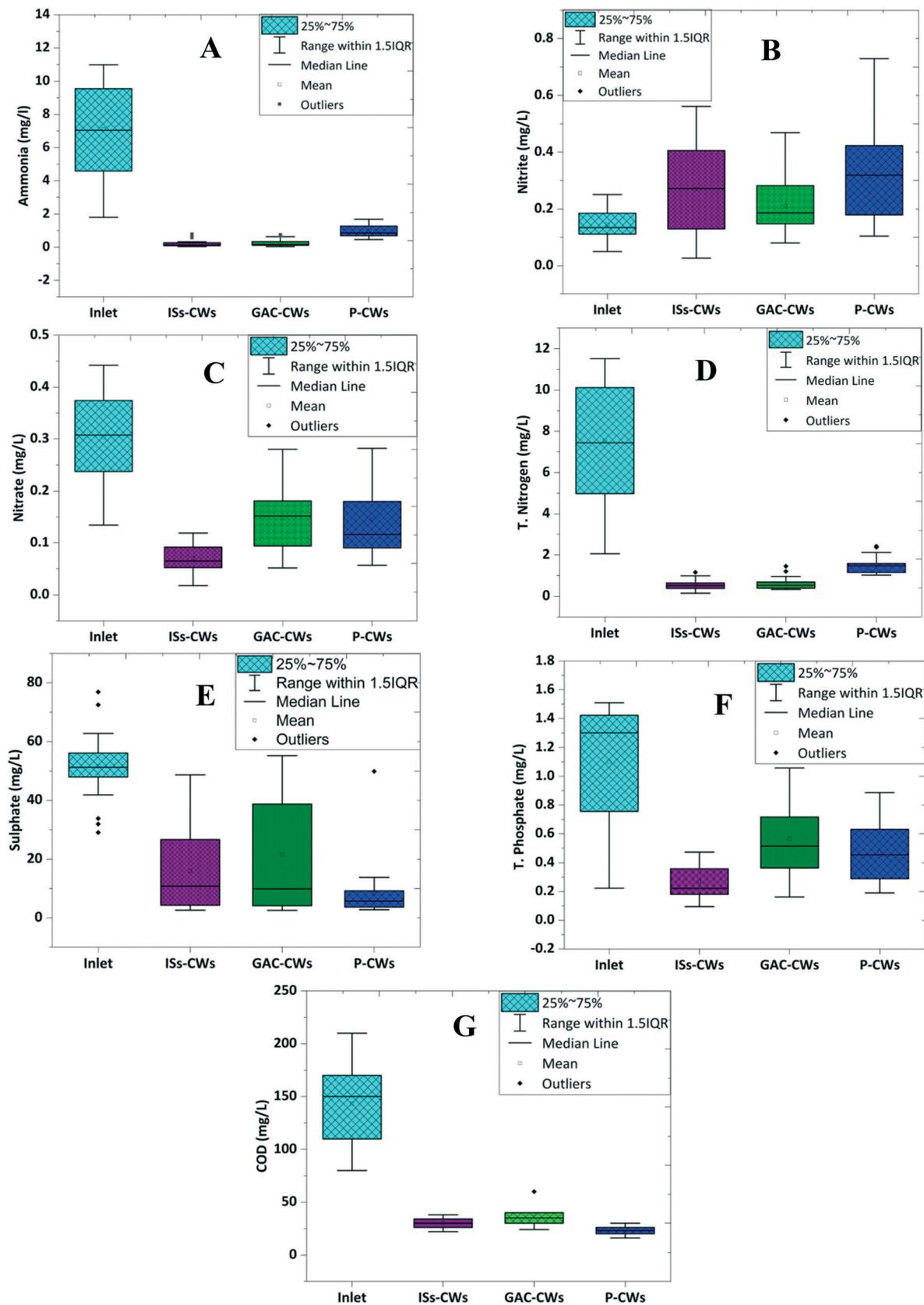


Fig. 2 Box plot distribution of pollutant in inlet and outlet concentration of A: ammonia; B: nitrite; C: nitrate; D: total nitrogen; E: sulfate; F: total phosphate and G: COD in P-CWs, GAC-CWs and ISS + GAC-CWs.

Table 3 Pollutant removal kinetic models in various contaminants and different constructed wetlands

CWs	Kinetic models	Pollutant removal kinetic				
		Ammonia	Nitrite	Nitrate	T. Nitrogen	T. Phosphate
	SS ²	25.716	19.105	5.482	39.824	10.797
	ISS-R ²	0.9788	0.9771	0.9951	0.993	0.9903
	rEL-ISS	0.8033	0.9262	0.8295	0.9468	0.9876
	R ²					
	SS ²	30.703	6.266	87.220	35.203	36.538
	GAC R ²	0.9741	0.9925	0.993	0.9892	0.9714
	rEL-GAC	0.7585	0.9897	0.8138	0.9572	0.9085
	R ²					
	SS ²	84.241	37.977	104.642	104.585	20.437
	P-R ²	0.9915	0.9876	0.9821	0.9987	0.9881
	rEL-P R ²	0.9525	0.9174	0.8349	0.9887	0.9881

After ρ_{FO} was determined, the daily C_t value was predicted using a first-order decay equation.

Efficiency loss (EL) model. The efficiency loss model (EL) accounts for the efficiency of the process rate relative to pollutant concentration decline over time.²¹ Similar to the first-order decay rate model, the efficiency loss model assumes that the substrate concentration is significantly smaller than K_s , the system is well mixed, and has no significant influences from water losses or gains. However, the model assumes a power relationship represented by the coefficient α , in which the order is less than 1. The efficiency loss model (EL) can be expressed as:

$$\rho_{EL} = \left(\frac{(C_F)^{1-\alpha} - C_1^{1-\alpha}}{t_{F-1}} \right)^{\frac{1}{\alpha-1}} D \quad (8)$$

where ρ_{EL} is the mass transfer coefficient (cm d^{-1}) and α is a unitless constant that varies between 0 and 1.

To test the model, daily C_t values were then predicted using the ρ_{EL} and averaged α values were used to determine the removal coefficients. The observed NO_3^- -N concentrations were compared with the predicted daily NO_3^- -N

concentrations (C_t) using eqn (9):

$$C_t = \left(\frac{\rho_{EL}^{(\alpha-1)}}{D} t + C_{Applied}^{1-\alpha} \right)^{\frac{1}{\alpha-1}} \quad (9)$$

3. Results and discussion

3.1 Municipal wastewater characterization

The physicochemical parameters of municipal sewage wastewater and the discharges from the P-CWs; GAC-CWs and ISSs + GAC-CWs are summarized in Table 2. To some extent, the average lowest decrease in the TDS to 810.79 ± 64.47 from 947.54 ± 24.77 was found in the GAC-CWs. The average pH in the CWs was 7.6 ± 0.035 which was almost similar to the initial value of 7.53 ± 0.22 , indicating that not much was significantly affected during the wastewater treatment in CWs. The chemistry of the wetland water and biological processes are influenced by pH.²³ All the CWs successfully maintained $\sim 3.0 \text{ mg L}^{-1}$ of DO sufficiently required for the occurrence of aerobic processes, whereas it was $1.30 \pm 0.18 \text{ mg L}^{-1}$ in the influent discharge. In the CW environment, the aerobic and anaerobic zones frequently coexist, and with hydraulic fluctuation, the dissolved oxygen concentration can change. Nitrification is declined and denitrification is stopped when DO is $< 1 \text{ mg L}^{-1}$ and $> 0.2 \text{ mg L}^{-1}$ in water, respectively.²⁴

3.2 Nitrogenous component removal and the kinetic study

The elimination of nitrogen in CWs depends on nitrification and denitrification.¹ Nitrification, the serial oxidation of NH_4^+ -N to NO_2^- -N and then to NO_3^- -N are the main microbial processes. The nutrient treatment performance of the P-CWs, GAC-CWs, and ISSs-CWs is presented in Table 2. The current outcomes showed much improvement in ammonia removal performance, due to higher average pH of 7.6 ± 0.035 in all the CWs (Table 2). Significant differences in the ammonia

Table 4 Pollutant removal rate kinetic models in various contaminants and different constructed wetlands

CWs	Kinetic models	Unit	Pollutants				
			Ammonia	Nitrite	Nitrate	T. Nitrogen	T. Phosphate
ISSs-CWs	J_0 -ISSs	$\text{g m}^{-2} \text{ h}^{-1}$	0.11 ± 0.0065	-0.008 ± 0.003	0.008 ± 0.006	0.267 ± 0.168	0.024 ± 0.021
	C_t -ISSs		27.47 ± 17.250	3.877 ± 1.887	0.903 ± 0.89	7.195 ± 3.977	3.562 ± 1.934
	ρ_{FO} -ISSs	cm h^{-1}	0.02 ± 0.002	0.003 ± 0.002	0.005 ± 0.002	0.012 ± 0.003	0.004 ± 0.002
	C_t		882.37 ± 502.183	1.743 ± 1.064	5.257 ± 3.699	355.216 ± 237.245	16.316 ± 13.896
	rEL-ISSs	cm h^{-1}	1.75 ± 2.03	3.25 ± 2.08	0.58 ± 0.71	6.47 ± 4.72	3.15 ± 2.33
GAC-CWs	J_0 -GAC	$\text{g m}^{-2} \text{ h}^{-1}$	0.26 ± 0.164	-0.004 ± 0.002	0.004 ± 0.004	0.264 ± 0.166	0.014 ± 0.013
	C_t -GAC		3.21 ± 1.891	2.824 ± 1.555	1.879 ± 1.061	7.915 ± 4.503	6.111 ± 4.113
	ρ_{FO} -GAC	cm h^{-1}	0.02 ± 0.002	0.002 ± 0.002	0.002 ± 0.002	0.011 ± 0.002	0.001 ± 0.002
	C_t		663.99 ± 440.243	1.620 ± 1.030	3.474 ± 2.354	322.446 ± 216.122	11.684 ± 9.035
	rEL-GAC	cm h^{-1}	2.61 ± 2.34	2.42 ± 1.72	2.49 ± 2.67	7.26 ± 5.21	5.28 ± 4.78
P-CWs	J_0 -P	$\text{g m}^{-2} \text{ h}^{-1}$	0.23 ± 0.147	-0.008 ± 0.003	0.004 ± 0.004	0.226 ± 0.145	0.014 ± 0.017
	C_t -P		11.05 ± 6.695	4.453 ± 2.291	2.017 ± 1.092	18.216 ± 10.331	6.431 ± 3.473
	ρ_{FO} -P	cm h^{-1}	0.01 ± 0.002	0.003 ± 0.002	0.001 ± 0.002	0.006 ± 0.002	0.001 ± 0.002
	C_t		216.49 ± 134.454	2.212 ± 1.342	3.413 ± 2.410	163.069 ± 106.568	12.174 ± 10.091
	rEL-P	cm h^{-1}	9.89 ± 7.81	3.59 ± 2.48	2.72 ± 2.83	16.78 ± 11.48	5.89 ± 4.08

Table 5 Comparison of work carried out using the classical CWs/integrated CWs

Types CWs	HRT (h) or (D)	Pollutants inlet/outlet (mg L ⁻¹) and removal performance (%)				References
		Ammonia inlet/outlet conc. (mg L ⁻¹)/removal (%)	Nitrate inlet/outlet conc. (mg L ⁻¹)/removal (%)	Total nitrogen inlet/outlet conc. (mg L ⁻¹)/removal (%)	COD inlet/outlet conc. (mg L ⁻¹)/removal (%)	
P-CWs	48 h	7.11 ± 2.66/0.98 ± 0.36/74.59%	0.30 ± 0.09/0.15 ± 0.08/35.81%	7.56 ± 2.71/1.49 ± 0.40/65.05%	143 ± 36.02/30.17 ± 4.28/66.4%	This study
GAC-CWs	48 h	7.11 ± 2.66/0.25 ± 0.20/92.56%	0.30 ± 0.09/0.15 ± 0.06/34.71%	7.56 ± 2.71/0.61 ± 0.29/84.05%	143 ± 36.02/38.58 ± 11.81/ 58.8%	This study
ISs + GAC-CWs	48 h	7.11 ± 2.66/0.20 ± 0.19/93.88%	0.30 ± 0.09/0.09 ± 0.03/63.37%	7.56 ± 2.71/0.54 ± 0.24/84.70%	143 ± 36.02/23.08 ± 3.78/72.7%	This study
UASB	8 h	31 ± 5.5/33 ± 5/	—	61 ± 20/55 ± 16	620.9 ± 189/241 ± 66/	50
SSF-CWs	5 D	31 ± 5.5/24 ± 6.6/	—	61 ± 20/40 ± 15	620.9 ± 189/53 ± 19/	50
FWS-CWs	10.8 D	31 ± 5.5/16 ± 5/	—	61 ± 20/32 ± 16	620.9 ± 189/73 ± 26/	50
SF-CWs	10 D	9.15 ± 1.75/0.19 ± 0.12/97.92%	15.92 ± 1.6/2.55 ± 2.2/83.98%	25.07 ± 2.02/2.55 ± 1.32/89.81%	275.35 ± 18.2/10.68 ± 5.1/96.12%	51
ST	5.6 D	44 ± 15/45 ± 15/-4.00%	0.33 ± 0.1/0.31 ± 0.1/6.1%	55.33 ± 15/49.31 ± 14/11.00%	588 ± 84/348 ± 82 41/41.00%	52
SSF wetland	7 D	44 ± 15/10.7 ± 376/76.00%	0.33 ± 0.1/0.2 ± 0.08/12.90%	55.33 ± 15/3 ± 0.6/69.00%	588 ± 84/74 ± 16/ 78.00%	52
UASB	3 D	—	—	—	492/152/69.10%	53
FWS-CWs	2 D	—	—	—	-/47.0/68.2%	53
SSF-CWs	1 D	—	—	—	-/21.4/53.0%	53

CWs (constructed wetlands), P-CWs (plant), GAC-CWs (granular activated carbon), ISs + GAC-CWs (iron scraps + granular activated carbon), UASB (up-flow anaerobic sludge blanket), SSF-CWs (subsurface flow), FWS-CWs (free water surface), SF-CWs (surface flow), ST (septic tank), HRT (hydraulic retention time) h (hour), D (days).

discharge concentration ($p < 0.05$), with the highest variation of 0.129% in P-CWs, were observed between the CWs. Moreover, despite having the highest variance in the influent ammonia (7.23%), the CWs showed significant removal performance ($p < 0.05$). Kim *et al.*, observed that instead of the biological oxidation of ammonia to nitrogen gas, the physicochemical factors such as diffusion through the membrane and volatilization of ammonia due to increasing pH had dominance in its removal.²⁵ The linear Pearson value of 0.858 (R^2) of ammonia in the ISs + GAC-CWs showed a better removal of 93.88% compared to that in the other media, GAC-CWs (92.56%) and P-CWs (74.59%). It strongly demonstrated that the substrate (GAC and ISs) played an important and positive role in $\text{NH}_4^+\text{-N}$ removal (Fig. 2A). Moreover, the results also demonstrated that the iron-carbon micro-electrolysis in the ISs-CWs and GAC-CWs systems achieved better ammonia removal than the P-CWs. Also, the pores within the granular activated carbon might promote the growth of microbial communities to remove $\text{NH}_4^+\text{-N}$. The lowest sum of the square value of the ammonia in the ISs-CWs confirmed its narrower distribution (25.716), followed by GAC-CWs (30.703) and P-CWs (84.241) (Fig. 2A and Table 3). In general, $\text{NH}_4^+\text{-N}$ removal in CWs relies primarily on the nitrification process in which ammonia-oxidizing bacteria play a vital role.²⁶ The equal mass transfer coefficient (ρ_{FO}) of $0.02 \pm 0.002 \text{ cm h}^{-1}$ in the ISs-CWs and GAC-CWs showed almost equal ammonia removal efficiencies (Table 4). The high mass transfer and stable adsorption capacity of the activated carbon (AC) for ammonia removal make it the most commonly used physical adsorbent.^{27,28} The quite good ammonia removal efficiencies (>75%) in all the CWs might be due to the improved DO

level ($\sim 3.0 \text{ mg L}^{-1}$), which was sufficiently more than that required for nitrification in the CWs. The strict aerobic nitrifiers work at free dissolved oxygen (DO) of 1.0 mg L^{-1} or more for the nitrification process.²⁹ Additionally, the ISs as a strong reductive, catalytic agent, supports micro-electrolysis, promotes adsorption, and flocculation.¹² A little lesser DO level in ISs-CWs might be due to its consumption in the iron corrosion processes. A little higher $\text{NH}_4^+\text{-N}$ removal rate by the ISs-CWs than by the GAC-CWs was recorded. Also, the differences in ammonia removal efficiency in the CWs ($F = 17.81$ under $p < 0.01$) and the value for homogeneity (equality) of the slope ($F = 1.472$ under p (same) = 0.2315) were confirmed by the ANCOVA assessment. The oxygen released from the roots was sufficiently more than the amount needed for O_2 demanding substances in the aerobic degradation, and the DO level raised to 1.5 mg L^{-1} was important for nitrification.³⁰ So, the DO level recorded in the present study might be representing a better oxidizing situation for the growth of nitrifying microorganisms. The significantly ammoniacal nitrogen reduction/removal rate was found to be about 90% in ISs-CWs and GAC-CWs. The $\text{NH}_4^+\text{-N}$ reduction rates in ISs-CWs, GAC-CWs, and P-CWs were found to be $70.99 \pm 43.80 \text{ g m}^{-2} \text{ h}^{-1}$ (93.88%), $70.20 \pm 43.60 \text{ g m}^{-2} \text{ h}^{-1}$ (92.56%), and $62.42 \pm 39.06 \text{ g m}^{-2} \text{ h}^{-1}$ (74.59%), respectively.

The average inlet nitrite concentration, its distribution, and removal across all the CWs are presented in Fig. 2B and Table 2. The highest accumulation level of $0.32 \pm 0.16 \text{ mg L}^{-1}$ was observed in the P-CWs, followed by ISs + GAC-CWs and GAC-CWs (Table 2). $\text{NO}_2^-\text{-N}$ can accumulate rapidly in the absence of sufficient organic carbon, limiting the denitrification in CWs.³¹ The principal intermediate formed

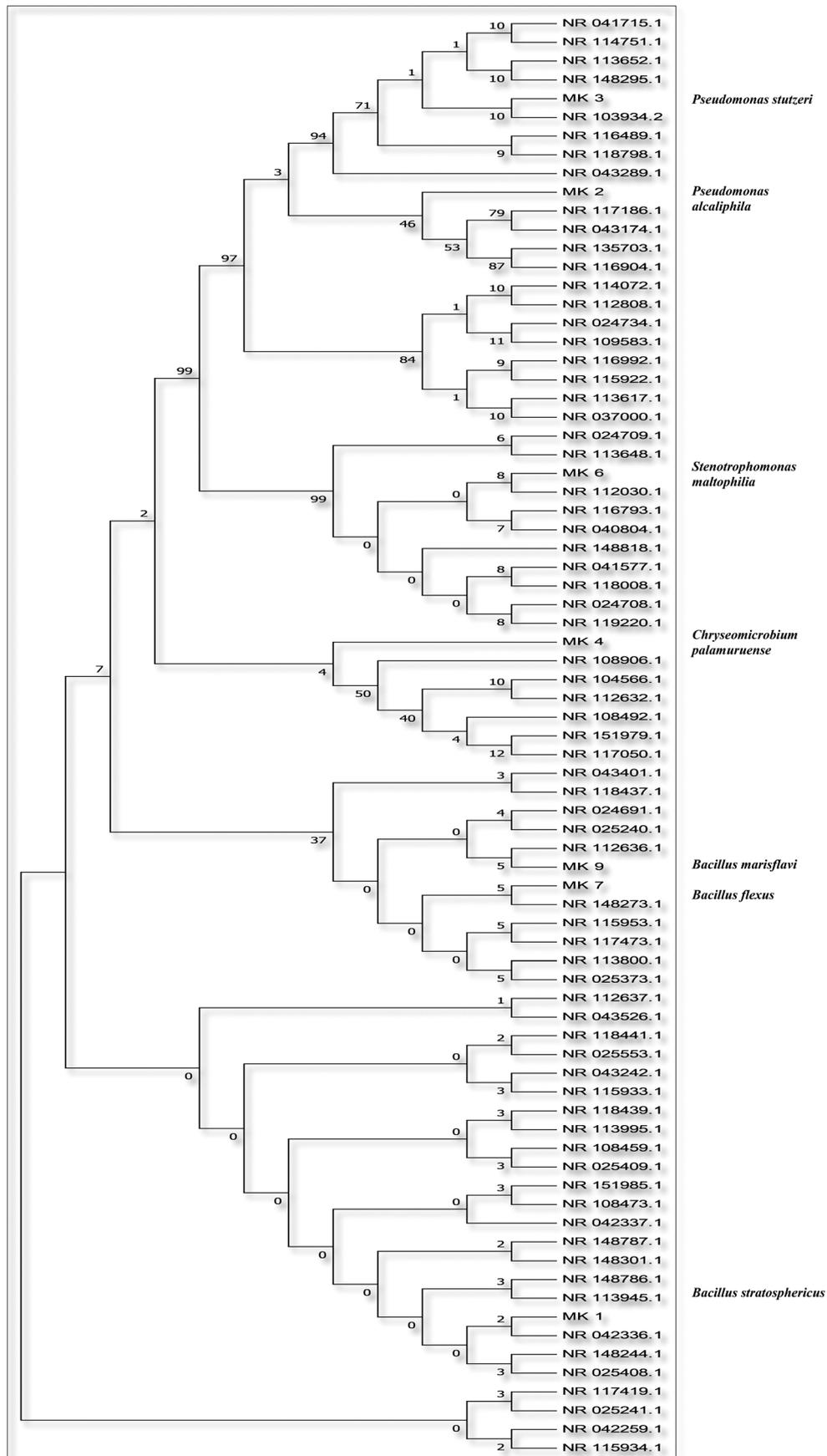


Fig. 3 Isolated microbial strain molecular combined phylogenetic tree.

either by nitrification or denitrification was NO_2^- -N.²⁶ This means that the use of CW to treat tailwater from WWTPs with low C/N ratios may cause the accumulation of NO_2^- -N in the system, which might present potential risks because of the NO_2^- -N toxicity.³² This difference in the NO_2^- -N accumulation may be due to the variable nitrogen conversion rates in the various mixture ratios of the matrix in the CWs. Similarly, as a result of the higher oxidation ability of NO_3^- -N than that of NO_2^- -N, a transient accumulation of NO_2^- -N was recorded.³³ The maximum average nitrite accumulation in P-CWs may be due to the higher ammonia oxidation rate linked to NO_2^- oxidation.

The lowest level to 0.903 ± 0.89 of the model's predicted value (C_T -ISs) confirmed the relatively higher average removal efficiency of 63.37% of nitrate with narrower discharge distribution in the ISs-GAC-CWs (Fig. 2C and Table 2). This may be due to the higher mass transfer rate of $0.005 \pm 0.002 \text{ cm h}^{-1}$ in the ISs + GAC-CWs. Though the predicted value (C_T -GAC) of 1.879 ± 1.061 was double, the average nitrate removal efficiency and pollutant removal rate were found to be very similar in ISs + GAC-CWs and GAC-CWs. The higher values of the coefficient of correlation (<0.8) for the observed and predicted models of the efficiency loss models showed stronger statistical results. Further, the lowest value of 0.58 ± 0.71 of the coefficient of efficiency loss confirmed the good nitrate removal efficiency of the ISs-CW (Table 4). The ISs + GAC-CW system underpredicted the efficiency loss value whereas the GAC-CW and P-CW over-predicted these values. The lowest level of $2.91 \pm 0.53 \text{ mg L}^{-1}$ of O_2 in the ISs-GAC-CWs might be responsible for providing the denitrifying conditions for the microorganisms. However, the lesser removal efficiency of the P-CWs showed that the plants contributed very little to the removal of NO_3^- -N. The porous activated carbon with maximum surface area and the DO consumption by the ISs provided an anoxic condition for the microbial biofilm establishment, which might be the probable explanation for the significant reduction in NO_3^- in the GAC-CWs and ISs + GAC-CWs.

The mechanisms involved in TN removal in CWs include volatilization, ammonification, nitrification/denitrification, plant uptake, and matrix adsorption.^{34,35} The TN removal performance recorded in the CWs operated at the average initial concentration of $7.56 \pm 2.71 \text{ mg L}^{-1}$ is presented in Table 2 and Fig. 2D. The lowest value of 6.47 ± 4.72 of the efficiency loss coefficient indicated the highest performance to be 84.70% removal with a minimum discharge concentration of $0.54 \pm 0.24 \text{ mg L}^{-1}$ of TN in the ISs + GAC-CWs. The average TN removal efficiency and pollutant removal rate were found to be similar in ISs-CWs and GAC-CWs.

The enhanced treatment of nitrogenous components in the ISs-CWs may be due to the improved micrometabolism by iron-carbon integration in comparison to that in the P-CWs. In the ISs-CWs, the iron scraps-activated carbon micro-electrolysis process converts the organic compounds to easily biodegradable organics, which offers additional usable

sources of carbon for denitrification and improves the elimination of nitrogen.³⁶ Furthermore, GAC provides perfect conditions for the development of microbial biofilm that could promote denitrification. There was no significant variance in the outflow of total nitrogen concentrations and pollutant removal efficiency in the GAC-CWs and ISs + GAC-CWs, respectively (Table 2). This study proves that TN can be efficiently removed by iron-carbon micro-electrolysis operation by enhancing the variety, disbursement, and metabolic roles of bacterial groups by iron in combination with treatment structures.³⁷

3.3 Sulphate removal efficiency

SO_4^{2-} is a natural part of domestic wastewater and its reduced compounds are considered to be effective inhibitors of plant growth and other microbial activities.³⁸⁻⁴⁰ The average sulfate concentrations of $9.16 \pm 10.02 \text{ mg L}^{-1}$ and $15.98 \pm 13.33 \text{ mg L}^{-1}$ in the P-CWs and ISs + GAC-CWs, respectively, were lesser as compared to that in GAC-CWs, which was $23.90 \pm 18.79 \text{ mg L}^{-1}$ (Table 2 and Fig. 2E). The significant value of 0.044 for linear relation for sulfate concentration in the P-CWs showed its removal efficiency to be 75.34%, fed at the initial average concentration of $51.57 \pm 10.93 \text{ mg L}^{-1}$ in the influent (Table 2). In comparison to GAC-CWs, the better performance of the ISs + GAC-CWs might be due to Fe^0 oxidation to $\text{Fe}^{2+}/\text{Fe}^{3+}$ involved in the SO_4^{2-} removal. The DO measured in all the CWs was near to the root zone sampling port; moreover, it could be lesser at the bottom of the CWs. The lesser DO at the bottom may favour the anaerobic microbial activity, responsible for the removal of the sulfate. The processes of the sulfur cycle inside the rhizosphere and their function in the dynamic system of processes of transformation are essential for a better understanding of the rhizosphere "black box" and for efficient design and operation of wetland systems.⁴¹

3.4 Total phosphate removal efficiency

The phosphorus removal process involves filler adsorption, chemical action, plant and algae absorption, combination with organics, natural assimilation, and excessive microorganism aggregation, of which phosphorus adsorption by fillers is considered to be the most efficient.⁴² The TP average concentrations of $1.1 \pm 0.38 \text{ mg L}^{-1}$ were found in the influent (Table 2). The average TP effluent concentrations found in the P-CWs, GAC-CWs and ISs + GAC-CWs were $0.47 \pm 0.20 \text{ mg L}^{-1}$ ($R^2 = 0.882$), $0.55 \pm 0.26 \text{ mg L}^{-1}$ ($R^2 = 0.002$) and $0.26 \pm 0.11 \text{ mg L}^{-1}$ ($R^2 = 0.972$), respectively (Table 2 and Fig. 2F). The highest removal rate of $6.50 \pm 5.68 \text{ g m}^{-2} \text{ h}^{-1}$ (60.82%) in the ISs-CWs might be due to its elimination either by adsorption to the ions/hydroxides of metals or being taken up by plants or fixed in the minerals of clay.⁴³ The confirmed absorption of phosphorus has also been reported by the decomposition of litter (dead plants), organic matter in wetlands and wetland plants, thereby reducing the levels in wetlands.⁴⁴ The excellent adsorption ability of the

pores in the activated carbon traps and locks nutrients such as phosphorous inside the pores.⁴⁵ A similar removal rate of $3.83 \pm 3.45 \text{ g m}^{-2} \text{ h}^{-1}$ (<40%) recorded in the GAC-CWs and P-CWs was almost half of that in the ISs-CWs ($6.50 \pm 5.68 \text{ g m}^{-2} \text{ h}^{-1}$). The improved performance in the ISs-GAC-CWs system might be due to the coagulation and precipitation of iron(III) and iron(II) formed by the internal electrolysis system.³⁶ Phosphorus combines with metal ions such as Fe, Al and Ca in the substrate to form phosphate precipitates, which can be removed by filtration and sedimentation.³⁶ The TP removal performance by the CWs ($F = 10.8$, $p < 0.01$) and the value for the homogeneity (equality) of the slope ($F = 0.014$, p (same) = 0.906) were explained by the ANCOVA evaluation. The study reported also noted that the Fe^{2+} released by the galvanic reaction of the iron-carbon granular charge layer formed precipitates of iron phosphate, improving the elimination efficiency of $\text{PO}_4^{3-}\text{-P}$.⁴⁶ The elimination of total phosphate from wastewater by the CWs may be affected by media, wetland vegetations, microbial immobilization, and precipitation.^{35,47,48}

3.5 COD removal efficiency

The organic matter in CWs can usually be removed by matrix adsorption and metabolic decomposition by microorganisms where physical adsorption is the main removal mechanism.³⁶ The average COD of $143 \pm 36.02 \text{ mg L}^{-1}$ in the influent was within the permissible limits (Table 2). This lower level of the initial COD resulted in a very narrow discharge distribution in the effluents of all CWs (Fig. 2G). A quite high value of R^2 (0.91) showed the highest average COD removal efficiency of 72.7% with the smallest discharge of $23.08 \pm 3.78 \text{ mg L}^{-1}$ in the ISs + GAC-CWs. The lowest discharge of COD in the effluent of ISs + GAC-CWs may be due to the involvement of Fe in the oxidation of the organic matter in the presence of diverse media. The organic compounds degraded by the micro-electrolysis function to settleable organics were quickly removed by deposition and filtration due to the coagulation effect of Fe^{2+} . The Fe-C integrated CWs supply iron(II) and electrons for high chemical activity, which in turn convert the structural properties of several organic compounds, causing the organics to interrupt and open the chemical rings present in the effluent.⁴⁹ However, it is challenging to compare the pollutant removal efficiencies in the CWs due to the variations in configuration, construction, and operation. A comparative study considering the application of this work (CWs) and other classical CWs is presented in Table 5.

4. Microbial community characterization during wastewater treatment

The 16S rDNA sequence, having a 99% similarity, analysis of the phylogenetic tree of the isolated microbial strains is presented in Fig. 3. The scope of water treatment in natural wetlands or CWs depends on the nature of the wetlands, the

microbial population, and the types of plants involved. The microbial consortiums are the most responsible components in the CWs to improve the water quality in natural wetlands and CWs.⁵⁴ The *Bacillus* and *Pseudomonas* genera are most common in soils, whereas in aquatic environments *Pseudomonas* is more common.⁵⁵ In the biological processes, certain microorganisms such as *Goebacteraceae* sp.,⁵⁶ *Pseudomonas* spp., and *Shewanella* spp.⁵⁷ are capable of denitrification and might be considered as heterotrophs, autotrophs, and heterogenotrophs⁵⁸ based on the source of energy they use for their metabolic activities. The presence of *Bacillus stratosphericus* has been reported for the first time in the CWs. *B. stratosphericus* which is highly tolerant to Fe (ref. 59) has also been isolated from soils⁶⁰ and estuarine sediments.⁶¹ Even under salt stress conditions, ferrous ions have been found to optimize the microbial community structure and promote the enrichment of specific bacteria for wastewater treatments.¹

Conclusion

Vertical flow constructed wetlands with various substrate beds as an ecologically friendly pollutant removal technology have shown strong potential for pollutant removal at an average ambient temperature of about 29 °C. The gravel-based P-CWs, GAC-CWs, and ISs + GAC-CWs can be successfully implemented as an appropriate technology to solve water and wastewater problems. The ISs + GAC-CWs are efficient in the removal of ammoniacal nitrogen, nitrate, total nitrogen, total phosphate, and COD. In ISs + GAC-CWs, the pollutant removal efficiencies of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, total nitrogen, total phosphate, and COD were found to be 93.88%, 63.37%, 84.70%, 60.82%, and 72.7%, respectively. *Bacillus stratosphericus* was isolated from the effluent of the constructed wetlands. So, in the future researchers can use *Bacillus stratosphericus* strain for the enhancement of bioelectricity generation (microbial fuel cell) and bioremediation of organic and inorganic compounds.

Conflicts of interest

There are no conflicts to declare.

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Development and Conflicts in Northeast India

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Asian Development Bank's Infrastructure Development Projects in Sikkim

SHRADDHA RAI & MANISH

The developmental debate in the 21st century is incomplete without addressing the action and prominence of new developmental mechanisms referred to as International Financial Institutions (IFIs). Although several states and non-state actors have set up and opened space for constructive debate on development, the speed and volume, these institutions are shaping and operating in line with the current development model make it imperative for study. Therefore taking Sikkim as a case wherein the operation of IFIs such as Asian Development Bank (ADB/Bank) is prominent, what this article will explain in brief is the ADB, and its engagement in the state of Sikkim and in that context will try to explore if the assistance/funds provided by it will work for the development of the state or not.

INTRODUCTION

IFIs, established or created by more than one country, provide grants and loans for economic and social development. It includes 'public banks' such as the World Bank (WB) and International Monetary Fund (IMF) and 'regional development banks' such as the Asian Development Bank (ADB), European Bank for Reconstruction and Development, African Development Bank, Inter-American Development Bank and others. Since their inception, these institutions have played important yet debatable roles in assisting impoverished countries by providing aid, grants and loans, giving professional advice and technical assistance (TA). Their reach and operations have become almost universal and have emerged as critical in contemporary times. Although addressing the case of each institution is essential, the focus of this article



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Assessment of dose–response relationship between ozone dose and groundnut (*Arachis hypogaea* L) cultivars using Open Top Chamber (OTC) and Ethylenediurea (EDU)



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ABSTRACT

Ground-level ozone is a serious environmental problem due to its direct oxidative effects on plant and indirect effect as greenhouse gas. Both of these scenario affected plant productivity and food security. A field experiment was conducted for two consecutive seasons to evaluate the dose–response relationship between ozone doses and groundnut (*Arachis hypogaea* L) cultivars (TG-37A, TPG-41, TAG-24, GG-20, and Dh-86) using OTC and EDU (to minimize ozone effects). Ozone negatively affects the growth, metabolism and yield of groundnut cultivars. Enhanced level of ozone reduces the leaf area and dry mass of plant parts while plant height was increase as compare to EDU supplemented plant and ambient ozone. In general the effects of ozone was profound at juvenile stages of groundnut cultivars than vegetative > and reproductive stages. Obtained yield was maximum in EDU treated plants followed by ambient ozone >and enhanced ozone treated plots in all the cultivars. Among the groundnut cultivars, cultivars TG-37A showed highest yield reduction (–53.90%) while, cultivar TAG-24 showed least yield reduction (–7.56%) by enhanced ozone exposure during first year of the experiment. The study concluded that the cultivar Dh-86 was most sensitive at 75 ppb level of ozone while TAG-24 shows least sensitivity and can be used for cultivation in ozone prone areas to minimize the agricultural yield loss to ozone.

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1. Introduction

Ozone is a kind of secondary pollution in the tropospheric environment and caused phytotoxic effects on plants. Tropospheric ozone arises through the photochemical reaction of the precursor gases including carbon monoxide, methane, nitrous oxides, and volatile organic compounds (Paoletti et al., 2014) arisen from motor vehicles, conventional brick-making kilns, and fossil fuels combustion (Chakraborty et al., 2015; Brauer et al., 2016). Ozone in the troposphere is considered a major threat to global crop yield production (Tai et al., 2014; Ainsworth, 2017) consequently, estimated in the range from 14 to 26 billion US\$ of economic yield loss (Mills et al., 2011; Avnery et al., 2013). Ozone pollution directly affects crop productivity by oxidative damage to cells and indirectly through as greenhouse effect and global warming (Ainsworth, 2017).

Food security is a global concern for the 21st century. Thus investigate the crop productivity under ozone exposure and to identify the inter-specific resistance is an important way to control the yield loss. Approximately, the rising levels

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of ozone lost 3.7% of global and 10% of regional rice yield loss (Ainsworth, 2017). A bottom-up modeling study by Ghude et al. (2014) quantified the potential impact of ozone on cotton, soybeans, rice and wheat crops, predicted yield loss which can feed 94 million people living below poverty line in India. The response of ozone to crops based on genetic makeup of the plant group, developmental stage of the crop, and ozone concentration. The sensitivity to ozone for grain crops was highest during flowering and seed maturity stages (Yi et al., 2018).

EDU (ethylene diurea) is a synthetic chemical considered as antiozonants due to its provided protection to plant against ozone stress without its own effect (Feng et al., 2010; Manning et al., 2011). EDU protection to ozone induced plant injury was first reported by Carnahan et al. (1978) on bean plants. The actual action and prevention mechanism of EDU against the phytotoxic effect of ozone is still remaining elusive (Paoletti et al., 2009; Manning et al., 2011). Researchers suggested that EDU may facilitated the scavenging of the ozone activity by mediating antioxidants defence reaction in the plant (Manning et al., 2011; Pandey et al., 2015). The application of EDU in plant is flexible which is applied with soil drench or foliar spray. However, this approach assumes that EDU has an ozone specific, but no constitutive effect on plants.

Groundnut is one of the important leguminous crops, grown mainly for its essential food oil produced from the seeds. India is the second largest producer of groundnut in Asia. In the year 2005, annual groundnut seed production was estimated to 5.9 million tons that produce about 1.5 million tons oils (Akhtar et al., 2010; Alexandratos and Bruinsma, 2012). In India, the Gujarat state produces about 2.5 million ton of groundnut that is approximately 25% of oilseed of groundnut (Misra, 2017). However, recent reports suggested a loss in groundnut productivity under climatic influences. Moreover, no significant study was seen for groundnut yield loss in relation to ozone pollution.

Therefore, the presented work was conducted with an objective to assess the dose–response relationship between ozone dose and crop responses. We hypothesized that the (1) variable cultivar responded differently to the ozone exposure, and (2) crop response to ozone is growth stage and ozone dose dependent.

2. Materials and methods

2.1. Crop selection and experimental design

Groundnuts (*Arachis hypogaea* L) is a commonly grown crop of Gujarat (India) during the Rabi season (winter crop). Five cultivars (cv. TG-37 A, TPG-41, TAG-24, GG-20 and Dh-86) of groundnut were chosen for the presented study. 50 seeds of each cultivar were sown in plots of metre² on 25 January 2017 and the same date for the second year (2018). Randomized Block Design (RBD) was applied for the experiment in which each blocks contain three replicate with different treatments viz. enhance ozone (75 ppb), ambient ozone and EDU supplemented (to minimize ozone effect).

Each plot was mixed with 250 g vermicompost during field preparation and was applied with NPK in the ratio of 40:40:20 kg/ha. Plots were regularly irrigated to ensure sufficient water supply and weeds were managed. Open Top Chamber (OTC) of 4 × 4 × 3.5 m sized was established for each treatment at the field of Central University of Gujarat, Gandhinagar, India (23.2156° N, 72.6369° E) for conducting this experiment. OTCs were made up of multi layered clear polycarbonate sheet (3 mm thick) to provide maximum available sunlight. Ozone concentration and ambient temperature were monitored with the help of ozone sensor (Ambetric, TC800D) and temperature sensor (HK Tempsensor) during the study period. Average temperature during the first year (2017) study period was varied between 30.94 °C (during winter days) to 38.52 °C (during hot days) while in second year (2018) of experiment, ambient temperature varied between 33.43 °C (during winter days) to 38.77 °C (during hot days). Average ambient ozone concentration in first year experiment was 13.89–22.42 ppb day⁻¹ while, during the second year it was 26.38–28.13 ppb day⁻¹ (Table S1). For ozone enhancement, concentration was maintained 75 ppb (varying between 55 and 85 ppb) during peak hours (11 a.m. to 3 p.m.) from seed germination to harvesting. Supplemental ozone was provided by ozone generator (Eltech Eng., India). For EDU treatment, 1L plot⁻¹ of 500 ppm EDU was applied as soil drench at every 10th day from seed germination to maturity in both year of experiments. EDU dose was decided on the basis of the best result of the experiments performed by the previous researcher (Agrawal et al., 2005; Manning et al., 2011; Rathore and Chaudhary, 2019, 2021).

Plants were sampled at juvenile stage (20 days after sowing), vegetative stage (40 days after sowing) and reproductive stage (65 days after sowing). Final harvesting was done at the maturity of crops. Triplicates of plants from each plot were taken for growth, biomass and biochemical analysis.

2.2. Plant growth & biomass

After sampling of plants, plants were washed by distilled water and dried by placing them in two layers of filter paper. Leaf area of sampled plants were measured by graphical method while, root and shoot length was measured by a regular metre scale. The fresh and dry weight of plant part was estimated with electric balance (Milton-MA224i, accuracy max. 220 g d = 0.0001 g). Dry weight of the same tissue was recorded after drying it in hot air electric oven 80 degree centigrade till a constant weight achieved.

Growth indices were calculated by the formulae of Hunt et al. (2002) for determination of biomass production and pattern of allocation.

2.3. Biochemical changes

Chlorophyll and carotenoids contents were estimated with the help of 80% of acetone by the formulae of [Maclachlan and Zalik \(1963\)](#) and [Duxbury and Yentsch \(1956\)](#), respectively. Total chlorophyll was estimated by adding the values of chlorophyll *a* and *b*. Total carbohydrate (mg g⁻¹ fresh leaf) was determined by colorimetric copper method of [Somogyi \(1952\)](#) using 80% ethanol. The total amount of carbohydrate was calculated against purified glucose standard. Determination of protein (mg g⁻¹ fresh leaf) was explained by [Lowry et al. \(1951\)](#) using phosphate buffer as an extracting solution. Standard was prepared with the help of different concentration of BSA (Bovine Serum Albumin) solution.

2.4. Yield characteristics

Crop was harvested after maturing the plants to determine the yield characteristic. Yield was presented as pod weight and seed weight (g m⁻²). Test weight was quantified using 1000 seed weight. Harvest index was calculated with the help of total plant biomass using the following formula:

$$\text{Harvest index (HI \%)} = (\text{Economic yield/Biological yield}) \times 100$$

2.5. Statistical tool

The data recorded for each parameter were subjected to analysis of principle component analysis (PCA) using Origin Pro 2019 software. The least significant difference was calculated at 0.05% probability level to determine the significant differences among the mean values using Duncan's Multiple Range Test by SPSS (SPSS Inc., version 17.0).

3. Results

3.1. Morphological characteristics

3.1.1. Leaf area and leaf area index

Ozone significantly reduced leaf area of all the selected experimental cultivars at each sampling stage ([Fig. 1](#)). The effect was higher under the enhanced ozone than ambient ozone exposure. Leaf area reduction was higher at the juvenile stage than the vegetative stage and minimum reduction was found at the reproductive stage in all selected cultivars. While in ambient conditions reduction of leaf area followed the same trends as enhancing ozone except for cultivar TG-37 A shows trends vegetative > juvenile >and reproductive stages. LAI also showed the value corresponding to leaf area under both level of ozone ([Fig. 1](#)). Among the cultivars, GG-20 showed higher reduction of leaf area followed by TAG-24 > TPG-41 > TG-37 A > and Dh-86.

3.1.2. Plant height and absolute growth rate (AGR)

A surprising effect of ozone exposure was seen on the height of tested groundnut cultivars. Enhanced level of ozone enhances plant height while, ambient level of ozone reduces height of all the experimental cultivars ([Fig. 2](#)). Every day growth of plant height calculated as AGR also showed reduction under ambient ozone exposure and increased under enhanced ozone exposure ([Fig. 2](#)). Ozone induced increase of height was maximum at reproductive stage except in cultivar Dh-86 where it was found maximum at vegetative stage. AGR also showed higher effect of enhanced ozone was during vegetative to reproductive stage on experimental cultivars except Dh-86.

3.2. Dry mass allocation and accumulation

Dry mass allocation and accumulation to different plant parts of tested groundnut cultivars was also influenced by ozone exposure ([Fig. 3](#) and S1). Higher the dose will have higher the impact. Above ground portion of groundnut cultivars was more affected by ozone regardless the dose as evident by increased RSR under ozone exposure. Percent decrease of root dry mass was higher at juvenile stage in cultivar TG-37 A and TPG-41, at vegetative stage in cultivar TAG-24 and GG-20 and, at reproductive stage in cultivar Dh-86. Similarly, percent reduction of shoot mass was maximum at juvenile stage in cultivar TG-37 A, TPG-41 and in cultivar GG-20, at vegetative stage in cultivar TAG-24 and in cultivar Dh-86, however germplasm of cultivar GG-20 showed maximum reduction at reproductive stage. Among the cultivars, TAG-24 having maximum percent difference of RSR under ozone exposures.

3.3. Biochemical changes

3.3.1. Total chlorophyll and carotenoids

The photosynthetic pigments (chlorophyll and carotenoids) of tested cultivars were significantly affected by ozone exposure ([Fig. 4](#)). Reduction of photosynthetic pigments was severe with higher dose of ozone. Trend of reduction was variable among the cultivars and by dose although, age wise reduction trend of chlorophyll was inversely proportionate to carotenoids in general. Chlorophyll and carotenoids of TPG-41 parent plants were least affected by ambient ozone exposure, however, percent reduction under enhanced ozone was in line with other cultivars of groundnut.

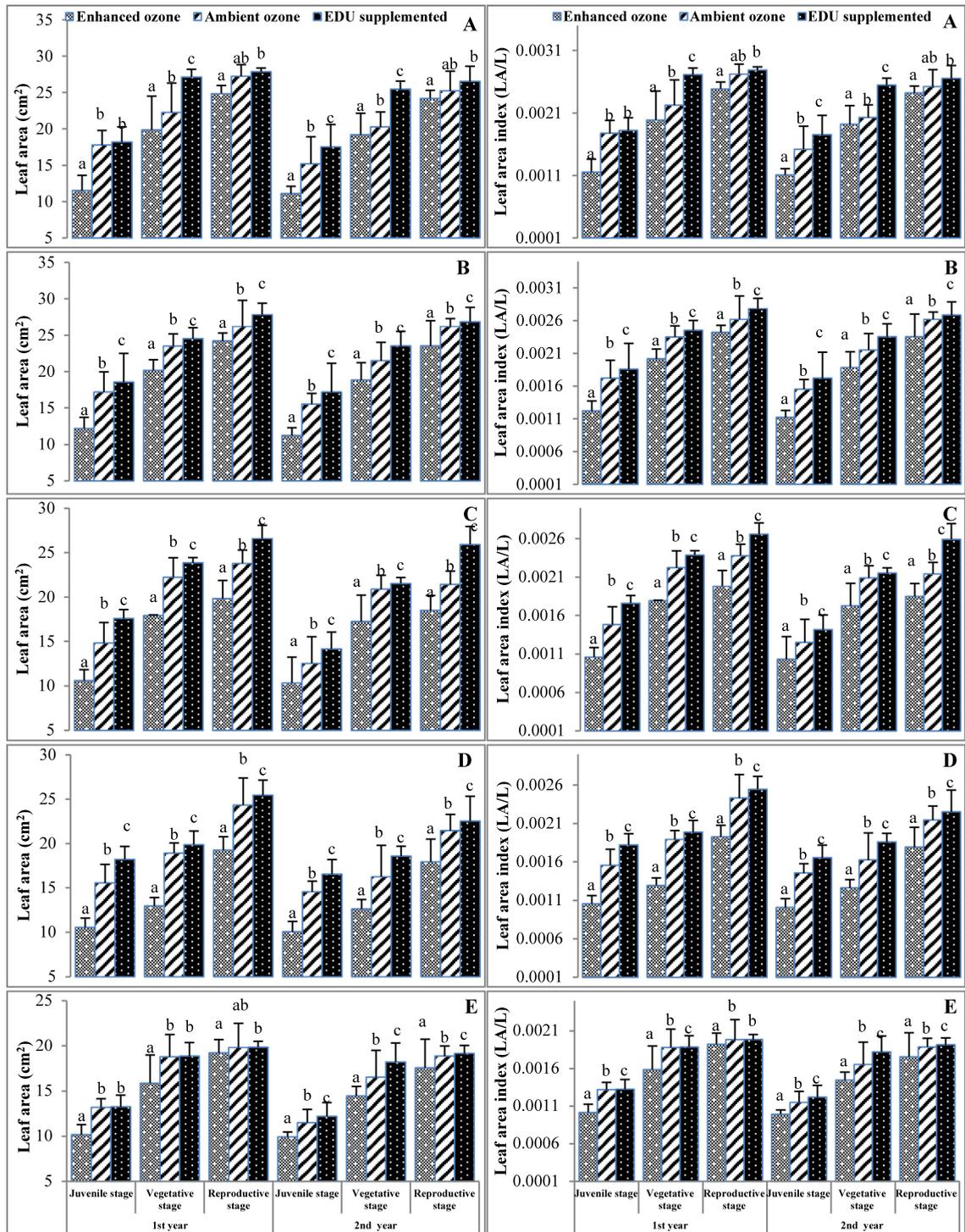


Fig. 1. Effect of ozone on leaf area (cm^2) and leaf area index (LA/L) of groundnut cultivars (A) TG-37 A, (B) TPG-41, (C) TAG-24, (D) GG-20 and (E) Dh-86 (Mean \pm standard deviation of three replicates presented by thin vertical bars, Value within each column followed by the same letter are not significantly different ($p < 0.05$) using Duncan's Multiple Range Test).

3.3.2. Total carbohydrate and protein contents

Similar to photosynthetic pigments, carbohydrate and protein content of groundnut cultivars were also significantly reduced under both ozone doses with higher severity under enhanced ozone exposure (Fig. 5). Reduction of carbohydrate

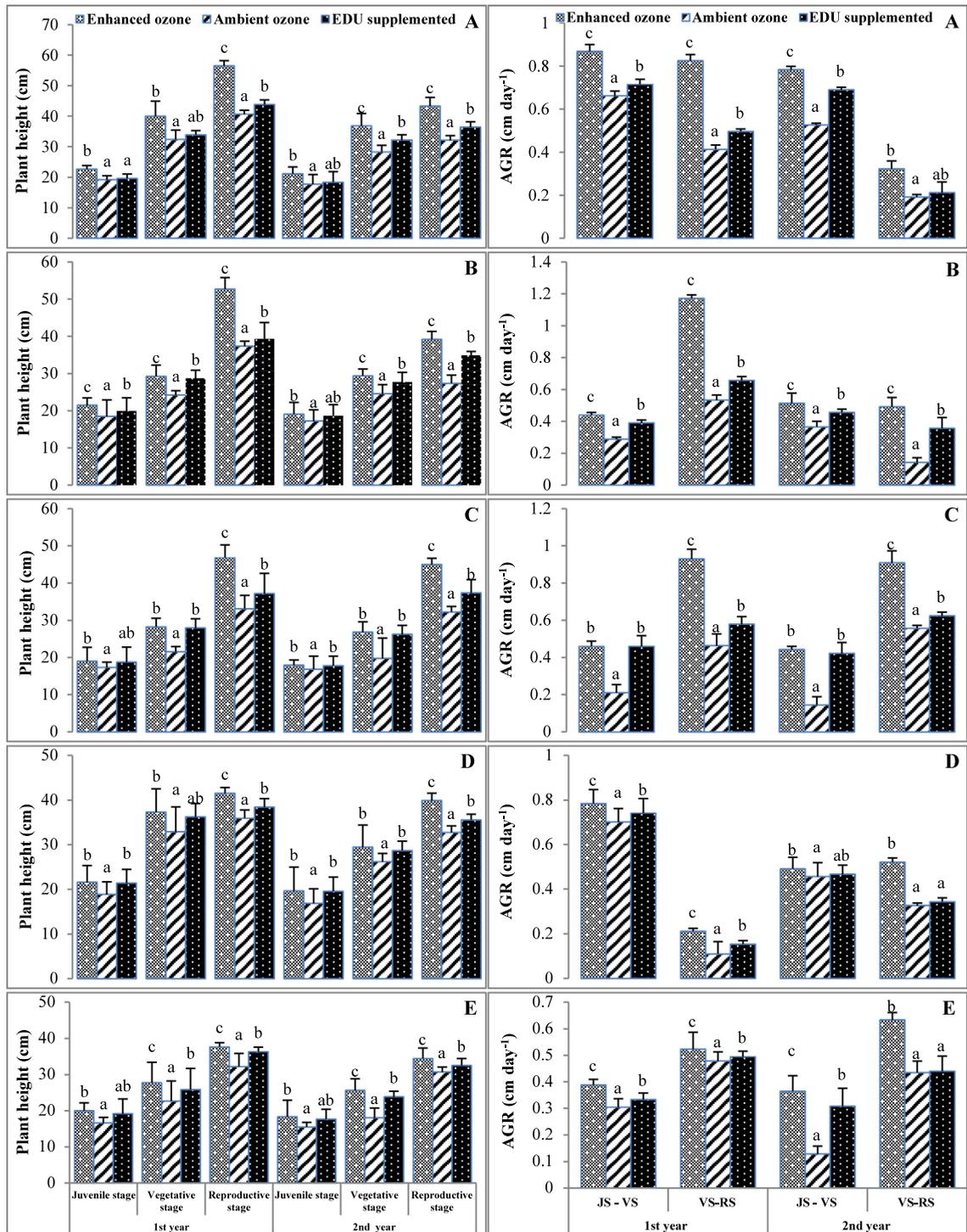


Fig. 2. Effect of ozone on plant height (cm) and absolute growth rate (JS – juvenile, VS – vegetative stage and RS – reproductive stage, cm day⁻¹) of groundnut cultivars (A) TG-37 A, (B) TPG-41, (C) TAG-24, (D) GG-20 and (E) Dh-86 (Mean ± standard deviation of three replicates presented by thin vertical bars, Value within each column followed by the same letter are not significantly different ($p < 0.05$) using Duncan's Multiple Range Test).

was maximum at vegetative stage followed by reproductive stage for cultivar TG-37 A and TPG-41, at reproductive stage followed by juvenile stage for cultivar TAG-24, reproductive stage followed by vegetative stage for cultivar GG-20 and, at

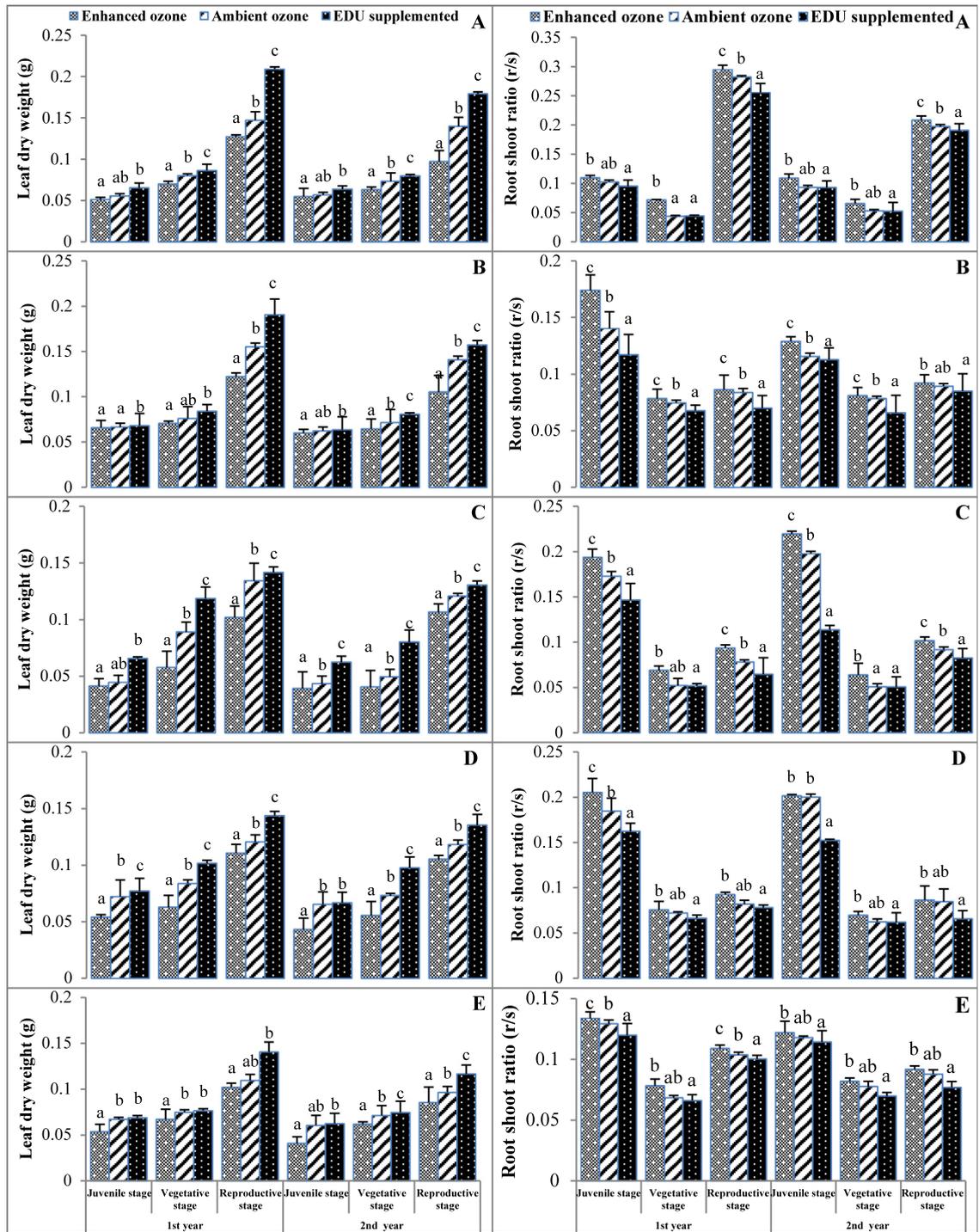


Fig. 3. Effect of ozone on leaf dry weight (g) and root shoot ratio (r/s) of groundnut cultivars (A) TG-37 A, (B) TPG-41, (C) TAG-24, (D) GG-20 and (E) Dh-86 (Mean \pm standard deviation of three replicates presented by thin vertical bars, Value within each column followed by the same letter are not significantly different ($p < 0.05$) using Duncan's Multiple Range Test).

juvenile stage followed by reproductive stage for cultivar Dh-86, except germplasm of cultivar TPG-41 and GG-20, where it was found maximum at reproductive stage followed by vegetative stage and, juvenile stage followed by vegetative stage, respectively. Percent reduction of protein was highly diverse among the cultivars growth stage and ozone dose.

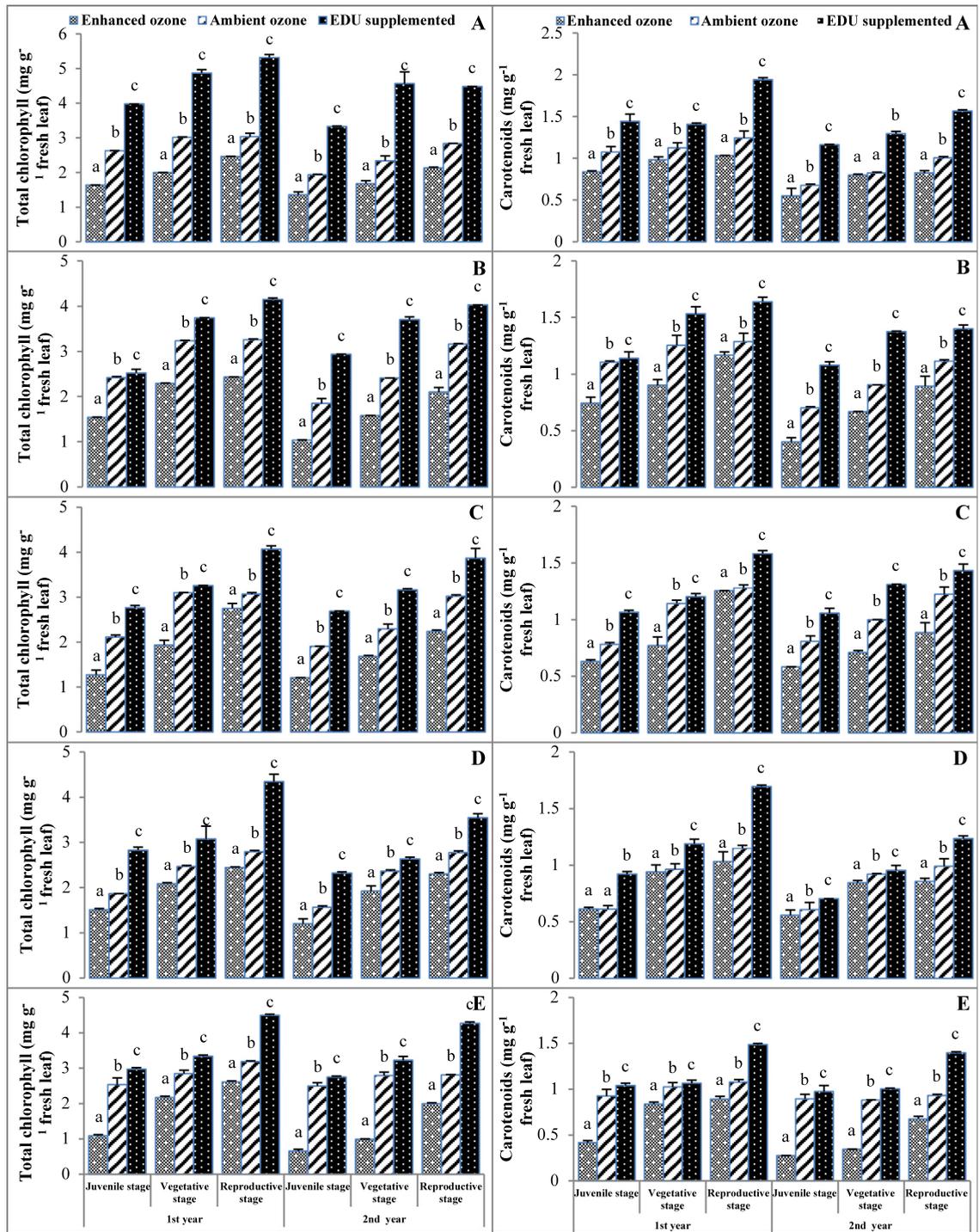


Fig. 4. Effect of ozone on total chlorophyll (mg g⁻¹ fresh leaf) and carotenoid contents (mg g⁻¹ fresh leaf) of groundnut cultivars (A) TG-37 A, (B) TPG-41, (C) TAG-24, (D) GG-20 and (E) Dh-86 (Mean ± standard deviation of three replicates presented by thin vertical bars, Value within each column followed by the same letter are not significantly different (*p* < 0.05) using Duncan's Multiple Range Test).

3.4. Yield characteristics

Groundnut yield was measured as pod weight and quality was measured as seed weight and test weight (Fig. 6). Elevated ozone caused negative effects on pod and seed weight of groundnut cultivars as compared to ambient ozone

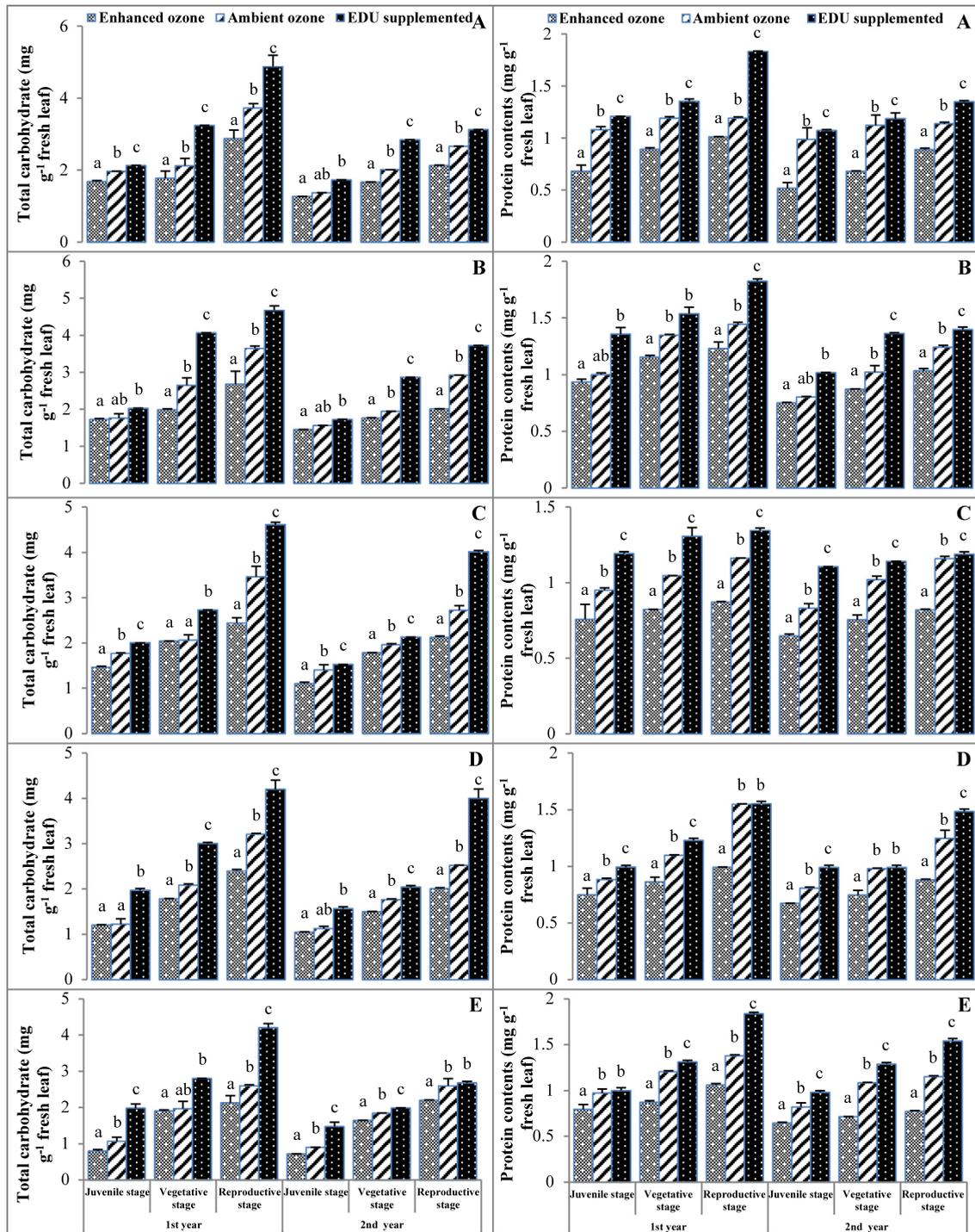


Fig. 5. Effect of ozone on total carbohydrate (mg g⁻¹ fresh leaf) and protein (mg g⁻¹ fresh leaf) of groundnut cultivars (A) TG-37 A, (B) TPG-41, (C) TAG-24, (D) GG-20 and (E) Dh-86 (Mean ± standard deviation of three replicates presented by thin vertical bars, Value within each column followed by the same letter are not significantly different ($p < 0.05$) using Duncan's Multiple Range Test).

and EDU application increased the weight of seed and pod. Total pod weight was count as the total yield of plants and average reduction trends in both years of the experiment were found Dh-86 >TAG-37 A > GG-20 > TAG-24 > and TPG-41 under enhance ozone. While minimum reduction of yield was noted in cultivar TAG-24 (-7.56%) in parental plants. Qualitatively, the seed weight (g m⁻²) of TAG-24 was the least affected among the tested cultivars under ambient and

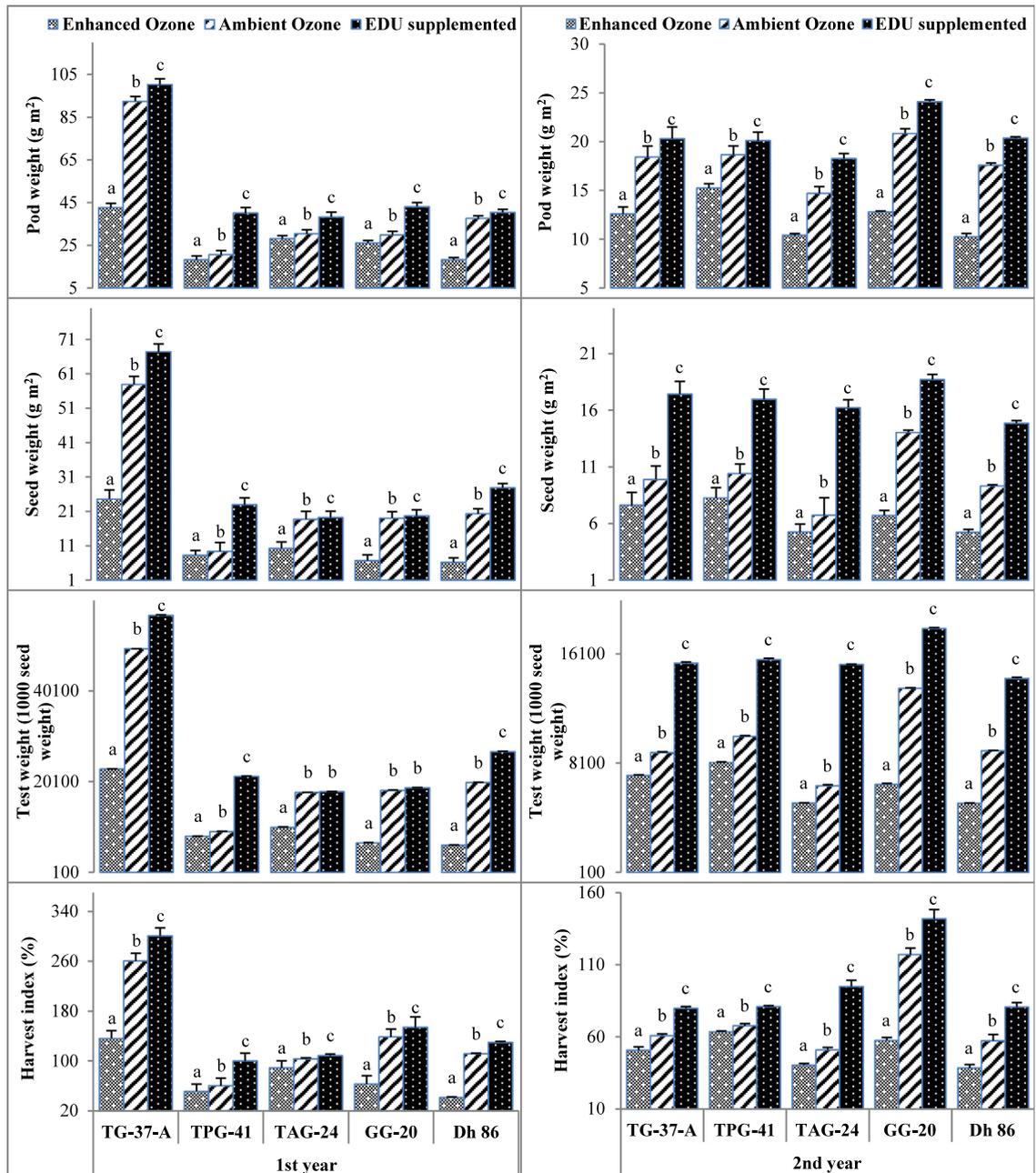


Fig. 6. Effect of ozone on pod weight (g m^{-2}), seed weight (g m^{-2}), test weight (1000 seed weight) and harvest index (%) of groundnut cultivars (Mean \pm standard deviation of three replicates presented by thin vertical bars, Value within each column followed by the same letter are not significantly different ($p < 0.05$) using Duncan's Multiple Range Test).

enhance ozone in the parental plant. The yield was significantly lower in germplasm of all the tested cultivars than the parent plants, however; the ozone effect was less evident in germplasm. The higher reduction of seed weight was found in cultivar Dh-86 (−69.29%) in the first year of the experiment. While cultivar TPG-41 (−12.05%) shows the minimum reduction of seed weight during same year of the experiment. Test weight of groundnut cultivars was following the same trends as seed weight. Maximum reduction of test weight was found in cultivar Dh-86 (−69.17%) and minimum in cultivar TPG-41 (−10.96%) during first year experiment. Harvest index of groundnut cultivar was in line with yield and cultivar TAG-24 was least affected under elevated ozone while in ambient ozone cultivar TG-37 A was least affected.

3.5. Principle component analysis (PCA)

PCA analysis of groundnut cultivar TG-37 A shows the percentage of variance bi-plot at PC1 (71.14%) and PC2 (13.70%) with eigenvalue 11.38 for PC1 and 2.19 for PC2. The higher loading value was found at PC1 in leaf dry weight and carbohydrate showed strong relation. While seed weight, test weight and harvest index was also showed a strong relation with loading value 0.26. Leaf area, LAI, shoot dry weight and pod weight were slightly correlated with above parameters and strongly correlated with each other's. EDU treated plants show positive loading values at both PCs and enhance ozone treated plant shows negative loading values. Loading values of ambient ozone plants show positive values at reproductive > and vegetative stages and negative value at juvenile stages (Fig. 7). The percentage variation of cultivar TPG-41 was higher at PC1 (72.13%) with eigenvalue 11.54, while, variation at PC2 was found (14.47%) with eigenvalue 2.31. Loading of PCAs for cultivar TPG-41 shows a strong relation between leaf area, leaf area index, leaf and root dry weight. Carbohydrate, shoot dry weight, test weight and seed weight was also showed strong relation with each other's and slightly correlated with above parameters. Harvest index and pod weight was strongly correlated and slightly correlated with protein contents. Score plot of PC1 with treatments showed positive values at reproductive and vegetative stages of plants and negative values were shown in all treatment at juvenile stages (Fig. 7). The higher variation was found in cultivar TAG-24 at PC1 (72.95%) and PC2 (15.15%) with eigenvalue 11.67 and 2.42 respectively. Within the parameters, leaf area, LAI, carotenoids, shoot dry weight, seed weight and test weight were strongly correlated with each other. While Total chlorophyll, pod weight and harvest index were slightly correlated with the above parameters. The maximum score plot value was found in EDU treated plant at the reproductive stage (7.29) for parent plant and the minimum value was noted at the juvenile stage (-4.76) in enhancing ozone-treated germplasm of TAG-24 (Fig. 7). EDU and ambient ozone-treated plant showed positive value at vegetative and reproductive stages while enhanced ozone-treated plant show positive values at the reproductive stage. Plant at juvenile stages showed negative values in all treatments. For cultivar GG-20, leaf area, LAI, root dry weight and protein contents were strongly correlated. While total chlorophyll, carotenoids, seed weight, test weight and harvest index were also strongly correlated and less correlated with leaf area, LAI, and leaf protein. The variance of cultivar GG-20 was found at PC1 (78.92%) and PC2 (9.55%) with 12.62 and 1.52 eigenvalue at PC1 and PC2. Treatment wise score value of cultivar GG-20 followed the similar trend to cultivar TAG-24. The maximum value was noted at the reproductive stage (7.58) in EDU treated plant from the first year of experiments and minimum in enhance ozone-treated plant at the juvenile stage (-4.80) in the germplasm (Fig. 7). The score of cultivar Dh-86 was 71.08% at PC1 and 15.18% at PC2 while eigenvalue was 11.37 and 2.42 at PC1 and PC2. Cultivar Dh-86 showed strong relation with leaf area, leaf area index, protein, pod and seed weight, test weight and harvest index with loading plot value 0.26 while, carbohydrate was slightly correlated with loading value 0.27. Treatment wise score value of this cultivar was also followed the same trends as above cultivars. The higher score value was found in EDU treated plant than ambient ozone > and enhance ozone (Fig. 7). Overall EDU application improves plant growth and yield of groundnut cultivars. On the basis of obtaining the result of PCAs ozone tolerant cultivars shows strong relation with the growth, physiology, and yield of crops.

4. Discussion

4.1. Morphological characteristics

Plant morphological responses to ozone dose were differing among the cultivars at different growth stages, although, higher the dose caused higher effect in all the cultivars. Ozone induces premature leaf senescence and reduction in leaf area (Wittig et al., 2009; Zhu et al., 2011). Similarly, meta-analysis of Feng et al. (2019) find reduced plant height and leaf area of poplar under ambient and enhanced ozone dose. Surprisingly, we find increased stem length of groundnut under enhanced ozone and reduced stem length under ambient ozone dose than EDU treatment. Moreover, Yi et al. (2018) reported that the sensitivity of crop was highest during the flowering and maturity stage. However, result of the presented study suggested that pattern of response also vary among the ozone dose and growth stage. All the cultivars for enhanced ozone showed maximum reduction of leaf area and LAI at juvenile stage. While, other growth parameters responded variably under ambient and enhanced ozone exposure. However, all the cultivars under both ozone exposures showed a common pattern that ozone effect was minimized when plants are reaching to vegetative stage.

4.2. Dry matter allocation

Dry matter of groundnut cultivars was decreased due to enhance ozone than the ambient level of ozone dose. Analysing of principle component (PCAs) clearly indicate that the variation of dry matter depend on ozone doses. The EDU applied plant part mass was positively correlated and ozone exposed plant shows negative correlation. Different crops have widely different responses to ozone due to genetics and developmental stages relative to concentration levels (Feng et al., 2008). For example, grain crop sensitivity is highest during the flowering and seed maturity stage (Mills et al., 2007; Yi et al., 2018). This is true for the groundnut cultivars too. Most of the experimental cultivars showed ozone induced reduction of shoot and root mass were maximum at juvenile stage while, for leaf mass it was maximum at reproductive stage for cultivar TAG-37 A, TPG-41 and Dh-86 at vegetative stage for cultivar TAG-24 and GG-20. Reduced mass and increased size

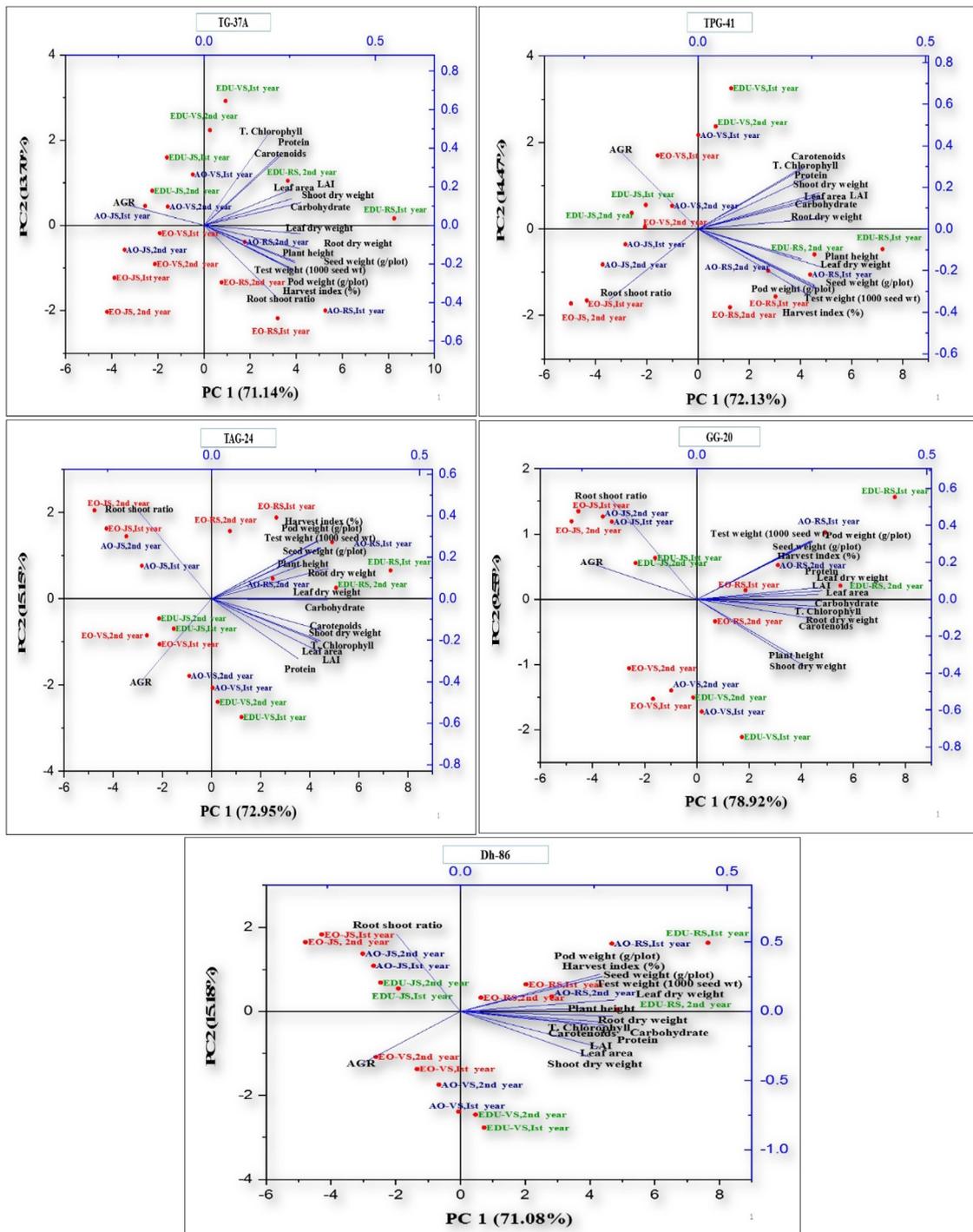


Fig. 7. Principle component analysis (PCA) correlation bi-plot of growth, biochemical and yield responses to ozone exposure and EDU treatments. Symbol represent the standardized scores on PC1 (x-axis) and PC2 (y-axis) for the ozone exposure and EDU treatment of *Arachis hypogaea* L. (cv. TG-37 A, TPG-41, TAG-24, GG-20 and Dh-86). Vector coordinates represent the correlations between standardized variables and principle components (PCs).

under enhanced ozone will resulted into weaker stem. Our previous studies (Rathore and Chaudhary, 2019) also found increased stem length and reduced stem mass of castor cultivars at 75ppb of ozone. Feng et al. (2019) also reported reduced basal diameter of poplar plants under enhanced ozone.

Analysing biomass allocation through RSR (root to shoot ratio) or SRS (shoot to root ratio) was considered advantageous as it involved whole plant into one parameter (Poorter et al., 2012). The increased root/shoot ratio under ozone-exposed plants suggested higher effect of above ground plant part and allocation of photosynthate towards underground plant part. Thus nutrient uptake properties and transport, translocation and partitioning of carbohydrates between the source (leaves) and sink organs (roots and grains) are often disturbed (Zheng et al., 2013). Rising ozone may cause cellular damages to plant leaves, which reduces photosynthetic production of carbohydrates and reduce above and below ground part of plants (Rathore and Chaudhary, 2019; Ghosh et al., 2021). Most of the cultivar shows higher values of root shoot ratio at vegetative and juvenile stages than the reproductive stage suggesting higher sensitivity and more allocation towards underground parts at early stage in cultivars of groundnut.

4.3. Biochemical changes

Air pollution including ozone-induced degradation in photosynthetic pigments was observed by a number of workers in different plant species and cultivars of similar species (Chaudhary and Agrawal, 2015; Chaudhary and Rathore, 2018a,b, 2019; Feng et al., 2016; Sarkar and Agrawal, 2012; Shang et al., 2017; Tiwari and Agrawal, 2018). Presented study also finds that the photosynthetic pigments of all selected groundnut cultivars were highly affected by ozone pollution. Effect was much distinct under enhanced ozone than ambient ozone for all the cultivars. Reduction trend of carotenoids was inverted to chlorophyll. Chlorophyll seems to be the primary target and affected significantly much higher than carotenoids. Castagna et al. (2001) suggested that the ozone initiates the destruction of total chlorophyll by preventing the synthesis of this pigment and suggested any reduction in plant pigments may serve as an adaptation against stress. Reduced number of light-harvesting antennae complexes may protect PSII from further photo-inhibition. Further, photosynthetic heterogeneity exist among leaves of a single plant and that both photosynthetic performance and photoprotective capacity influenced by the time of emergence, the age of the leaf, and the age of the plant itself (Horton, 2012). Chlorophyll pigments exist in highly organized state, and under stress they may undergo several photochemical reactions such as oxidation, reduction, pheophytinisation and reversible bleaching. Hence, any alteration in chlorophyll concentration may change the morphological, physiological and biochemical behaviour of the plant (Ghosh et al., 2021). In presented study ozone pollution caused adverse effects on chlorophyll and carotenoids contents at juvenile stages than vegetative > and reproductive stages of all cultivars. While cultivars GG-20 showed a higher reduction of carotenoids content at reproductive stages and TAG-24 at vegetative stages. Carvalho et al. (2015) suggested that the photosynthetic activity changes during the vegetative stage, and as the plant gets older, its tolerance to stress increases.

Carbohydrate metabolism is the major metabolic pathway in plants (Xing et al., 2015), with plant carbohydrates helping a major functional role as compatible solutes, including hexoses, disaccharides, sugar alcohols, and complex sugars, which accumulate during stress (Chen et al., 2018). The negative effect of ozone disrupted the membrane integrity, can cause chlorophyll loss, reduces the photosynthesis rate and affects the biomass, carbohydrate and protein of plants (Chaudhary and Rathore, 2020). While, EDU application minimizes the effect of ozone and increased the concentration of chlorophyll, carotenoids, and plant metabolites such as carbohydrate and protein contents (Gupta et al., 2018). Cultivar TPG-41 shows a higher concentration of total chlorophyll contents and also found an increasing level of carbohydrate and protein contents while cultivar Dh-86 shows minimum values. Reduction of carbohydrate content was higher in cultivars TG-37 A, TPG-41 and GG-20 at reproductive stages than vegetative > and juvenile stages while, cultivar Dh-86 was highly affected at juvenile stage than reproductive > and vegetative stages and cultivar TAG-24 shows higher reduction of total carbohydrate at reproductive > juvenile > and vegetative stage. Result of the study shows that the plant affected high at juvenile stage is sensitive to ozone and which plant affected high at reproductive stages is tolerant to ozone in nature. Maximum reduction of carbohydrate was found in cultivar Dh-86 and minimum reduction in cultivar TPG-41. Minimum reduction of protein contents was found in cultivar TPG-41 while higher increment of protein content due to EDU application was noted in sensitive cultivars.

4.4. Yield characteristics

The O₃ tolerance in groundnut depends more on the specific potential of each cultivar than the stress-induced physiological and biochemical adjustments to avoid and counter stress-induced oxidative damage. The higher dose of ozone (enhanced ozone) caused higher reduction of yield of groundnut cultivars than > ambient level of ozone and > EDU supplemented plants. The defensive mechanisms are an integral part of plant which provides protection against various type of stress (Chaudhary and Rathore, 2018a, 2019; Singh and Rathore, 2019). It means that plants invested most of its resources for their active defence and provide tolerance to plants. This study demonstrated that the sensitivity to ozone for cultivar TAG-24 is least with lesser yield loss under futuristic ozone exposure while, cultivar TG-37 A and Dh-86 was highly sensitive for future ozone level. However, low yield reduction under ambient ozone for TG-37 A and Dh-86 suggested that the yield loss to ozone is dose dependent. Yield reduction to ozone was higher at lower dose itself in cultivar TAG-24 > GG-20 and > TPG-41, and changed little with dose enhancement. Although, least reduction of seed quality and harvest index in cultivar TAG-24 suggested its tolerance to ozone exposure followed by GG-20 and > TPG-41.

4.5. Principal component analysis

Results of the present study were scattered and PCA can be a useful tool to provide important information. PCA can be used for any experiments to extract important information such as; correlation, effects and assessment, from large multivariate data by reducing the dimensionality (Xu et al., 2016; Rathore and Chaudhary, 2021). PCA analysis of all selected growth and biochemical parameters of groundnut cultivars shows positive correlation with ozone dose suggesting its reduction with increased ozone. Cultivars having ozone tolerance represent positive values at all ages while sensitive cultivars such as Dh-86 treated with ozone shows positive relation only at reproductive stage. As argued earlier, growth and biochemistry of cultivar Dh-86 is highly correlating with treatments suggested its highest sensitivity for ozone pollution while negative PCA score of cultivar TAG-24 and TPG-41 suggested reduced responsiveness for ozone pollution. Different doses of ozone and cultivars play variable result of the study. Elevated ozone negatively correlated with all cultivars and EDU application shows positive correlation in all selected cultivars. A study was also reported that the EDU application shows positive score value at all growth stage of groundnut cultivars (Rathore and Chaudhary, 2021). Overall the selected cultivars show strong relation with each other in both year of experiment. Cultivar TPG-41 and TAG-24 shows positive values and strongly correlated with each treatment. While cultivars GG-20 and Dh-86 show negative values maximum score values in the second year of the experiment were noted in cultivar TG-37 A (8.24) under EDU treated plant. Result of the study shows that the cultivar TAG-24 is less responsive to ozone dose while cultivar Dh-86 shows highly sensitive to ozone.

5. Conclusion

The presented study demonstrated that the higher dose of ozone caused negative effects on growth, biomass and yield of groundnut cultivars than ambient ozone. The germplasm of groundnut does not show any adaptability of ozone. Moreover, ozone induced loss was higher in early growth stage in groundnut cultivars than later growth stages where plant developed some tolerance to ozone. On the basis of both year yield reduction cultivar Dh-86 was the most sensitive cultivar and cultivar TAG-24 was least sensitive in nature. However, yield reduction was also ozone dose dependent and high sensitive variety for enhanced ozone showed low yield loss under ambient ozone. Further, reduction of yield is a cumulative response of physiological activity of plants to ozone dose and depends on the genetic makeup of cultivars. The study recommended more research on this line for more conclusive evidence.

CRedit authorship contribution statement

Indra Jeet Chaudhary: Conceptualization, Methodology, Software, Data curation, Writing - original draft, Visualization, Investigation. **Dheeraj Rathore:** Supervision, Software, Validation, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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Assessment of Groundwater Quality and Associated Human Health Risk of Central Gujarat, India

Swayam Siddha and Paulami Sahu*

The groundwater quality of Vishwamitri River Basin (VRB), Central Gujarat, is assessed to evaluate its competency to meet the agricultural and drinking standards, and to determine associated human-health risk by consumption of this groundwater, if any. The overall groundwater quality for drinking is expressed by water quality index. Various indices like sodium adsorption ratio, salinity hazard, soluble sodium percentage, magnesium adsorption ratio, and Kelly's ratio represent groundwater's agricultural suitability. A health risk assessment model is used to measure non-carcinogenic human health hazard. Results reveal that i) trace metals like lithium, manganese, molybdenum, strontium, thallium, vanadium and zinc are present in groundwater indicating contamination of groundwater by hazardous industrial and agricultural wastes; ii) groundwater of about 78% of VRB area is unsuitable for drinking; iii) positive values of Schoeller index of maximum groundwater samples indicate that ion exchange of Na^+ and K^+ with Ca^{2+} and Mg^{2+} is the dominant mechanism to control the quality of groundwater; iv) SAR values indicate high to very high salinity of groundwater and therefore, it is unsuitable for irrigation; and v) the health risk assessment model discloses that the risk of gastrointestinal disorder is higher in children than adults; and oral ingestion is more dangerous than dermal exposure.

the profuse amount of hazardous and trace elements into the environment and cause groundwater contamination.^[6] This contamination consequently may lead to severe environmental problems as well as health hazards to the life forms. In the modern era, water quality issues are a grave concern in many agrarian countries like India, Nepal, Myanmar, Nigeria, Brazil, Indonesia, Pakistan, Thailand, etc., of developing and the underdeveloped world.^[7,8] Therefore, assessment of groundwater quality for irrigation should be given maximal attention before adopting it for the same.

The groundwater quality and quantity are the major highlights in the research field across the length and breadth of the globe. According to these studies, during the last two decades, every part of the world has documented triumphs in accessing improved drinking water (IDW), sanitation, and personal cleanliness with the sustainable development goals (SDGs).^[9–12] As per the report, Sub-Saharan Africa; Latin America, and the Caribbean; West Asia and North Africa; East Asia; and South-East Asia had

24%, 65%, 90%, 94%, and 50% of their approaches to IDW, respectively.^[11,13] Intake of polluted groundwater together with compromised hygiene and sanitary conditions are the primitive causes of million annual deaths globally.^[10] Studies also reveal that 80% of all waterborne ailments like epidemic cholera, diarrhea, dysentery, enteric fever, poliomyelitis, dracunculiasis, and dermal infections in developing countries occur due to intake of polluted water, unsanitary conditions, and open defecation.^[10,11,14] Therefore, assessment of the drinking water quality happens to be the top-drawer for the supply of clean water in a sustainable manner for the wellbeing of human health in integrity with hydro-environment.^[15,16]

India also witnesses chronic water scarcities, both qualitatively and quantitatively, due to the booming population, agricultural development and industrial activities.^[17] Among 29 states of India, Gujarat and Rajasthan, semi-arid and arid states, respectively, are severely affected by the inadequacy of water. While taking account of the quality of water, Gujarat is one of the most severely affected states of the country. $\approx 89\%$ of the total water requirement for irrigation in India is sufficed by the groundwater as reported by the Central Ground Water Board.^[18] Shallow groundwater which is generally used for irrigation in Gujarat is saline in nature and causes salt accretion in the soil. Additionally, excess use

1. Introduction

The groundwater is assumed as a treasurable and the purest source of drinkable water abundant for human consumption. The biotic, actinic, and material states of groundwater ascertain its quality criteria as its competency factor for precise intentions like drinking, agriculture, domestic, and industrial activities.^[1–4] The quality of groundwater mostly depends on its geogenic sources such as weathering and erosion of bedrock, rock-water interaction, and also its duration of persistence in the aquifer system.^[5] In the recent times, population explosion, industrial growth, mining activities, prodigious use of chemical plant foods (manures), acaricides, insecticides in the agricultural sector, and various other anthropogenic activities have led to the release of

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Assessment of ozone toxicity on cotton (*Gossypium hirsutum* L.) cultivars: Its defensive system and intraspecific sensitivity

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ABSTRACT

Anthropogenic activities help the ozone formation at the troposphere which causes toxic effects on plants and humans. Ozone is a highly reactive gas that enters in plants through stomata and initiates the overproduction of ROS which causes oxidative stress in plants that lead to the destruction of membranous lipids, proteins, impaired the production of sugars and other metabolites and ultimately damage the cell. Presented study was conducted to assess the ozone toxicity on the biomass accumulation of cotton (*Gossypium hirsutum* L.) cultivars and the role of antioxidative activity in intraspecific sensitivity among the tested cultivars. Results showed that the ozone exposed plants have higher accumulation of H₂O₂ and MDA correspond to the EDU supplementation which increase the membrane permeability and adversely influence the protein, starch, and biomass accumulation and allocation of the experimental cotton cultivars. On the basis of biomass reduction, cotton cultivar ADC1 is the most sensitive cultivar, while cultivars G. Cot.21 > GADC-2 and G. Cot.13 is moderately sensitive and cultivar V-797 is the least sensitive to ozone stress. Activated defense mechanism such as enhanced activity of antioxidative compounds and enzymes detoxify the ROS by scavenging H₂O₂ and protects plants against damage. However, activation of defence is variable among the cultivars and corresponded to the biomass loss. Study concluded that the ozone sensitivity among the cotton cultivars depends on the scavenging of ROS. Further, study recommended cultivar ADC-1 as an assessment tool for ozone and cultivar V-797 for cultivation at ozone prone areas to minimize the agricultural loss.

1. Introduction

Ground level ozone is widely recognised as the most damaging air pollutant to vegetation due to its phytotoxicity and prevalence at high concentrations over rural/agricultural regions (Ainsworth et al., 2012). Ozone is expected to have an adverse effect on plant growth including reduced biomass accumulation, inhibition of photosynthetic processes, and impairment of organelles, and formation of oxidative contents in physiological processes even at a relatively small concentration (Wang et al., 2015; Rathore and Chaudhary, 2019, 2021; Chaudhary and Rathore, 2021).

Aerobic organism generates ROS as by-products of natural metabolic pathways. However, greatly accelerated production of ROS and its reaction products has been widely identified and tested under various stress conditions including drought (Zou et al., 2020), salinity (Luo et al., 2021), heavy metals (Singh and Rathore, 2019), temperature (Zafar et al., 2020), nutrient deficiency and excess (Singh and Rathore, 2018), UV radiation (Agrawal and Rathore, 2007), atmospheric dust

(Chaudhary and Rathore, 2018, 2019) and ozone (Rathore and Chaudhary, 2021). Reactive oxygen species act as signalling molecules for growth and development. Plants have maintains an equilibrium between the generation of ROS and metabolic activity (Gill et al., 2012; Anjum et al., 2012; Bhandari et al., 2020). However, the exceeded generation of ROS induces oxidative stress when necessary antioxidant amounts are decreases in plants (Singh and Rathore, 2018) which resulted into reduced membrane integrity, impaired metabolic functioning and ultimately permanent damage to the cell (Bhattacharjee et al., 2005; Foyer et al., 2005; Rathore and Chaudhary, 2021).

Antioxidant systems counteract stress-induced ROS aggregation (Mittler et al., 2004). Various antioxidative defense mechanisms scavenge reactive oxygen species molecules in steady-state circumstances (Foyer et al., 2005). Antioxidative enzymes especially superoxide radicals are formed when oxygen produced in chloroplasts during photosynthesis and accepts electrons passing over photosystems (Bhandari et al., 2020). Similarly, proline is listed as the most effective non-enzymatic antioxidant that can help counter the special effects of

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ROS (Chen et al., 2005). Numerous biotic and abiotic stresses may disturb the equilibrium among the generation and scavenging of ROS (Mittler et al., 2011). These disruptions in equilibrium induce a rapid rise in intracellular stages of ROS, which can deregulate cell functioning (Bhattacharjee et al., 2005). The higher ROS production in plants under ozone exposure causes significant crop loss worldwide (Ainsworth et al., 2012). Stress tolerant plant species thus be identified and grow to reduce the agricultural loss.

Cotton is an industrially important crop cultivated in tropical and subtropical regions of more than 80 countries (Singh and Kairon, 2001) is the primary source of the world's industrial textiles and stock feed and are a major source of cooking oil for much of the world (Deguine et al., 2008). The largest exporting countries in 2017 for cotton (raw cotton, cotton yarn, thread and woven fabrics) were China (USD 15.1 billion), the United States (USD 7.6 billion), and India (USD 4.7 billion) (Workman, 2020a, b). Due to the higher temperature condition for cotton cultivation, the area is also prone to the production of ozone hence, the cotton productivity loss to ozone. However, no study on cotton in relation to ozone pollution is seen in the literature.

Therefore, the presented study was conducted to assess the ozone toxicity on the biomass accumulation of cotton (*Gossypium hirsutum* L.) cultivars and the role of antioxidative potential in intraspecific sensitivity among the tested cultivars. Study hypothesized that the sensitivity of cotton is dependent on the inherent potential of ROS scavenging.

2. Materials and methods

2.1. Experimental crop, design, and field management

Cotton cultivar V-797, G. Cot. 13, ADC-1, G. Cot. 21, GADC-2 were selected for the present experiment. Selection of the crop was based on the local growing conditions. 100 seeds of each cultivar was sown in each plot (1 m² size) in triplicate. Thinning was conducted after emergence of seedlings to maintain 5 plants per plot. Plants regularly irrigated by ground water. During field preparation, each plot was mixed with 250 g vermicompost and recommended dose of NPK in the ratio of 40:40:20 kg/ha, and the same doses of fertilizers were applied at 25, 102, and 152 days after sowing of plants. Experiment was arranged in randomized block design with three treatments i.e. enhance ozone, ambient ozone and EDU supplement. Open Top Chambers (OTCs) of 4 × 4 × 3.5 m sized was established for each treatment at the field of Central University of Gujarat, Gandhinagar, India (23.2156° N, 72.6369° E) for conducting this experiment. OTCs were made up of multilayered clear polycarbonate sheet (3 mm thick) to provide maximum available sunlight. Ambient ozone and temperature during study period was measured using ozone sensor data logger (Ambetric, TC800D) and temperature sensor (HK Tempensor). During first year of experiment, ambient ozone was ranging between 39 ppb (during summer) to 13 ppb (during winter) while it was highly fluctuating during second year and reached 79 ppb during summer month. Ambient ozone and temperature is presented in Fig.S1. Average ozone concentration for ozone enhancement was maintained 75 ppb (varying between 57 and 84 ppb) during peak hours (11 a.m.–3 p.m.) from the seed germination till harvesting. Supplemental ozone was provided by ozone generator (Eltech Eng., India). EDU was provided 500 ppm as soil drench at every 15th day. EDU dose was decided on the basis of best result of the experiments performed by previous researcher (Agrawal et al., 2005; Manning et al., 2011; Rathore and Chaudhary, 2019, 2021). For controlling the insects the insecticides pyriproxyfen and methomyl were used.

2.2. Total biomass of plants

Sampled plant parts was separated and washed using double distilled water to remove the excess soil and kept in dry air oven at 80 °C till a constant weight achieved. Total biomass of plants parts was measured using a single pan electric balance (Milton-MA224i d = 0.0001 g).

2.3. Sugar and starch contents

Sugar was determined using 80% ethanol solution following the method of Somogyi (1952). Fresh leaf sample (50 mg) was crushed with ethanol and centrifuged at 3500x for 15 min. Pellets were washed by 80% ethanol four times and then with deionized water and centrifuged at each washing. After final washing and centrifugation 1 ml of supernatant was taken and mixed well with 1 ml of copper reagent. The solution was heated for 10 min and cooled to room temperature for 30 min before taking OD (optical density) at 500 nm to quantify total soluble sugars. To determine total reducing sugar, 0.5 ml aliquot was diluted with 1 ml of phenol (5% reagent) and kept it at room temperature for 10 min. The solution was added with 5 mL of H₂SO₄, and the solution was kept on a water bath for 10 min before taking OD 480 nm to estimate the SS (soluble Sugar). Pellets left behind extracting process were washed twice by perchloric acid (52%) and centrifuged, and finally washed by double distilled water and centrifuged. The mixture makeup to 50 ml using deionized water. 1 ml of supernatant from the solution was taken for determination of starch following the method described for soluble sugars to estimate the starch. RS, SS, and starch was calculated against the standard curve prepared from purified glucose.

2.4. Total proteins

The contents of protein in fresh leaf were measured by the Lowry et al. (1951) using the phosphate buffer as extracting solution. The sample was centrifuged, and the supernatant was used for protein determination. Total protein content was measured against the standard curve prepared using BSA (Bovine Serum Albumin). The quantity of protein is presented in mg g⁻¹ fresh leaf.

2.5. Membrane permeability (MP)

Membrane damage was measured in terms of electrolytes leaked out from the sample as proposed by Blum and Ebercon (1981). For this, twenty pieces of 1 cm circular shape sample was kept in a beaker with 10 ml of distilled water for 3 h to measure the conductivity (mS/cm) using electrical conductivity (EC) meter.

2.6. Hydrogen peroxide and MDA contents

H₂O₂ was estimated by the method of Velikova et al. (2000). For this, 0.25 g of leaf sample was homogenised in 5 ml 0.1% TCA and centrifuged. 500 µL of supernatant from the centrifuged solution was added with 500 µL (10 mM) of potassium phosphate buffer and 1 mL of potassium iodide (1 M) and mixed well. The solution was kept in room temperature for 20 min before observing the OD at 390 nm.

MDA contents were determined by using 5% TCA with the proposed method of Heath and Packer (1968). For this, 250 mg fresh leaf was homogenised with 5 ml TCA (5%) and centrifuged at 4800x for 10 min 500 µL supernatants of the solution was then added with 2 ml thiobarbituric acid (0.5%). This mixture was warmed at 95 °C for 50 min and immediately cooled in an ice bath before taking OD at 600 nm and 532 nm.

2.7. Non enzymatic antioxidants

2.7.1. Carotenoids

For carotenoids content 100 mg leaf sample was homogenised in 10 mL 80% (v/v) acetone. After homogenization, the solution was kept at 4 °C for overnight and OD was measured at 663 nm and 645 nm after bringing the solution at room temperature. Total carotenoids in fresh leaf mg g⁻¹ was calculated by following the formula of Duxbury and Yentsh (1956).

2.7.2. Phenol and flavonoids

Phenol was determined by the method described in Mallick and Singh (1980) using 250 mg leaf sample homogenised in 70% acetone as extracting solution. The solution was centrifuged at 6000x for 10 min before adding 5 ml of 20% sodium carbonate. Final volume of the solution was made 10 ml with distilled water before taking the OD at 750 nm.

Flavonoids was estimated using 100 mg of leaf sample homogenised in 100 ml of ethanol and acetic acid solution (99:1, v/v). After homogenization, this solution was boiled (2 min) and gently cools to room temperature. The optical density was reads from 250 to 350 nm (Cameron et al., 1943).

2.7.3. Proline

Proline content in 500 mg fresh leaf was determined following the method as explained in Plummer (1979) using 10 ml of 3% sulphosalicylic acid. The solution was filtered by Whatman filter paper before adding 2 ml of ninhydrin and 2 ml of glacial acetic acid. The solution was stored at 80 °C for 1 h 4 ml of toluene was added in the solution after cooling and mixed by a stirrer for 15 min. The OD was taken at 520 nm and the standard curve was made using purified proline and presented as mg g⁻¹ fresh leaf.

2.8. Enzymatic antioxidant activities

Enzymatic activities were estimated using potassium phosphate buffer. For this, 0.25 g leave was homogenised in 5 mL of cold potassium phosphate buffer (50 mM, 7.8 pH). The mixture of the solution was centrifuged for 20 min at 12000x at 4 °C. Solution was kept at –20 °C for purpose of the CAT, SOD, POD, and APX estimation:

2.8.1. Superoxide dismutase (SOD)

For SOD activity Van Rossum et al. (1997) procedure was followed. 50 µL supernatant of stored solution was taken and added with 400 µL deionized water, 250 µL (50 mM) potassium phosphate buffer (pH 7.8), 100 µL L-methionine, 100 µL triton-X, 50 µL nitro blue tetrazolium (NBT), 50 µL riboflavin gradually. The absorbance of the mixture was obtained at 560 nm.

2.8.2. Peroxidase (POD) and catalase (CAT) activity

The procedure suggested by Chance and Maehly (1955) was used to assess POD and CAT activity. For POD, 100 µL of supernatant was added with 1.8 mL (50 mM) potassium phosphate buffer, 100 µL (20 mM) guaiacol and 100 µL (40 mM) H₂O₂. The absorbance of the solution was detected at 470 nm every 20 s for 3min. For CAT, 100 mL supernatant was added with 1.9 mL potassium phosphate buffer (50 mM), and 1 mL (5.9 mM) H₂O₂ and the OD was measured at 240 nm every 20 s for 2 min.

2.8.3. Ascorbate peroxidase (APX) activity

APX activity was estimated following the method explained in Nakano and Asada (1981). For this, 3 mL mixture of phosphate (100 mM), EDTA-Na₂ (0.1 mM), ascorbic acid (0.3 mM), and H₂O₂ (0.06 mM) was added to 100 mL supernatant. The OD of the solution was recorded for 30 s intervals at 290 nm. Under assay conditions, one element of APX produces 1 M of ascorbate reacted min⁻¹.

2.9. Statistical analysis

The study was organised in a RBD (randomized block design) with three factor arrangement. The data documented for each parameter was expressed in mean of triplicate with standard deviation using Microsoft excel 2010. DMRT test applied between the treatments to check the significant variation among the same group using SPSS (SPSS Inc., version 17.0) software. The least significant difference and correlation between treatment, cultivar and age factors was calculated with the help

of PCA using Origin Pro2020 software.

3. Results

3.1. Total biomass and root shoot ratio (RSR)

Elevated ozone reduced total biomass of all cotton cultivars than ambient ozone. However, application of EDU suppressed the ozone induced biomass reduction (Fig. 1). Higher reduction of biomass was noted in cultivar ADC-1 followed by cultivars G. Cot. 21 > GADC-2 > G. Cot. 13 > and V-797. Total biomass of cotton plants was highly affected in second year of experiment than first year of experiment. Maximum reduction was noted in cultivar G. Cot. 21 (50.9%) at vegetative stage in first year of experiments and minimum in cultivar V-797 (10%) at juvenile stage in second year of experiment. However, RSR significantly increased by ozone enhancement while found lower under EDU supplementation in all the experimental cultivars (Fig. 1). Maximum RSR enhancement was observed at reproductive stage and least affected at juvenile stage in all the cotton cultivars tested.

3.2. Primary metabolites

3.2.1. SS, RS and starch

Elevated ozone reduced total soluble sugar of experimental plants than ambient ozone while, increased in EDU supplemented plants (Fig. S2). While, reducing sugar was increased under elevated ozone and reduced by EDU treatment (Fig. S2). Maximum reduction of soluble sugar was found in cultivar G.Cot.21 followed by GADC-2 > G.Cot.13 > and V-797. Soluble sugar of cultivar ADC-1 was least affected. Cultivars V-797 and ADC-1 were highly affected at juvenile stage than reproductive > and vegetative stages, cultivars G.Cot.13 and GADC-2 were shows higher reduction at reproductive stage than juvenile > and vegetative stages and cultivar G.Cot.21 was highly pretentious at vegetative > reproductive > and juvenile stages. In case of total reducing sugar, all cultivars showed higher increase at reproductive stage than vegetative > and juvenile stages except cultivar G.Cot.21 which showed higher increasing value at vegetative stage than reproductive > and juvenile stages. Second year of experiment showed more increase of reducing sugar by ozone exposure than first year of experiments. Among the cultivars, trend of increase in total reducing sugar was G.Cot.21 > V-797 > ADC-1 > G.Cot.13 > and GADC-2.

Similar to SS, starch was also reduced under elevated ozone exposure than ambient level of ozone while, EDU application increased the starch content in cotton leaf (Fig. 2). Reduction of starch content was maximum in cultivar V-797 (–68.30%) at vegetative stage and minimum in cultivar G.Cot.13 (–17.90%) at juvenile stage during first year of experiment. As compared to first year of experiment cultivars G. Cot.21, ADC-1 and GADC-2 shows higher reduction in second year of experiments while, cultivars V-797 and G.Cot.13 showed higher reduction during first year of experiment. Among the tested cotton cultivar, overall higher to lower reduction trends was V-797 > GADC-2 > G.Cot.21 > ADC-1 > and G.Cot.13

3.2.2. Soluble protein

Leaf protein of selected experimental cultivars was found least under enhanced level of ozone than ambient ozone > and EDU supplemented plants (Fig. 2). Trend of reduction for total soluble protein was ADC-1 > G.Cot.21 > GADC-2 > G.Cot.13 > and V-797. Ozone sensitive cultivars ADC-1 and GADC-2 show higher reduction of total protein contents at juvenile stage than reproductive > and vegetative stage.

3.3. Oxidative stress

3.3.1. Hydrogen peroxide and MDA contents

Hydrogen peroxide generation was higher in enhanced ozone treated plant than ambient ozone while, EDU supplemented plants having least

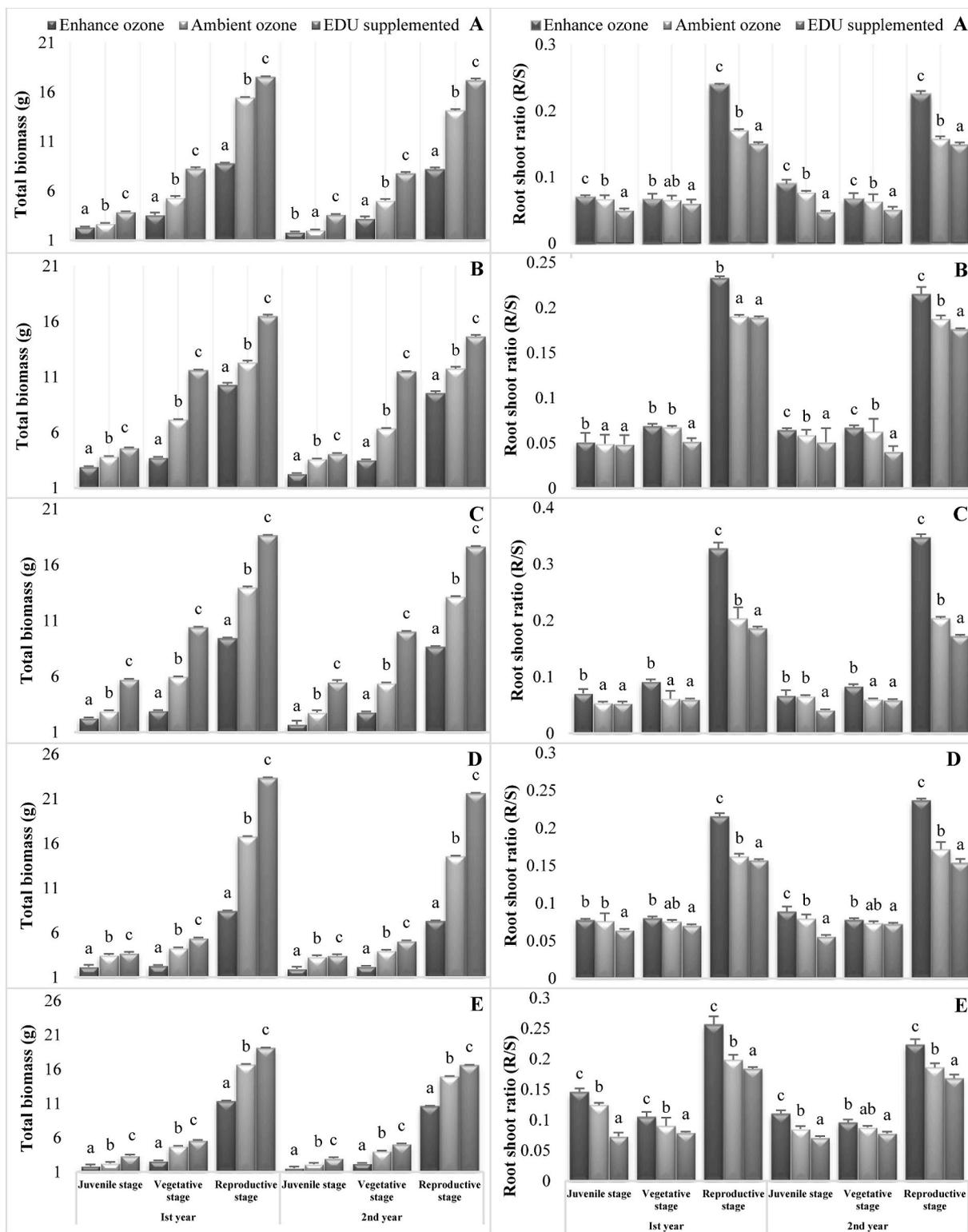


Fig. 1. Effect of ozone on total biomass (g) and root shoot ratio (R/S) of cotton cultivars (A) V-797, (B) G. Cot-13, (C) G. Cot-21, (D) ADC-1 and (E) GADC-2 (Mean ± standard deviation of three replicates presented by thin vertical bars, Value within each column followed by the same letter are not significantly different ($p < 0.05$) using Duncan's Multiple Range Test).

content (Fig. 3). Cultivars wise higher production of hydrogen peroxide was found in GADC-2 > G.Cot.21 > V-797 > ADC-1 >and G.Cot.13. Production of hydrogen peroxide was noted maximum at reproductive than vegetative >and juvenile stage except cultivar G.Cot.21 which showed higher production of hydrogen peroxide at vegetative stage than reproductive >and juvenile stages.

Similar to hydrogen peroxide, elevated ozone also increased the MDA content of leaf than ambient ozone with least content in EDU supplemented plants (Fig. 3). Maximum effect of elevated ozone on MDA contents was seen at reproductive stage than vegetative >and juvenile steps in genotypes V-797, G.Cot.13 and G.Cot.21. Cultivar ADC-1 shows higher MDA content at juvenile than vegetative >and

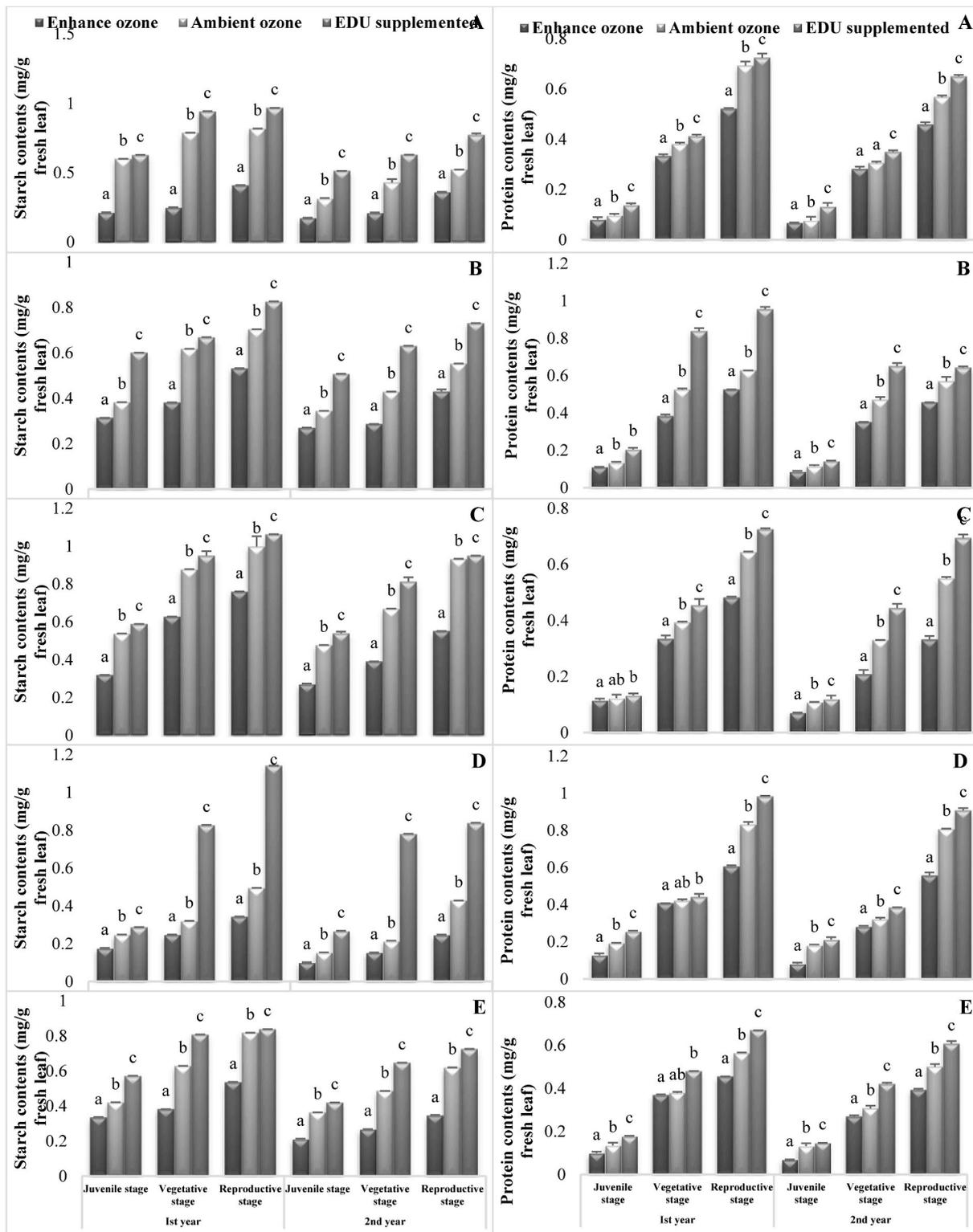


Fig. 2. Effect of ozone on starch contents (mg g^{-1} fresh leaf) and protein contents (mg g^{-1} fresh leaf) of cotton cultivars (A) V-797, (B) G. Cot-13, (C) G. Cot-21, (D) ADC-1 and (E) GADC-2 (Mean \pm standard deviation of three replicates presented by thin vertical bars, Value within each column followed by the same letter are not significantly different ($p < 0.05$) using Duncan's Multiple Range Test).

reproductive stage and cultivar GADC-2 shows higher value of MDA at vegetative than reproductive and juvenile stage. Overall higher value of MDA contents in selected cultivar was noted in GADC-2 followed by G.Cot.21 > V-797 > G.Cot.13 > and ADC-1.

3.3.2. Membrane permeability

Membrane permeability measured as an electrolyte linkage was increased by ozone exposure in cotton leaves (Fig. 4). The maximum leakage was found in elevated ozone than ambient ozone at all stages of plant growth. The membrane permeability of cultivar G.Cot.21 and ADC-1 was greatly affected at juvenile stage than reproductive and

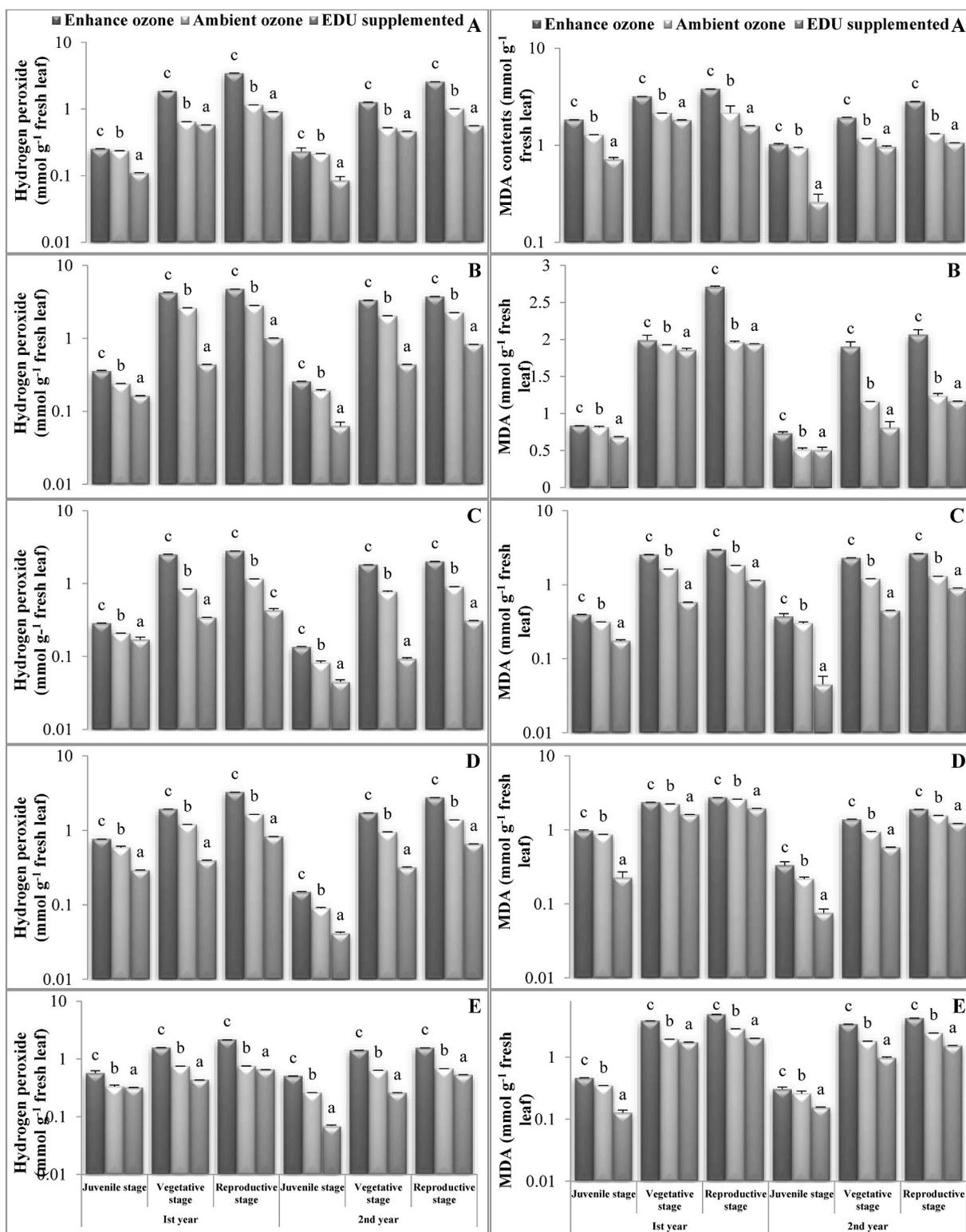


Fig. 3. Effect of ozone on hydrogen peroxide (mmol g^{-1} fresh leaf) and MDA contents (mmol g^{-1} fresh leaf) of cotton cultivars (A) V-797, (B) G. Cot-13, (C) G. Cot-21, (D) ADC-1 and (E) GADC-2 (Mean \pm standard deviation of three replicates presented by thin vertical bars, Value within each column followed by the same letter are not significantly different ($p < 0.05$) using Duncan’s Multiple Range Test).

vegetative stage, cultivar V-797 showed the higher effects at reproductive than vegetative and juvenile stage, cultivar G.Cot.13 was highly affected at juvenile stage followed by vegetative and reproductive stage while, cultivar GADC-2 showed increasing trends from juvenile to reproductive and vegetative stage. Trend of least to higher membrane permeability was GADC-2 > V-797 > ADC-1 > G.Cot.21 > and G.Cot.13 in

experimental cotton cultivars.

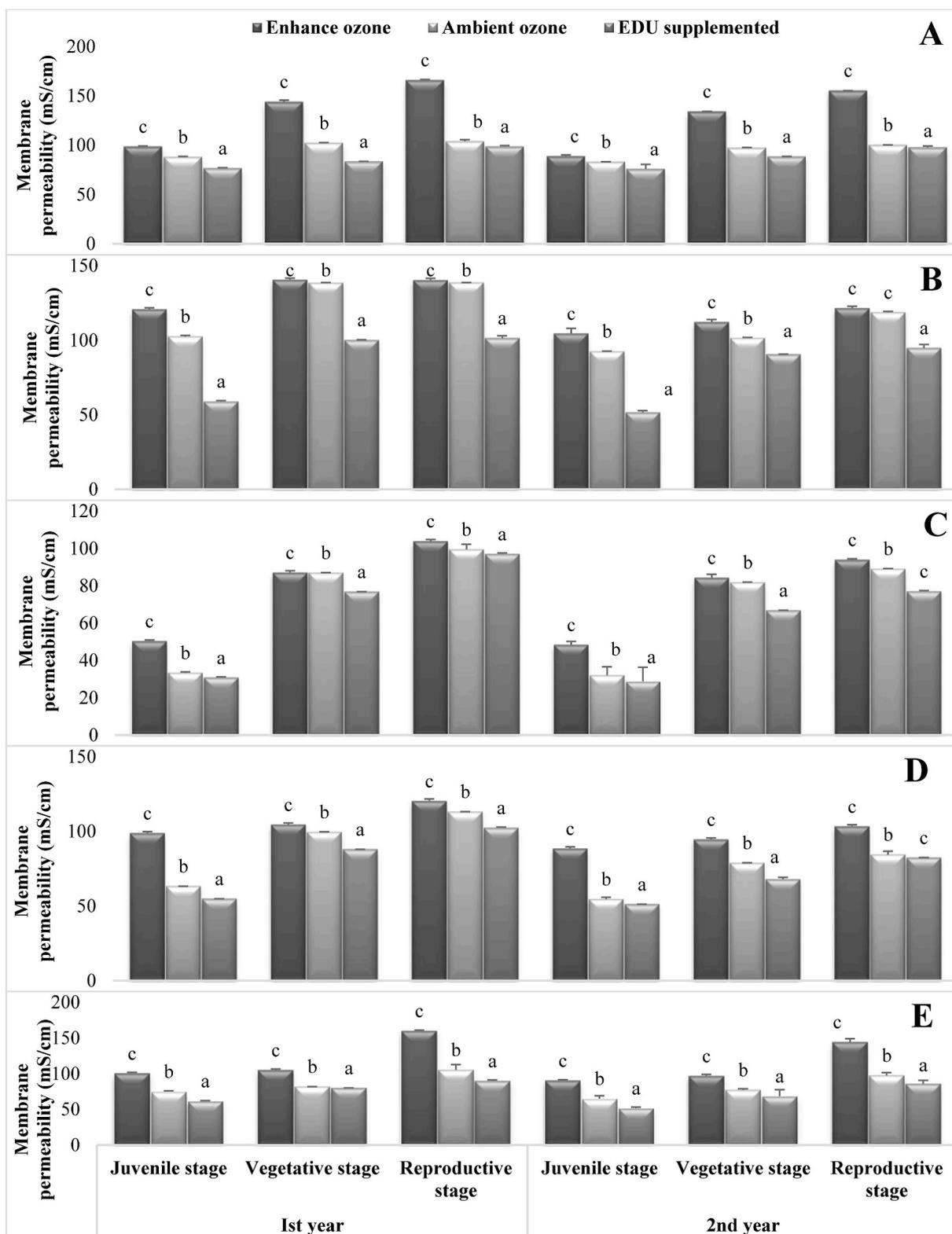


Fig. 4. Effect of ozone on membrane permeability (mS cm^{-1}) of cotton cultivars (A) V-797, (B) G. Cot-13, (C) G. Cot-21, (D) ADC-1 and (E) GADC-2 (Mean \pm standard deviation of three replicates presented by thin vertical bars, Value within each column followed by the same letter are not significantly different ($p < 0.05$) using Duncan's Multiple Range Test).

3.4. Antioxidative defense

3.4.1. Nonenzymatic antioxidants

3.4.1.1. Carotenoids contents. Carotenoids content of experimental

cotton cultivars was negatively influenced by elevated ozone while effect was minimized by EDU treatment (Fig. 5). Reduction of carotenoids under enhanced ozone exposure was observed maximum in cotton cultivar G. Cot.21 while, least in cultivar V-797. Maximum reduction of carotenoid content was noted at juvenile growth stage than vegetative

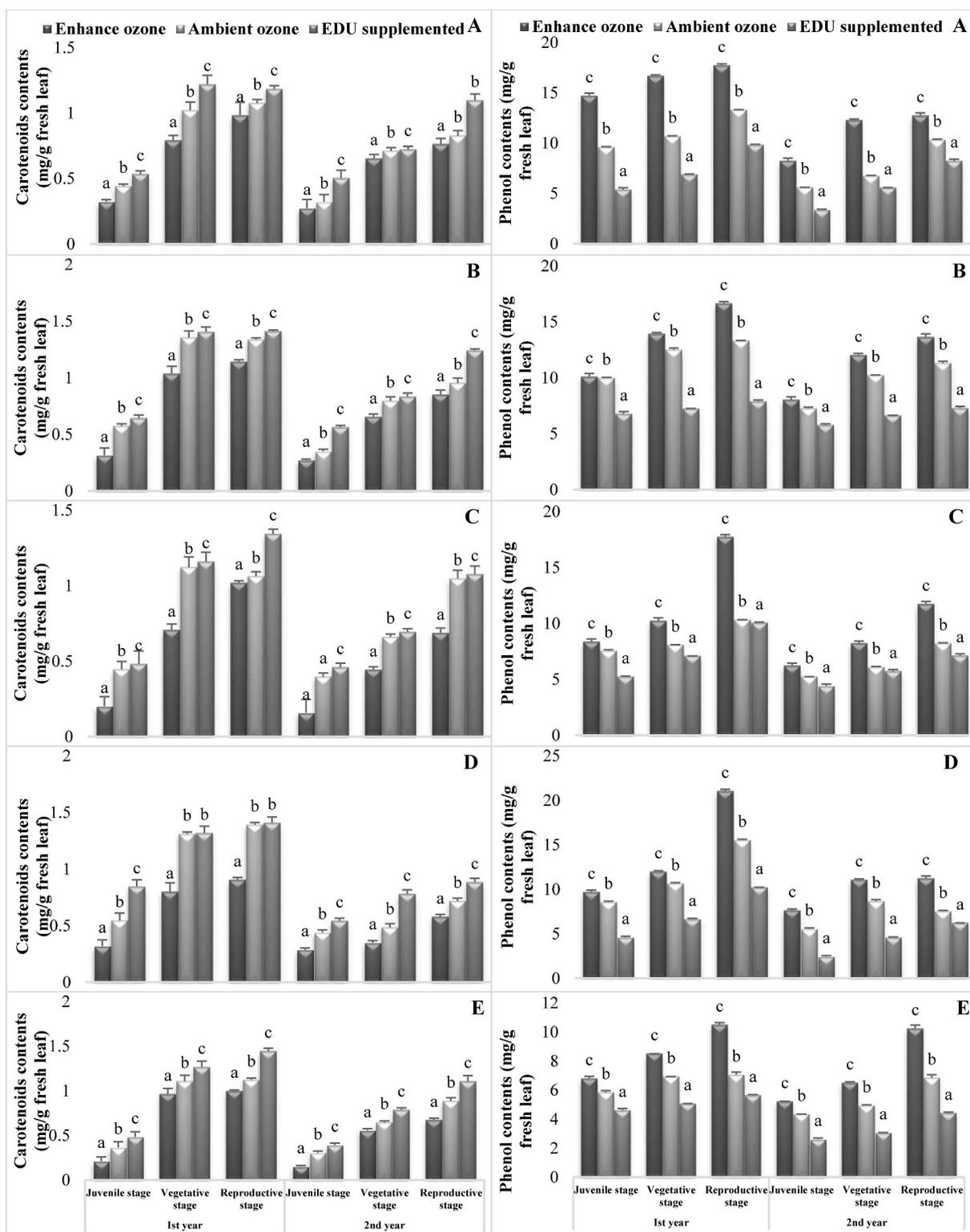


Fig. 5. Effect of ozone on carotenoids contents (mg g^{-1} fresh leaf) and phenol contents (mg g^{-1} fresh leaf) of cotton cultivars (A) V-797, (B) G. Cot-13, (C) G. Cot-21, (D) ADC-1 and (E) GADC-2 (Mean \pm standard deviation of three replicates presented by thin vertical bars, Value within each column followed by the same letter are not significantly different ($p < 0.05$) using Duncan's Multiple Range Test).

> and reproductive growth stage in all the selected cultivars except cultivar G.Cot.21 and GADC-2 which showed higher reduction at juvenile stage followed by reproductive > and vegetative growth stage.

3.4.1.2. Phenol and flavonoids. Cotton cultivar V-797 having naturally higher phenol content while, cultivar G.Cot.13 have minimum phenolic

content (Fig. 5). Exposure of ozone increased phenol content in all the experimental cultivars which was found maximum in enhanced ozone exposed plants followed by ambient ozone > and EDU supplemented plants. Ozone induced increase in phenol higher at reproductive stage than vegetative and juvenile stage of cotton cultivar G.Cot.13, G.Cot.21 and GADC-2, cultivar V-797 showed higher phenol content at juvenile

growth stage than vegetative >and reproductive stage and cultivar ADC-1 showed higher value at reproductive stage than juvenile >and vegetative stages.

Flavonoid content was observed as absorbance per mg fresh leaf and found reduced under ozone exposure (Fig. 7). However, EDU application enhances flavonoids (Fig. 6). Sensitive cultivars showed higher

reduction of flavonoids contents at juvenile than vegetative > and reproductive stages. Higher reduction of flavonoids contents was found in cultivar G. Cot.13 (–43.6%) at vegetative stage in second year of experiment while, cultivar ADC-1 shows lower reduction (–0.21%) of flavonoid content at vegetative stage in first year of experiments. Cultivar wise higher to lower reduction trends of flavonoids content was

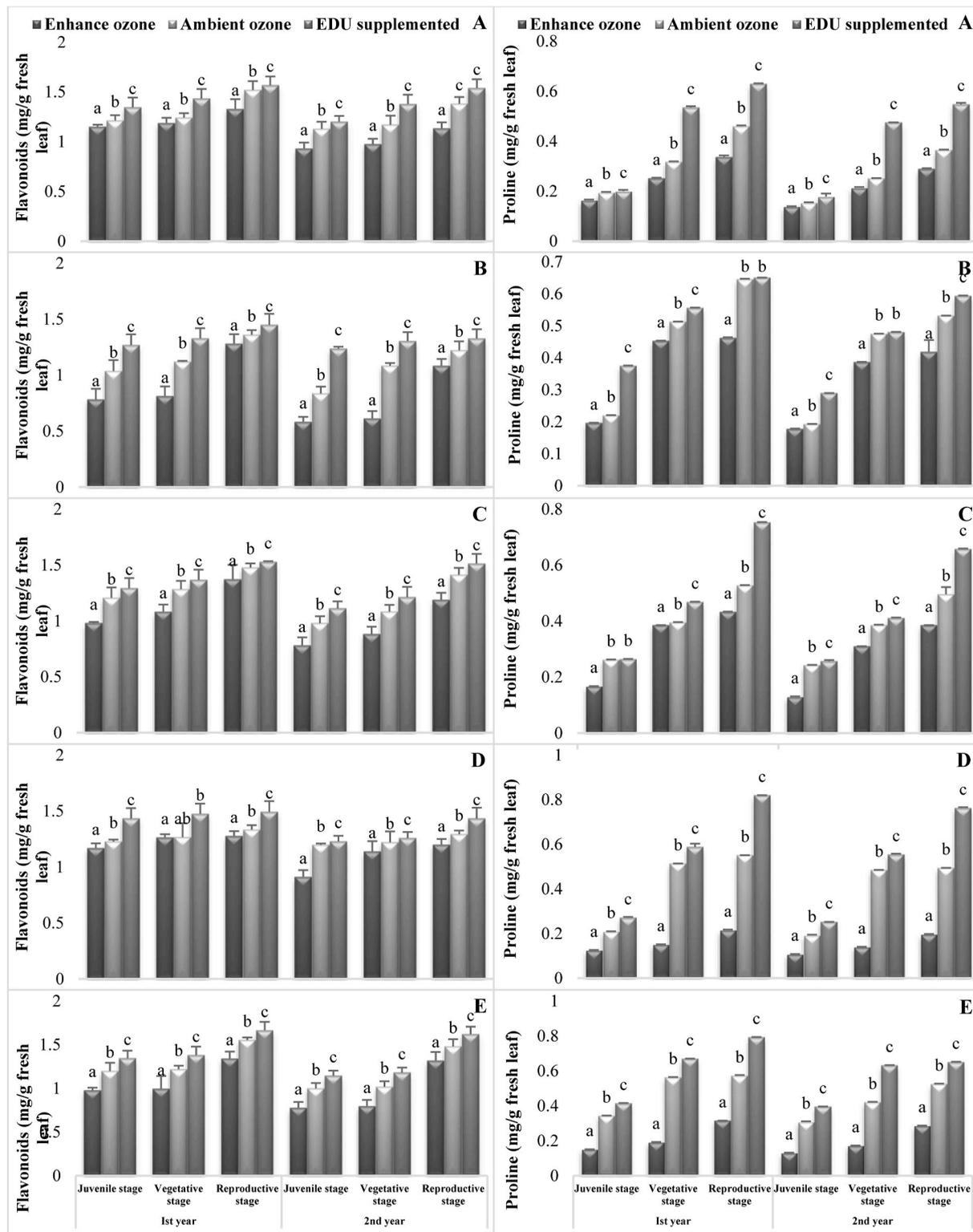


Fig. 6. Effect of ozone on flavonoids contents (A mg g⁻¹ fresh leaf) and proline contents (mg g⁻¹ fresh leaf) of cotton cultivars (A) V-797, (B) G. Cot-13, (C) G. Cot-21, (D) ADC-1 and (E) GADC-2 (Mean ± standard deviation of three replicates presented by thin vertical bars, Value within each column followed by the same letter are not significantly different (p < 0.05) using Duncan’s Multiple Range Test).

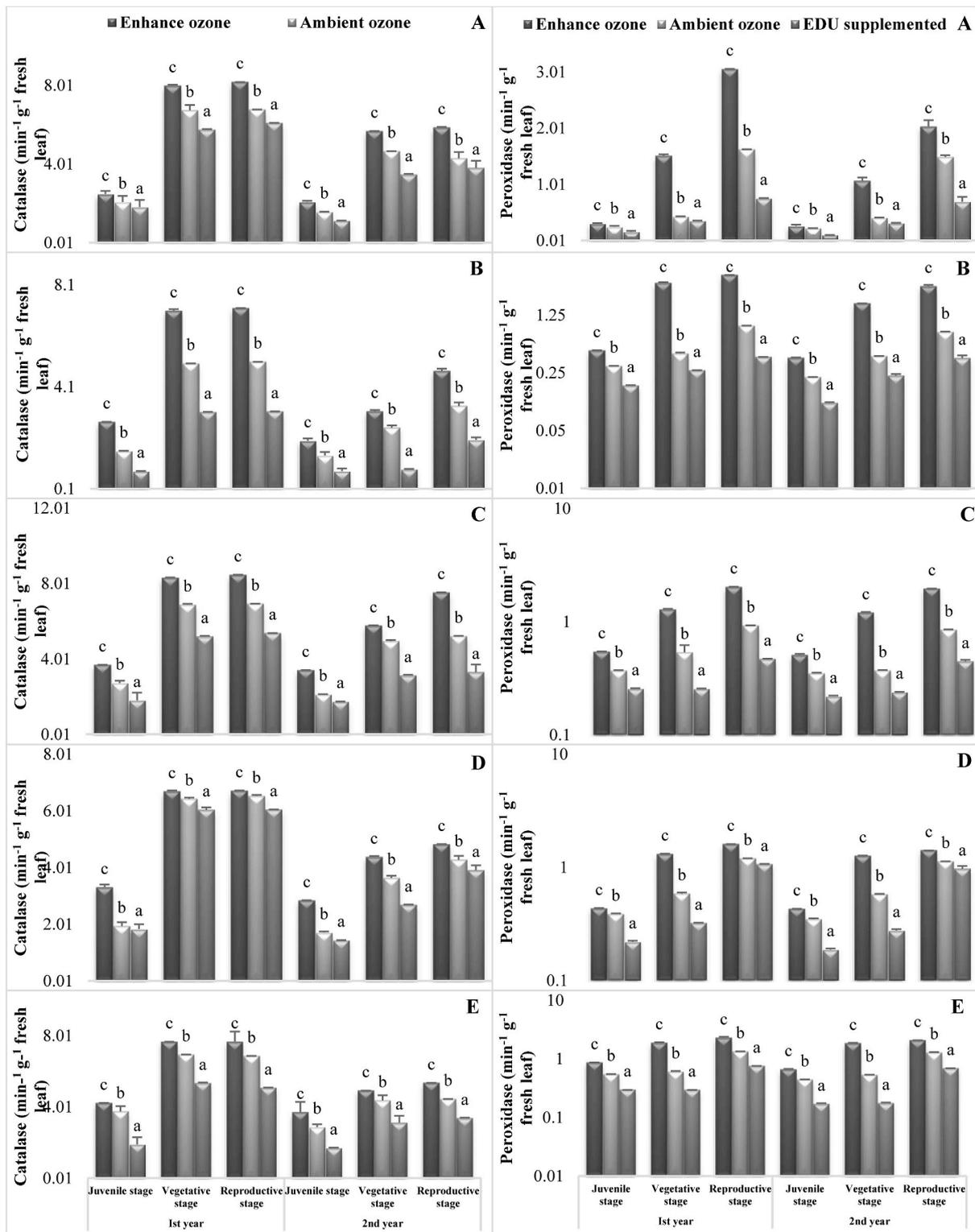


Fig. 7. Effect of ozone on catalase ($\text{min}^{-1} \text{g}^{-1}$ fresh leaf) and peroxidase ($\text{min}^{-1} \text{g}^{-1}$ fresh leaf) of cotton cultivars (A) V-797, (B) G. Cot-13, (C) G. Cot-21, (D) ADC-1 and (E) GADC-2 (Mean \pm standard deviation of three replicates presented by thin vertical bars, Value within each column followed by the same letter are not significantly different ($p < 0.05$) using Duncan's Multiple Range Test).

G.Cot.13 > GADC-2 > V-797 > G.Cot.21 > and ADC-1.

3.4.1.3. Proline. Proline content was also found less in cotton cultivars exposed to elevated level of ozone than ambient ozone and increased EDU supplemented plants (Fig. 6). Proline content was highly affected at reproductive stages in cultivars V-797 and G.Cot.13, cultivars ADC-1

and GADC-2 was highly affected at vegetative stages in both year of experiments while, cultivar G.Cot.24 showed maximum reduction at juvenile stages in both year of experiments. Among the tested cotton cultivars, cultivar ADC-1 having maximum (-71.2%) ozone induced reduction at vegetative stage in second year of experiment while, cultivar G.Cot.21 having least (-2.4%) ozone induced reduction at

vegetative stage in first year of experiment.

3.4.2. Enzymatic antioxidants

3.4.2.1. Catalase and peroxidase activities. Elevated ozone enhanced the activity of catalase and peroxidase in experimental cotton cultivars while, EDU supplemented plants had lower activity of these enzymes (Fig. 7). Catalase activity of experimental cotton cultivars was higher at juvenile phase in both year of experiment except cultivar V-797 which showed higher catalase activity at reproductive phase. Among the selected cultivars, cultivar G.Cot.13 had maximum catalase activity (71.9%) at juvenile stage and cultivar ADC-1 had minimum catalase activity (4.05%) at vegetative stage during first year of experiment. Cultivar wise trend of higher to lower catalase activity was noted in cultivar G.Cot.13 > G.Cot.21 > ADC-1 > V-797 > and GADC-2 respectively.

Peroxidase activity of cotton cultivars follows the trends similar to catalase activity while, age wise variability was observed higher at vegetative stage and lowest at juvenile stages in all selected cultivars (Fig. 7). Enhanced level of ozone increased peroxidase activity in cultivar G.Cot.13 (312%) at vegetative stage while minimum activity was found in cultivar ADC-1 (12.5%) at juvenile stage.

3.4.2.2. Superoxide dismutase and ascorbate peroxidase activities. Enhanced level of ozone of presented experiment also enhances the activity of SOD and APX while, EDU application reduces the activity of these enzymes (Fig. 8). SOD activity was maximum in cultivars G.Cot.13 followed by V-797 > ADC-1 > G.Cot.21 > and GADC-2. Higher SOD activity was noted at vegetative stage than juvenile >and reproductive stage in cultivars G.Cot.13 and G.Cot.21, cultivars GADC-2 shows higher activity at vegetative than reproductive > and juvenile phase and, cultivar V-797 and ADC-1 shows higher activity of SOD at juvenile phase followed by vegetative >and reproductive phase.

Similarly, APX was also increased in elevated ozone and reduced under EDU treatment in experimental plant cultivars (Fig. 8). Maximum activity of APX was found in cultivar G.Cot.13 followed by cultivars G. Cot.21 > GADC-2 > V-797 >and ADC-1. Higher increase of APX was noted at vegetative stage in cultivars G.Cot.13, ADC-1 and GADC-2 while cultivar V-797 shows higher increment at juvenile stage and G. Cot.21 shows higher increasing values of APX at reproductive stage. Maximum increasing value of APX was noted in cultivar G.Cot.13 (262%) at vegetative stage and minimum in cultivar GADC-2 (11%) at reproductive stage.

3.5. Analysis of principle component (PCA)

Total variation of PCAs was found in cotton cultivar V-797 from 45.56% to 31.61% for PC1 and PC2 with eigenvalue 8.65 to 6.00 (Fig. 9). A biplot graph of PC1 and PC2 was prepared for correlation between parameters and treatments showed negative score value for enhanced and ambient ozone at juvenile and vegetative stages while, positive score values at reproductive stages. However, EDU treated plants showed negative score at the juvenile stage and positive score at vegetative and reproductive stages for both PCs. Loading plots of cultivar V-797 for biomass of plant was slightly correlated with protein and TSS at PC1. In cultivar V-797, biomass and physiology such as root shoot ratio, hydrogen peroxide, MDA, membrane permeability, phenol, SOD, POD, and TRS all have positive scores on both PCs. Variability in cultivar G. Cot.13 was 51.07% (eigenvalue 9.70) for PC1 and 36.10% (eigenvalue 6.85) for PC2. The PCA biplot for this cultivar looks a lot like the cultivar V-797 (Fig. 9). However, variability within the treatment was differing in first year of experiment. Biplot score value for all the treatments was negative at juvenile phase while, positive at vegetative and reproductive phases. Proline content showed maximum loading values at both PCs (0.26 for PC1 and 0.17 for PC2). The factor disturbing

the deviation of biomass and enzymatic antioxidant CAT and RS showed strong correlation. MP, and SOD were also strongly correlated with each others. While, POD and starch was strongly correlated to each other. Overall antioxidative potential and biomass and primary metabolites of cultivar G. Cot.13 showed positive values at both PCs. Variability for cultivar G. Cot.21 was 57.81% (Eigenvalue 10.98) for PC1 and 31.44% (Eigenvalue 5.97) for PC2 (Fig. 9). The fact that the EDU treatment's PC value was on the right side of component 1 indicated a strong correlation. The majority of the data produced from the results were grouped on the PC1 and PC2 positive sides. Treatment wise cultivar G. Cot.21 showed similar patterns as cultivar G. Cot.13. Higher score value (4.75) was found in EDU treated plant at reproductive stage in first year of experiment. While higher loading plot value was found in MP (0.27). The phenol loading value was positively associated with treatment at both PCs (PC1 (0.22) and PC2 (0.23)). Plant height, biomass, MDA, flavonoids and starch showed strong correlation. While RSR, proline, TSS and APX was also showed strong relation. Oxidative potential of cultivar G. Cot.21 and antioxidant showed positive values at both PCs. Variability at PC1 and PC2 for cultivar ADC-1 were 53.56% (eigenvalue 10.17) and 30.71% (eigenvalue 5.83), respectively (Fig. 9). In cultivar ADC-1, all parameters including RSR, H₂O₂, MDA, MP, phenol, TRS, and enzymatic activities (CAT, SOD, POD, and APX) shows negative loading values at PC2. While root shoot ratio, hydrogen peroxide, MDA, MP, phenol, TRS, and enzymatic activities (CAT, SOD, POD, and APX) had positive loading values at PC1 too. MP was positively associated with TSS and TRS, while biomass, hydrogen peroxide, and phenols were all highly correlated. Protein, APX, and CAT all have a close relationship with one another. GADC-2 had a difference of 53.28% at PC1 and 35.38% at PC2, with eigenvalues of 10.12 and 6.72, respectively, at PC1 and PC2 (Fig. 9). Cultivar GADC-2 showed positive relation of all parameters at PC1. Protein and MDA content of cultivar GADC-2 showed strong relation while biomass was slightly correlated with these parameters. Hydrogen peroxide, POD and flavonoids of this cultivar was also showed strong relation with each others. Plant height, TSS and carotenoids was strongly correlated while phenol, starch and SOD was highly correlated. Higher loading plots value was noted in APX and TRS (0.27). Antioxidant such as enzymatic and non enzymatic activity was showed strong correlation. But for juvenile stages in both procedures, improved ozone treatment score values were found on the negative side of both PCs, and ambient ozone and EDU applied plants shows positive side score values. The PCs score of atmospheric ozone at vegetative growth phases for all cultivars in this research was closer to the plot's middle, indicating the strongest correlation.

4. Discussion

4.1. Effects of ozone on cotton cultivars

Increased concentration of ambient ozone affects the vigour of sensitive plants. Most important consequence of ozone is seen as the reduction of productivity (Einig et al., 1997). Ozone induced crop loss is well documented (Agrawal et al., 2005; Ainsworth, 2016; Ainsworth et al., 2012; Rathore and Chaudhary, 2019) suggested the severity of this pollutant for global economic development and to achieve the sustainable development goal. Plant biomass accumulation is key characteristic to assess the overall plant development and resistance under the prevailing environmental condition. The ozone levels of the presented study is high enough to cause toxicity and reduce crop productivity (Ainsworth, 2016; Ashrafuzzaman et al., 2017) as seen by reduced biomass accumulation of all the cotton cultivars. However, the ozone induced biomass reduction was differed among the cultivars and at diverse growth stages suggesting that the sensitivity to ozone is cultivar centric and dependent on their growth stages. Broadly, all the experimental cultivars showed higher sensitivity at early growth stage and developed resistance to the ozone stress with growth. However, potency of the developed resistance depends on the genetic make-up of the cultivar.

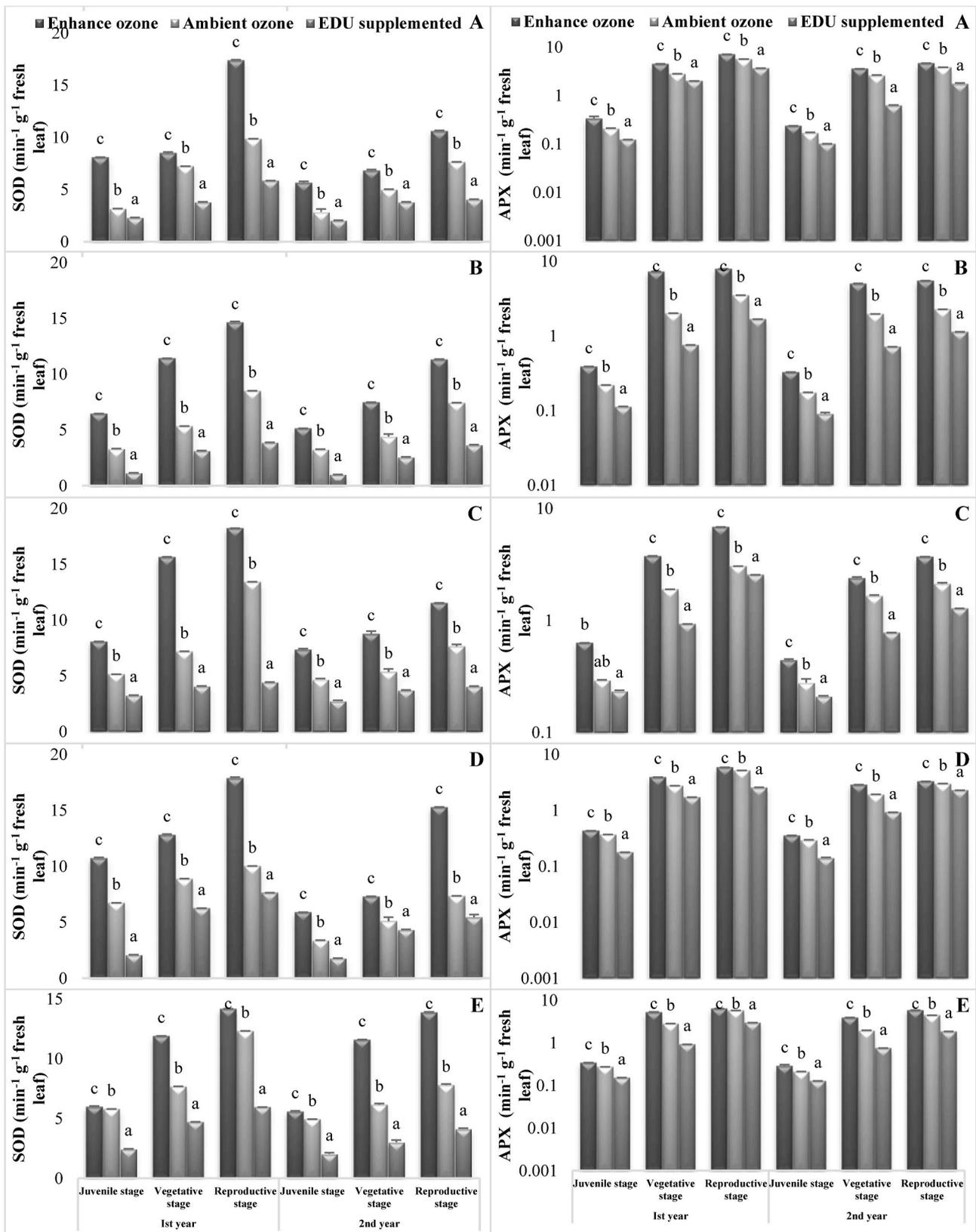


Fig. 8. Effect of ozone on SOD ($\text{min}^{-1} \text{g}^{-1} \text{fresh leaf}$) and APX ($\text{min}^{-1} \text{g}^{-1} \text{fresh leaf}$) of cotton cultivars (A) V-797, (B) G. Cot-13, (C) G. Cot-21, (D) ADC-1 and (E) GADC-2 (Mean \pm standard deviation of three replicates presented by thin vertical bars, Value within each column followed by the same letter are not significantly different ($p < 0.05$) using Duncan's Multiple Range Test).

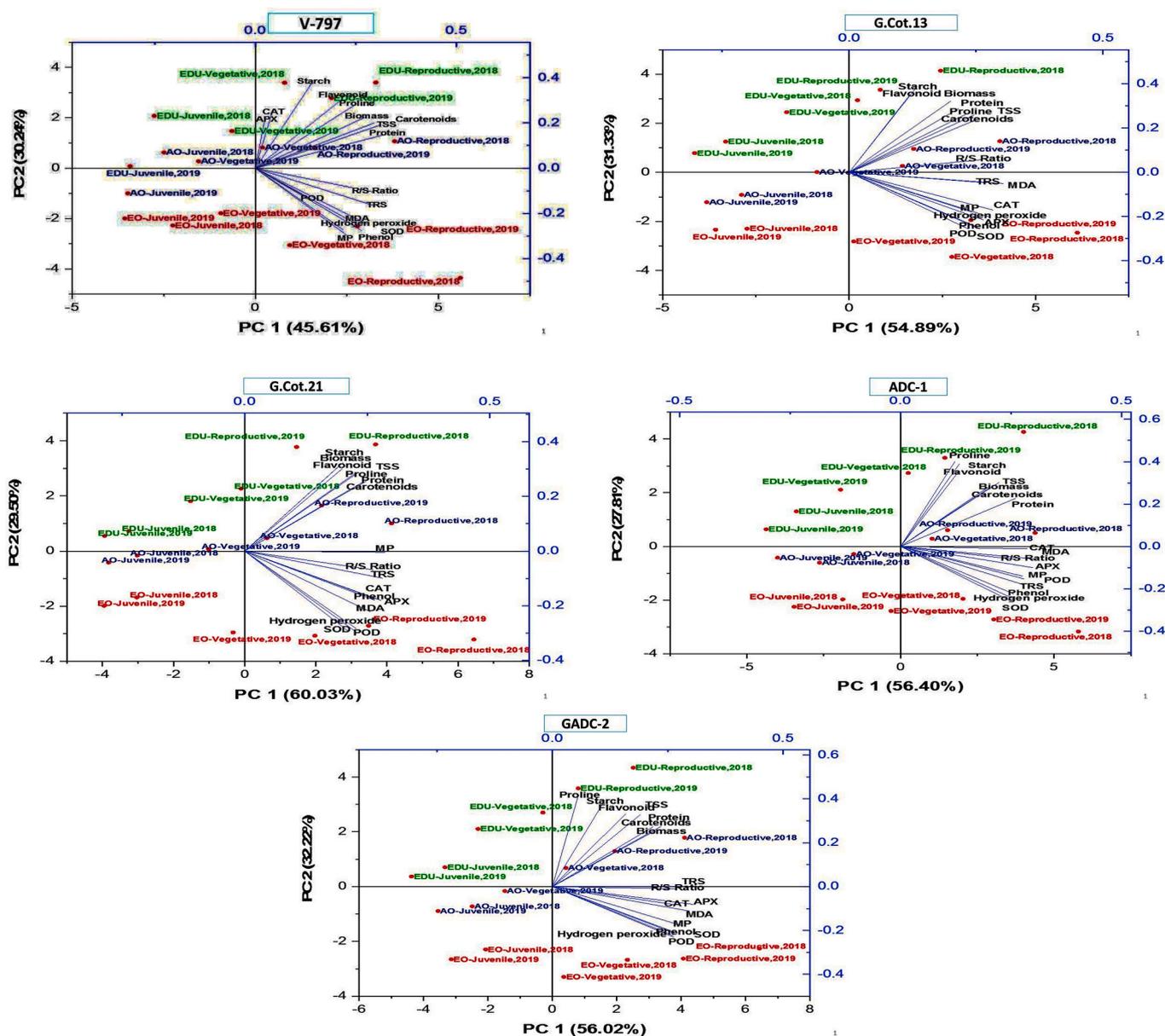


Fig. 9. Principle component analysis (PCA) correlation bi-plot of growth, biomass and biochemical responses to ozone exposure and EDU treatments. Symbol represent the standardized scores on PC1 (x-axis) and PC2 (y-axis) for the ozone exposure and EDU treatment of *Gossipium hirsutum* L. (cv. V-797, G. Cot.13, G. Cot.21, ADC-1 and GADC-2). Vector coordinates represent the correlations between standardized variables and principle components (PCs).

Feng et al. (2016) recommended that the special effects of ozone are dependent on the plant’s growing stage based on their observations from winter wheat. Moreover, increased RSR under ozone suggested more effect on above ground part or higher allocation of photosynthate towards the below ground part under ozone stress. However, Zheng et al. (2013) suggested that the elevated ozone can change soil properties and affect plant physiology. As a consequence, nutrient absorption properties as well as transfer and translocation of carbohydrates among source such as leaves to sink organs roots and grains are frequently delayed by ozone. Chen et al. (2018) also reported decreased biomass of *Phoebe bournei* and *P. zhennan* under ozone exposure however, no significant effect of ozone on RSR was seen in either species. Higher reduction of biomass of cultivar ADC-1 under ozone exposure suggested its higher sensitivity to ozone while, cultivar V-797 is less sensitive to ozone with least biomass reduction.

Detrimental effect of ozone on carbon metabolism, carbon allocation including changes in the balance of structural and non-structural carbohydrate is another major consequence of ozone pollution (Sild et al.,

2002; Chen et al., 2018). All the experimental cultivars used in the presented study showed reduction in soluble sugars, starch and protein while, increase in reducing sugar. Least affected soluble sugar of ADC-1 suggested it’s less partitioning for repair and detoxification thus higher sensitivity to ozone. This result supported the hypothesis of Gupta et al. (2021) which suggested increased sugar catabolism as the partitioning of sugars due to detoxification and repair of damage caused by oxidative stress. Ahsan et al. (2010) explained that reduction in photosynthesis under long term ozone exposure mobilize starch to sugar for plant’s energy requirement. This is in line with increased reducing sugars and reduced starch of ozone cultivars under ozone exposure. Singh and Rathore (2020) suggested role of transitory starch for defence under abiotic stress however, presented study has not found any direct correlation of starch reduction with ozone tolerance. Moreover, ozone induced ROS damages many protein (Fiscus et al., 2005) might be the reason for reduced protein of experimental cotton cultivars.

4.2. Ozone induced oxidative stress

Generation of ROS in plant cells is a natural process. However, generation of ROS increased in the internal tissue of plants many fold under stress condition (Rathore and Chaudhary, 2021). Ozone is a strong oxidant that enters through the stomatal opening and produced enormous amount of ROS in internal parts of plant cells (Tiwari and Agrawal, 2018; Rathore and Chaudhary, 2021). Higher production of H₂O₂ in the plant cells, damage cell membrane and produced higher level of MDA contents and finally caused oxidative damage to the cell (Birben et al., 2012; Sharma et al., 2012; Chaudhary and Rathore, 2020). Present study with cotton cultivars showed increased concentration of hydrogen peroxide and MDA under exposure of ozone. The concentration was greater in elevated ozone fumigated plants than ambient ozone and, EDU supplemented plants have lower hydrogen peroxide in experimental cultivars. Moreover, increased permeability of the cotton cultivars under ozone was possibly due to higher lipid peroxidation (MDA) of the all cultivars. The production of H₂O₂ also increased with age of plants when exposed to atmospheric and acute ozone, meaning that they are all ozone sensitive. The least heterogeneity at a later growth stage, on the other hand, indicated that cultivars had effective ROS scavenging potential at earlier growth stages, decreasing the likelihood of a long-term cumulative effect. This is further confirmed by the decreased MDA with plant age. Caregnato et al. (2010) explained that the plant growth and biomass were decreased due to MDA aggregation, which is caused by lipid peroxidation induced by oxidative stress. Higher membrane permeability was found in sensitive germplasm of genotype ADC-1 at a juvenile phase. This appearance is additional confirmation by less biomass of germplasm of genotype ADC-1 (Fig. 1). Cultivar V-797 shows higher biomass and moderate membrane damage represents tolerance in nature. While cultivar G.Cot.13 was the second most tolerant cultivar against ozone stress.

4.3. Antioxidative defensive system

4.3.1. Non enzymatic antioxidants

Plants having self-defensive mechanism which is responsible for the protection of plants to sustain under stress conditions. Antioxidants of plants perform a vital role in the defense by scavenging the ROS and are able to counteract the critical effects on plants (Barna et al., 2012). Secondary metabolites such as phenolic and flavonoids compounds are the first line of defence in plants toward environmental stress (Lin et al., 2016; Chaudhary and Rathore, 2020). According to Manning et al. (2011), ozone and reactive oxygen species attack phenolics and ascorbate of plants. Presented study also found the reduction of phenolics in cotton cultivars that can be explained as its consumption by either or both of these. Flavonoids act as free radical neutralizers and ROS scavengers (Lvdal et al., 2010) which was found higher under ozone stress in present experiment. In the current experiment, germplasm with less apoplastic ascorbate had a greater susceptibility to ozone pollution and further react as reduction of plant biomass. Proline, an osmolyte and a scavenger of ROS (Anjum et al., 2011; Liang et al., 2013), was higher in EDU supplemented plants than ambient > and enhanced ozone suggesting its potential role under ozone stress. Plants with higher proline content is found to be more resistant to ozone stress in presented experiment as the reduction of proline was higher in sensitive cultivar ADC-1 while tolerance cultivar V-797 > and G. Cot.13 showed a lower reduction rate. Proline's protective role in ozone stress is further supported by the lower reduction of proline content in enhanced ozone exposed plants.

4.3.2. Enzymatic antioxidants

Enzymatic antioxidants work as the eliminator of free radicals by breaking them down. The occurrence of cofactors in plants such as mineral metals (Cu, Zn, Mn and Fe), enzymes function as antioxidants by converting toxic oxidative products to H₂O₂ and water (Nimse and Pal,

2015). In the presented study elevated ozone increases the activity of antioxidative enzymes (SOD, CAT, APX, and POD) of cotton cultivars. Enzymatic activity plays an important role to eliminate ROS (Rathore and Chaudhary, 2021). Sensitive cultivars of cotton shows higher activity of CAT at juvenile phase than vegetative > and reproductive phases. While, ozone tolerance cultivars shown higher activity at reproductive phase than vegetative > and juvenile phases.

Activity of SOD in the cotton plants of the presented experiment was satisfactorily found greater under elevated ozone > and ambient ozone. SOD plays important role in plants provides protection to plants against stress by catalyzing the ROS into oxygen and H₂O₂ which is scavenge by catalase and peroxidases (Tripathy and Oelmüller, 2012; You and Chan, 2015; Singh and Rathore, 2018). Dismutase can be led to hydrogen peroxide, which can then be converted to oxygen and water by catalase activity (Day, 2009; Weydert and Cullen, 2010; Rathore and Chaudhary, 2021). Activity of SOD and CAT was increased and presented higher correlation with H₂O₂ and MDA of plant at all growth phases. Rathore and Chaudhary (2019) reported higher increase of CAT, POD, SOD, and APX in castor cultivars.

Catalase is a type of protein that is restricted to peroxisomes in cells and it is responsible for removing H₂O₂ formed by oxidases peroxisome (Schrader and Fahimi, 2006). The heme of peroxidases including APX and thiol-dependent peroxidases were also used by plants (Foyer and Noctor, 2009). According to accessible information, the most common H₂O₂ rising peroxidase called APX found in plants (Ishikawa and Shigeoka, 2008), which is utilises the ascorbic acid by the donor of hydrogen (Agrawal and Rathore, 2007). APX was reported in the stroma, thylakoid membrane and microbody called sAPX, tAPX and mAPX respectively, (Yoshimura et al., 2000; Yadav and Sharma, 2016). In the present experiment, tolerant cultivars of cotton having higher activity of APX at the initial growth stage compare to the later growth stage may be due to the ozone dose. While, the minimum activity of APX was found at the initial stage in sensitive cultivars. In general, increased concentration of ozone increased ROS generation, MDA content and membrane permeability. To counteract this condition, activity of antioxidative enzymes viz. CAT, POD, SOD, and APX was also increased. However, this activated defense was not sufficient to protect the plant from generated ROS which in turn resulted into loss of plants biomass in all the cotton cultivars.

4.4. Analysis of effect of ozone and it's correlation with defense system

PCAs define that the variability of plant growth with treatment and age. PCAs analysis confirmed that the ozone tolerant cultivars showed strong relation with biomass, protein, carbohydrate and plant antioxidants. Statistical analysis, however, indicates that there is a lot of variance with different therapies at different periods of development (Singh and Rathore, 2018; Rathore and Chaudhary, 2021). By reducing the dimensionality, PCA has also elaborated details regarding cultivars, treatments, and plant ages with reference from wide dispersed results (Xu et al., 2016). Among all the cotton cultivars tested, biomass, stress markers and metabolites are clustered close to the EDU treatment at positive side of PC1 while, ROS and its markers and antioxidative enzymes clustered at negative side of PC1 close to ozone treatment explaining strong correlation between ozone stress, ROS generation and antioxidative potential of the plants. Conclusively, increasing ozone increased enzymatic activity, phenolic compound, membrane permeability and MDA contents in selected cotton cultivars while, decreases total plant biomass, and primary metabolites such as sugar and protein (Fig. 9). PCA biplot showed However cluster analysis of presented experiment not showing any conclusive evidence of intraspecific variation, the PCA associated biplot showed a strong correlation among total biomass, sugar, proline, flavonoid, and protein of leaves in all selected genotypes of cotton at the reproductive stage, (Fig. 9). Moreover, tolerant cultivar V-797 showed positive relation with defensive mechanisms and biomass accumulation. While, correlation between

parameters were relatively low in sensitive cultivars suggesting that the ozone pollution affect on growth and physiology of cotton cultivars is age and cultivars dependent.

5. Conclusion

The findings of presented study confirmed that the elevated ozone induced a greater decrease in biomass than ambient ozone, while EDU reduces the ozone toxicity. Further, the experimental cotton cultivars of presented study showed intra-specific variations for ozone sensitivity. Moreover, the sensitivity was depending on plants growth stages. However, developed antioxidative defence reduces the ozone toxicity. On the basis of both year biomass reduction cultivar ADC-1 as the most sensitive cultivar, cultivars G. Cot.21 > GADC-2 and G. Cot.13 is moderate sensitivity and cultivar V-797 is the least sensitive cultivar for ozone stress. Study concluded that the sensitivity to ozone in cotton cultivars depend on the scavenging of ROS. Further, study recommended cultivar ADC-1 as an assessment tool for ozone and cultivar V-797 for cultivation at ozone prone areas to minimize the agricultural loss.

Contribution

D. Rathore conceived the idea. D. Rathore and I.J. Chaudhary designed the study. I.J. Chaudhary performed the experiments and analyzed the data. D Rathore and I.J. Chaudhary wrote the manuscript. Both authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.plaphy.2021.06.054>.

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આત્મનિર્ભર ભારત

મુખ્ય લેખ

ગ્રામીણ ઉત્પાદનોનું ભૌગોલિક સંકેત સાથે જોડાણ
જી. આર. ચિતાલા, કાનેજી મણી અને સુરેન્દ્ર બાબુ

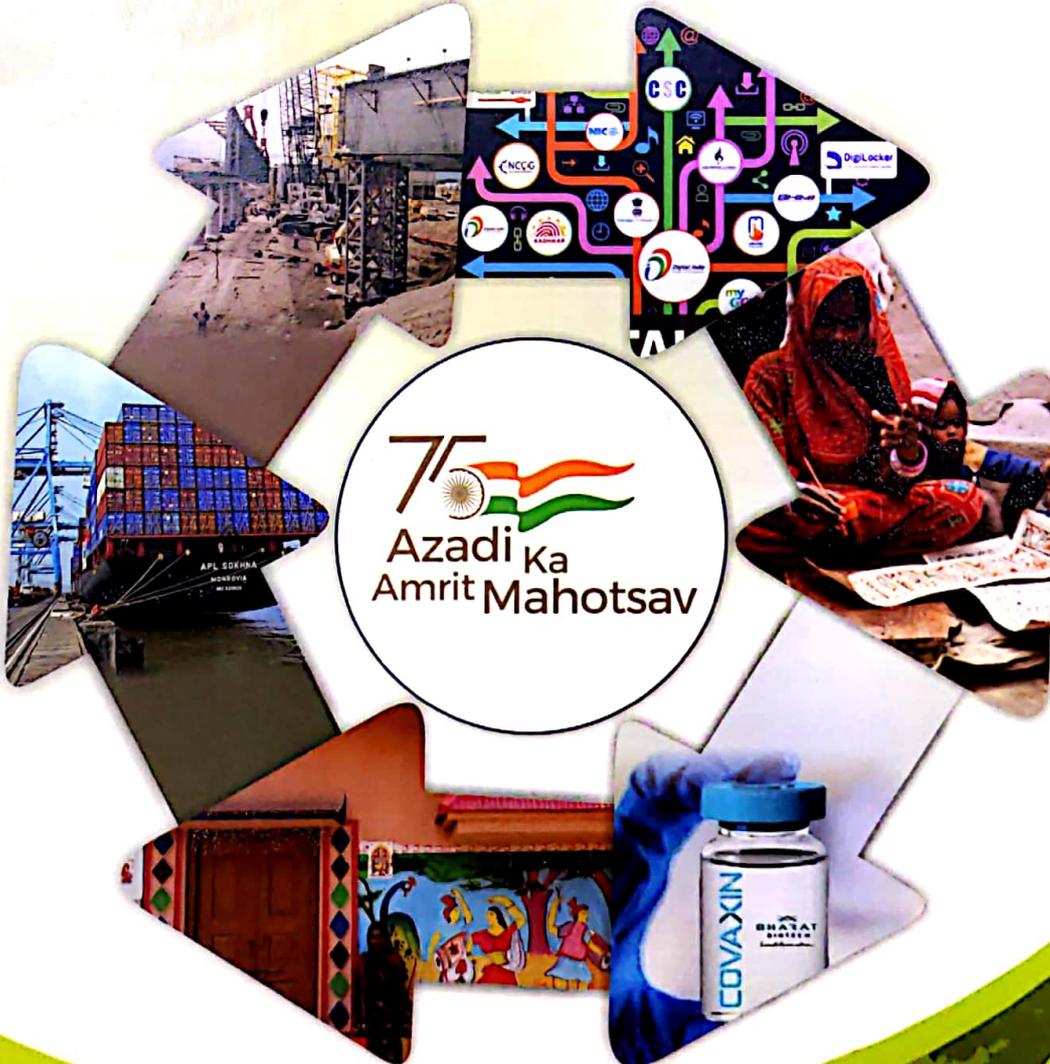
વિશેષ લેખ

‘ઉત્કૃષ્ટતાના કૈન્દ્રો’ તરીકે PSU
પ્રવીણ કુમારી સિંઘ અને ત્રિશલજિત સેઠી

ફોકસ

‘હર ઘર જલ’
યુગલ જોશી

ઊર્જા ક્ષેત્રમાં આત્મનિર્ભરતા
ડૉ. અમિયા કુમાર મોહપાત્રા અને
તમન્ના મોહપાત્રા

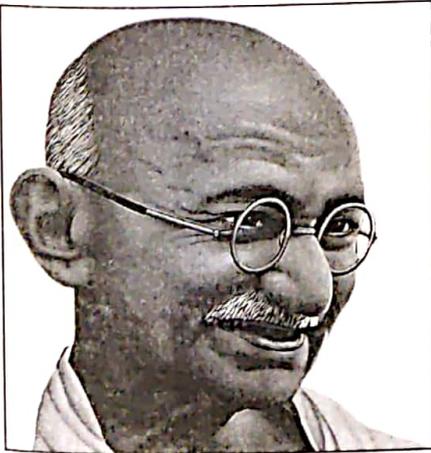


આઝાદીના આંદોલનમાં ગુજરાતી સાહિત્યકારોનું યોગદાન

ડૉ. રાજેશ મકવાણા

ગાં

ધીજી દક્ષિણ આફ્રિકાથી ભારત આવે છે અને સમગ્ર દેશમાં એક ચેતનાની લહેરના ઊઠે છે. ચંપારણમાં ખેડૂતોની સમસ્યાને દૂર કરવા માટેના સત્યાગ્રહથી માંડીને આઝાદીના સમયકાળ સુધી એમનું બહુમૂલ્ય યોગદાન રહ્યું છે. એક લાંબા સમયપટ પર દૃષ્ટિ કરીએ તો હવે ૧૯મી સદીનો અંત આવવાની સાથે અંગ્રેજોનું સામ્રાજ્ય સ્થિર થઈ ગયું. આ તબક્કે ભારતના વિચારક્ષેત્ર અને કાર્યક્ષેત્રમાં ગાંધીજીનું આગમન મહત્વની ઘટના છે. દક્ષિણ આફ્રિકાના સત્યાગ્રહ પછી ઈ.સ. ૧૯૧૫માં ગાંધીજી સ્વદેશ આવ્યા ત્યારથી ૧૯૪૦ સુધીના સમયકાળમાં ખાસ કરીને ગુજરાતી ભાષા સાહિત્ય તેમજ વિવિધ ક્ષેત્રમાં એમનો વ્યાપક પ્રભાવ પડે છે. ગાંધીજીના પ્રભાવને કારણે મોટા ભાગનું કાર્ય ગુજરાતી ભાષામાં થયેલું છે, જેના કારણે સાહિત્ય અને કવિતામાં પણ ભાષાની પકડ મજબૂત બને છે. 'કોશિયો સમજી શકે તેવી સીધી-સાદી, સરળ ભાષા'ને સાહિત્યમાં સ્થાન મળ્યું અને લોકબોલીનો ઉદય થયો. કવિતાક્ષેત્રે નવાં પરિમાણો ઊભાં થવા લાગ્યાં. દલિત, શોષિત,



યોજના, ડિસેમ્બર-૨૦૨૧

પીડિતની વ્યથાને ઉજાગર કરવાનું કામ ભાષા-સાહિત્ય-કવિતા દ્વારા થયું. જીવન અને સાહિત્યના આ પારસ્પરિક પ્રબળ પ્રભાવના કારણે સ્વાતંત્ર્ય સંગ્રામ અને સાહિત્ય વચ્ચેનો સંબંધ સમજવો સરળ બને છે. સ્વાતંત્ર્ય સંગ્રામ પ્રજાના સૌથી પ્રબળ પુરુષાર્થનું પ્રતીક બની રહે છે. આથી સ્વાતંત્ર્ય સંગ્રામ દેશના સમાજના જીવનનો એક મુખ્ય પ્રવાહ બની રહે છે. આથી સ્વાતંત્ર્ય અર્થે ઉત્કટ ભાવના, વિચાર અને કલ્પના સાહિત્યમાં સહેજે પ્રતિબિંબિત થાય છે અને નિબંધ, કવિતા અને નાટક જેવાં તમામ સ્વરૂપોમાં તે ભાવ પ્રગટ થતા રહે છે. ગાંધીજી ગુજરાતી ભાષાના સર્જક હતા. એમના વિચારો સમકાલીન સર્જકોએ ઝીલ્યા અને ગાંધીયુગ અસ્તિત્વમાં આવ્યો. ગાંધીજીની વિચારધારાને કેન્દ્રમાં રાખીને મેઘાણી, સુંદરમ્, ઉમાશંકર, રા.વિ. પાઠક, ર.વ.દેસાઈ, શ્રીધરાણી, ઈન્દુલાલ ગાંધી વગેરે અનેક સર્જકોએ સમકાલીન ઘટનાને પોતાની કવિતા, નવલકથા, ટૂંકીવાર્તા જેવા સાહિત્યસર્જનમાં વાચા આપી હતી. મેઘાણી રચિત 'સિંધુડો'ને તો અંગ્રેજ સરકારે પ્રતિબંધિત કરેલ. આવી અનેક ઘટના એ સમયમાં બની હતી કે જેણે આઝાદીના આંદોલનમાં પ્રભાવક અસર ઊભી કરી હતી. ગાંધીજીના વ્યક્તિત્વ અને વિચારોએ ગુજરાતી સાહિત્યને પ્રભાવિત કર્યું હતું. ગાંધીયુગના સાહિત્યમાં દેશપ્રેમ, આઝાદીની તમન્ના દેશ માટે કંઈક કરી છૂટવાની ભાવના પ્રતિબિંબિત થઈ છે. ગાંધીજીના સત્યાગ્રહો અને એની પ્રભાવક અસરથી કાકાસાહેબ કાલેલકર, કિશોરલાલ મશરૂવાળા, મહાદેવભાઈ દેસાઈ, સ્વામી આનંદ વગેરેએ ચિંતનાત્મક ગદ્ય ક્ષેત્રે મહત્વપૂર્ણ યોગદાન આપ્યું છે. ગાંધી વિચારધારાના સર્જનમાં ગાંધીજી પ્રબોધી

ભાવનાઓ વ્યક્ત થઈ છે. ડૉ. શાંતિલાલ મ. દેસાઈ નોંધે છે કે, '૧૯૩૦ની ઘાંડીકૂચે અને તે વેળાના સત્યાગ્રહે સાહિત્યના સીમાડાનો ભારે વિસ્તાર કર્યો તેમ નવલાં ખેડાણ કર્યાં અને સાહિત્યમાં ગાંધીયુગને મધ્યાહને પહોંચાડ્યો. ગાંધીયુગનાં મંડાણ કેવળ રાજકારણમાં જ નહીં, પરંતુ સાહિત્યમાં પણ થયાં.' રમણલાલ વસંતલાલ દેસાઈના વ્યક્તિત્વ ઘડતરમાં ગાંધીજીના સ્વાતંત્ર્ય પ્રાપ્તિના કાર્યક્રમોનો મહત્વનો ફાળો રહ્યો છે. એમની નવલકથાઓમાં ગુજરાતી પ્રજાના મધ્યમ વર્ગના જીવન અને પાત્રો માટે જાણીતી છે. ૧૯૩૦ના મીઠાના સત્યાગ્રહ દ્વારા આખા દેશમાં વ્યાપી ગયેલ ગાંધીજીના વ્યક્તિત્વ વિચારણાને કાર્યક્રમોનું દસ્તાવેજ નિરૂપણ કરતી 'દિવ્યચક્ષુ' નવલકથામાં રાષ્ટ્રીય ઉત્થાન નિમિત્તે સત્યાગ્રહ, અસ્પૃશ્યતા નિવારણ, અહિંસા વગેરે બાબતોને સાંકળી લીધી છે. રમણલાલ વ. દેસાઈની લોકપ્રિય નીવડેલી પ્રસ્તુત નવલકથામાં સ્વદેશી આંદોલન, સ્ત્રી-સ્વાતંત્ર્ય, સભા-સરઘસો, પોલીસના અત્યાચાર, અંગ્રેજ અમલદારોની જોહુકમી વગેરે એ જમાનાની અનેક પ્રવૃત્તિઓને સ્થાન મળ્યું છે. 'ગ્રામલક્ષ્મી' રમણલાલ વ. દેસાઈની ૧,૨૩૩ પાનાંમાં વિસ્તરેલી આદર્શવાદી નવલકથા છે. તત્કાલીન ભારતની દુર્દશા માટે પરાધીનતા ઉપરાંત ગામડાંની અવદશાનું ચિત્ર આપ્યું છે. એમની દોઢસો જેટલી વાર્તાઓમાં દાંપત્યજીવનની પ્રસન્નતા, વેદના, પ્રેમની ભગ્નતા, કવિ-કલાકારનું માનસ, સામ્યવાદી વિચારોથી રંગાયેલાં પાત્રોની ભાવનાઓ આદિ વિવિધ વિષય અને ભાવનું નિરૂપણ છે, રામનારાયણ વિશ્વનાથ પાઠક ઉપર ગાંધીવાદી વિચારોનો ઊંડો પ્રભાવ હતો,

તેમણે વિવેચન, કવિતા, નાટક અને ટૂંકીવાર્તા જેવા સ્વરૂપોમાં મહત્વપૂર્ણ યોગદાન આપ્યું છે. સુંદરમ્, સ્નેહરશ્મિ, કરસનદાસ માણેક વગેરેને એમણે કવિતા શિક્ષણ આપેલું. ઉમાશંકર જોષીએ જેમને 'ગાંધીયુગના સાહિત્યગુરુ' તરીકે અને યશવંત શુક્લએ જેમને 'ગુજરાતી ટૂંકી વાર્તાના સૌથી ઊંચા શિખર' તરીકે ઓળખાવ્યા છે તેવા રામનારાયણ વિ. પાઠક એ 'દ્વિરેફ' ઉપનામથી વાર્તાઓ, 'શેષ' ઉપનામથી કાવ્યો અને 'સ્વૈરવિહારી' ઉપનામથી હળવી શૈલીનાં લખાણો-નિબંધોનું સર્જન કર્યું હતું. ગાંધીયુગના અગ્રણી કવિ, વાર્તાકાર, વિવેચક અને પૂર્ણયોગના સાધક તરીકે સુંદરમ્નું નામ પ્રસિદ્ધ છે. ૧૯૮૫માં શ્રી સુંદરમ્ને પદ્મભૂષણથી સન્માનિત કરવામાં આવ્યા હતા. એમની કવિતાઓ 'ક્રોધા ભગતની કડવી વાણી', 'કાવ્યમંગલા', 'વસુધા', 'યાત્રા', 'વરદા', 'મુદિતા', 'લોકલીલા', 'દક્ષિણા-૧,૨' જેવા વીસેક કાવ્યસંગ્રહમાં સંગ્રહિત છે. તેમણે ભારતની સ્વતંત્રતાની ચળવળમાં ભાગ લીધો હતો અને થોડો સમય જેલમાં રહ્યા હતા. તેઓ અમદાવાદમાં સ્ત્રીઓના કલ્યાણ માટે કામ કરતી સંસ્થા 'જ્યોતિસંઘ' સાથે ૧૯૩૫થી ૧૯૪૫ સુધી સંકળાયેલા હતા. ૧૯૪૫માં શ્રી અરવિંદના સંપર્કમાં આવ્યા અને પૌરિચેરી ખાતે સ્થાયી થયા હતા. રમેશ ત્રિવેદી નોંધે છે કે, '૧૯૨૦ના સમયમાં ગાંધીજીના સત્યાગ્રહનું મોજું દેશવ્યાપી બની રહ્યું હતું તેમાં સુંદરમ્ ભીજાયા વગર ન જ રહે. ગાંધી

થેલો ખભે ભેરવી 'રઝળપાટે' નીકળી પડેલા આ યુવકને 'એક ખેતરની વાડ પાસેથી 'બુદ્ધનાં ચક્ષુ' મળી આવ્યું હતું.' સુંદરમ્ની કવિતામાં રાષ્ટ્રીય ભાવનાનું અભિનવ રૂપ પ્રગટે છે. જૂની વિચારસરણીને તોડીને નવસર્જન તરફ આગળ વધવા હાકલ કરતું એ જમાનામાં બહુ જ પ્રખ્યાત થયેલું આ ગીત છે.

ઘણુંક ઘણું ભાંગવું, ઘણ ઉઠાવ, મારી ભુજા!
ઘણુંક ઘણું તોડવું, તું ફટકાર ધા, ઓ ભુજા!
અનંત થર માનવી હૃદય-ચિત્ત-કાર્યે ચઢ્યા
જડત્વ યુગ જીર્ણના, તું ધધડાવી દે ધાવત્યાં.

ગાંધીયુગના આદર્શોનું મૂલ્ય અને તત્કાલીન કપરી વાસ્તવિકતા પરત્વેની પ્રતિક્રિયામાં એમના સર્જનનાં મૂળ પડેલાં છે. ઊર્મિશીલતા, રંગદર્શિતા, રહસ્યમયતા અને લયમધુરતાની સામગ્રી એમના કાવ્યજગતને પોતીકી વિશિષ્ટતા અર્પે છે. અલબત્ત, સ્વાધીનતા અને દેશભક્તિનો સૂર એમના પ્રારંભના સંગ્રહોમાં પ્રમુખ છે,

મારી નાવ કરે કો પાર?
કાળા ભમ્મર જેવાં પાણી,
જુગ જુગ સંચિત રે! અંધાર;
સૂર્યચંદ્ર નહિ, નહિ નભજ્યોતિ,
રાત દિવસ નહિ સાંજ સવાર!
મારી નાવ કરે કો પાર?

ઉમાશંકર જોષી ગુજરાતી સાહિત્યના જાણીતા કવિ અને લેખક હતા. ૧૯૬૭માં ભારતીય અને ખાસ કરીને ગુજરાતી સાહિત્યમાં તેમના ઉમદા પ્રદાન માટે શાનપીઠ પુરસ્કારથી સન્માનિત કરવામાં આવ્યા હતા. તેમના જીવન ઉપર રવીન્દ્રનાથ ટાગોર અને મહાત્મા ગાંધીજીની ભારે અસર હતી. ઉમાશંકર જોષી ગાંધીયુગના પ્રધાન

સાહિત્યકાર હતા.

ભોમિયા વિના મારે ભમવા'તાડુંગરા,
જંગલની કુંજકુંજ જોવી હતી;

જોવાં'તાં કોતરો ને જોવી'તી કંદરા,
રોતાં ઝરણાંની આંખ લહોવી હતી.

ઉમાશંકરે વિશ્વશાંતિ, ગંગોત્રી, 'નિશીથ', પ્રાચીના, આતિથ્ય, વસંતવર્ષા, મહાપ્રસ્થાન, અભિજ્ઞા, ધારાવચ્ચ એમના કાવ્ય સંગ્રહ છે. એમની તમામ કાવ્ય રચનાઓનો સંગ્રહ 'સમગ્ર કવિતા' નામે થયો છે.

મળતાં મળી ગઈ મોંઘેરી ગુજરાત
ગુજરાત મોરી મોરી રે.

ભારતની ભોમમાં ઝાઝેરી ગુજરાત
ગુજરાત મોરી મોરી રે.

ઉમાશંકર જોષી ગાંધીયુગના પ્રભાવક પ્રવક્તા તો છે જ સાથે નર્મદ અને ગાંધીને સ્મરીને ગુજરાતી જીવનના આગામી પડકારોનો એક ભાવિ નકશો પણ દોરે છે. 'તારી હાક સૂણીને કોઈ ના આવે તો એકલો જાને', જેવી રવીન્દ્રવાણી અને 'કલમને ખોળે માથું' મૂકવાની પ્રતિજ્ઞાની ભાષામાં છલકાતી ભાષા અને સંસ્કૃતિપ્રીતિ ઝગમગાટવાળી સમૃદ્ધિમાં ન ભુલાય માટે પોતાની આગવી વાણીને મૂર્ત કરે છે.

'નર્મદની ગૂજરાત દોહલી રે જીવવી,
ગાંધીની ગુજરાત કપરી જીવવી,
એક વાર ગાઈ કે કેમ કરી ભૂલવી?
ગુજરાત મોરી મોરી રે'.

કવિ કહે છે જ્યાં નર્મદના સમયમાં ગુજરાતની પરિસ્થિતિ સામાજિક રીતે ખૂબ કથ ડેલી હતી. એમણે જે પુરુષાર્થ કર્યો હતો તે ખૂબ મહત્વનો હતો એટલે તે સમયે ગુજરાતમાં જીવન દુર્લભ હતું. તો વળી ગાંધીયુગમાં અંગ્રેજોનું શાસન હતું. ત્યારે પ્રજાના જીવનમાં ઉદ્ભવેલું સંઘર્ષભર્યું વાતાવરણ ઘણું કઠિન હતું. એટલે જીવન જીવવું ઘણું મુશ્કેલભર્યું હતું.



‘ભારતની ભોમમાં ઝાઝેરી ગુજરાત
ગુજરાત મોરી મોરી રે.

મળતાં મળી ગઈ મોંઘેરી ગુજરાત
ગુજરાત મોરી મોરી રે...’

ગુજરાતી ભાષાનું ગૌરવ દર્શાવતી ઉમાશંકર
જોષીની આ અમર રચના...

જે જન્મતાં આશિષ હેમચન્દ્રની
પામી, વિરાગી જિનસાધુઓએ

જેનાં હિંચોળ્યાં મમતાથી પારણાં,
રસપ્રભા ભાલણથી લહી જે

નાચી અભંગે નરસિંહ-મીરાં-
અખા તણે નાદ યડી ઉમંગે

આયુષ્મતી લાડલી પ્રેમભટ્ટની
દ્રઢાય ગોવર્ધનથી બની જે,

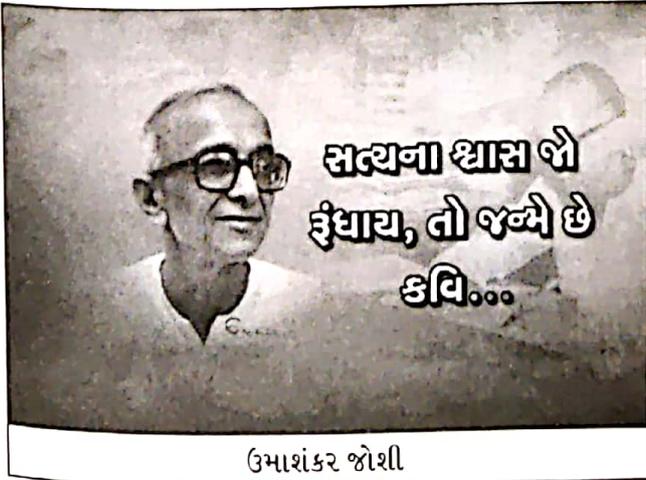
અર્ચેલ કાન્તે, દલપત પુત્રે
તે ગુર્જરી ધન્ય બની ઋતંભરા

ગાંધીમુખે વિશ્વમાંગલ્યધારી
‘ઘરે ઘરે વીર ગાંધી જગાવો ને બારણે બારણે

બુદ્ધ’ની હાકલ કરનાર આ કવિ
મૂડીવાદીઓને પડકાર ફેંકે છે :

‘ભૂખ્યાજનોનો જઠરાગ્નિ જાગશે,
ખંડેરની ભસ્મકણી ન લાધશે’

૧૮૯૭માં ગુજરાતના ચોટીલા ગામમાં
જન્મેલા ઝવેરચંદ મેઘાણી પચાસ વર્ષની વયે



ઉમાશંકર જોષી

અવસાન પામ્યા. પોતાને ‘પહાડનું બાળક’
તરીકે ઓળખાવનાર ઝવેરચંદ મેઘાણીનું
આઝાદીના આંદોલનમાં ઐતિહાસિક
યોગદાન રહ્યું છે. ગોળમેજી પરિષદમાં
હાજરી આપવા ગાંધીજી જ્યારે લંડન
જવાના હતા તે વખતે ‘રાષ્ટ્રીય શાયર’
ઝવેરચંદ મેઘાણીએ રચેલ ‘છેલ્લો કટોરો’
આઝાદીના જંગ વખતે પ્રજાનો મિજાજ કેવો
હતો, મરી મીટવાની-જાન ફના કરવાની
કેવી તમન્ના હતી અને ગાંધીજી પ્રત્યે પ્રજાનો
કેવો ભાવ હતો એનું ડૂબલું નિરૂપણ કરે છે.
‘દેખી અમારાં દુઃખ નવ અટકી જજો, બાપુ!
સહિયું ઘણું, સહીશું વધુ: નવ થડકજો,
બાપુ!’ ઘણું બધું કહી જાય છે. Marie
Ravenal de La Coste કૃત
‘Somebody’s Darling’ ગીતનું
મેઘાણીએ કરેલું ગુજરાતી રૂપાંતર એટલે
‘કોઈનો લાડકવાયો’. યુદ્ધભૂમિમાંથી
લાશનો ખડકલો આવે છે તેમાં એક લાશને
હજી કોઈએ ઓળખી નથી એ પણ કોઈ
માતાનો લાડકવાયો છે એ વ્યથા આ કાવ્યનું
સંવેદન કેન્દ્ર બને છે.

રક્ત ટપકતી સો સો ઝોળી સમરાંગણથી
આવે,

કેસરવરણી સમરસેવિકા કોમલ સેજ
બિછાવે;

ઘાયલ મરતાં મરતાં રે! માતની આઝાદી
ગાવે.

કોની વનિતા, કોની માતા, ભગિનીઓ ટોળે
વળતી,

શોણિતભીના પતિ-
સુત-વીરની રણશૈયા
પરલળતી;

મુખથી ખમ્મા ખમ્મા
કરતી માથે કર મીઠો
ધરતી.

ઝવેરચંદ મેઘાણીની
અવિસ્મરણીય કૃતિ
‘કસુંબીનો રંગ’ ખૂબ જ
લોકપ્રિય બનેલ છે.
સ્વાધીનતા કાજે શૂરતા

પ્રગટાવતું, આઝાદી માટે આહલેક ગજાવતું
ગૂર્જરજનોનું જાણીતું આ કાવ્ય યુગવંદનાને
ખરા અર્થમાં ચરિતાર્થ કરે છે.

લાગ્યો કસુંબીનો રંગ,

રાજ, મને લાગ્યો કસુંબીનો રંગ!

જનનીના હૈયામાં પોઢંતા પોઢંતા પીધો
કસુંબીનો રંગ;

ધોળાં ધાવણ કેરી ધારાએ ધારાએ પામ્યો
કસુંબીનો રંગ રાજ

૧૯૩૦માં ધોલેરા સત્યાગ્રહ સંગ્રામ માટે
રચેલાં શૌર્યગીતોના સંગ્રહ ‘સિંધુડો’ની પંદર
રચના મેઘાણી પોતે જ પોતાના બુલંદ કંઠથી
રજૂ કરતાં, જેની ધારી અસર થતી અને
નવયુવાનો પોતાના પ્રાણ ન્યોછાવર કરવા
માટે તત્પર થતાં, એટલે જ અંગ્રેજ સરકારે
‘સિંધુડો’ સંગ્રહ અને રચનાને રજૂ કરવા પર
પ્રતિબંધ ફરમાવેલો. સિંધુડો શૌર્ય રસનો
પ્રકાર છે. અંગ્રેજ સરકારે તેની જેટલી પણ
નકલો હતી એ જપ્ત કરી હતી.

મા તારી કોણ ગાશે,

પલ પલ ઇતિહાસ ભરી બારમાસી?

મે તો શ્રદ્ધા વિહોણે,

પ્રથમ મહૂર્તે કીધી’તી કરુણ હાંસી.

બોટાદની અદાલતમાં જજની સામે ‘છેલ્લી
પ્રાર્થના’ કાવ્ય ગાયું અને ‘હજારો વર્ષની
અમારી વેદના’ સાંભળીને એમની આંખમાં
પણ આસું વહેતાં હતાં.

‘તારા નામમાં, ઓ સ્વતંત્રતા, મીઠી આ શી
વત્સલતા ભરી!

મુરદાં મસાણેથી જાગતાં—એવી શબ્દમાં શી
સુધા ભરી!’

જેવાં કાવ્યો જેને અનુસર્જન કહી શકાય તે
પ્રકારનાં છે. ટાગોરનાં કેટલાંક કાવ્ય પરથી
મેઘાણીએ અનુસર્જનો કર્યા જે મેઘાણીએ
‘સ્વીન્દ્રવીણા’માં ગ્રંથસ્થ કર્યાં છે. આ
સંગ્રહનાં કાવ્યોમાં મેઘાણી મોટા ભાગે ભાવ
કે પરિસ્થિતિનું કાઠું તો મૂળ કાવ્યનું રાખે છે,
પણ તેને ઘાટ પોતીકો આપે છે.
‘સ્વીન્દ્રવીણા’નાં કાવ્યોની ભાષા, લય;

પ્રતીક વગેરેમાં સૌરાષ્ટ્રના લોકજીવનની છાપ જોવા મળે છે. 'કુરબાનીની કથાઓ' એ મેઘાણીનું પુસ્તક ટાગોરની 'કથાઓ કાહિનીની' વાર્તાઓના અનુસર્જન રૂપ છે. 'યુગવંદના' કાવ્યગ્રંથનું તો શીર્ષક સૂચક છે. કારુણ્ય અને માનવતાના ભાવ ગાનાર મેઘાણી આપણા રાષ્ટ્રીય શાયર અવશ્ય છે. મેઘાણીએ એ યુગને, રાષ્ટ્રપિતા ગાંધીજીના સ્વરાજ્ય આંદોલનના એ સમયના, કાળદેવતા, જીવનદેવતાની તેમણે કરેલી વંદના છે. અહીં મેઘાણીની કવિતા દોઢલું માનવજીવન, શોષણ, સમાજજીવન, મુક્તિ માટેનો સંગ્રામ, વીરોની શહીદી વગેરે વિષયો-યુગનાં પરિબળોને ઝીલે છે. ડો. દિલાવરસિંહ જાડેજા નોંધે છે કે, 'રાષ્ટ્રીય શાયર'નું બિરુદ પામેલા મેઘાણીની રાષ્ટ્રીય અસ્મિતા પ્રગટ કરતી કવિતાનો રંગ 'હૃદયના સર્વ ભાવ, જેમાં નિયોવાયા હો' તેવો કસુંબી જણાય છે. મેઘાણીની કસુંબલ કવિતાનાં બે પાસાં છે. સ્વાતંત્ર્ય ઝંપના વ્યક્ત કરતાં ઉદામ મનોવૃત્તિવાળાં કાવ્યોમાં એક પાસું તથા પીડિતો પ્રત્યે ઉત્કટ સહાનુભૂતિ પ્રગટ કરતાં કાવ્યોમાં મેઘાણીની કવિતાનું બીજું પાસું દેખાય છે.'^૩

કૃષ્ણલાલ શ્રીધરાણીનો જન્મ ૧૬-૯-૧૯૧૧ના રોજ થયેલો. માધ્યમિક શિક્ષણ દક્ષિણામૂર્તિમાંથી લીધું. ૧૯૨૯માં ગૂજરાત વિદ્યાપીઠ, અમદાવાદમાં જોડાયા. કવિ, નાટ્યકાર તરીકે એમનું મહત્વપૂર્ણ યોગદાન રહ્યું છે. ૧૯૩૦ની ઐતિહાસિક દાંડીકૂચના એક સૈનિક તરીકે એમની પસંદગી થઈ. ધરાસણા જતાં કરાડીમાં એમની ધરપકડ થતાં સાબરમતી અને નાસિકમાં કારાવાસની સજા થાય છે. ૧૯૩૦ની ગાંધી-દાંડીકૂચમાં ઓગણીસ વર્ષના તરુણ શ્રીધરાણીએ ભાગ લીધો અને તેમણે રચી તે કવિતા 'સપૂત':

'આવવું ન આશ્રમે - મળે નહીં સ્વતંત્રતા!
જંપવું નથી લગીર - જો નહીં સ્વતંત્રતા!
સ્નેહ, સૌખ્ય સૌ હરામ- ના મળે સ્વતંત્રતા!

જીવવું મર્યાસમાન- ના યદી સ્વતંત્રતા!'

કૃષ્ણલાલ શ્રીધરાણીની પ્રથિતયશ કવિતા બે છે. એક 'સર્જકશ્રેષ્ઠ આંગળાં' ને બીજી 'આઠમું દિલહી.' એમની કવિતામાં રાષ્ટ્રભક્તિ અવનવા રૂપ ધારણ કરે છે. શ્રીધરાણીની કવિતા આરંભમાં એક સર્જનાત્મક, સ્વયંસ્ફુરણાની સહજતા અને સુંદરતા સાથે સ્ફુટ થાય છે. 'અમે તો સૂરજના છડીદાર અમે તો પ્રભાતના પોકાર' એમની કવિતા પ્રણય, પ્રકૃતિ અને પ્રભુતાના સનાતન વિષયના સૌંદર્યની અભિવ્યક્તિ છે. ટાગોરના રહસ્યવાદને એ ગુજરાતીમાં લઈ આવે છે અને ગાંધીજીનાં દર્શનને તેમ જ સ્વાતંત્ર્ય ચળવળને તે બિરદાવે છે. એમની કવિતામાં રાષ્ટ્રભક્તિ અવનવાં રૂપ ધારણ કરે છે. 'સર્જકશ્રેષ્ઠ આંગળાં'માં અંગ્રેજોએ કાપી નાખેલાં ઢાકાની મલમલના સર્જક કારીગરોનાં આંગળાંની કરુણતા દ્વારા રાષ્ટ્રપ્રેમ ને સ્વરાજ્ય ભાવના જગાવી છે, તેઓ શિવાજીનું 'સ્વરાજ્યરક્ષક' તરીકેનું વ્યક્તિત્વ ખડું કરે છે. ગાંધીજીને તેમણે કાવ્યાત્મક અંજલિ આપી છે. ગાંધીજી ભારતમાની આંખનાં આંસુ છે. નર્મદ, ખબરદાર, મહાદેવ દેસાઈ, લલિત, મહારાણીશંકર શર્મા, ત્રિભોવન ગૌ. વ્યાસ, કલ્યાણજી વિ. મહેતા, ચંદ્રવદન મહેતા, ન્હાનાલાલ વગેરે સર્જકોની રચનાએ પણ આઝાદીના આંદોલનને પ્રભાવક બનાવવામાં ફાળો આપ્યો હતો. ઈચ્છાલાલ ઈ. દેસાઈ પાસેથી 'લડતનાં ગીતો' નામે સંપાદન મળે છે, જેમાં આઝાદીના આંદોલનમાં દેશભક્તિન, અને રાષ્ટ્રપ્રીતિની કવિતાનું રસજાં કરનાર કવિઓની કવિતા સમાવવામાં આવી છે. ઉષા ઉપાધ્યાય 'શબ્દસૃષ્ટિ'ના સ્વાતંત્ર્ય સુવર્ણજયંતી વિશેષાંકમાં નોંધે છે એ વિધાન સાથે સમાપન કરીએ કે, 'ગાંધીયુગની કવિતામાં રાષ્ટ્રીયતા 'વિષય સંદર્ભે એ સમયની કવિતાનો વિચાર કરતાં કેટલીક નોંધપાત્ર હકીકતોનો પણ અચૂક નિર્દેશ

કરવો ઘટે. આ સમયગાળાની કવિતા વૈયક્તિ આસ્વાદ માટે નહીં, પરંતુ સામૂહિક પ્રેરણાને સમૂહના પ્રતિભાવરૂપે લખાઈ હોવાથી કાવ્યનું હાર્દ સ્વરૂપ તેને સવિશેષ ફાવ્યું છે. ગુણ પરત્વે આ કવિતા પ્રસાદ અને ઓજસ ગુણની છે તો રસ પરત્વે વીર, કરુણ અને ક્વચિત અદ્ભુત રસની છે. હેતુપ્રધાન હોવાના કારણે એમાં પ્રાસંગિકતાનું તત્ત્વ સવિશેષ હોવા છતાં એમાંથી એ સમયના ભારતવર્ષનાં કોટિ કોટિ હૃદયોની ઊછળતી ઊર્મિઓનો સચ્ચાઈભર્યો ધ્વનિ પ્રગટે છે ને તેનું મૂલ્ય સહેજેય ઓછું નથી.'

સંદર્ભ સૂચિ:

- ૧) 'રાષ્ટ્રનો સ્વાતંત્ર્યસંગ્રામ અને ગુજરાત', શાંતિલાલ મ. દેસાઈ, પ્રકાશક: યુનિવર્સિટી ગ્રંથ નિર્માણ બોર્ડ, અમદાવાદ. સાતમી આવૃત્તિ ૨૦૨૧, પૃ. ૨૬૮.
- ૨) 'અર્વાચીન ગુજરાતી સાહિત્યનો ઇતિહાસ', રમેશ ત્રિવેદી, પ્રકાશક: આદર્શ પ્રકાશન, અમદાવાદ, દ્વિતીય પુન: મુદ્રણ ૨૦૦૧, પૃ. ૨૧૧.
- ૩) 'શબ્દસૃષ્ટિ' આઝાદીનો અમૃત મહોત્સવ-દીપોત્સવી વિશેષાંક, દિલાવરસિંહ જાડેજા સંપાદક: જયેન્દ્રસિંહ જાડેજા, પ્રકાશક: ગુજરાત સાહિત્ય અકાદમી, ગાંધીનગર. ૨૦૨૧, પૃ. ૩૧૩.
- ૪) 'શબ્દસૃષ્ટિ' સ્વાતંત્ર્ય સુવર્ણજયંતી વિશેષાંક, ઉષા ઉપાધ્યાય, સંપાદક: હર્ષદ ત્રિવેદી, પ્રકાશક : ગુજરાત સાહિત્ય અકાદમી, ગાંધીનગર. ૧૯૯૮, પૃ. ૫૮.

લેખક ગાંધીનગર સ્થિત સેન્ટ્રલ યુનિવર્સિટી ઓફ ગુજરાતના સેન્ટર ફોર ગુજરાતી લેંગ્વેજ એન્ડ લિટરેચરમાં પ્રોફેસર અને અધ્યક્ષ છે.
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બહુશ્રુત વાચક ડૉ. આંબેડકર

ડૉ. બાબાસાહેબ આંબેડકરના પિતાશ્રી રામજીરાવ સકલાલ ઈસ્ટ ઈન્ડિયા કંપનીમાં શિક્ષક

હતા. કબીરપંથી હતા એટલે ઘરનું વાતાવરણ શિસ્તમૂલક હતું. પરિવારમાં ભજન, અભંગ અને દોહાનું નિયમિત પઠન થતું. એમના પિતાજીને તો અનેક અભંગ મોઢે હતા અને તે અસ્ખલિત રીત ગાઈ સંભળાવતા. પિતાજી પાસેથી એમને મુક્તેશ્વર અને તુકારામ જેવા સંતકવિની અનેક રચના સંભળવા મળતી. ડૉ. બાબાસાહેબને અભ્યાસકાળમાં વિષયસંલગ્ન પુસ્તકો કરતા ઈતર વાંચનમાં વિશેષ રુચિ હતી. આર્થિક સ્થિતિ નાજુક હોવા છતાં પણ પિતાશ્રી એમનો વાચનશોખ પુરો કરતા. નવા પુસ્તકો ખરીદવા જીદ કરતા અને તેઓએ એમના આત્મકથાના બેઈટીંગ ફોર વિસામાં નોંધ્યું છે કે, મેં પિતા પાસે પુસ્તક માંગ્યું હોય અને સાંજ સુધીમાં એ પુસ્તક ન લાવી આપ્યું હોય તેવું ભાગ્યે જ બનતું.

ડૉ. બાબાસાહેબને નાની ઉંમરે પુસ્તકો વાંચવાનો શોખ લાગ્યો હતો. એ એટલી હદે હતો કે કયા પુસ્તકના કયા પાના પર કઈ મહત્વની વાત લખેલી છે એ તરત જ ચીંધી બતાવતા.

એમણે વિવિધ દેશના ઇતિહાસ, કાવ્યો, નાટકો, નવલકથાઓ, ટીકાત્મક, ગ્રંથો, નૃવંશશાસ્ત્રીય પુસ્તકો વગેરેનું ઊંડું વાચન તો અમેરિકામાં અભ્યાસકાળમાં જ કરેલું હતું. કોઈ એક લેખકનું અર્થસભર પુસ્તક વાંચવા મળતું તો એના બધા જ પુસ્તકો પર નજર ફેરવી દેતા. અને સંદર્ભગ્રંથો પણ જોઈ લેતા. ઘણીવાર અભ્યાસમાં એટલા તલ્લીન બની જાય કે જમવાનું પણ સુદ્ધા ભૂલી જતા. એક તબક્કો એવો આવ્યો કે, એમણે જમવાનું એક ટાઈમ જ કરી નાંખ્યું. બપોરના સમયે ચા બિસ્કીટથી ચલાવી લેતા. અને આખો દિવસ ગ્રંથાલયમાં વાચન કરતા. આ એમની અનોખી જ્ઞાન સાધના હતી.

રવિવાર કે રજાના દિવસે તેઓ જૂનાં પુસ્તકોની દુકાનની મુલાકાત લેતા. ત્યાંથી અલભ્ય પુસ્તકો ખરીદતા. શિષ્યવૃત્તિ મર્યાદિત હતી એટલે એકાદ દિવસ ભોજન કરવાનું માંડી વાળે અને એમાંથી વધેલા પૈસાથી પુસ્તકો ખરીદતા. અન્ય ખર્ચ પર કાપ મૂકી અને પૈસા બચાવી

- ડૉ.રાજેશ મકવાણા

પ્રોફેસર અને અધ્યક્ષ, ગુજરાતી ભાષા સાહિત્ય કેન્દ્ર, ગુજરાત કેન્દ્રીય વિશ્વવિદ્યાલય, ગાંધીનગર

રાખતા. કરકસરની આદત તો પહેલાથી હતી. ખોટા ખર્ચ ન કરવાની શીખ બાળપણમાં મળેલી પરંતુ જ્યારે મનગમતું પુસ્તક જુવે ત્યારે પોતાનો કરકસરનો સ્વભાવ ભૂલી જતા અને કોઈ પણ ભોગે એ પુસ્તક ખરીદી લેતા.

પોતાના સંશોધન અભ્યાસમાં પણ એટલું વિશાળ વાંચન કરેલું. કાર્ડ બનાવેલા કયો સંદર્ભ ક્યાં છે, એ માત્ર કાર્ડની ટીપ્પણી પરથી જ શોધી લેતા.

માહિતી અને પ્રસ્ારણ વિભાગ દ્વારા પ્રકાશિત પુસ્તકમાં ડબલ્યૂ એન. કુબેર નોંધે છે કે આંબેડકર કહેતા કે લોકોએ મારો સામાજિક બહિષ્કાર કર્યો ત્યારે આ પુસ્તકોએ મને તેમનાં હેયામાં સ્થાન આપ્યું, જેમ પ્રેમી એની પ્રેમિકા માટે જે રીતે વાત કરે તે રીતે આંબેડકર તેમના પુસ્તકો વિશે વાત કરતા.

પંડિત માલવિયાજીએ એમનો ગ્રંથભંડાર માંગ્યો ત્યારે એમણે કહેલું કે, જો મારાં પુસ્તકો મારાથી દૂર જાય તો મારો પ્રાણ જ નીકળી જાય. પુસ્તકો પ્રત્યે તીવ્ર અનુરાગ હોવા છતાં પોતાના આ ગ્રંથો એમણે મુંબઈમાં સિદ્ધાર્થ કોલેજ અને ઔરંગાબાદની મિલિંદ કોલેજ શરૂ કરી એમાં ભેટમાં આપી દીધેલા.

ડૉ. બી.આર. આંબેડકરનો અંગત લાઈબ્રેરીમાં ઇતિહાસ વિષયક ૨૩૦૦ પુસ્તકો, મહાપુરુષના જીવન વિષયક ૧૨૦૦ પુસ્તકો, અર્થશાસ્ત્ર અને રાજકારણના ૩૦૦ પુસ્તકો, સાહિત્યમાં ૧૧૦૦ પુસ્તકો, ભાષાવિજ્ઞાનના ૬૩૦ પુસ્તકો, ધર્મ અને ધર્મશાસ્ત્રોના ૨૦૦૦ પુસ્તકો, દર્શનશાસ્ત્રનાં ૬૦૦ પુસ્તકો, બૌદ્ધધર્મ તથા પાલી સાહિત્યના ૨૦૦૦ પુસ્તકો, સંસ્કૃતના ૨૦૦૦ પુસ્તકો, હિન્દીના ૫૦૦ પુસ્તકો, યુદ્ધ વિષયક ૫૦૦ પુસ્તકો, સમિતિના અહેવાલો ૧૧૦૦૦ તથા એન્સાઈક્લોપિડીયાના ૨૦ ભાગ હતા.

ડૉ. બી.આર. આંબેડકરના જીવનઘડતરમાં ગ્રંથોનો અમૂલ્ય ફાળો રહ્યો હતો. તેઓ કહેતા કે ગ્રંથોની સોબત માટે તે હું સર્વસ્વનો ત્યાગ કરવા તૈયાર છું. ભારતની સર્વ સંપત્તિ મારા ગ્રંથ પ્રેમ સામે તુચ્છ છે.

परामर्श : प्रो. काशीनाथ सिंह
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डॉ. के. सी. शर्मा
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प्रो. माधव हाड़ा

सम्पादक : पल्लव

सहयोग : गणपत तेली, भँवरलाल मीणा

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राजेश जोशी : कविता में किस्सागोई का महीन हुनर

लगभग पचास वर्षों की काव्य यात्रा में राजेश जोशी ने हिंदी कविता के गोलार्ध की एक परिक्रमा पूरी कर ली है। इस काव्य यात्रा में राजेश जोशी स्त्री, दलित, आदिवासी और हाशिए पर जीवन जी रहे मनुष्यों की बहुविध चिंताओं और संघर्षों से साझेदारी करते हुए सांप्रदायिकता, भ्रूणहत्या, बेदखली, विस्थापन, प्रकृति और पर्यावरण के विनाश, असमान और असंतुलित विकास से पैदा हुए हालातों में छटपटाते 'महाजीवन' की महीन पड़ताल करते हैं। एक कवि के रूप में उनकी कोशिश बेजुबान लोगों के दुख और गुस्से को उतने ही ताप भरे शब्दों में अभिव्यक्त करने की रही है। हाशिए का जीवन जी रहे लोगों की छोटी-छोटी खुशियों और मोटे-मोटे आँसुओं को उतने ही पारदर्शी शब्दों में अभिव्यक्त करने के लिए वे बेचैन दिखते हैं। वे अपने समय की विकृतियों और विडंबनाओं को फूली हुई नसों वाले वीभत्स और दैत्याकार राक्षसों की तरह कविताओं में दिखाना चाहते हैं, ताकि उनसे आसानी से घृणा और नफरत की जा सके। वे जानते हैं, कि 'जहर' को जहर की तरह बिल्कुल नहीं दिया जाता किसी समाज को, इसलिए अनेक औषधियों और उपचार की नई-पुरानी पद्धतियों में जहर के इस्तेमाल के आधार पर सत्ताधारियों द्वारा जहर के पक्ष में आम राय बनाने के षड्यंत्र का बखूबी पर्दाफाश करते हैं। जीवन की सुंदरता को कुरूप करने वाली कोशिशों के प्रति बनी एक प्रकार की 'मौन सहमति' को 'महान कला मूल्यों' की आड़ में छिपाने वाले यशः प्रार्थी रचनाकारों की आत्मा में गुदगुदी और कानों में फुसफुसा रहे 'डॉलर' को बेनकाब करने से भी वे नहीं बचते। साथ ही हजारों प्रजाति की चिड़ियों, नदियों, पेड़ों, पहाड़ों और पठारों की दुर्गम भाषा के काव्यानुवाद की चुनौती स्वीकार करते हैं। राजेश जोशी आग, पानी, हवा, आकाश और धरती को बचाने वाले, जिंदगी के हाशिए पर अनाम और चुपचाप जीवन जी रहे धरती के 'नमक' को इस पृथ्वी पर थोड़ी सी जगह देने का संवेदनात्मक आह्वान करते हैं।

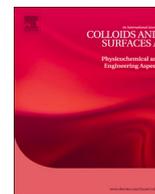
युवा जोशी और तेवर से पगी 'समरगाथा' से आरंभ करके संयत और गंभीर स्वर और कलेवर वाले पहले कविता संग्रह 'एक दिन बोलेंगे पेड़', 'मिट्टी का चेहरा', 'नेपथ्य में हैंसी', 'दो पक्षियों के बीच' से गुजरते हुए 'चौंद की वर्तनी' पढ़ता कवि सत्तर पार की ठहरी हुई उम्र की दहलीज पर 'जिद' और 'उल्लांघन' को आमादा नजर आता है तो उसकी चिंता और बेसब्री बेसबब नहीं है। अपनी पहली लम्बी कविता 'समरगाथा' में बतर्ज धूमिल, राजेश जोशी ने पूछा था कि--

"क्या इलाज है आदमी को आदमी से/ एक दर्जा कम कर देने वाली
इस साजिश का?/ इस अर्थशास्त्र का/ जो निक्कमों की अंटी में पैसा
और श्रमिकों की जेब में भूख डालता है?/ जो चालाकी की ज्यादा
और पसीने की कीमत कम आँकता है?"

अपनी काव्य यात्रा के पाँच दशक पूरे करते हुए भी कवि का अपनी चिंताएँ दुहराना और प्रतिबद्धताओं को पुनः रेखांकित करना हमारी कथित विकास यात्रा पर गंभीर सवाल खड़े करता है। अपने सद्यःप्रकाशित संग्रह 'उल्लांघन' में राजेश जोशी कवि कर्तव्य को परिभाषित करते हुए लिखते हैं--

"मैं एक कवि हूँ
और कविता तो हमेशा से ही एक हुक्म उदूली है

संजीव कुमार दुबे : समीक्षक एवं अध्यापक।
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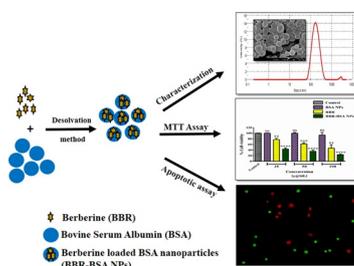
Bovine Serum Albumin Nanoparticles for the Efficient Delivery of Berberine: Preparation, Characterization and *In vitro* biological studies



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GRAPHICAL ABSTRACT



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ABSTRACT

Berberine (BBR) is a naturally occurring plant-derived isoquinoline alkaloid. It has been reported to exhibit multiple pharmacological properties including anticancer activity. However, its low water solubility and bioavailability restrict its clinical use for cancer treatment. Hence, to overcome these limitations and enhance its therapeutic efficacy, in the present study we have encapsulated BBR in Bovine Serum Albumin nanoparticles (BSA NPs) by desolvation method. The average particle size of synthesized nanoparticles were found to be 116 and 166 nm for BSA NPs and BBR-BSA NPs respectively. The FESEM images of nanoparticles implied that prepared nanoparticles are of spherical shape and size was validated. The drug entrapment efficiency of prepared nanoparticles was found to be 85.65 % with a drug loading capacity of 7.78 %. The FTIR spectra, XRD patterns and thermograms of DSC and TGA confirms the synthesis of BBR-BSA NPs and its entrapment in amorphous form. The stability studies of BBR-BSA NPs suggests that nanoparticles were quite stable in aqueous solution of pH 7.4 and degraded under acidic condition (pH 5). The *in vitro* cytotoxicity assay and Trypan blue assay proved that the prepared BBR-BSA NPs were selectively toxic towards breast cancer cells and kill the cells more efficiently as compared to pure BBR. The cellular uptake studies and AO/EtBr staining suggest that BBR-BSA NPs were therapeutically more effective and improve the anticancer activity of BBR by delivering it to target site, for potential therapeutic use. All the above information collectively suggests that BBR-BSA NPs may emerge as a novel paradigm for treatment of breast cancer.

1. Introduction

Cancer is a major global health catastrophe threatening the health of millions worldwide. Nowadays, Cancer has become one of the most

common public health issues due to increased prevalence, morbidity and mortality caused by complications. GLOBOCAN 2018 data suggests that the global cancer burden has increased to 18.1 million cases and 9.6 million cancer deaths. In females Breast cancer is the most

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commonly diagnosed cancer and the leading cause of cancer death. In the year 2018, 2.1 million breast cancer cases has been diagnosed worldwide [1]. The current treatment regimen involves surgery, chemotherapy, radiotherapy, hormone therapy and combinations of these approaches for treating breast cancer patients. The current treatment regimen are not sufficiently satisfactory, as resistance against chemotherapy, toxicity, non-specific delivery of anticancer drug and side-effects are the major obstacles. So always there is a pressing need of research progress to provide novel therapeutic agent of nature origin with good efficacy and smart strategic delivery leading to less toxicity and high selectivity.

Berberine (BBR) is a natural, isoquinoline alkaloid compound derived from the plants of Berberis genus. It is found in the roots, rhizomes and stem bark of the plants. Berberine plant extracts has been used in Chinese and ayurvedic medicine for many years together [2]. It has been reported that Berberine exhibit multiple pharmacological functions such as: anti-inflammatory [3], anti-oxidant [4], anti-diabetic [5], antihypertensive [6], antidepressant [7], antidiarrheal [8], antimicrobial [9], neuroprotective [10], hepatoprotective [11], hypolipidemic [12], pro-apoptotic [13], anti-cancer [14]. Berberine shows its anticancer activity via regulating different proapoptotic, antiapoptotic and intrinsic signaling pathways [15–17]. Though it has multiple pharmacological properties, its major drawbacks are poor water solubility, low bioavailability and limited intake in the gastrointestinal (GI) tract hindered its potential applications and resulting in low effective concentration of BBR delivering at target site [18]. To surmount all these challenges of drug, biodegradable delivery vehicles act as the infallible solution. Very recently protein biomolecules has been materialized as a potent drug delivery vehicles by overcoming several limitations of conventional therapy like poor aqueous solubility, lack of bioavailability and poor therapeutic efficacy; thus proteins are the perfect and the best choice for drug delivery. Proteinaceous delivery systems and specifically the albumin-based drug delivery systems have the properties of intrinsic accumulation at the tumor site, because of the immature, highly permeable and leaky vasculature called enhanced permeation and retention effect (EPR effect) [19]. Additionally, number of reports has been published mentioning that tumors are a site of albumin catabolism and also studies have suggested that the hypoalbuminemia evident in cancer patients is a result of albumin catabolism by the tumor [20–21].

Among proteins biomacromolecule, Bovine serum albumin/Human serum albumin based nanoparticles have extensively been investigated for their clinical applications because of their abundance in blood, ready availability, easy purification, nontoxicity, biodegradability, low-immunogenicity, greater accumulation at tumor site, showing significant biocompatibility, allowing ease of delivery by injection and thus an ideal candidate for nanoparticle formation [22]. The protein often increases the apparent solubility of hydrophobic drugs in the plasma and influences the circulation, metabolism and efficacy of drugs. The breakthrough innovation and market approval of the paclitaxel-albumin nanoparticles (Abraxane®) has intrigued researcher to use albumin drug carrier for targeted drug delivery in cancer therapy. Abraxane is an example of nanometer albumin-bound technology (nab™), for the treatment of advanced non-smallcell lung cancer and metastatic breast cancer; Abraxane® is being approved by the U.S. Food and Drug Administration (FDA) [23]. Studies have shown that Abraxane® has greater therapeutic index than Paclitaxel alone [24]. Recently, Abraxane® has also been employed for the first-line treatment of patients with metastatic adenocarcinoma of the pancreas in combination with gemcitabine and for patients with metastatic non-small cell lung carcinoma, in combination with carboplatin [23]. So, albumin has widely been in use as a nano-drug delivery vehicle and acquiring special attention. Now a days scientist and researchers have developed a lot of albumin-based drugs/imaging agents, which are present in the market and many are going through clinical trials for different applications [25,26]. All these factors has increased the level of interest in

BSA as a nano-drug delivery vehicle for different biomedical applications. Albumin (Mol. wt. 66.5 kDa) is a most abundantly present plasma protein. It possess unique characteristics of extreme stability over a pH range of 4 to 9, thermally stable [27], maintains plasma colloidal osmotic pressure, transport nutrients to different parts of cells, maintains plasma pH and solubilizes hydrophobic fatty acid molecules. Its unique drug delivering nature provides enhanced aqueous solubility to the albumin-bound hydrophobic drugs in plasma and ameliorate the pharmacokinetics of bound-ligand in physiological environment [28]. So, we have picked BSA as a nano-carrier to encapsulate Berberine for efficient delivery and improved therapeutic efficacy.

However, nanoparticulate polymeric drug delivery systems in association with BBR have not been investigated for the treatment of Breast cancer. To our knowledge no studies has been done on entrapment of Berberine in Bovine Serum Albumin nanoparticles and its application for Breast Cancer cells. So, here in the present study, to improve the anticancer efficacy of Berberine, we have encapsulated Berberine within BSA nanocarrier, to form BBR-BSA NPs. BSA was selected as a delivery vehicle to safe guard the active component BBR from proteolytic degradation and provide long circulation life in the bloodstream. The physicochemical characterization of BBR-BSA NPs were ascertained by using FESEM, FTIR, DLS, XRD, DSC, TGA measurements. Further, the pH stability and time-dependent stability of the synthesized nanoparticles were analyzed. Moreover the *in vitro* cytotoxicity of the BBR-BSA NPs was evaluated in MDA-MB-231 cell line in order to evaluate their anticancer potential.

2. Materials and Methods

2.1. Materials

Berberine (BBR, ≥99%), Bovine serum albumin (BSA, ≥99%), Glutaraldehyde,3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Coumarin-6 (C6), Dimethyl sulfoxide (DMSO), Acridine orange (AO) and Ethidium bromide (EtBr) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Fetal Bovine Serum (FBS), Dulbecco's modified essential medium and Ham's F-12 (DMEM/F12) medium, Penicillin-streptomycin-amphotericin B cocktail and 0.25% Trypsin EDTA were purchased from Thermo-fisher scientific (Waltham, MA, USA). MDA-MB-231 cell line was procured from NCCS (Pune, India).

2.2. Synthesis of BSA NPs and BBR-BSA NPs

BSA NPs and BBR-BSA NPs were synthesized by desolvation method (Figure S1) with slight modification [29]. To form BSA NPs, 200 mg of BSA was dissolved in 2 mL of Milli-Q water and stirred at 500 rpm at room temperature (25 °C) for 10 minutes. Then 8 mL of ethanol was added to the BSA solution drop-wise (1 mL/min.) with continuous stirring (500 rpm) at room temperature till the solution appears milky. Further, 235 µL of glutaraldehyde (8% v/v) was added to the solution as a crosslinker. The reaction was stabilized by continuous stirring for 24 h. The resulting nanoparticles were purified by three cycles of centrifugation at 10,000 rpm for 10 min and then pellets were redispersed in 1 mL of Milli-Q water and sonicated for 5 min at Room Temperature. Resultant solution was stored at –80 °C overnight then lyophilized for further use. For the formation of BBR-BSA NPs, 20 mg of BBR was dissolved in 8 mL of absolute ethanol. 200 mg of BSA was dissolved in 2 mL of Milli-Q water and pH of the solution was adjusted to 7.4 using 1 M NaOH solution. Then the BBR solution was added dropwise (1 mL/min) to the BSA-solution under continuous stirring condition (500 rpm). The rest of the steps are similar to the synthesis of BSA NPs. Synthesized BSA NPs and BBR-BSA-NPs were lyophilized and stored at 4 °C for further use.

2.3. Characterization of Nanoparticles

The hydrodynamic particle size distribution was analyzed by DLS at 25 °C by using Zetasizer, NanoS90, Malvern, U.K. The morphology and shape of the lyophilized BSA NPs and BBR-BSA NPs were evaluated by Field emission scanning electron microscopy (FESEM: JSM7600 F, Jeol Asia) operated at an acceleration voltage of 10 kV. Dispersed particle solution was applied dropwise on silicon wafers and allowed to dry, further placed onto a carbon tape coated with gold under vacuum for 2 min. After which images were captured and the size of the nanoparticles was measured by ImageJ software.

The FTIR spectra of the nanoparticles were recorded with FTIR spectrophotometer (Nicolet iS5, Thermo Scientific, USA) over the range of 4000 to 400 cm⁻¹. 2 mg of pure BSA, pure BBR, BSA NPs, and BBR-BSA NPs were taken for the FTIR analysis. The XRD patterns of pure BBR, BSA NPs and BBR-BSA NPs were recorded using X-ray diffractometer (PANalytical, Almelo, the Netherlands). The operating voltage was 40 kV, the operating current was 30 mA, the start angle 2θ was 5° and the finishing angle was 50°.

The thermodynamic properties of BSA NPs and BBR-BSA NPs were evaluated by Differential Scanning Calorimetry (DSC 6000, Perkin Elmer, USA). The samples were scanned over the temperature range between 30 °C to 300 °C at a heating rate of 10 °C/min under nitrogen condition at a flow rate of 20 mL/min. For reference, an empty aluminum pan was used. TGA was performed on a TGA machine (TG/DTA 7300, Exstar). For TGA analysis, the product was placed in an aluminum pan and heated from 30 °C to 700 °C under nitrogen flow at a heating rate of 10 °C/min.

The stability of BBR-BSA NPs was evaluated by measuring the hydrodynamic diameters using Zetasizer Nano ZS 90 (Malvern Instruments) up to 8 days after the dispersion of nanoparticles in the aqueous media and PBS (pH 7.4 and pH 5.0).

2.4. Determination of the Berberine content and encapsulation efficiency (EE%)

For loading of BBR on BSA NPs, 8 mL BBR solution was (1 mg/mL in ethanol) added dropwise into the 2 mL aqueous solution of BSA. After overnight incubation in the dark at RT, BBR-BSA NPs were centrifuged at 10,000 rpm for 10 min. The unloaded drug content in the supernatant was determined by measuring the BBR concentration in the supernatant using a UV-Vis spectrophotometer. Drug encapsulation efficiency (EE) and drug loading capacity (LC) were calculated as follows:

Encapsulation Efficiency (%)

$$= \frac{\text{Initial amount of BBR} - \text{Free BBR in supernatant}}{\text{Initial amount of BBR}} \times 100$$

$$\text{Loading Capacity (\%)} = \frac{\text{Initial amount of BBR} - \text{Free BBR in supernatant}}{\text{Total amount of initial BBR and BSA}} \times 100$$

2.5. *In vitro* cell culture and cell cytotoxicity studies

MDA-MB-231 cell line was purchased from NCCS (Pune, India) for the cytotoxic study of synthesized BBR-BSA NPs. The cells were maintained in DMEM/F12 medium supplemented with 10% FBS, 100 U/mL streptomycin and penicillin at 37 °C in a CO₂ incubator.

2.5.1. MTT assay

To assess the *in vitro* anticancer activity and cell viability of the BBR-BSA NPs, we tested the cytotoxicity of BBR-BSA NPs on MDA-MB-231 breast cancer cell line by MTT assay. 5 × 10³ cells/well were seeded in 96-well culture plate and kept in a CO₂ incubator for 24 h. Further, Cells were treated with BBR, BSA NPs and BBR-BSA NPs. After the

incubation period, media from the wells were discarded and 100 μL MTT solution (0.5 mg/mL) was added to each well and kept for 4 h, at 37 °C in a CO₂ incubator in dark. After completion of the incubation period, MTT was removed and formazan crystals were dissolved by adding 100 μL of DMSO in each well. Absorbance was recorded at 570 nm using a multimode ELISA plate reader (Synergy H1, USA). Absorbance measured is directly relative to the %cell viability.

2.5.2. Trypan Blue Assay

Further confirmation of cell cytotoxicity, was done by Trypan Blue assay which is the most common method used to calculate the live cells and dead cells in a cell suspension. This assay is most reliable and fast method, works on the basis that cells having intact cell membranes, prohibit the entry of Trypan Blue dye whereas dead cells with compromised cell membrane accumulates with dye, which gives blue colour. 20,000 cells/well were seeded in 24 well plates. After 24 h of incubation, cells were treated with different concentration (25 μg/mL and 50 μg/mL) of BBR, BSA NPs and BBR-BSA NPs respectively. Then cells were trypsinized by EDTA-Trypsin. The cell suspension was centrifuged at 1250 rpm for 10 min. at 4 °C. The supernatant was discarded and cells were dissolved in 50 μL PBS. An equal amount of Trypan blue dye was added and the mixture were incubated for less than three minutes. Then cells were counted by hemocytometer.

2.5.3. Apoptosis study

For the study of apoptosis, 2.0 × 10⁴ cells/well (MDA-MB-231) were seeded in 24 well plates. After 24 h, cells were treated with two different concentrations (25 μg/mL and 50 μg/mL) of BBR, BSA NPs, and BBR-BSA NPs respectively. After completion of the treatment period cells were trypsinized and centrifuged for 5 min. at 1800 rpm. Pellet was redispersed in PBS and taken for further examination. 5 μL of Ethidium bromide (EtBr) dye (1 mg/mL) and Acridine orange (AO) dye (1 mg/mL) were mixed with 10 μL of cell suspension on a clean glass slide. Cells were observed by a fluorescence microscope (BX53 F, Olympus) at 200X magnification.

2.5.4. Cellular uptake study

To analyze the internalization of BSA NPs into MDA-MB-231 cells, coumarin-6 was incorporated into BSA NPs by the same protocol followed during the preparation of BBR-BSA NPs. The cells were seeded into 35 mm plates at a density of 2 × 10⁴ cells/well, and kept for incubation for 24 h. After which cells were incubated with coumarin-6 loaded BSA NPs (C6-BSA NPs) and free coumarin-6 (C6) solution with 10 μg/mL concentration for different time points (0.25 h, 0.5 h, 1 h, 2 h, 4 h and 8 h). After fixed time intervals, plates were washed with cold PBS thrice and observed under the microscope. Finally, the fluorescence images of cells were captured by a camera attached with fluorescent microscope (Olympus) for determination of intracellular uptake of the different Coumarin-6 loaded BSA NPs and compared with plain Coumarin-6.

2.6. Statistical analysis

All experiments were performed in triplicate and acquired data are expressed as mean ± SD. Statistical significance was analyzed using the student t-test for two groups. A probability (p) of less than 0.05 was considered statistically significant.

3. Results and Discussion

3.1. Preparation and physicochemical characterization of BSA NPs and BBR-BSA NPs

The significant success of Abraxane (Albumin-bound form of paclitaxel) has elevated the focus of research on protein nanoparticles and its applications for decades [30]. Previously, entrapment of many drugs

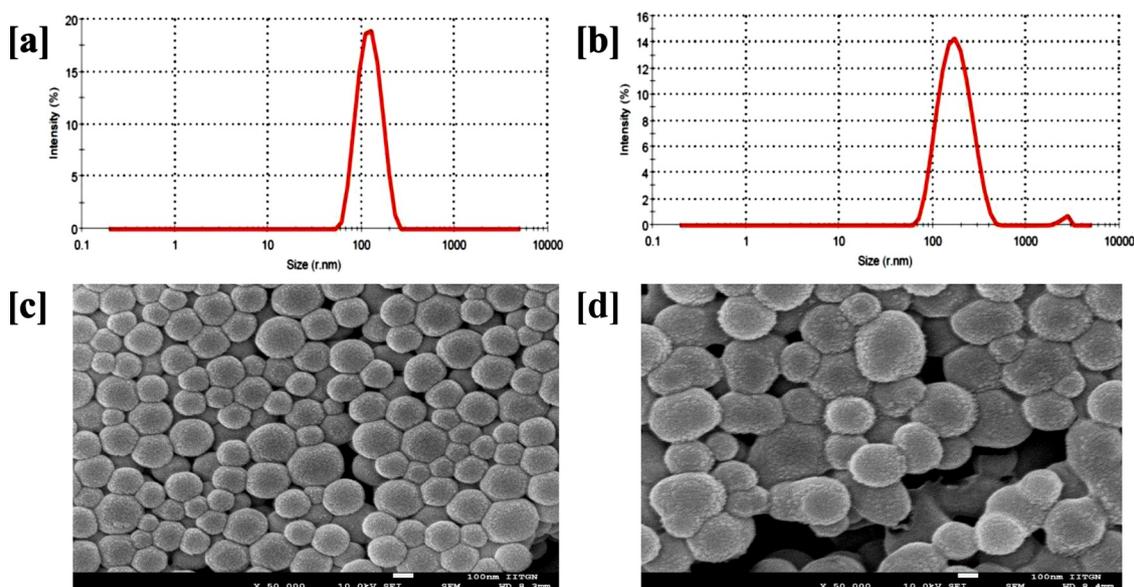


Fig. 1. The hydrodynamic size distribution of BSA NPs (a) and BBR-BSA NPs (b) determined by DLS. FESEM images of BSA NPs (c) and BBR-BSA NPs (d).

by BSA nanoparticles has been done by desolvation process, as it endows the drug with high dispersibility and biocompatibility [31–34]. In the current study we have synthesized BSA NPs and BBR-BSA NPs successfully by desolvation method, using ethanol as the desolvating agent [29,32]. Figure S1 represents the process of preparation of BBR-BSA NPs, which enhances the solubility as well as the anticancer activity of BBR. Nanoparticles prepared were stable in water and cell culture medium, as evidenced by *in vitro* analysis. The average size and morphology of nanoparticles was determined by DLS and FESEM respectively. The average hydrodynamic size of BSA NPs and BBR-BSA NPs was found to be 116 nm and 166 nm, respectively from DLS (Fig. 1a and 1b). Fig. 1c and 1d represents the FESEM images of BSA NPs and BBR-BSA NPs respectively, which provides information about the shape and surface morphology of nanoparticles. The average size distribution of BSA NPs and BBR-BSA NPs were found to be 104 nm and 110 nm respectively by FESEM (Figure S2). The images demonstrate a spherical morphology for the nanoparticles, which corroborates with the literature published [35]. The hydrodynamic diameter obtained from DLS is relatively higher than the diameter obtained from FESEM, which could be due to the presence of water molecules surrounding the BBR-BSA NPs during DLS measurement as compared to FESEM measurement in the dried state. The nanoparticles synthesized are of desirable sizes, which is in line with the previous published report [36]. Previous literature reveals that nanocarrier size (≤ 400 nm) plays crucial role in drug delivery and advantageously accumulate at the tumor site via enhanced permeability and retention (EPR) effect [37–40].

The molecular characterization of BSA NPs and BBR-BSA NPs obtained were done by FTIR spectroscopy. This technique has been used to evaluate the chemical and conformational changes that occur when nanoparticles (NPs) are formed or when they interact with other compounds through the slight shift in characteristics bands in the spectral regions of amide I and amide II [41]. Fig. 2a shows the FTIR spectra of pure BBR, pure BSA, BSA NPs and BBR-BSA NPs. A major band was observed at 3280 cm^{-1} for pure BSA (amide A, related to N-H stretching), 2970 cm^{-1} (amide B, N-H stretching of NH_3^+ free ion), 1643 cm^{-1} (amide I, C = O stretching), 1515 cm^{-1} (amide II, related to C-N stretching and N-H bending vibrations), 1392 cm^{-1} (CH_2 bending groups) and 1260 cm^{-1} (amide III, related to C-N stretching and N-H bending) (Figure S3b). The most intense bands are associated with the secondary structure and conformation of proteins. The spectra of BSA NPs and BBR-BSA NPs exhibited these characteristic bands of the protein and BBR structure shifted slightly [33,42–44]. A small shift of the

observation bands, was observed when compared to pure BSA with BSA NPs and BBR-BSA NPs. The related changes in the amide I, II and III bands confirm the formation of albumin NPs (BSA NPs) and BBR-loaded NPs (BBR-BSA NPs).

The characteristic peaks of pure BBR were observed at 3467 cm^{-1} (O-H stretching), 1634 cm^{-1} (C = N stretching), 1505 cm^{-1} (aromatic C = C vibrations), 1227 cm^{-1} and 1104 cm^{-1} (sulfate ions) which confirms the presence of BBR (Figure S3a). The FTIR spectrum of freshly synthesized BBR-BSA NPs showed significant peaks at 1648 cm^{-1} and 1528 cm^{-1} which revealed the presence of amide I and amide II bond of BSA. The characteristics peaks of BBR at 1233 cm^{-1} and 1100 cm^{-1} confirmed the presence of sulfate ions of BBR (Figure S3d). Therefore, the resulting FTIR peaks confirm the successful encapsulation of BBR into the BSA NPs [35].

XRD is conducted to examine the encapsulation of BBR into BSA NPs. Fig. 2b exhibits the powder XRD diffractograms of pure BBR, BSA NPs and BBR-BSA NPs. The XRD pattern of pure BBR shows sharp and intense peaks at 2θ angles of 8.7° , 9.2° , 13.0° , 14.1° , 16.3° , 20.5° , 24.7° , 25.5° and 26.4° which indicates the crystalline nature of pure BBR in the native form [45]. However, these crystalline characteristics peaks of BBR were not observed in the diffractogram of BBR-BSA NPs. These results suggest that BBR is successfully encapsulated either in an amorphous form or molecularly dispersed in the matrix of the BSA NPs [46–47]. To confirm it further we performed the DSC and TGA analysis. Fig. 2c represents the DSC thermograms of BBR, BSA NPs and BBR-BSA NPs. It is observed that pure BBR gives an endothermic peak at $\sim 186^\circ\text{C}$, however that peak is not observed in BBR-BSA NPs but a single peak at 244°C is observed, which indicates the transformation of physical state of drug during the process of nanoparticle formation. So, the DSC data corroborates the XRD data of successful encapsulation of BBR into BSA NPs in their amorphous form and reduction of crystallinity increase the amorphous property of drug within nanoparticles [32]. Fig. 2d represents the TGA thermograms of pure BSA, pure BBR and BBR-BSA NPs. Total weight loss of pure BSA and BBR observed was 75% and 50%, respectively. After the loading of BBR, the total weight loss of BBR-BSA NPs observed was 81%. The increment in the total weight loss may be the result of the organic component of the drug after loading in the BSA NPs [48].

Entrapment efficiency (%EE) and Loading capacity (%LC) of prepared BBR-BSA NPs were observed to be 85.65% and 7.78%, respectively. The %EE and %LC were calculated using UV-Vis spectrophotometer (Table 1).

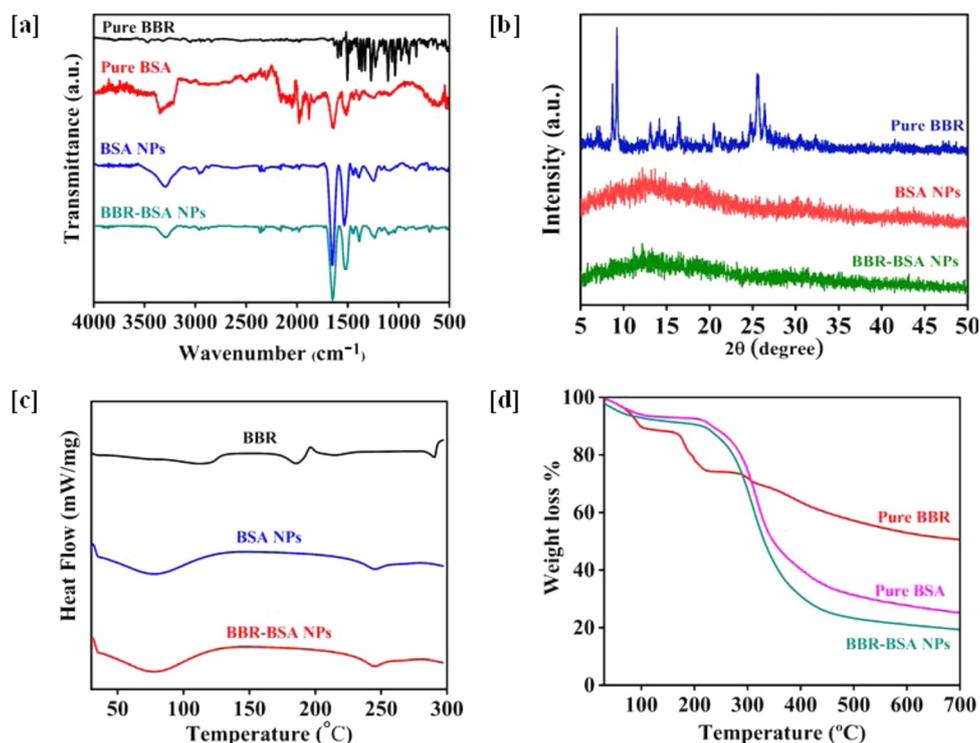


Fig. 2. FTIR spectra of Pure BBR, Pure BSA, BSA NPs and BBR-BSA NPs (a). XRD patterns of pure BBR, BSA NPs and BBR-BSA NPs (b). DSC Thermograms of Pure BBR, BSA NPs and BBR-BSA NPs (c). TGA thermograms of Pure BBR, Pure BSA, and BBR-BSA NPs.

Table 1

%EE and %LC of BSA NPs and BBR-BSA NPs.

SAMPLES	PARTICLE SIZE (nm)	PDI	%EE	%LC
BSA NPs	116	0.038	-	-
BBR-BSA NPs	166	0.184	85.65	7.78

Various factors such as particle size, stability, molecular weight and blood circulation time plays significant roles in the accumulation of nanoparticles at the tumour site [49]. So, we have examined the stability of the synthesized BBR-BSA NPs at regular time intervals up to 8 days. Fig. 3a, represents the time dependent size stability of BBR-BSA NPs at physiological pH. It depicts that there was no significant change observed in the size of BBR-BSA NPs over the period of 8 days, which suggests that the BBR-BSA NPs are quite stable at room temperature in aqueous solution. Further the effect of pH on BBR-BSA NPs was studied, as shown in Fig. 3b. The particle size of BBR-BSA NPs was monitored at physiological pH (PBS, pH 7.4) and acidic medium (PBS, pH 5.0, which mimics the tumor pH condition) for 8 days. The stability of BBR-BSA NPs was maintained for 8 days at pH 7.4. The size of BBR-BSA NPs was remained unchanged up to 8 days at pH 7.4, indicating its thermodynamic stability in aqueous medium. On the other hand, the size of NPs increased in acidic condition (pH 5.0), which is attributed to the fact of formation of large aggregates helping to release the drug at target site. The stability analysis reveals that the synthesized nanoparticles were highly stable in physiological buffer, which implies that

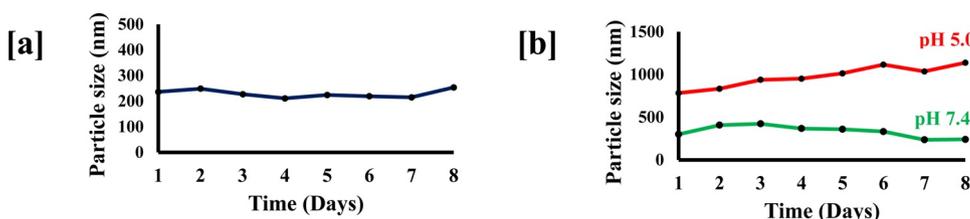


Fig. 3. Stability analysis: Time-dependent stability of the BBR-BSA NPs using particle size analysis over a period of 8 days with respect to different time intervals (a) and pH stability at different pH (b).

the studied nanoparticles may show prolonged circulation in vivo [50]. From all these observations, it is predicted that the synthesized BBR-BSA NPs will have high stability in the blood circulation and it will trigger the release of BBR, when it sees the acidic pH environment at the tumor site; thus it can be effective for cancer therapy. Extended circulation of high molecular weight nanoparticles in the blood subsequently results in tumor targeting by the enhanced permeability effect and by minimizing macrophage uptake [51].

3.2. In vitro cytotoxic study of BSA NPs and BBR-BSA NPs

3.2.1. Anti-proliferative assay

For drug delivery and other biomedical uses, toxicity is a critical parameter to consider when evaluating their applications. Keeping the biomedical and pharmaceutical applications in mind, these biocompatible nanoparticles were synthesized intentionally to interact with cells without any adverse effect. To assess the *in vitro* proliferation rates of cells and potential anticancer activity of the nanoparticles, we investigated the cytotoxicity of the synthesized nanoparticles (i.e. BSA NP and BBR-BSA NPs) and Berberine by MTT assay on highly aggressive breast cancer cell line MDA-MB-231 cells.

While performing experiments, we made sure that at each concentration, enough quantity of BBR-BSA NPs were used, so that the free BBR concentration was fairly comparable to that of the entrapped one, which enabled us for proper evaluation. Fig. 4 represents the viability of MDA-MB-231 breast cancer cells after incubating with the varying

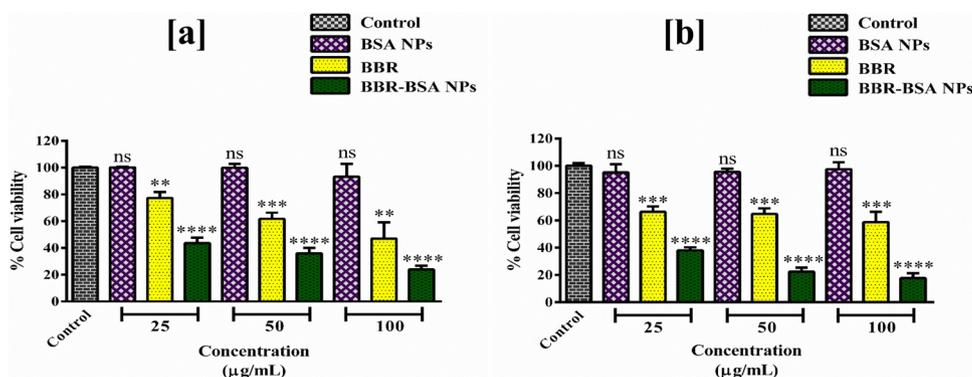


Fig. 4. Effect of BBR-BSA NPs on cell viability in MDA-MB-231 cells. MDA-MB-231 cells were treated with different concentrations (25 µg/mL, 50 µg/mL and 100 µg/mL) of blank BSA NPs (BSA NPs), Pure Berberine (BBR) and Berberine loaded BSA NPs (BBR-BSA NPs) for 24 h (a) and 48 h (b) respectively. All time points were followed by MTT assay. Control cells were left untreated. The cell growth data shows mean \pm SEM (n = 3); *p \leq 0.05, ** p \leq 0.01, *** p \leq 0.001, ****p \leq 0.0001 vs. respective controls.

concentrations of BSA NPs, BBR and BBR-BSA NPs for 24 h and 48 h. Importantly, it was observed that the BSA NPs showed no cytotoxicity which was evident from more than 95% of cell viability of the cells incubated with blank BSA NPs and indicated the non-toxic and highly biocompatible nature of the carrier. However, BBR and BBR-BSA NPs showed a significant decrease in the viability of the MDA-MB-231 breast cancer cells respectively in both dose and time-dependent manner, coincides with the result reported earlier for different cell lines [35,52]. In addition, it was observed that BBR-BSA NPs showed higher cytotoxicity as compared to pure BBR at all the concentration studied for all the time points (24 h and 48 h) in MDA-MB-231 cells, which implies that the anticancer activity of BBR loaded BSA NPs is phenomenally significant, effective and successfully released and remained functional in the cancer cells.

3.2.2. Trypan Blue Assay

Further, to assess the no. of viable and/or dead cells in the cell suspension, the Trypan blue assay was performed. It's a simple, very inexpensive and reliable test, which provides information about the cell membrane integrity.

Fig. 5 shows the cytotoxicity of BBR, BSA NPs and BBR-BSA NPs after 24 h and 48 h of incubation at different concentrations (25 µg/mL and 50 µg/mL). BBR-BSA NPs at both the concentrations shows 20 % to 30 % higher cell death as compared to pure BBR. Moreover BSA NPs did not show any significant percent of cell death at both the concentrations (25 µg/mL and 50 µg/mL) for both the incubation periods of 24 h and 48 h. So, Trypan blue assay suggested that BBR-BSA NPs induce more inhibitory or anti-proliferative effect than BBR in MDA-MB-231 breast cancer cells, which further corroborate the MTT assay results.

3.2.3. Apoptosis Assay

Acridine orange/Ethidium bromide (AO/EtBr) assay is mainly used to differentiate between live, apoptotic and necrotic cells [53]. The apoptotic study was performed to determine the changes at cellular

level after treatment with BBR, BSA NPs, and BBR-BSA NPs using AO/EtBr staining method [54]. In this method, AO dye stains nuclei of live cells that have intact cellular membranes while EtBr stains the nuclei of dead cells having compromised cellular membrane. AO emits green fluorescence whereas EtBr emits red fluorescence showing live and dead cells population. Fig. 6 indicates the treatment of MDA-MB-231 cells with BSA NPs, BBR and BBR-BSA NPs at different concentrations (25 µg/mL and 50 µg/mL) for 24 h and 48 h respectively. Treatment of MDA-MB-231 cells with BSA NPs did not exhibit any signs of apoptosis or necrosis at all the time points for all the concentrations used and all the cells appeared uniformly green in colour with intact nuclear organization and cellular morphology. On the other hand, the cells treated with BBR and BBR-BSA NPs exhibited typical features of cell death, for which uniform red fluorescence was observed in cells at 24 h and 48 h time points for both the concentrations (25 µg/mL and 50 µg/mL). The number of dead cells were maximum for BBR-BSA NPs at 48 h with 50 µg/mL concentration, which implies BBR-BSA NPs trigger higher apoptosis in the MDA-MB-231 cells than pure BBR. Collectively, these results suggest that BBR-BSA NPs is able to induce apoptosis in a time and concentration dependent manner in MDA-MB-231 cells, which corroborates our antiproliferative assay and suggest BBR-BSA NPs kill cells more effectively as observed in the apoptotic study.

3.2.4. Cellular uptake study

Cellular uptake studies were performed to get insight of the intracellular trafficking potential of synthesized nanoparticles, i.e. BBR-BSA NPs. For that Coumarin-6 (C6) loaded BSA NPs (C6-BSA NPs) were separately synthesized and treated on MDA-MB-231 breast cancer cells for different time intervals (0.25 h, 0.5 h, 1 h, 2 h, 4 h and 8 h). Fig. 7 shows the fluorescence images of MDA-MB-231 cells on treatment with C6 and C6-BSA NPs for mentioned time intervals. Cells incubated with C6-BSA NPs shows higher fluorescence intensity than cells incubated with only C6 at all the time points studied. C6-BSA NPs showed higher cellular uptake than plain C6 in time dependent manner. The results

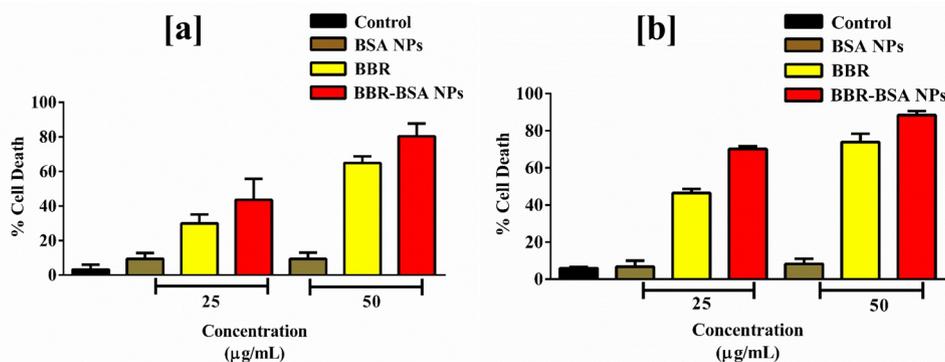


Fig. 5. Trypan blue exclusion assay showing the effect of different concentrations (25 µg/mL, 50 µg/mL) of blank BSA NPs (BSA NPs), Pure Berberine (BBR) and Berberine loaded BSA NPs (BBR-BSA NPs) on MDA-MB-231 cells at 24 h (a) and 48 h (b) respectively as compared to control cells. Control cells were left untreated.

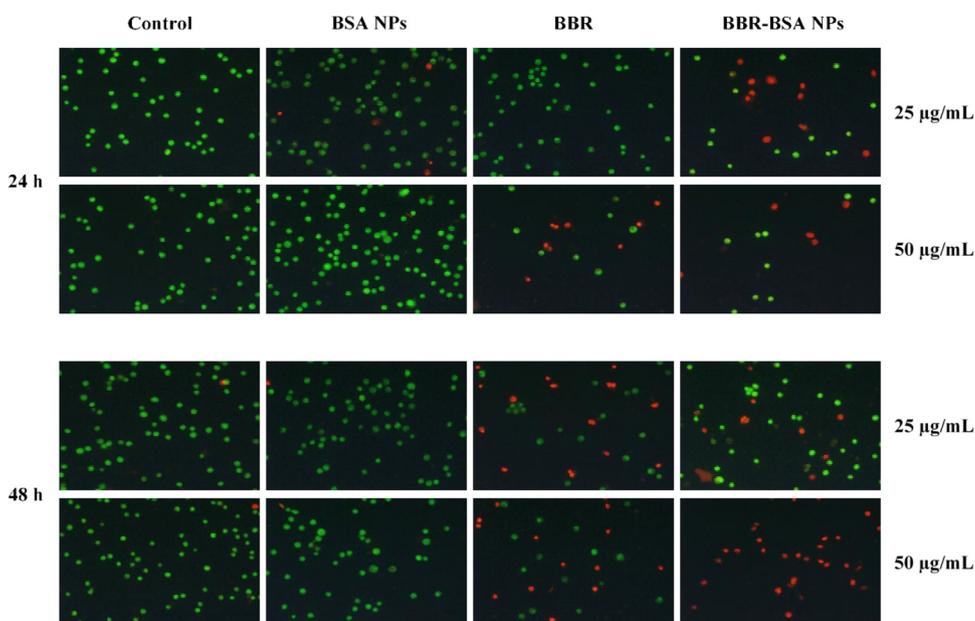


Fig. 6. Apoptosis study by Acridine orange/Ethidium bromide (AO/EtBr) staining. Effect of BBR-BSA NPs on apoptosis in MD-AMB-231 cells. MDA-MB-231 cells were treated with two different concentrations (25 µg/mL and 50 µg/mL) of blank BSA NPs (BSA NPs), Pure Berberine (BBR) and Berberine loaded BSA NPs (BBR-BSA NPs) for 24 h and 48 h respectively, followed by fluorescence microscopy using AO/EtBr staining assay. Control cells were left untreated. All the photomicrograph (n = 3) were acquired at 200X magnification.

suggest that BBR-BSA NPs could effectively improve the delivery of hydrophobic molecules into the MDA-MB-231 breast cancer cells and therefore it could be responsible for higher cytotoxicity of BBR-BSA NPs against these cells than BBR. It was observed that BSA NPs entrapped coumarin formulation showed time-dependent cellular uptake by an increase in the intensity of Coumarin with the increase in incubation time. The BSA NPs entrapped drug showed pronounced uptake in comparison to pure drug.

Higher fluorescence intensity was observed in coumarin-6-BSA NPs (0.5 µg/mL) than only coumarin-6 (0.5 µg/mL) in a time-dependent manner (Fig. 7b), which may confirm the higher uptake of BBR-BSA NPs in the MDA-MB-231 cells. The results suggest that BSA NPs could enhance the delivery of BBR into the MDA-MB-231 cells at lower concentrations and therefore it could enhance the anti-proliferation activity of BBR.

4. Conclusion

In the present study, we have successfully developed/formulated a biocompatible, nontoxic, novel anticancer Berberine delivering system i.e. BBR-BSA NPs. The nanoparticles were synthesized by desolvation method and characterized successfully. The particle size, morphology and stability analysis indicated that the BBR-BSA NPs were quite stable,

spherical in shape and nearly monodisperse in nature. The results of DLS and FESEM implies that the size of synthesized nanoparticles were within the suitable and optimal range for drug delivery applications. The MTT assay further supports the nontoxic and biocompatible nature of the BSA nanocarrier (BSA NPs), at the same time BSA NPs sufficiently enhanced the solubility and anticancer activity of pure Berberine against breast cancer cells, when entrapped (BBR-BSA NPs). The apoptosis and cellular uptake study proved that the BBR-BSA NPs were more cytotoxic towards MDA-MB-231 breast cancer cells whereas the results of higher intracellular uptake studies suggest that BBR-BSA NPs could effectively improve the anticancer activity at the lower dose of BBR. Overall, all the experiments performed, clearly demonstrates that BBR-BSA NPs could be useful as a potent delivery system for intracellular release of Berberine and increase its anticancer efficacy. The current study uses Bovine Serum Albumin as a proof-of-technology examination. Further, Human Serum Albumin, which is the human analog of BSA would be considered as a probable delivery system to avoid any immunologic consequences for *in vivo* trials. Thus, our study proposes that the chemotherapeutic potential of Berberine could be better utilized by loading it in a protein-based carrier for future clinical applications.

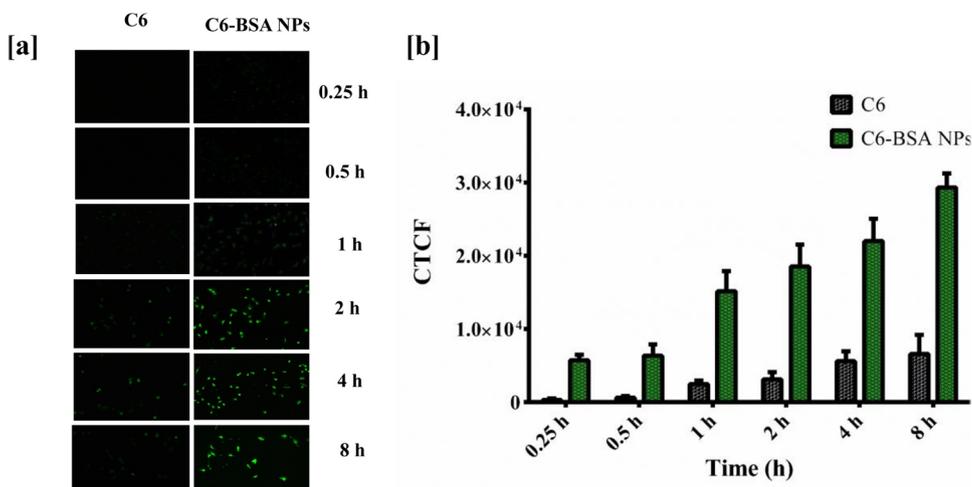


Fig. 7. Cellular uptake study in breast cancer cell line (MDA-MB-231). Cells were treated with native Coumarin-6 (C6) (0.5 µg/mL) and Coumarin-6 loaded BSA NPs (C6-BSA NPs) (0.5 µg/mL) at different time intervals of 0.25, 0.5, 1, 2, 4 and 8 h. All the images were taken by fluorescence microscopy (a). The quantitative cellular uptake analysis of C6 and C6-BSA NPs (b). (Scale bar = 50 µm).

CRedit authorship contribution statement

Raghu Solanki: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing - original draft. **Krunal Patel:** Methodology, Software, Visualization. **Sunita Patel:** Supervision, Conceptualization, Funding acquisition, Project administration, Resources, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

Authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.colsurfa.2020.125501>.

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श्रुति शब्दसृष्टि

सर्प : १०० भाग (१००) : १०० भाग (१००) : १०० भाग (१००) : १००

नवी विद्युत् संस्था
पुणे - ४११००४

નાંકા, ઉબાલીમ રાહોડ, જયંત મહેતા, રમણભાઈ ડી. પટેલ, તરનીમ જાની, મુલાજી શાહેસ્ત્રજી, મનેશચંદ્ર કંસારા, દીપક બારપોલીકર, યોગેશ પટેલ, પ્રહ્યાભાઈ પટેલ, કુમુદ પોપટ, પન્ના નાયક, પીતિ સેનગુપ્તા, ભદ્રા વડગામા, બાનુગંધર વ્યાસ, ચીતમ વિનય કવિ અને રોહિત પંડ્યા આદિ વાર્તાકારો કાર્ય કરી રહ્યા છે.

અહમદ ગુલ એમનામાંના જ એક વાર્તાકાર. મુળે એ ગઝલકાર છે. એમણે સાતેક ગઝલસંગ્રહ આપ્યા છે. 'ઉપવન', 'પમરાટ', 'મીન પડપાપા કરે', 'મીનનું તેડું', 'પાપટ્રી', 'સંગતિ' અને 'મીનાલય'. એમની ગુજરાતી ગઝલોનું અંગ્રેજી રૂપાંતર અરોમા નામથી પ્રકાશિત છે. 'નૂરાની મુલાકાતો' નામનાં સસ્મરણો લખ્યાં છે. 'યોર્કશાયર(યુ.કે.)માં ગુજરાતી ભાષા-સાહિત્યનો આલેખ' એ સંપાદન પણ કર્યું છે.

'અજ્ઞાપ્યાં' વાર્તાસંગ્રહમાં કુલ ચૌદ વાર્તાઓ છે. આ ચૌદ વાર્તાઓમાં સ્થળાંતર કે વતનપુરોધાને લગતી આઠેક વાર્તાઓ છે. જ્યારે વતનને યાદ કરતાં કરતાં વતનની સ્મૃતિઓને સંભારવાનો પ્રયત્ન બીજી છ વાર્તાઓમાં છે. અહમદ ગુલ આ વાર્તાઓમાં જે બપાન કરે છે તે આમ જોવા જવ તો સામાન્ય સાહિત્યનાં - એટલે કે ટ્રાયસ્પોરાનું નથી એવા - સાહિત્યનાં લક્ષણો ધરાવે છે. ગુજરાતી સાહિત્યમાં મુસ્લિમ સમાજ અને સંસ્કૃતિને વ્યક્ત કરે એવું સાહિત્ય ઓછું છે. અહમદ ગુલ આ વાર્તાઓમાં એ અપુરવને પૂર્ણ કરવાનો પ્રયત્ન કરે છે. એમાંય વિદેશમાં રહીને દેશ અને સંસ્કારવારસો ભાષા, ભાષાનો લહેકો આદિ એવાં ને એવાં તાજાં વાર્તાઓમાં પ્રતિબિંબિત થાય છે.

અહીં જે વાર્તાઓ છે એનું કેન્દ્ર માનવસહજ સંવેદના છે. એ વિદેશમાં હોય કે દેશમાં... એનું સંવેદનવિશ્વ બદલાતું નથી. પણ વિદેશનું વાતાવરણ એનાં સ્વપ્નો, એની કશુંક આનંદપ્રદ મેળવી લેવા માટેની મથામણો - સંઘર્ષો વગેરે આ વાર્તાઓમાં જોવા મળે છે.

'શોધ' વાર્તામાં બ્રિટનના હવામાનની વાતથી શરૂઆત કરીને લેખક એક કરુણ અંત પાસે વાર્તા પૂરી કરે છે. અહીં વાતાવરણની, પ્રકૃતિની કૂરતા જાણે હુસેનનાં બધાં સ્વપ્નો શેળી નાખે છે. શિયાળામાં બ્રિટનનું વાતાવરણ. ઉત્તર ઈંગ્લેન્ડના યોર્કશાયર પરગણાની વાત. અહીં એશિયન લોકો એક ઘરમાં પાંચસાત જણ સાથે રહે. સવારે નોકરી કરવા જાય પછી સાંજે પાછા કરે ત્યારે વાતાવરણ બદલાઈ ગયું હોય. વાહનો બંધ થઈ ગયાં હોય. એવા સંજોગોમાં આબીદ ને હુસન બેશુદ્ધ જેવી દશામાં મળે. એની સારવાર કરે ને છેવટે એને માનસિક અસંતુલનની હોસ્પિટલમાં ભરતી કરવો પડે છે.

'હુસેન આ દેશમાં એક વર્ષ પહેલાં આપણી માફક ઉજ્જવળ ભવિષ્ય માટે આવ્યો એનું એકમાત્ર રહેવાનું ઘર ગીરવે મૂકેલું. અને પત્ની અને ત્રણ બાળકોને મૂકીને અહીં આવેલો. આવ્યા પછી એના બદનસીબે કામ ન મળતાં પીમે પીમે તજાવમાં રહેવા લાગ્યો અને છેલ્લા બેએક મહિનાથી તો એના મગજનું સંપૂર્ણ સમતોલપણું ગુમાવી બેઠેલો... આજના હિમવર્ષાના દિવસે એ માનસિક અસંતુલન દર્દીઓની ચિકિત્સા માટેની હોસ્પિટલમાં પહોંચી જતાં એના ઉજ્જવળ ભાવિની શોધનો અંત આવી ગયો.' (પૃ. ૩૩. અજ્ઞાપ્યાં).

આ વાર્તામાં ઈંગ્લેન્ડના લોકોની માનવતાનો પણ આછો ચિતાર છે, પણ બદનસીબ હુસેનના જીવનમાં કોઈ પરિવર્તન આ દેશમાં આવવાથી થતું નથી એ દારુણ વેદના પણ પ્રતિબિંબિત થાય છે.

કોને ડાયસ્પોરિક સાહિત્ય કહીશું એ વિષે બહુ જ અન્યતર્યા મતો છે. દેશમાં રહેલી સર્જક અને વિદેશમાં રહેતો સર્જક જે લખે છે તેમાં ઘણી બધી સમાનતા જોવા મળે છે. કાર્ત્તીક માંજી વિભાગો પાડી શકાતા નથી. તેમ છતાં જે વિદેશમાં લખાય છે તે ડાયસ્પોરિક સાહિત્ય સ્વીકારાતું રહ્યું છે. 'મનુષ્યની ચેતનામાં અમુક વલણો તો દુનિયાના કોઈ પણ ખૂણે મળ્યા હોવાના.' એમ કહીને શ્રી સર્વક જિવેદી વિદેશમાં લખાતા ફેશી પ્રકારના સાહિત્યને સ્વીકારવાની તૈયારી જતાવી છે.

એ રીતે જોતાં અત્યંત ગુલાની વાર્તાઓમાં જે એ રંગ દેખાય છે તેને ડાયસ્પોરિક સંવેદન ગણવામાં કયો વાંધો ન હોવો જોઈએ. વિપુલ કલ્યાણીએ કરેલા સંપાદનમાં સંપાદકીય પ્રસ્તાવનામાં સંપાદકોને ડાયસ્પોરિક સાહિત્ય વિશે પોતાના વિચારો રજૂ કર્યા છે. એ મુજબ ડાયસ્પોરિક ગણવા માટે ખરેખર જે જરૂરી છે તે લક્ષણોનો ખ્યાલ આવે છે.

ડાયસ્પોરા સાહિત્ય એટલે 'જે સર્જકો પહેલાં, કોઈ સમયે આફ્રિકામાં જઈ ગયા હતા અને રાજકીય પરિસ્થિતિ બદલાતાં, ત્યાંથી સ્થળાંતર કરીને યુરોપ-અમેરિકા જઈને ફરી વસ્યા હતા— એ સર્જકોનાં, પોતાના મૂળ વતન તેમ જ આફ્રિકા-નિવાસનાં સંસ્મરણો-ભાવનાઓને વ્યક્ત કરતું સાહિત્ય.'

'વિદેશવાસી થયા પછીય, મોટે ભાગે પોતાનાં દેશ-પ્રદેશ અને સમાજ-સંસ્કૃતિની અવિસ્મૃત પરંપરામાં થસતા રહીને વતન-સુરાધાને જીવતા સર્જકોનાં સ્મરણ-સંવેદનોને વ્યક્ત કરતું સાહિત્ય.'

'સ્વદેશ છોડીને વિદેશ વસી, સ્થિર થવા ચાહતા અને એ માટેની મજબૂત મથામલ કરતાં કરતાં વસવાટી ભૂમિ અને તેની સંસ્કૃતિનો પ્રભાવ ઝીલી-સ્વીકારીને, એ પ્રક્રિયાની પીઘ અને આનંદથી સમૃદ્ધ થયેલા સર્જકની લાગણીઓને વ્યક્ત કરતું સાહિત્ય.'

'વસવાટી દેશ-પ્રદેશની આબોહવા, એના પરિવેશમાં પગ ખોડીને ઝિભેલા અને સ્વદેશ-વિદેશની સાંસ્કૃતિક-સાહિત્યિક પરંપરામાં આદાન-પ્રદાનથી સમૃદ્ધ થયેલા સર્જકોનાં સંવેદનોને નિરૂપતું સાહિત્ય'.

આટલી બાબતોનો આધાર લઈ બ્લોગ લેખક ચિરાગ ઠક્કર ડાયસ્પોરિક સાહિત્યની એક વ્યાખ્યા આપવાનો પ્રયત્ન કરે છે.

'એક ભૂમિ અને/અથવા સંસ્કૃતિમાં જન્મ અને ઉછેર પામીને બીજી ભૂમિ અને/અથવા સંસ્કૃતિમાં સ્થિર થયેલા કે સ્થિર થવા મથતા સર્જકના સંવેદનતંત્રમાં એ ભૂમિ અને/અથવા સંસ્કૃતિઓના અવનવા મિશ્રણ થકી સર્જાતી ઊચલપાચલ એટલે ડાયસ્પોરિક સાહિત્ય'.

આ વાર્તાસંગ્રહ 'અજણ્યા'માં અત્યંત લુપ્તાત ગુલા વિદેશ વસવાટની જે વાર્તાઓ લઈને આવે છે તેમાં આ સંવેદન પ્રગટ થયું છે. ગુજરાતી સાહિત્યમાં અને ગુજરાતી ડાયસ્પોરિક સાહિત્યમાં પણ મુસ્લિમ સમાજની વાર્તાઓ બહુ જુજ જોવા મળે છે. અને તેમાંય કોઈ ને કોઈ કારણસર વિદેશની ભૂમિ પર વસવાટ કરવો પડ્યો હોય, ત્યાં સમૃદ્ધ પણ થયા હોય છતાં મૂળ વતન પ્રત્યેની સંવેદના જોર કરતી હોય તેવું જેમ અન્ય કોમ કે જાતિને લાગુ પડે તેવું જ આ મુસ્લિમ સમુદાયને પણ લાગુ પડે છે તે 'અજણ્યા'ની વાર્તાઓમાંથી પરસાર થતાં અનુભવાય છે.

અલબત્ત બ્રિટનમાં વાર્તાસર્જકોની સંખ્યા ઓછી નથી. શાંતશીલા ગઘર, વલ્લભ શબ્દસૃષ્ટિ : ઓગસ્ટ : ૨૦૨૧ & ૧૦

બ્રિટિશ-ગુજરાતી વાર્તાકાર અહમદ ગુલની વાર્તાઓમાં ઢાયસ્પોરિક સંવેદન

દીપક ભાનુશંકર ભટ્ટ

[Abstract/સાર : ગુજરાતી કથાસાહિત્યમાં આપણા સમાજના ઘણા વર્ગોને હજુ સ્થાન નથી મળ્યું. એવા એક વર્ગ મુસ્લિમ ગુજરાતી સમાજને ધ્યાનમાં લઈને બહુ ઓછું કામ થયું છે. એમાંય ઢાયસ્પોરા સાહિત્યમાં ગુજરાતથી વિદેશમાં વસતા મુસ્લિમ સમાજનું પ્રતિનિધિત્વ પણ નજીવું છે. અહમદ ગુલ જે બ્રિટનમાં રહીને ગુજરાતી ગઝલ-કાવ્યસ્વરૂપમાં સર્જન કરે છે, તેમણે ૨૦૧૨માં 'અજબલ્યા' ગ્રીર્ષક સાથે એક વાર્તાસંગ્રહ પ્રગટ કર્યો. આ વાર્તાસંગ્રહમાં એમણે સામાન્ય લોકોના જીવનમાં બનતી અસામાન્ય ઘટનાઓને ધ્યાનમાં લઈ વતનથી દૂર રહીને જીવન વિતાવતાં પાત્રોની સંવેદના વ્યક્ત કરી છે. તેમની આ વાર્તાઓ બ્રિટનના સ્થાનિક 'ઓપિનિયન' અને અન્ય સામયિકોમાં પ્રકાશિત થઈ છે.

આ વાર્તાઓમાં બ્રિટિશ-ગુજરાતી મુસ્લિમ સમાજના પ્રશ્નો, મુકાબલાઓને વાચા મળતી જોઈ શકાય છે. ડ્રે. બાબવંતભાઈ જાની કહે છે તેમ : 'અંગ્રેજ સભ્યતા અને સંસ્કૃતિ સંદર્ભે મુસ્લિમ સમાજ કેટલું અનુકૂળ વલણ દાખવે છે અને પ્રતિકૂળ પ્રશ્નોનો કેવી રીતે સામનો કરે છે એનો પ્રતિપાંચ એમની પ્રકાશિત અનેક વાર્તાઓમાં દષ્ટિગોચર થાય છે.' અહમદ ગુલ મુસ્લિમ સમાજનું ચિત્ર આલેખે છે ત્યારે ભારતીય કથાનકનાં રૂપકો પ્રયોજે છે. મુસ્લિમ-અંગ્રેજ મિત્રો વચ્ચે હળવાનું-મળવાનું, સાથે રહેવાનું અને અને વિભિન્ન સાંસ્કૃતિક વિચારધારાને કારણે વિસંવાદિતા સર્જાય એને ખુબ જ સારી રીતે અભિવ્યક્તિ આપે છે .

'અજબલ્યા' વાર્તાસંગ્રહમાં ચૌદ વાર્તાઓ છે. એમાં બ્રિટનમાં વસતા ગુજરાતી મુસ્લિમ સમાજના ઢાયસ્પોરિક સંવેદનને અહમદ ગુલ પ્રસ્તુત કરે છે. મુસ્લિમ સમાજને કેન્દ્રમાં રાખીને લખાતી વાર્તાઓ આમંચ વિરલ છે, તેમાંય આ વાર્તાઓ તો વતનની યાદને, વતનની ભાષામાં લખાયેલી વાર્તાઓ છે એટલે તેનું મહત્વ જુદું છે.]

પૂર્ણ પ્રપત્ર

ગુજરાતી 'ઢાયસ્પોરિક સાહિત્ય' કે સ્થળાંતરનું સાહિત્ય કોને કહેવાય એ વિશે સતત મઘામણ ચાલ્યા જ કરે છે. ગુજરાતી ઢાયસ્પોરા વિશે એ મંઘન ચાલ્યું છે. ગુજરાતી સાહિત્યમાં જોકે દલિતો લખે એ દલિત સાહિત્ય, સ્ત્રીઓ લખે એ નારીચેતનાનું સાહિત્ય ને એવા વિભાગો છે. એમ જ વિદેશમાં વસતો કોઈ પણ ગુજરાતી જે લખે તે ઢાયસ્પોરિક (ગુજરાતી) સાહિત્ય કહેવાય એમ મનાતું થયું છે. ને ત્યાં સુધી કે દેશ-વિદેશના પ્રવાસ કરે તે જે પ્રવાસઅનુભવ વિશે લખે તેને પણ ઢાયસ્પોરિક કહેવાનો ચાલ છે.

શબ્દસૃષ્ટિ

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વર્ષ : ૩૮, અંક : ૮, ઓગસ્ટ : ૨૦૨૧, સર્વમ અંક : ૪૫૩



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પ્રતિભાવ, ૯૫

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આ અંકના સાહિત્યકારો, ૧૦૨



Carrier-free resveratrol nanoparticles: Formulation development, *In-vitro* anticancer activity, and oral bioavailability evaluation

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ABSTRACT

Resveratrol nanoparticles (RNP) formulation was prepared with 0.5% w/v of SDS and confirmed by TEM, XRD, DSC, XRD, FTIR and NMR. The prepared RNP were evaluated for physicochemical properties like solubility, stability, dissolution profile and partition coefficient. The anticancer activity of prepared RNP formulation was determined against HCT 116 human colorectal cancer cells by cell viability and apoptosis studies. An *in vivo* pharmacokinetic study in SD rats showed that, the AUC of the RNP was significantly higher ($p < 0.0001$) than the pure RSV. The plasma C_{max} was found to be $0.23 \pm 0.02 \mu\text{g/mL}$ for RSV and $0.62 \pm 0.09 \mu\text{g/mL}$ for RNP. An increase in AUC and C_{max} suggested that, the developed formulation was more bioavailable than the pure RSV.

1. Introduction

Solubility is the major hurdle for many potential bioflavonoids and synthetic drugs and creates hurdles in their product development and commercialization [1]. Various bioflavonoids suffer from less water solubility and poor bioavailability due to their weakly acidic or weakly basic nature [2]. These molecules produce several important health benefits, but the therapeutic efficacies of these molecules are limited due to their poor physicochemical properties and hence unfavourable pharmacokinetic (PK) profiles after administration. Among the various type of nanocarriers, the preparation of drug nanoparticles (DNPs) using a size reduction technique looks like a better approach, particularly when the solubility and bioavailability enhancement are primary objectives [3]. Further, a significant decrease in the crystallinity of drugs is observed during the preparation of DNPs, which also helps to improve the physicochemical properties of drugs [4].

Resveratrol (RSV) is a naturally occurring, BCS-II polyphenolic compound and prominently found in grapes, dark berries, red wine or peanuts etc [5]. The RSV is well reported to show the potential therapeutic benefits [6] however, it has poor water solubility (30 $\mu\text{g/mL}$) and low oral bioavailability with a partition coefficient of 3.1 which limits its pharmacological activities [7]. Therefore, we have prepared carrier-

free, amorphous RSV nanoparticles (RNP) formulation for the enhancement of solubility, dissolution and oral bioavailability of RSV. The prepared RNP was explored by TEM, XRD, DSC, FTIR and NMR. Then, *in-vitro* dissolution, cytotoxicity and pharmacokinetic studies were investigated.

2. Experimental section

The carrier-free RNP were prepared by the ultra-nanoprecipitation method. All these experimental details can be found in [Supplementary Material](#).

3. Results and discussion

3.1. Preparation and characterization of resveratrol nanoparticle (RNP)

Tables S1 and S2 show the particle size, polydispersity index and zeta potential of RNP with 0.5% w/v of SDS showing the particle size (76 nm), monodispersity (PDI ~ 0.212) and good zeta potential (-18 mV). Fig. 1a-c shows the TEM image and Selected Area Electron Diffraction (SAED) pattern of the final optimized RNP formulation. The suspended RNP particles were almost spherical and well dispersed with a particle

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size ~ 8 nm Fig. 1b illustrates the SAED pattern of prepared RNP where no diffraction spots were observed, suggesting the amorphous nature of the RNP. Fig. 1d represents the PXRD patterns of RSV and RNP formulation where RSV showing the peaks at 2θ angles 6.7° , 13.2° , 16.4° , 19.1° , 22.2° , 23.5° , 25.3° , 28.4° , 31.6° , 33.0° , 33.8° and 38.4° which suggested the crystalline nature of RSV [8]. Interestingly, the diffraction peaks of RSV were not observed in RNP spectra which indicated the amorphous nature of the formulation. Fig. 1e depicts the DSC thermogram, which showed a sharp endothermic peak at 263°C . The DSC thermogram of RNP shows the two different endothermic peaks at 246 and 235°C but with peak shifting. The shifting of the sharp endothermic peak of RSV further confirmed the change in the physical nature of the RNP. Fig. 1f showed the FTIR spectra of RNP which shows the peaks at 3268 cm^{-1} (O–H stretching), 1608 (C = C stretching of aromatic ring), 1585 and 1465 cm^{-1} (C = C trans double bond stretching), 1390 cm^{-1} (C–O–C stretching), 1155 cm^{-1} (phenolic C–O stretching), 962 cm^{-1} (trans C = C stretching), 833 cm^{-1} (=C–H vibration bands of phenolic ring), 616 – 517 cm^{-1} (=C–H vibration bands of trans double bond). [9]. Fig. 1g shows the ^1H NMR spectra of RSV, SDS, physical mixture (RSV + SDS 1:1 w/w), and RNP. The FTIR and ^1H NMR spectra of RNP showed all protons like pure RSV with a very slight shift confirming the removal

of SDS and no change in RSV after formulating as drug nanoparticles.

3.2. Physicochemical properties of resveratrol nanoparticles (RNP)

Solubility and partition coefficient (log P) of RSV and RNP are shown in Fig. 2a–b. The solubility of RSV in water was $36.6 \pm 1.92\text{ }\mu\text{g/mL}$ with Log P value of 3 whereas RNP had the solubility of about $321.43 \pm 7.07\text{ }\mu\text{g/mL}$ with 2.5 Log P value. From the observed solubility data, it was observed that the solubility of RSV was significantly (8.7 times, $p < 0.0001$) increased with decreasing Log P value after nanoparticle formation. The reduction in particles and the amorphous nature of the RNP might be responsible for solubility enhancement and a decrease in the Log P value of RSV [10]. The dissolution profile was higher for RNP than the pure RSV in both the media. After 2 h, dissolution of RSV and RNP in 0.1 N HCl was found to be 11.4% and 51.3%, respectively. In phosphate buffer pH 6.8, dissolution of RSV and RNP was 30.4% and 91.01%, respectively after 30 min (Fig. 2c–d) which may be due to reduction in particle size, decrease in hydrophobicity and amorphous nature of RNP [10]. Table S3 shows the change in physicochemical properties of the RNP formulation after three months of storage. No significant changes were observed in particle size, PDI and zeta potential of RNP. The

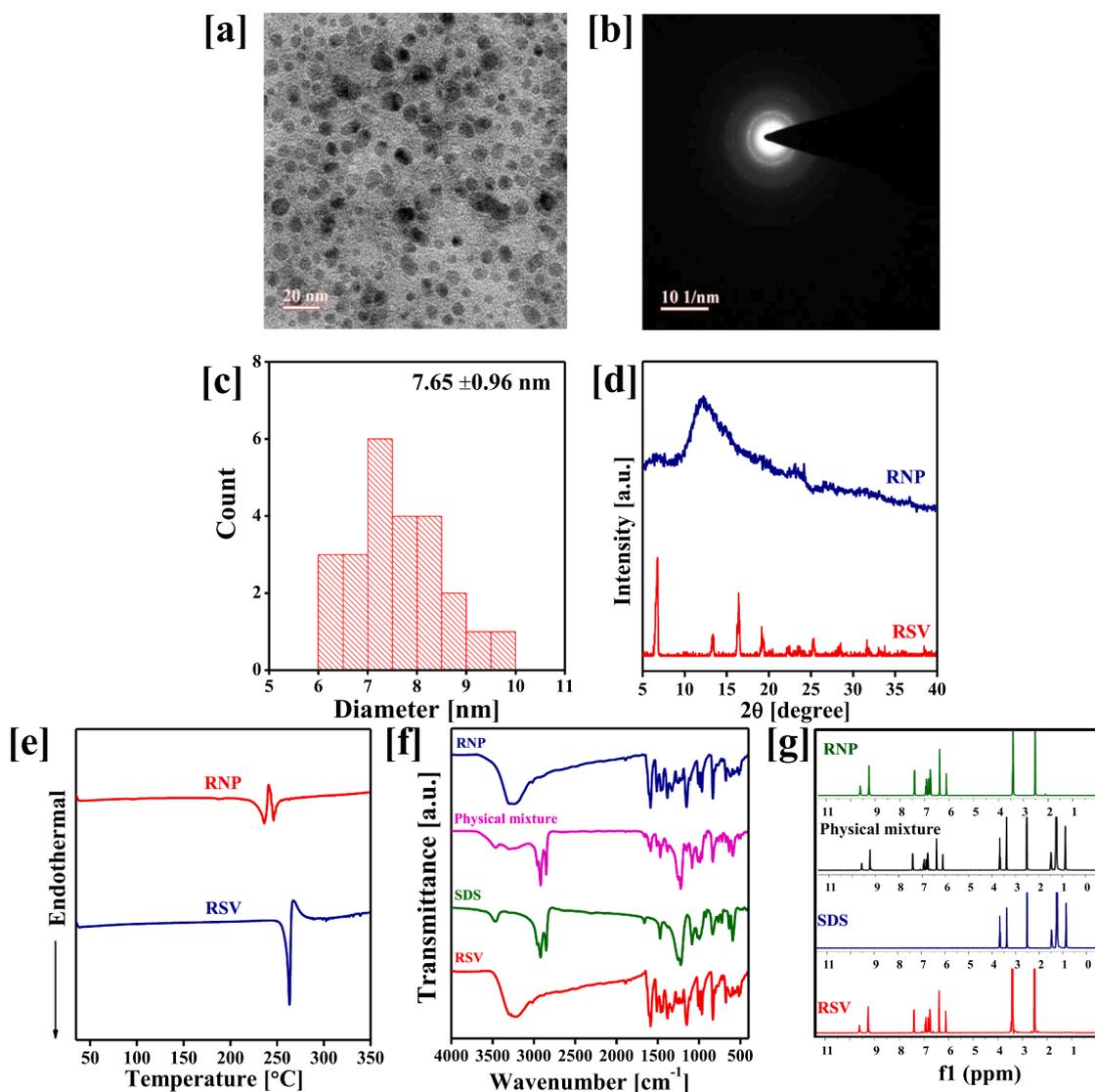


Fig. 1. Characterization: (a) TEM image of RNP, (b) SAED pattern of RNP, (c) Size distribution of RNP, (d) PXRD patterns of RSV and RNP (e) DSC scans of RSV and RNP, (f) FTIR spectra of RSV, sodium dodecyl sulphate (SDS), physical mixture (RSV + SDS 1:1 w/w) and RNP and (g) ^1H NMR spectra RSV, SDS, RSV + SDS and RNP.

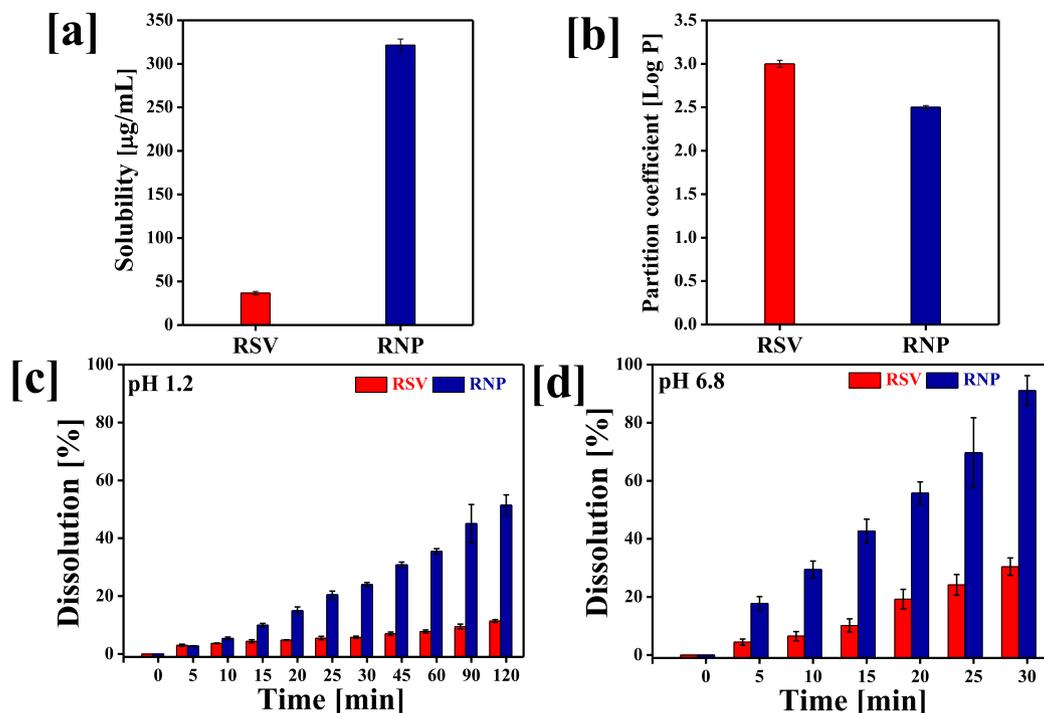


Fig. 2. (a) Solubility of RSV and RNP in water, (b) partition coefficient (Log P) of RSV and RNP; (c and d) *In vitro* dissolution of RSV and RNP in 0.1 N HCl (pH 1.2) and phosphate buffer (pH 6.8).

observed RSV content in RNP was $99.1 \pm 0.4\%$. Therefore, it can be concluded that the prepared surfactant-free RNP formulation was stable at refrigeration conditions.

3.3. *In vitro* cytotoxicity study of RNP against human colorectal cancer cells

Fig. 3a-b showed the *in vitro* cytotoxicity of RSV and RNP against HCT 116 cancer cells. The half-maximal inhibitory concentrations (IC_{50}) of RSV and RNP were found to be $37.2 \pm 1.2 \mu\text{g/mL}$ and $32.6 \pm 0.8 \mu\text{g/}$

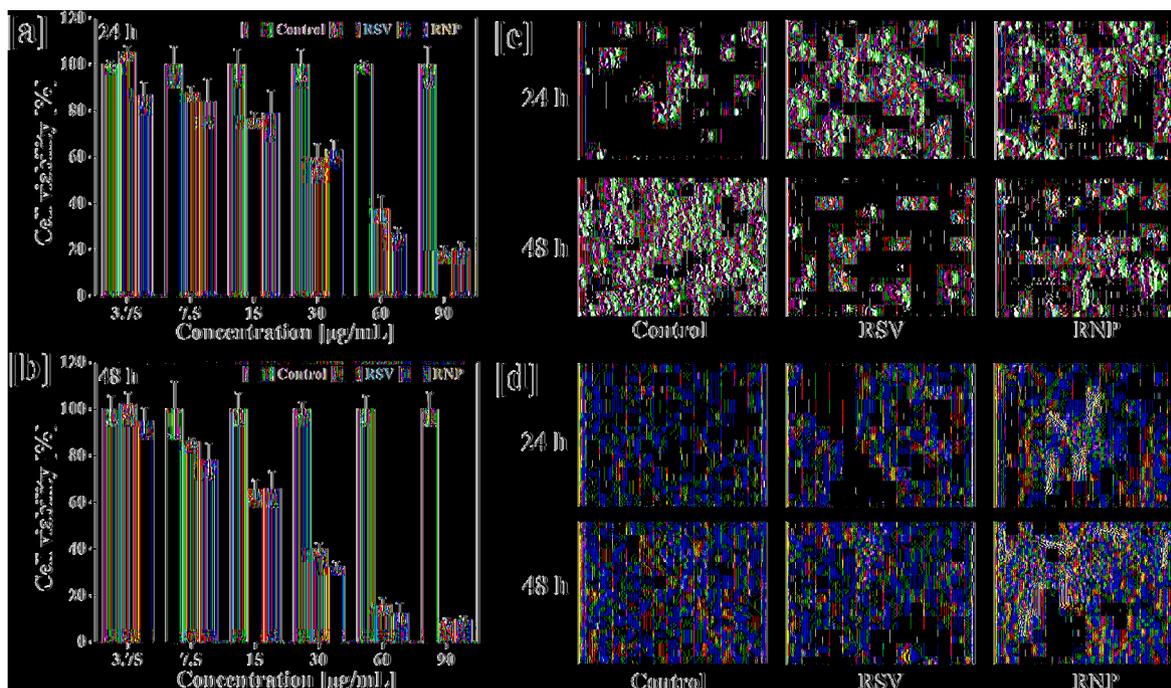


Fig. 3. (a and b) Cytotoxicity of RSV and RNP against HCT 116 colorectal cancer cell lines at 24 h and 48 h, respectively, (c) Acridine orange-ethidium bromide staining of HCT 116 colorectal cancer cell lines after exposure of RSV and RNP, and (d) Hoechst 33,342 staining for the detection of apoptotic nuclei of HCT 116 colorectal cancer cells after incubation with RSV and RNP. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mL after 24 of incubation, respectively. The IC₅₀ values were decreased to 23.5 ± 2.8 µg/mL for RSV and to 19.7 ± 3.5 µg/mL for RNP after 48 h (Fig. S1). Fig. 3c shows the AO/EB staining assay, as the time of incubation was increased, the number of necrotic cells was increased while the number of live cells (with green fluorescence) was decreased. It was found that the induction of apoptosis was more in RNP-treated cells than RSV-treated cells. Fig. 3d, the bright nuclei (chromatin concentration) represent the late apoptosis or necrosis cells. The results showed the HCT 116 cells treated with RNP showed more bright nuclei which further confirmed that the prepared RNP formulation was more toxic to the human colorectal HCT 116 cells than the native RSV.

3.4. Pharmacokinetic study

Fig. S2 shows the RSV plasma concentration versus time profile, and the pharmacokinetic (PK) parameters are listed in Table S4. The AUC of the RNP (2.32 ± 0.28 µg/ml × h) was 2.36 times significantly higher ($p < 0.0001$) than the pure RSV (0.98 ± 0.03 µg/ml × h). The plasma C_{max} was found to be 0.23 ± 0.02 µg/mL for RSV and 0.62 ± 0.09 µg/mL for RNP. The increased AUC and C_{max} values suggested the higher absorption of the RSV after administration as RNP. This demonstrated that the as-made RNP had greater anticancer activity and bioavailability as compared to pure RSV.

4. Conclusion

The RNP formulation prepared using 0.5% w/v of SDS exhibited the lowest particle size (81 nm) distribution with 0.202 PDI and -15.4 mV zeta potential values. Solid-state characterization of the amorphous RNP confirmed by the PXRD and DSC analysis and the complete removal of surfactant from the nanoparticle surface confirmed by the ¹H NMR and FTIR spectroscopy. The carrier-free and amorphous RNP significantly enhance the water solubility, dissolution, stability with decreasing hydrophobicity as compared to crystalline RSV. Therefore, the amorphous RNP exhibited better *in vivo* performance compared to raw RSV powder. The increase in C_{max} and AUC confirmed the increase in oral bioavailability due to enhance solubility, amorphous nature, and better dissolution profile of RSV from the RNP formulation.

CRedit authorship contribution statement

Ashok Kumar Jangid: Conceptualization, Methodology, Writing - original draft, Software. **Krunal Patel:** Investigation. **Poonam Jain:** Data curation, Software, Formal analysis. **Sunita Patel:** Validation. **Kanakaraju Medicherla:** Formal analysis. **Deep Pooja:** Writing - review & editing, Visualization. **Hitesh Kulhari:** Conceptualization, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.matlet.2021.130340>.

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Comparative Study of Critical Thinking Ability among Teacher Educators on the basis of Gender

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Abstract

Critical thinking is an important skill that promotes self-reflection in the work done by anyone. The present study was conducted to understand the influence of gender on the critical thinking ability of teacher educators. The total number of teacher educators was 30 which were selected out of which 16 were males and 16 were females from three Teacher Education Institutions of Gandhinagar. Test on critical thinking ability, developed by the researchers was used to test the critical thinking ability of teacher educators. The finding of the study showing that there is no influence of Gender on Critical Thinking ability among teacher educators. The study gives suggestions towards designing classroom pedagogy for teacher education based on critical thinking and also towards the further research that needs to be carried on under this domain.

Keywords : Critical Thinking, Gender, Teacher Educators.

Introduction:

In ancient India, education and religion were closely linked to each. The character formation with the proper development of the moral feeling along with preparing students to become a useful member of society was an important aim of education during that time. In the classical Indian handbook of Ayurveda called Charaka Samhita, the author Acharya Charaka said following words, "One who has acquired the knowledge (given by the authoritative text) based on various reasons and refuting the opponent's view in debates, does not get fastened by the pressure of opponent's arguments nor does he get subdued by their arguments" (Vaidya, 2016). According to Nagasena, "When scholars talk a matter over one with another, then there is a winding up, an unraveling, one or other is convicted of error, and he then acknowledges his mistake; distinctions are drawn, and contra-distinction; and yet thereby they are not angered" (Vaidya, 2016).

The National Curriculum Framework (2005) also visualizes teachers in the role of "facilitator who encourages learners to reflect, analyze and interpret in the process of knowledge construction". It also recognizes the fact that a sensitive and informed teacher is one who "is able to engage children through well-chosen tasks and questions so that they are able to realize their developmental potential". The NCF, 2005 also suggests that student-teachers, teacher educators or regular teachers should critically examine the curriculum, syllabi, and textbook on a regular basis.

Critical Thinking is one of the important skills which is much discussed in the field of Teacher Education. The National Curriculum Framework for Teacher Education, 2009 recommends teachers to critically engage with the theory and practice for developing a professional approach in the process of teacher education. The relevance of Critical Thinking can

be understood going back to the era of Socrates where he emphasized seeking reasons, obtaining evidence, questioning assumptions and analyzing concepts to justify one's claim. To understand the significance of Critical thinking in the field of teacher education, it is important to understand its meaning and definition given by various philosophers. John Dewey defines critical thinking as "active, persistent and careful consideration of a belief or supposed form of knowledge". From being active, he meant the active involvement of the individual in the process of knowledge construction rather than learning in a passive way from somebody. Building on the idea of Dewey, Edward Glaser, co-author of one of the widely used tests of critical thinking adds more emphasis to the evidence-based inquiry.

According to Mason (2008), Robert Ennis (1996) propagates the idea of critical thinking to be based on skills such as becoming reasonable and a reflective thinker in the work one chose to do. McPeck (1981) argues that critical thinking is specific to a discipline which depends on a thorough knowledge of the subject content.

Teacher Educators are required to be flexible and reflective, innovative, dynamic in their responses where they can locate, map and translate the different discourses in their arena of practice. The most significant aspect of critical thinking is raising a question which is the central aspects of both learning and knowledge creation. Therefore, the assessment of critical thinking would require an individual to be open for multiple solutions and have the motivation to examine the complex content deeply rather than just simply recalling facts or restating the answer.

Rationale:

The attitude of prospective teacher educators towards critical thinking would help in bringing a desired social change in the formal Indian education system. It would also help them understand the need for collaborative planning and practice in their workspace which would help them grow as professionals. Facione (1990) defines critical thinking as "purposeful, self-regulatory judgment which results in interpretation, analysis, evaluation, and inference, as well as an explanation of the evidential, conceptual, methodological, criteriological, or

conceptual considerations upon which that judgment is based. Different Policies and recommendations on Indian education system highlight the needs towards improving quality in the education system. It is felt that the current practices in the education system fail to establish the relationship between the content taught in the classroom and its implication in the real world. Similar problems are being faced in the preparation of teacher educators where they end up spending more time "decorating their lesson-plan rather than reading and reflecting on what to teach, why and how" (Report of the High-Powered Commission on Teacher Education constituted by the Hon'ble Supreme Court of India, August 2012). Annual Status of Education Report (ASER), 2019 and National Achievement Survey (NAS), 2018 clearly highlights the concerns related to quality in the Indian education system. This, in turn, raises the question on the quality of teacher education which "acts as a bridging agency between school education and society" (Sharma & Rawat 2014).

Critical thinking has a positive influence on the academic achievement of an individual that enhance their confidence level. Sherafat (2016) conducted a study to find out the effect of critical thinking on academic achievement among secondary and senior secondary students. The findings of the study suggest that higher the critical thinking levels, the better is the academic achievement scores. It means critical thinking positively affects academic achievement of (secondary and senior secondary) students. The study also found that the age or the educational level does not pose an influence on critical thinking as secondary students were found performing better than the senior secondary students.

There are different studies that suggest different ways to enhance critical thinking. Patel (2010) found that the instructional strategies based on thinking tools and strategies were effective in enhancing fluency, flexibility and originality dimensions of creative and critical thinking among primary school teachers. Ojha (2018) conducted a study to develop the critical thinking skills of students in history through an inquiry-based approach in the classroom. The findings suggest that the inquiry approach was more effective than memorizing the facts in which students learned to appreciate the complexities,

uncertainties, and ambiguities inherent in historical issues and problem. Gopalakrishnan (2016) conducted an experimental study to see the effectiveness of Educational Ergonomics Programme (EEP) in terms of critical thinking amongst students of XI grade. The findings suggest that critical thinking of the group who underwent the EFP program was enhanced at the end of the treatment. There are many studies conducted to see the influence of different interventions in enhancing the critical thinking of students studying at different levels of school education or higher education either in a subject or around the overall developmental domain. Also, there are studies that talk about the need for developing teaching competency and professionalism among teachers or teacher educators, but it is difficult to find the study that provides an example of the practice of enhancing critical thinking abilities among teacher educators.

There can be different factors that can affect critical thinking of an individual. A research conducted on “critical thinking tendencies and factors that affect critical thinking of higher education students” gives a result that shows that female student were seen to be better than male students on the dimensions of analyticity, open-mindedness, and truth-seeking. Whereas, inquisitiveness was the only dimension that male students were better than female students (Arselen, Gulveren, and Aydin (2014). Gender is defined as a “social construction of sexed bodies and an analytical tool for providing a conceptual bridge to past and present relationships between men and women” (Pearson and Rooke, 1993).

In India, from 1991 to 2014, female to male teacher ratio has increased from 41 to 88 in primary school and 44 to 90 in secondary school as per the data of the Indian Human Resource Department. In urban areas, female teachers have outnumbered male teachers with many big schools having just 5 to 10% male teachers (Nair, 2017). But how far have they attained the sense of autonomy and decision-making is a question to inquire about. The percentage of female teachers are increasing rapidly compared to male teachers. Despite that, it would be difficult to say whether they are able to think critically in their profession that demands them to be a reflective thinker. Therefore, a comparative study of critical

thinking on the basis of gender became a chance to reflect on the process that one has gone through or going through to become teacher, educators.

Statement of the Problem

Our current Indian education system emphasized heavily on memorizing the answer to the question rather than testing the critical thinking skills mentioned in almost all the report based on the Indian education system. In India, around 77% of teachers are female and every year they are becoming part of this profession in a large number. Therefore, a study was conducted on, “Comparative Study of Critical Thinking Ability among Teacher Educators on the basis of Gender”.

Significance of the Study

To ensure the quality of education system in India a good teacher educator is required for preparing a good teacher which would, in turn, be reflecting in the learning level of students. In the 21st century, it is important to have an education system that promotes critical thinking which would lead towards a maximum of innovation and creativity to excel in a worldwide scenario.

Objective

The objective of the study was

To study the influence of gender on critical thinking of teacher educators.

Hypothesis

Ho was framed by researchers:

Ho - There is no significant difference between the mean scores of critical thinking of male and female teacher educators.

Delimitations of the Study

Delimitations of the study were:

- The study was delimited to the English Medium Teacher Education institutions only.
- The study was conducted in the institutions of Gandhinagar city of Gujarat only.

Methodology

The present study was descriptive in nature. In the study, the researchers used Survey method.

Sample

In the present study, a sample of 32 teacher educators was taken, out of which 16 were males

and 16 were females. The sample consists of Teacher Educators, Research Scholar and Master of Education (M.Ed.) students among the age group of 21 to 50 years. The institutions selected for the purpose were English medium and the name of the institutions are Indian Institute of Teacher Education, Education department of Kadi Sarva Vishwavidyalaya and School of Education Central University of Gujarat, located in Gandhinagar city.

Sampling techniques

The sampling technique used by the researchers was purposive.

Tool

For the study, a test based on critical thinking ability was prepared by the researchers. The language of test was English. The dimensions of critical thinking that were considered while developing a test were- analyzing arguments, assumptions, deductions, inferences and interpreting information.

Data Analysis, Result, and Discussion

Mann Whitney 'U' test was used for data analysis which summary of results is given in Table 1.

Table 1. Summary of Mann Whitney 'U' test for comparing Critical thinking and Gender

** not significant at 0.05 level of significance

From table 1, it is clear that the value of Mann-Whitney 'U' is 102.50 for which the value of two-tailed significance is 0.335, which is greater than 0.05 level of significance, therefore 'U' value 102.50 and Z-value 0.964 is not significant at 0.05 level of significance. Hence, there is no significant difference between the scores of critical thinking of male and female teacher educators. In this view, the null hypothesis, "There is no significant difference between the mean scores of critical thinking of male and female teacher educators" is not rejected. The data in table 1, clearly shows that the value of scores of critical thinking of male is 18.09 which is not significantly higher than the value of scores of critical thinking of female which is 14.91. Therefore, it can be concluded that critical thinking of teacher educators is not influenced by gender.

Salahshoor and Rafiee (2016) conducted a study to investigate the relationship between critical thinking and gender among Iranian (English as Foreign Language) learners. A result of a standardized test revealed that males and females were not significantly different from one another in applying critical thinking skills. Leach (2011) in his study explored the five dimensions of critical thinking based on gender, college and academic discipline. The result of the study shows that there is no significant difference found among gender aspect, however, a student within a certain academic discipline is found to perform better in some areas of critical thinking. The study also indicated that the development of critical thinking is highly dependent on the environment and instruction by teachers. Another study regarding gender and problem solving administered under PISA (Programme for the International Student Assessment) by the Organisation for Economic Cooperation and Development (2009) found that gender differences in problem-solving for adolescents were few and insignificant.

The above studies suggest that the role of gender is not much in determining the critical thinking ability of an individual. However, the other factors that can play a major role in the critical thinking of an individual are

Critical Thinking	Gender	N	Mean Rank	Sum of Ranks	Z	Mann-Whitney 'U'	Sig.
	Male	16	18.09	289.80	.964	102.50	0.335**
	Female	16	14.91	238.50			

the academic environment and teacher's facilitation at the school and university level. Afsahi and Afghari (2017) conducted a study with thirty students of Master of Arts (M.A.) to study the relationship between Mother Tongue, Age, Gender and Critical Thinking. The result of the study indicates that there is a significant relationship between mother tongue and critical thinking but there is no significant relationship between age, gender, and critical thinking level.

The reasons behind getting the above result were maybe because of the similar educational or academic attainment by both the male and female teacher educators. They have either completed or were pursuing a Master of Education (M.Ed.) degree, that means, they all were at a certain level of education. Therefore, there was no significant difference found in the

relationship between critical thinking and the gender of the teacher educators.

Conclusion

The finding of the study showed that critical thinking is not influenced by gender. The studies display that it is not gender that determines the critical thinking of an individual but the environment in which one is living. Another important point stated by almost all the study is the significance of critical thinking in one's academic or personal life as it demands self-reflection and self-regulation at every end of life. Similarly, the beliefs, practices, and attitude of teachers educators are really significant to improve the educational scenario of the country as they are the ones who would prepare teachers to cope with their professional life challenges and provide a student-friendly environment which would, in turn, shape the motivation and achievement of students. At last it can be concluded that there is no relationship between gender and critical thinking.

Implications of the study

The study can give direction for further researches which are going to explore or experiment towards improving the existing classroom practices in the field of teacher education. It can also be useful in designing a classroom pedagogy of teacher education. It can also give an insight to the teachers who are teaching at a different level of schools and colleges to stop generalizing the student's choice of subject with their gender.

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Cyberspace, Social Media and National Security Concerns: Indian Perspective

Dr. Rajneesh Kumar Gupta

Introduction

The phrase -Struggle for Existence, used by Charles Darwin in his famous book *Origin of Species* (1859) suggest that there has been continuous battle in different living beings and it is the law of the nature that only fittest survives in this world. These conceptions are useful to examine security dimensions of human life. It is a well-established fact that security remains a core concern throughout history of human civilization. Being a multifaceted in nature it has many dimensions- physical, political, economic, social, cultural, and so on. Traditionally, political security aspects have been in the domain of international affairs with the prime priority of safety and security of the nation-state. Those have been studied with the state centric approach in which interstate relations were units of analysis. Conventionally, issues of national security were addressed in two different dimensions- external and internal. Whereas, matters of foreign aggression and guard of national boundaries were considered matters of external affairs in the purview of diplomacy and defense establishments; maintenance of law and order within the boundaries of state were in the jurisdiction of police forces and other law enforcement agencies.

However, advancement in the communication and transportation technologies clubbed with changed realities of the dynamic world order has brought new challenges to the phenomenon of national security. In our age communication technology has brought different parts of the world much closer to each other. Pace of information is so fast that any happening in one part of the world is known to the people living in any other region of the globe in seconds by clicking websites. Similarly, one can travel any part of the earth in less than twenty-four hours. In the post cold war era most of the nations are pursuing policies of liberalization, privatization, and globalization in which role of state has changed from controller to regulator and facilitator. This has resulted in tremendous growth in transnationalism. Now, we see fundamental growth in multinational companies, transnational banks and international migration leading to the idea of 'global village' and 'global citizenship'. Emergence of avenues

of virtual communication and networking has changed the world dramatically. In present generation we are interconnected through social media beyond political boundaries and nationalities. The cyberspace is giving us opportunity to explore any information without hindrances. This scenario provides ample scope of personal networking and growth in enhancement of our knowledge and skills. On the other hand, it has also brought tremendous challenges to national security. Cyberspace and social media is misused by enemy nations, anti-national elements and antagonistic groups to spread social tensions, communal violence, sexual crimes, and destabilize nations. The present paper examines these aspects and argues that notion of national security needs to be redefined and reshaped to meet the changed realities.

Cyberspace

The term 'cyberspace' was coined by science fiction writer William Gibson in his short story 'Burning Chrome'. He later popularized the concept in his debut novel *Neuromancer* in 1984. Though, there is no standard definition of the term it is used to describe the entire virtual world of computers (Aiyengar: 2010). Cyberspace is the notional environment in which communication over computer networks occurs. There has been means of telecommunication and public broadcasting prior to the emergence of computer networking and internet in the form of telegraph, radio, and television. The first modern computers were designed in the 1930s and by mid 1960s those were available for public use in the form of personal computers. In August 1962 J.C.R. Licklider floated the idea of "Galactic Network". However, fundamental changes occurred only after the evolution of world wide web which is today practically a synonymous to the internet and paved the way for the era of cyberspace. Tim Berners-Lee, a British scientist, invented the World Wide Web (WWW) in 1989. It was originally conceived and developed to meet the demand for automated information-sharing between scientists in universities and institutes around the world. Since, its inception it has passed to many phases and has brought revolutionary changes in the world (Figure-1).

Data Protection and Right to Privacy

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Abstract

With the development of information and technology, our lifestyle has become such that one cannot live without using the internet, mobile phones, laptops, android phones applications etc. Due to this, human beings become exposed and knowingly or unknowingly share their personal data on these platforms. In this way, their right to privacy which is one of the aspects of the right to live with dignity, comes at stake. Privacy being a basic human right, we must recognize that a vision protective of information privacy in cyberspace will be singularly hard to maintain. The protection of the privacy interests of netizens is in their hands. If netizens refuse to disclose their data, marketers have to employ technical measures which can be legally prevented. Objectives: The paper attempts to identify ways by which privacy is at risk to breach. The paper also aims to analyze the legal provisions available to maintain the privacy of any human being. Methodology: The paper is based on secondary information extracted from court cases, bare act of cyber-related laws, the constitution of India and press releases. The article will follow an analytical approach to understand the status of privacy in Indian laws. Main Findings: The major finding of the paper is that the unavailability of a specific law, rules for privacy is exist in the Indian legal system Implications: Digital revolution is now on full pace. Things are being virtual, digital life replacing physical one. People appreciate the digital avatar of social media because of breach of privacy if users do not follow due safety measures. This study has importance recognizing the importance of privacy in the course of maintaining the dignity of people. This study also inevitable in the legal sphere to identify legal remedies in case of breach of privacy. Novelty of Study: This study is original as it attempts to create an awareness regarding how privacy of an individual is important and should be kept unopened for strangers.

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ઢાયસ્પોરા સંદર્ભઃ ડાહ્યાભાઈ પટેલની વાર્તાઓ પૈકી પ્રણયજીવનનું મનોસંઘર્ષ નિરૂપણ

સંગીતા બી. ચૌધરી
સંશોધક, પીએચ.ડી. છાત્રા,
ગુજરાતી વિષય

ઢાયસ્પોરા શબ્દ કથાનક સાહિત્ય સાથે સંકળાયેલ છે. એને દરેક ભાષાએ, દરેક સાહિત્યએ સ્વીકૃત કર્યું. તેને પશ્ચિમીવાદનું ગણી શકાય. એ પશ્ચિમીકરણના સાહિત્યમાંથી ઉદ્ભવેલ છે. અને ત્યાંથી જ આપણે ત્યાં ૨૦મી સદીમાં ઊતરી આવેલું છે, એમ કહી શકાય. 'ઢાયસ્પોરા' શબ્દ 'ગ્રીક' ભાષામાં સૌ પ્રથમ જોવાં મળે છે. અને એ ગ્રીકભાષામાં જ પહેલો વહેલો પ્રચલિત થયો. આશરે અઢી વર્ષો પૂર્વેના સમયગાળા દરમ્યાન 'જયુઈશ પ્રજા'ના લાક્ષણિક રૂપે અસ્તિત્વમાં આવ્યો. અને એ 'ઢાયસ્પોરા' શબ્દ ધીમે ધીમે સાહિત્ય જગતમાં પ્રસરવા માંડયો. 'ઢાયસ્પોરા' ગ્રીકમાંથી ઊતરી આવેલ શબ્દની સંજ્ઞા પ્રયોજાય છે. એનો અર્થ ગુજરાતીમાં 'વિખેરવું', 'વિખરાયેલ', વિખરાવવું વગેરે થાય છે. અને 'ઢાયસ્પોરા' વિભાવના ગહન વિચારો સાથે સાહિત્યમાં ભળવા લાગ્યું. અને અનેક વિદ્યાશાખામાં દિશાઓ એ સંદર્ભ ખુલી ગઈ. ઢાયસ્પોરા સંદર્ભ સાહિત્યિક દષ્ટિ માંડી આદિકાળમાં ભટકતી માનવપ્રજાની પરિસ્થિતિ કેવી નિર્માય છે. તેના વિશે મનોમંથન કર્યું. એ પ્રજા જેમ જેમ સભ્ય થતી ગઈ તેમ તેમ માનવ વસાહતો બનતા ગયા. મનુષ્ય જગત નવી નવી ખોજમાં હરતું ફરતું રહ્યું. પોતાનું વિશ્વ કે સ્થાન ઊભું કરવા માટે સંઘર્ષ અને સાહસ કરવા પ્રયત્નશીલ બનવા લાગ્યું. વિજ્ઞાન તકનિકીનો વ્યાપ વધી ગયો. એક દેશમાંથી અન્ય દેશમાં જવા માટેની સુવિધા થવા માંડી એના પરિણામે અવરજવર મનુષ્ય વચ્ચે થઈ. વૈશ્વિકરણનો વિકાસ સંઘાતો ગયો. અને મનુષ્યજગત રાજકીય, સામાજિક, આર્થિક, શૈક્ષણિક કાર્ય સ્થાળતંતર કરવા લાગ્યું. પોતાનું વતન ત્યાગીને નવી ભૂમિના સ્થાયી બનવા પ્રયાસ કર્યો. પરંતુ એના અધિકારો મૂળવતનમાં તેમજ અન્ય અપનાવેલ ભૂમિના રાષ્ટ્રમાં પણ મળતા નથી. આવી, પરિસ્થિતિમાં જે પ્રજા હેરાન બની ગઈ તેને ઢાયસ્પોરા પ્રજા તરીકે ઓળખ આપી.

ઢાયસ્પોરિક સાહિત્ય ગુજરાતી સાહિત્યના મુખ્ય પ્રવાહની શાખા માની શકાય છે. તેની રચના વિદેશ સમાજ વચ્ચે થતી હોવાને કારણે એ વિષય વૈવિધ્ય શૈલી વિદેશના સામાજિક, રાજકીય, આર્થિક, શૈક્ષણિક, ભૌગોલિક પરિવેશના પરિબળો હેઠળના પ્રભાવથી સર્જાયા છે. આપણા ગુજરાતી સાહિત્ય ચિંતકો વિદેશના સામાજિક સાંસ્કૃતિક પરિવેશને અવલોકન કરી વિદેશસ્થિત વ્યક્તિના જીવનનો સંસ્કાર ઘટ્ટને વિદેશી ધરતી પર વસીને વિદેશી સમાજની તાસીર દર્શાવે છે. અને તેની વિશિષ્ટતાને જુદી જુદી દષ્ટિકોણથી નિહાળી સ્વાનુભવ દ્વારા ઉપસાવી આપવા સતત પ્રયત્નશીલ બન્યા. એમાંના મહાત્મા ગાંધીજી જેવા બ્રિટનની સાહિત્યિક પ્રદાનમાં વકિલાતનો અભ્યાસ કરવા ગયા. ત્યાં એમણે 'ઇન્ડિયન ઓપિનિયન' શરૂ કરીને ચલાવેલું. રસ્કિનના 'અન્ટુ ધિસ લાસ્ટ' ઉપરથી 'સર્વોદય', સોક્રેટિસના બચાવનામા ઉપરથી 'એક સત્યવીરની કથા' અને 'આરોગ્ય' વિશે સામાન્ય જ્ઞાન એમ લેખમાળાઓ પ્રગટ કરેલી તે કૃતિઓ મુખ્ય ધારામાં સ્થાન પામેલી છે. ઓસ્ટ્રેલિયાનું 'માતૃભાષા' પાકિસ્તાનનું 'વતન ગુજરાતી' પત્ર બ્રિટનની ગુજરાતી સાહિત્ય અકાદમી તેનું મુખપત્ર 'અસ્મિતા' અને સ્કુતિમંત વિચારપત્ર 'ઓપિનિયન', વગેરે સામયિકોએ ગુજરાતી ઢાયસ્પોરિક સાહિત્યના પોષણ-સંવર્ધનમાં સવિશેષ ડાળો આપ્યો છે.

આમ, ગુજરાતી ઢાયસ્પોરિક સાહિત્ય સંદર્ભ ખેડાણ થવા લાગ્યું અને એ સાહિત્યની માંગ ગુજરાતી સાહિત્યમાં કથાનક સાહિત્ય સ્વરૂપે આવશ્યક રીતે આવકાર મળ્યો અને એને 'ઢાયસ્પોરા' કથાનક સાહિત્યના તંતુઓને માનવતું બનાવી ગુજરાતી સાહિત્ય ચિંતકોએ, વિદ્વાનોએ, વિવેચકો, વાચકો વગેરેએ ગહન રીતે ઊંડો અભ્યાસ કર્યો. અને એની તાતી માંગ ઊભી કરી. એને સ્વીકૃત બનાવ્યું. આપણા ઘણા બધાં સર્જકો જેવાં બળવંત જાની, નિરંજન ભગત, સુમન શાહ, મણિલાલ હ. પટેલ, નૂતન જાની, ડાહ્યાભાઈ પટેલ, બળવંત નાયક, ભાનુ શંકર, ઓધવજી વ્યાસ, દીપક બારડોલીકર, પંકજ વોરા વગેરેનો સમાવેશ સાહિત્યની મુખ્ય ધારામાં મથામણ કરવા પ્રયાસ કર્યો છે. ભાષા પરિવર્તન પામે સંસ્કૃતિ પણ નવા રૂપે ઉદ્ભવે છે. એ સંદર્ભ ડાહ્યાભાઈ પટેલે ઊંડો અભ્યાસ કરી, અવલોકન કરી, અનુભવ મેળવી સ્વાનુભવ અને કાલ્પનિક સૃષ્ટિથી ઢાયસ્પોરિક સાહિત્ય ખેડવાના સારો પ્રયાસ કર્યો છે. ડાહ્યાભાઈ પટેલ ગુજરાતી હોવા છતાં પરદેશની ભાષાના લક્ષણોમાં આકર્ષાયને પોતાની સ્વાનુભૂતિમાં સર્જન શૈલીને ઉપસાવી આપવામાં કોઈ શંકા રાખી નથી.

ગુજરાતના જ જાણીતા વતની વિદેશમાં જઈને નિર્માણ પામેલા ડાહ્યાભાઈ પટેલ ત્યાંના પરિબળો, પરિસ્થિતિ સામાજિક સાંસ્કૃતિક પરિવેશના હેઠળ પ્રભાવિત બનેલા અને ગુજરાતી સાહિત્યમાં ઢાયસ્પોરિક દષ્ટિપાતથી કથાનક સર્જનશૈલી

વિકસાવી આપવા કોઈ ક્યાશ રાખી નથી. એમણે નવલકથા, નવલિકા વાર્તાઓ, કવિતાઓ વગેરેનું ખેડાણ આરંભ્યું. આમ, તેઓ ગુજરાતી સાહિત્યમાં કવિ તરીકે પહેલેથી જ ઓળખ પામી ચૂક્યા હતા. અને જેની અસર સમગ્ર વિશ્વ સાહિત્ય ભારતીય સાહિત્યમાં કથાનક સાહિત્ય પ્રકારે પ્રયોજાવવા લાગે છે. ત્યાર બાદ વિદેશી ગમન થયેલા ગુજરાતી સર્જકોએ આધુનિક ગાળાના સમયમાં એના વિશેની સમજ પામી. ડાયસ્પોરા (વિદેશ સ્થિત) સાહિત્ય સંદર્ભ ચર્ચા કરી. જેની નોંધ આપણે ત્યાં સાહિત્યિક ચિંતકો, વિદ્વાનો, ભાવક વર્ગે લીધી. અને એ ક્ષેત્રમાં સાહિત્યકારોએ શ્રી ગણેશ કર્યા.

‘ડાયસ્પોરા’ સંદર્ભે કથાવસ્તુને ગૂંથી નવલિકા વાર્તાઓ ઘણી બધી આલેખી છે. જેમ કે, ‘પદમાવતી વાર્તાસંગ્રહ’ (તીર્થસ્થાન), ‘પુર્નમિલન વાર્તાસંગ્રહ’ (પુર્નમિલન), ‘અમરપ્રેમી વાર્તાસંગ્રહ’ (અમરપ્રેમી), ‘અમરપ્રેમી આદીવાસીનું આક્રંદ’, આગમન (ખજાનો), ‘શર્મિષ્ઠા’ (લાખુ), પ્રાયશ્ચિત, જનમોજનમના પ્રેમી, પરિમલ, ત્યાગ, ‘શાલિની’ (અદ્ભૂત પ્રેયસી), ‘છેલ્લો અભિનય’ (ગતશ્રધ્ધા) વગેરે... નો સમાવેશ કર્યો છે. પરંતુ ભાવક તરીકેની મર્યાદામાં રહી સમગ્ર કૃતિઓ રચનાઓનું ઉલ્લેખ કરવા શક્ય નથી. તેથી ડાયસ્પોરાની કથાનક વાર્તા પ્રણયભાવ દર્શાવતી હોય એવા પાત્રસૃષ્ટિના સંબંધોનું વસ્તુ ગૂંથણ કરવા પ્રયાસ આદર્યો છે. વળી, એ સંબંધો વાચક વર્ગને આકર્ષે છે. અને પ્રણયજીવનના સંબંધોમાં મનો સંવર્ધનું અસ્તિત્વ કેવું ટકી રહ્યું છે તેની પ્રતીતિ કરાવી આપી છે. તે જોઈ શકાશે.

શર્મિષ્ઠા વાર્તા સંગ્રહ (૨૦૦૧) પ્રગટ થયો. એમાં ‘લાખુ’, ‘પ્રાયશ્ચિત’, ‘જનમોજનમના પ્રેમી’, ‘પરિમલ’, ‘ત્યાગ’ ઇત્યાદિ વાર્તાઓનો સમાવેશ થયેલ છે. એમાં પ્રણયભાવનાનું જીવનદર્શન અદ્ભૂત રીતે આલેખાયું છે. અને પાત્રો વચ્ચે પ્રણયત્રિકોણમાં મનો સંઘર્ષ કઈ રીતે નોખું પડી આવે છે. તેની અનુભૂતિ વાચક વર્ગને કરાવી આપી છે. આ વાર્તાઓમાં પાત્રો વચ્ચે સંવેદનાઓમાં મનોવેદના એકબીજા પ્રત્યે કઈ રીતે ઉભરાય છે તે અંગેની વાત નિખાલસ ભાવે નિરૂપવાનો પ્રયત્ન સર્જકે કર્યો. એમાં વિદેશ અને ગુજરાતના પ્રેમીજનોની પ્રણયભાવના પ્રબળ રીતે આલેખાયેલી છે. તેને જીવંત સાહિત્ય દ્વારા કર્યું છે.

શર્મિષ્ઠા વાર્તાસંગ્રહમાંથી ‘જનમોજનમના પ્રેમી’ અને ‘ત્યાગ’ વાર્તાનું કથાવસ્તુ પાત્રોસૃષ્ટિ દ્વારા પ્રણયનો મર્મ પ્રગટ કર્યો છે. એમાં વાર્તાના શીર્ષક હેઠળથી જ પ્રણયની મનોવેદના, સંવેદનાઓની કળા સ્પષ્ટ થતી હોય એ રીતે આપોઆપ ખીલી ઊઠે છે. તેની સ્પષ્ટતા કરી નિર્દેશાપણું વાચક વર્ગને કરાવી આપવામાં કોઈ અતિશયોક્તિ રાખી નથી.

‘જનમોજનમના પ્રેમી’ નામની વાર્તામાં સર્જકે અદ્ભૂત પ્રણયભાવનો મર્મ મૂકી આપ્યો છે. મનુષ્ય જગત સાથે જ ‘પ્રણય’ની દૃષ્ટિએ મિથ્યા અને નિરર્થક ગણ્યું છે. પ્રેમને વેરઝેર સાથે જોડ્યું છે. જાણે એકમેક બની રહેવા પ્રેમ સર્જાયો છે. પરંતુ એનું અસ્તિત્વ ટકાવવામાં આજ સુધી મનુષ્ય જીવન નિષ્ફળ નીવડ્યું છે. એમ કહી માની શકાય એમાં પેરિસની અદ્ભૂત રોમાંચક પ્રણયની કથા આલેખી છે. અને એના મુખ્ય પાત્રો રોઝ બેકોનેલ, કર્નલ બેકોનેલ, આલ્ફ્રેડ એ વચ્ચેનો પ્રણયત્રિકોણની રોચક કથા સર્જકે આલેખન કરી છે. રોઝ બેકોનેલ સુંદર યુવતી એ કર્નલ બેકોનેલની પત્ની તરીકે નિર્માણ પામી છે. કર્નલ બેકોનેલની મિલિટરીનો વધારે પડતો ગંભીર જડ વ્યક્તિ. જર્મન કે ફ્રાંસ વચ્ચેના આક્રમણ વખતે વારંવાર કહેતો કે ભવિષ્યની લડાઈમાં જર્મન લોકોને મારીને આવીશ અને એ વેર લઈને જ રહીશ. વેરના લઉં તો નોકરી ત્યજી દઈશ એમ પત્નીને જણાવતો.

રોઝ આલ્ફ્રેડ ને ખૂબ જ ચાહતી હતી. એ એનામાં મશગૂલ બનીને ફરતી. એ દૂર થવા ન માંગતી જનમોજનમ સુધી જીવનસંગિની અને પ્રિયતમા બનવા માટે આતુર બનતી જતી હતી. એની નોંધ એમણે નોંધપોથીમાં કરી અને જે રોઝના હૈયામાં ઉદ્ભવેલા પ્રણયના અંકુરો વિશેના સંવેદનો લખી રાખેલા. તે આલ્ફ્રેડને બતાવવા હતા. એ નોંધપોથી કદાચ કર્નલ બેકોનેલના હાથમાં આવશે તો તારી સ્થિતિ કેવી નિર્માણે ? એવા પ્રશ્નો થવા લાગ્યા. અને એક દિવસે બંનેની પ્રણયગાથા વિશે જાણ થતા જ નોકરીની બદલી લે છે. કર્નલ આલ્ફ્રેડ સામે તલવારના પડકાર ફેંકે છે. ત્યારે એ ફૂર, ખૂની પતિ પોતાની પત્નીના પ્રેમીને ગરીબાઈનું ભાન કરાવે છે. ત્યારે આલ્ફ્રેડ પણ જણાવે છે કે, એ પ્રેમના તંતુઓ વચ્ચે ગરીબાઈ હોતી નથી. સંબંધો પ્રણયના હોય છે ત્યાં કોઈ ભેદ નથી. રોઝ માને છે કે આવા કુલીન પૈસાવાળા પતિને છોડીને જવામાં જ હિતાવહ છે. અને કર્નલ બેકોનેલ આલ્ફ્રેડને તલવાર ભાંકી રહેલી નાંખે છે. એ રોઝના જ ખોળામાં માથું મૂકી અંતિમ શ્વાસ લે છે. એ વખતે આલ્ફ્રેડે કહ્યું ‘રોઝ એકવાર મરવાનું છે, તો એ મર્દની જેમ મરવું’ અને એ પોતાના પ્રણય ખાતર તલવાર ફેંકતો નથી. રોઝનો પ્રેમી મૃત્યુને ભેટે છે. રોઝ પણ એની સાથે નામશેખની અવસ્થા ધારણ કરે છે. ફૂર કર્નલ જગત માટે એક પાત્ર બનીને જ રહી ગયો.

આમ, આ પ્રણયકથામાં આલ્ફ્રેડ અને રોઝ વચ્ચેના સંવાદો સાથે જ જીવંત અને મર્મ બન્યા. બંનેનું પ્રણયજીવનમાં મનોસંઘર્ષના તત્વો એકબીજા વચ્ચે ટકરાયા હોય એમ જાણી શકાય. અને એકમેક બનવા રોઝની મનોભાવના કેટલી તીવ્ર હતી

તેની અનુભૂતિ દર્શાવી આપી છે. એ બંને વચ્ચે પ્રણય ભાવમાં બલિદાનની ભાવના દેખાય આવે છે. જ્યારે પતિ વેર લે છે. સર્જકે પ્રણય દર્શનમાં પ્રેમનું મૂલ્ય કેટલું છે. તેની મહત્તા દર્શાવવા પ્રયત્ન કર્યો છે.

‘ત્યાગ’ વાર્તામાં મુખ્ય પાત્રો રોબર્ટમેન, માર્યા, વિલ્સન છે. આ ત્રિકોણ તરીકે પ્રણયજીવન ઓળખાતું. એમાં રોબર્ટમેનનું વ્યક્તિત્વ સુંદર રીતે ઊપસી આવતું નજરે આવે છે. પ્રણયજીવનના ત્રિકોણ સંગમમાં તંતુઓ કઈ રીતે પ્રેમમાં બંધાય છે. તેની રજૂઆત અહીં જોઈ શકાય છે. યુધ્ધમાં મૃત વ્યક્તિ તરીકે રોબર્ટમેનને જાહેર કરવામાં આવ્યો. અને એ જ રોબર્ટમેન પાછો ફરે છે. ત્યારે માર્યા નામની યુવતી પ્રેમિકા એક વિલ્સન નામના યુવક ને વરી ચૂકી હતી. એની સાથે જીવન ગુજારતી હતી. જ્યારે એ જ માર્યા સાથે એવી દુઃખટના બને છે ત્યારે એ આકસ્મિક રીતે અપંગ બની જાય છે. અને એ અપંગ માર્યાને વિલ્સન પતિ ત્યાગી દે છે. પરંતુ માર્યા પણ અટૂલી એકલી નથી. એને ભૂતપૂર્વ પ્રેમી રોબર્ટસન અપંગ માર્યાને અપનાવી લે છે. પોતાની જીવનસંગિની પ્રેમીકા બનાવી લે છે. આવા પ્રેમી યુવકોને લગ્નની જરૂર તાતી હોતી નથી. અને સહજ ભાવે કુદરતના ખોળે સ્વીકૃત કરી સંકોચ જરા કરતા નથી. એમાનો રોબર્ટમેન પ્રેમી છે.

આવો જ બીજો કિસ્સો પ્રણયભાવનામાં ગુજરાતમાં બને છે. ત્યાં પણ ત્રિકોણ પ્રણયજીવનનું રચાયેલ પાત્રો છે. જગદીશચંદ્ર, મૃદુલા, પ્રો. નટવરલાલ. અહીં આઝાદીની ચળવળ બેતાળીસની સર્જાયેલી એમાં જગદીશચંદ્ર નામનો સુરતનો યુવક રાષ્ટ્રહિત માટે પોતાની કાયાનું મૂલ્ય ચૂકવે છે. ત્યાં એમને ગોળીબારથી શિકાર બની એક હાથ, એક પગ ગુમાવવાનો વારો આવી ચઢે છે. તેઓ એક પગ અને એક હાથ વિનાના બની ગયેલા. તેથી એણે ચાહેલી પ્રેમિકા મૃદુલાને પોતાની જીવનસંગિની બનાવી શકાતા નથી. પણ એનો અફસોસ કરતાં નથી. તદ્દઉપરાંત તેઓ પ્રો. નટવરલાલ સાથે લગ્ન કરાવી આપવા સફળતા અનુભવે છે. એ બંનેની પ્રણયગાથા પતિ જાણે છે. છતાં પત્ની તરીકે મૃદુલાને સ્વીકારી એ જ એમનું સૌથી મોટું ‘પ્રેમશૌર્ય’ ગણાય. અને ત્રણેયનું જીવન સાથે જ પસાર કરતાં જોવાં મળે છે. આવા પ્રકારનો પ્રણયભાવ જે રીતે ભાવ દર્શાવે છે.

આમ, બંને વાર્તામાં વિવશતા કડુણભાવના ત્યાગ, ઠૂર, વેરઝેર વગેરે માનવ મનના સંકુલ સંવેદનો સર્જક અને વાચકવર્ગના હૈયામાં ટકરાયા વિના રહ્યાં નથી. વાચકવર્ગને પોતાની જ પ્રણયગાથા સર્જ આપી હોય એ રીતે સર્જકે જીણવટથી રચનાઓનું નિરૂપણ કર્યું છે.

આમ, બંને દેશનું પરિવેશને કેન્દ્રમાં રાખી તેની અસર જીલીને સાહિત્ય સર્જન ડાયસ્પોરિક સંદર્ભે પ્રારંભ કર્યું. એમના વૈવિધ્ય સભર બનાવેલા સાહિત્યસર્જનમાં ભારતીય સમાજના પ્રશ્નો વિદેશ સમાજના પ્રશ્નો સામે કેવા સવલતોના મુકાબલા કરવા પડે છે તેનો પરિચય આપણને કરાવી આપ્યો છે. ત્યાંની પ્રણય સંવેદનાઓ અને આપણા ત્યાંની પ્રણય સંવેદનાઓ વચ્ચેનો ભેદ પણ જીણવટભરી નિરૂપવાનો પ્રયાસ કર્યો છે. આ પ્રકારની પ્રણયભાવનાઓ સૌથી વધુ આકર્ષિત બને પણ છે. તેને એમણે જોઈ છે. અને સાહિત્ય સૃષ્ટિના કથાનક સંદર્ભે મૂકી આપી સમૃધ્ધ બનાવવાના આશય સાથે આલેખન કર્યું છે.

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DEBATING LIBERALISM AND COMMUNITARIANISM: FROM THE PERSPECTIVE OF MINORITY RIGHTS

Dr. Jagannatham Begari

Abstract

The paper is an attempt to examine liberalism. It further evaluates the interrelation between liberalism and Communitarianism to understand the importance the minority rights and their dignity. The paper also proposes to understand Rawls's theory of justice. The paper also attempts to bring out the limitations of liberalism. Importantly the paper focuses explicitly on Justice. Further, it interrogates several stages of minority rights. It further examines the significance of participatory democracy which strive towards democratization. It also focuses upon multiculturalism to conceptualize the relevance of group rights. The paper discusses the approaches to minority-related issues. To examine critically, the paper intends to assess theoretical interventions and the relevance of communitarianism and the importance of minority rights in India.

Keywords: Liberalism, Communitarianism, minorities, multiculturalism, democracy, rights, justice, freedom, India

Introduction:

The ideologies i.e., socialism, conservatism, liberalism, nationalism and republicanism in 18th and 19th centuries offered the ideas of liberty, equality, and community. The notion of community took different forms from class solidarity to common ethnic cultural identity. For all theories and the philosophers, the community was one of the basic blocks. However, after the 2nd world war, the notion of community seemed to drop out of the picture. For instance, Theory of Justice by John Rawls interpreted the concepts of liberty and equality significantly. Though he did not reject explicitly the importance of community but paid less attention to it. Kymlika argued that for Rawls the community was not an important subject of ideological dispute. Liberal vision of politics does not include the principle of community and its characteristics (Kymlika, W. (2002: 208). Kymlika argued that the community has resurfaced in the last twenty years. Thus, communitarian theorists argue that the vitality of community did not theorize by liberalism (Kymlika, W. (2002: 208).

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Diaspora and Cultural Heritage: A Study of Indians in Kenya

Rajneesh Gupta

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Central University of Gujarat, Gandhinagar*

Introduction

Movement from one territory to another is a fundamental feature of human being. Anthropology documents nomadism as a stage preceding human settlement as communities. Even after their evolution as communities, throughout the civilization human beings have been experiencing temporary, seasonal, or permanent migration from their original habitat either voluntarily or forced. Migration of people is closely related to a variety of socio-economic, demographic, and political factors. History of human civilization is full of such migration, however, in modern history major waves of migrations originated in the colonial period. In post colonial era and especially since the beginning of 1990s the process of liberalization, privatization and globalization is giving further boost to the movement of people across the globe. Last few decades have seen tremendous growth in international migration.

International Organization for Migration estimates that the total number of international migrants in the world stood at 272 million in 2019 and they constitute about 3.5 per cent

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Diaspora Factor in India's Soft Power Diplomacy (With Special Reference to Indian Diaspora in USA)

Dr. Siba Sankar Mohanty
&
Dr. Nabin Kumar Khara

The notion of power is quite central to international politics. Though there are various dimensions of power, in the common parlance power exhibits one's ability to determine the movements of others in a pursued manner. Soft power is one dimension of power, which entails one's ability to influence others in achieving something. In the present era of international politics soft power is given prime importance by different countries. In the twenty-first century, different countries are using their soft power resources as important and influential means to fulfill their strategic objectives. In fact, soft power is considered as a major instrument for conducting public diplomacy. India has used its rich cultural heritage as a part of its public diplomacy. India possesses an enormous source of soft power due to civilizational values, rich cultural heritage, music and art, pluralism, democratic ethos, spiritualism and yoga, science and technology, and most prominently, the vibrant diaspora group who have taken India to every corner of the world. India has showed its potential of soft power for several years, even before the concept was analyzed by political scientists and other scholars. India, at different time periods in the past, has used both hard power as well as soft power. India has enough potential of applying its 'smart power', comprising of soft power and hard power elements.

Indian diaspora as a very significant asset of India's soft power can attribute its success to its rich civilizational and spiritual ethos and superb cultural traits and heritage, those they have maintained in the hostland, and also their brilliance in knowledge, intellectual, technological, economic and political capabilities. Indian diaspora has remarkable capabilities and strengths to cooperate with varied people and to adjust to different situations. Indian diaspora has tremendous economic capabilities. Especially in the countries like, USA the economic condition of Indian diaspora is quite better. So, India's fostering of this economic engagement and relation with its diaspora will definitely be an effective tool in engaging politically, economically and culturally the diaspora's host country. In a nutshell, Indian diaspora as India's soft power asset enhances India's cultural heritage, civilizational values, economic capabilities and political engagements. So,

the paper argues that India's widening global presence is felt significantly due to its effective use of soft power diplomacy, and Indian diaspora as a magnificent soft power asset is playing a tremendous role in the global image making of India.

Understanding the Concept of 'Soft Power'

The notion of power has always remained a deciding reality in international politics. Power has two dimensions, hard power and soft power. Both these variants of power attribute to foreign policy making, security, economy and international politics. Whereas dictum of hard power focuses on defence capabilities and military interventions, forceful diplomacy, economic restrictions and political interventions to fulfill national interests, on the contrary to this, soft power stresses on shared political values and peaceful processes for conflict resolution and economic coordination to attain common solutions. As Nye observes:

Soft power is the ability to get what you want through attraction rather than coercion or payments. When you can get others to want what you want, you do not have to spend as much on sticks and carrots to move them in your direction. Hard power, the ability to coerce, grows out of a country's military and economic might. Soft power arises from the attractiveness of a country's culture, political ideals, and policies. When our policies are seen as legitimate in the eyes of others, our soft power is enhanced (Nye 2004: 256).

Referring to Joseph Nye, Gomichon points out that soft power enables a change of behavior in others, without competition or conflict, by using persuasion and attraction (Gomichon 2013). Soft power of a country is determined by its ability to achieve expected outcomes in various international issues by appealing and reaching to other states and non-state actors and not through enforcement. In addition to soft power, strategies play a significant role in the international structure. India's soft power forms the base of its strategy.

Soft power resources are usually intangible that comprises ideas, knowledge, values, and culture which have long-term effects on societies. However, there are



Does Caste-based Social Stratification Moderate the Relationship Between Social Capital and Life Satisfaction? Evidence from India

Chinglen Laishram & Khaikholen Haokip

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Does Caste-based Social Stratification Moderate the Relationship Between Social Capital and Life Satisfaction? Evidence from India

Chinglen Laishram  and Khaikholen Haokip 

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ABSTRACT

This study examined if India's caste system, a form of social stratification that divides different social groups into ranked categories, moderates the relationship between social capital and life satisfaction. Using data extracted from the International Social Survey Programme's module on Social Network and Social Resources (N = 909), we conducted Principal Component Analysis and identified two distinct components of social capital – formal and informal. Using Multiple Hierarchical Regression, we examined the relationship between social capital (formal and informal) and life satisfaction, and it was found to be significant. We also found that the relationship is different for different castes. Average life satisfaction was lowest among the middle castes (Other Backward Classes), and not the lower castes (Scheduled Castes and Scheduled Tribes). Formal social capital was found to positively contribute to life satisfaction across all the caste categories, but heavy informal social capital was observed to reduce life satisfaction among upper castes.

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KEYWORDS

Caste; India; life satisfaction; social capital; subjective well-being

Introduction

Studies on self-reported happiness and life satisfaction, that is, subjective well-being (SWB), have cut across the social science disciplines, such as economics, psychology, and, more recently, sociology. Decades of empirical works undertaken on SWB have been pivotal in shaping policy formulations in several countries (Kroll 2011). Intergovernmental organizations like the United Nations have also emphasized SWB as a fundamental goal in the promotion of economic and social progress (UN Secretary General 2013). It is viewed that measuring happiness and life satisfaction addresses the limitations of assessing the progress of nations using Gross Domestic Product (GDP) (Diener and Seligman 2004; Kroll 2011; Ngamaba 2017).

The apparent growth and significance of SWB studies are also reflected in the number of empirical works that have been carried out till date. By mid-2020, for instance, more than 20,000 correlational findings are reflected in the World Database of Happiness (Veenhoven 1995). Of the various determinants of SWB that have been explored in the empirical studies, social capital has acquired particular salience. Social capital is shown to have significant implications, among others, on depression (Cao et al. 2015), self-rated

health (Yip et al. 2007), modern democracy (Fukuyama 2001), and stable government (Boix and Posner 1998). Concerning its implications in the field of Quality of Life (QoL) studies, social capital is often found to bear positive impact on individual's SWB. For instance, social capital was found to improve life satisfaction by advancing positive affect while at the same time limiting individual's negative affect (Zou, Su, and Wang 2018). Individual's social capital has also been found to have strong positive impact on life satisfaction where country level trust was high (Elgar et al. 2011). Similarly, formal social capital (i.e. civic engagement/volunteering) was observed to have positive bearings on life satisfaction, particularly among childless people (Kroll 2011).

Though various studies have examined the implications of social capital on life satisfaction, it is far from complete in the context of India, limiting our understanding on the utility of the concept. Existing studies have highlighted social capital not as a unitary concept but a multidimensional concept. Bjørnskov (2006), for instance, has questioned the validity of reducing various social capital measuring items into a unitary social capital index. In conducting Principal Component Analysis (PCA), Bjørnskov (2006) found that items for measuring social capital such as community organizational life, engagement in public affairs, community volunteerism, informal sociability, and social trust, load into three distinct facets of social capital – social trust, social norms, and associational activity. Indeed, it would be improper to assume that informal social connections, such as meeting with friends and family, will have similar function to engagement in formal organizational life. In the context of India, treating social capital as a multidimensional concept and empirically examining it by segregating into finer parts have not yet gained much traction. The bridging and linking dimensions of social capital, for instance, have not been implemented in studies that focus on the interface between caste and social capital (Vijayabaskar and Kalaiyaran 2014). Similarly, other dimensions of social capital, such as the formal and informal social capital or the structural and cognitive dimensions, have also not yet been employed in the context of India.

Further, social capital and its relationship with life satisfaction has often been reported to differ based on demographic attributes such as, inter alia, gender, age, marital status, and parental status. In the case of India, examining caste differences in the association between life satisfaction and social capital has been neglected in the existing literature. This extant gap continues to mark social capital studies as well as SWB studies, in the light of the fact that caste constitutes a prominent feature of Indian society.

Considering the neglect of SWB, social capital, and their interface with caste, the current study has threefold objective. First, we intend to identify finer parts of social capital and estimate their relationship with SWB. Second, we intend to examine caste differences in the average level of SWB and social capital. And third, we intend to examine how the relationship between SWB and forms of social capital are moderated by caste. The study has the potential to contribute to the enrichment of the previous work on social capital as well as on life satisfaction.

Social capital: theoretical aspects, and implications on SWB

Though the ideas that underlie social capital have a much longer intellectual history, the widespread popularity of the concept of social capital could be majorly attributed to the seminal



Effects of Fertilization with Textile Effluent on Germination, Growth and Metabolites of Chilli (*Capsicum annum* L) Cultivars

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Abstract

Nutrient deficiency in soil suppresses crop growth, yield and nutritional value of the products. Textile effluent, a rich source of several essential minerals (including Ca, Mg, Cu, Fe, Zn, Mn) required for plant growth, could be a viable option to supplement the nutrient availability of soil. Although presence of some toxic metals and organic compounds restrict its use as irrigation water, its controlled use as fertilizer was not studied so far. This study was undertaken to assess the utilization of textile industry effluent for its suitability and potentiality as nutrient supplement by applying it onto chilli (*Capsicum annum* L.) cropping system. The effects of textile effluent fertilization was assessed by the wet cotton method at room temperature for germination, and in pot experiment in field conditions for growth and biochemical analysis using four effluent dilution rates (i.e., 10%, 20%, 40% and 60% v/v). The results of the experiment showed no inhibitory effect of textile effluent on seed germination, while its fertilization as soil drench worked as nutrient supplement for growth in chilli cultivars. The fertilization with textile effluent enhanced the plant biomass by 110.9% and 124.5%, and the leaf area by 21.5% and 2.6% in chilli cultivar GVC-121 and GVC-101, respectively. The total carbohydrate and foliar protein were also favoured by fertilization with the effluent. Moreover, least proline accumulation under lower dose suggested reduced stress due to textile effluent fertilization. The study concluded that the lower dose of textile effluent fertilization can function as nutrient supplement for chilli cultivars and 20% (v/v) effluent dilution provides the most favourable results.

Keywords Textile effluent · Fertilization · *Capsicum annum* · Germination index · Growth · Metabolites

Highlights

- The application of textile effluent enhances germination of chilli cultivars.
- A low dose of textile effluent provides nutrients and increases growth.
- High dose of textile effluent delays germination and reduces growth.
- Application of wastewater to agricultural field may be a viable management option.

1 Introduction

Effluent of the textile industry is considered to be among the most polluted industrial effluents consisting of a high concentration of heavy metals and organic compounds (Singh et al. 2020; Singh and Rathore 2020). Besides, the textile industry is also known as a water intensive industry, consuming large quantities of water for various processes and discharging equally large volumes of wastewater containing a variety of pollutants (Kumar and Chopra 2013; Bhatia et al. 2018; Singh and Rathore 2019). The textile effluent is also characterized by the presence of organic dyes that develop strong colour, fluctuating pH, high chemical and biological oxygen demands, total dissolved solids, sulphate, and ammonical nitrogen (Kaushik et al. 2005; Grönwall and Jonsson 2017) which further complicate the problem of its management. The unimpeded disposal of textile effluent not only causes risks to human, animal and plant health but also possesses serious threats to soils and water bodies, and ecology of affected areas (Saratale et al. 2011; Forss et al. 2017; Singh et al. 2020). Fait et al. (2017) suggested that the uncontrolled disposal of waste could lead to distribution of metallic trace elements into the soil from top of the soil to deep up to one km through the soil-water interface. Textile effluent can induce mutations, and cause genotoxicity and oxidative damage, root growth retardation, mito-depression, and induction of chromosomal abnormalities in root meristematic cells (Hemachandra and Pathiratne 2016; Akhtar et al. 2016). Iqbal (2016), Abbas et al. (2018) and Iqbal et al. (2019) reviewed the available literature and concluded cytogenic and mutagenic effects of raw textile effluent on *Vicia faba*, *Vibrio Fischeri* and *Allium cepa*, respectively, and suggested biological treatment of the effluent before its use.

On the other hand, micronutrient deficiency in soil and plants is a global nutritional problem and is prevalent in many countries with different magnitude of severity. Micronutrient deficiencies in soil have been identified as one of the main factors affecting crop yield and food quality, which resulted in reduced nutrient in diet (Cakmak 2002; Singh et al. 2018). Widespread micronutrient deficiency increases the challenge to feed healthy food for 7 billion people which is expected to reach 9 billion by 2050 (FAO 2011). Therefore, sufficient key nutrients are required in soil for successful agricultural practice (Knez and Graham 2013). The industrial effluent contains several macro and micronutrients which the plants can uptake for their growth. Application of such effluent in agricultural fields may be a viable option to dispose industrial effluent, and would sustain agriculture in non-irrigated areas where the availability of fresh water is scarce (Kumar and Chopra 2013). Moreover, waste water can provide important nutrients, especially nitrogen and phosphorus and some micro-nutrients, which can increase soil fertility and enhance plant growth, crop production and quality of produce. It also reduces the requirements for commercial chemical fertilizers, and thus, it increases farmer's economic benefits (Papadopoulos and Savvides 2003). Fertilization by effluent may provide dual benefit to the environment as it reduces the requirement of chemical fertilizers besides resolving the problem of effluent disposal (Knez and Graham 2013; Kumar and Chopra 2013). Tran et al. (2016) also discussed his experimental result that the reuse of wastewater in agriculture offers cost savings over chemical fertilizers as it contains considerable amount of nutrient elements for plants.

Besides the presence of toxic heavy metals and organic compounds, metals necessary for plant metabolism as enzyme activators or regulators are present in textile effluent, e.g. Fe, Cu, Mn, Mo; however, these may cause toxicity if supplied in excess quantities (Kaushik et al. 2005; Singh and Rathore 2018). Use of wastewater reduces fertilizer and irrigation cost as it is freely available (Papadopoulos and Savvides 2003). Earlier researches with lower dose of

distillery effluent as irrigation provided positive results on seed germination, total sugars, starch, reducing sugars, and chlorophyll (Ramana et al. 2002; Kaushik et al. 2005; Hassan et al. 2013; Singh and Rathore 2018, 2020).

The studies conducted so far on the utilization of textile effluent in agriculture were focused on nutrient utilization during irrigation. However, the presence of high salinity and significant amounts of trace metals can harm the crop and soil by its continuous application as irrigation. Djehaf et al. (2017) reviewed the available treatment technologies for textile effluent and suggested its use for field application after treatment. Use of diluted treated wastewater could somewhat mitigate the side effects without additional treatment; it is considered that diluted wastewater can be used for soil fertilization (Bañón et al. 2011; Jeong et al. 2016). Singh and Rathore (2020) suggested to use diluted textile effluent during the field preparation to satisfy the need of micronutrients to plants. This would not only reduce the input of chemical fertilizer but also reduce the cost of textile effluent disposal.

Chilli (*Capsicum annum* L.) is an important commercial spice crop which is a rich source of vitamin 'C' and 'A' with plenty of minerals (Parvathi and Yurnus 2010). Global trade of chilli is around 400,000 metric tons. Alabi (2006) stated that chilli shows greater yield and quality when essential elements are provided in appropriate amounts. So far, no study has been conducted on the application of limited amount of textile effluent as fertilizer to provide nutrients from textile effluent on chilli crop. Therefore, the present study was conducted to assess the potential of textile industry effluent to supplement micronutrients during cultivation of chilli (*Capsicum annum* L. cv.GVC-101 and GVC-121) by fertilization with different concentration of effluent, and to identify the optimum effluent concentration for fertilization of chilli cultivars.

2 Materials and Methods

2.1 Effluent Collection and Characterization

Textile effluent originating from the Mangalam textile industry was collected in well cleaned unused plastic containers from a Green Environment common effluent treatment plants (CETP) located in Vatwa, Ahmedabad, India (Singh and Rathore 2020) before the treatment process. The collected samples were stored in refrigerator (4 °C) to avoid changes in their characteristics. As the textile effluent used in the present study was same to that of our previous studies (Singh and Rathore 2018, 2020), its physico-chemical characteristics, determined using Standard Methods (APHA 2012), were also similar (Table 1) as reported in Singh and Rathore (2018, 2020).

2.2 Germination Experiment

The wet cotton method was applied for the germination experiment. Cotton was moistened with 10 mL of water as control and with the same quantity of different concentrations of textile effluent resulting from 10%, 20%, 40% and 60% v/v dilution in water and kept in a Petri dish. Eight seeds of the two cultivars were placed in these Petri-dishes and were incubated at temperature of 30 ± 1 °C. Germination was recorded every 5 days from the date of sowing for 15 days at 11:00 am, and the emergence of the radicle was taken as a criterion of germination. All the experiments were carried out in three replicates (3 Petri dishes for each treatment) and the results were averaged.

Table 1 Physico-chemical Characteristics of textile effluent (obtained from Singh and Rathore 2018)

Physical Properties	
Temperature	38 °C
Colour	Brownish
Odour	Fishy
pH	6.86±0.1
Chemical properties	
Alkalinity (mEq L ⁻¹)	643±0.21
EC (µS cm ⁻¹)	438±0.43
TS (mg L ⁻¹)	2043±1.94
TSS (mg L ⁻¹)	97±1.63
TDS (mg L ⁻¹)	1909±1.08
COD (mg L ⁻¹)	1587±1.34
chloride (mg L ⁻¹)	693±0.51
sulphate (mg L ⁻¹)	286±0.37
Phosphate (mg L ⁻¹)	1.58±1.08
Phenols (mg L ⁻¹)	173.05±0.07
Total nitrogen (mg L ⁻¹)	80.13±1.5
Ca (mg L ⁻¹)	60.63±0.01
Mg (mg L ⁻¹)	ND
Elemental Properties	
Fe (mg L ⁻¹)	1.2±0.063
Zn (mg L ⁻¹)	2.16±2.26
Mn (mg L ⁻¹)	1.1±0.05
K (mg L ⁻¹)	23.44±0.87
Cr (mg L ⁻¹)	4.22±2.34
Pb (mg L ⁻¹)	ND
Cd (mg L ⁻¹)	1.3±0.004
Co (mg L ⁻¹)	0.9±0.01
Cu (mg L ⁻¹)	1.3±0.002
Al (mg L ⁻¹)	14.3±0.03
Ni (mg L ⁻¹)	1.05±0.33
Biological properties	
BOD ₅ (at 20 °C) (mg L ⁻¹)	688±1.08
CFU (mL ⁻¹ × 10 ³)	1.02 × 10 ⁶
MPN/100 mL	32.46

Seed germination for the various effluent concentrations was recorded and computed as germination percent. Speed of germination was analysed using the Eq. (1):

$$\text{Speed of germination} = \frac{\text{No. of seeds germinated}}{\text{No. of days from the first count}} + \dots + \dots + \frac{\text{No. of seeds germinated}}{\text{No. of days of the final count}} \quad (1)$$

Peak value and germination value were calculated using Eqs. (2) and (3) presented by Kaushik et al. (2005):

$$\text{Peak value} = \frac{\text{Cumulative percent germination on each day}}{\text{No. of days elapsed since initial imbibition}} \quad (2)$$

$$\text{Germination value} = \text{Peak Value} \times \text{germination percent.} \quad (3)$$

Seed Vigor index and delay index were obtained using the following Eqs. (4) and (5) explained in Abdul-Baki and Anderson (1973):

Vigor index = Germination percent

$$\times \text{mean of seedling length (root + shoot) at the end of incubation period.} \quad (4)$$

$$\text{Delay index} = \frac{\text{Delay in germination time over control}}{\text{Germination time for control}}. \quad (5)$$

2.3 Pot Experiment Material and Design

The pot experiment was conducted at the nursery of Gujarat Forest Research Foundation (GFRF), Gandhinagar, India (23.2362° N, 72.6766° E). Seeds of chilli (*Capsicum annum*) cultivars, i.e., GVC-101 and GVC-121 were obtained from Anand Agricultural University, Anand, Gujarat. Both cultivars are grown in winter-monsoon season, but are different in their morphology, biochemical characteristics, nutritional composition and yield potential (<https://www.aau.in>; Litoriya et al. 2014). Genetically, uniform seeds of chilli were sown in pots of 19 cm (diameter at top) × 18 cm (height) size. Pots were filled with equal amounts of slightly alkaline (pH 7.8, EC 0.53 μScm^{-1}) sandy loam soil of medium fertility (N, P, K values of used soil were 236.31, 78.63, 118.54 kg ha^{-1} , respectively). Trace element values of the experimental soil before addition of textile effluent were: Fe 60.6 ± 0.11 ; Zn 0.21 ± 0.01 ; Mn 1.28 ± 0.04 ; As 0.0028 ± 0.21 ; Cd 0.0 ± 0.0 ; Co ND; Al 92.8 ± 1.03 , Ni 0.30 ± 0.01 ; Pb 0.2 ± 0.003 (all values are mg kg^{-1} of soil) (Singh and Rathore 2020). Twelve seeds of chilli cultivars were sown in each pot. Four dilution rates of textile effluent (10%, 20%, 40% and 60% v/v, i.e., T1, T2 T3 and T4, respectively) were applied in soil as the basal fertilizer dose for micronutrient supplementation (Table 2). Fertilization with textile effluent was applied only once during the soil preparation. Pots were fertilized by textile effluent before the sowing of seeds and left for a week for proper mixing. A control set (without textile effluent fertilization) was also maintained for comparison. Ground water was applied for irrigation purpose in all treatments. After germination seeds were thinned to six seedlings per pot in all the pots, which were further thinned at each sampling period. The experiment was conducted under completely randomized design and replicated three times. For the various physiological, biochemical and elemental analyses, the chemicals were purchased from Sigma-Aldrich, Bangalore, India. Other acids, buffer components, major and minor salts were obtained from MERCK and Himedia Mumbai, India. All chemicals were purchased in highest purity and either of analytical or HPLC grade as required.

2.4 Plant Growth Analysis

Plants were randomly sampled in triplicate from all treatments at 30, 60, 90 and 120 days after sowing (DAS). After carefully washing with distilled water, plants were separated into roots, stem and leaves. Plant height (roots and shoot length) was measured by meter scale and leaf area was measured by graphical method and presented as cm^2 per plant. To determine biomass, plant parts were oven-dried at 80 °C till a constant weight was achieved. After drying, the plant

Table 2 Volume of textile effluent used

Treatment	Quantity of textile effluent (mL textile effluent/kg soil)
C	0
T1	110
T2	220
T3	440
T4	660

parts were weighed by weighing balance. Root to shoot ratio (RSR) was calculated according to Hunt and Burnett (1973).

2.5 Photosynthetic Pigments

For chlorophyll (Chl) and carotenoid determinations, 0.1 g leaf sample was placed in 10 mL of 80% acetone in a test tube and kept it overnight in a refrigerator at 4 °C. It was then homogenized and centrifuged at 6000×g for 15 min. The optical densities of the supernatant were measured at 480, 510, 645 and 663 nm using a spectrophotometer (Systronic, Model 2203). The contents of Chl *a*, Chl *b* and carotenoid were calculated as explained in Maclachlan and Zalik (1963) and Duxbury and Yentsch (1956), respectively. Total chlorophyll was obtained by adding the value of Chl *a* and Chl *b*.

2.6 Metabolites and Proline Content

For carbohydrate, 1 g of fresh leaf sample was crushed with chilled 70% ethanol and centrifuged at 4000 rpm for 10 min. After centrifugation, 1 mL of obtained supernatant was added with freshly prepared 4 mL of anthrone reagent and the mixture was allowed to stand in warm water for 8–10 min, then cooled rapidly and absorbance was read to estimate total carbohydrate content as described by Yemm and Willis (1954).

Protein was estimated by leaf extraction in 0.2 M phosphate buffer of pH 7 following the method of Lowry et al. (1951). Amount of protein present in the samples was expressed with the bovine serum albumin (BSA) as standard in $\mu\text{g mL}^{-1}$.

Proline content was estimated by extracting the leaf sample in 10 mL sulfosalicylic acids and the supernatant was used for acid-ninhydrin test (Bates et al. 1973) and expressed as $\mu\text{mol proline g}^{-1}$ FW.

2.7 Statistical Analysis

The data for different treatments are presented as mean value of three replicates, and standard errors. The significance of the data was analysed using two ways ANOVA, with growth stage and treatments considered as two factors. Statistical analyses were performed using the SPSS program (version 17.0) to compare the effect of textile effluent fertilization and plant age. Plant photosynthetic pigment content, biochemical characterisation, and biomass assay were compared by analysis of variance and multiple comparison tests. In case of significant changes, heterogeneous groups were distinguished on the basis of duncan multiple range test (DMRT) at $p < 0.05$.

3 Results

3.1 Effluent Characteristics

The used textile effluent was brownish in colour, deficient in dissolved oxygen, rich in total solids, total alkalinity, high in biological oxygen demand (BOD) and chemical oxygen demand (COD) and with considerable amounts of total nitrogen, phosphate, chlorides, sulphate, sodium, calcium, zinc, manganese, copper, nickel, ferrous, lead and cadmium (Singh and Rathore 2018). The concentration of total suspended solids (TSS), BOD, COD, and total dissolved solids (TDS) of effluent used in the study exceeded the prescribed limits of Indian irrigation standards (BIS 1991). Bhatia et al. (2018) also reported increased pollutant indices of textile effluent; however, nutrient content was not analysed.

3.2 Germination Experiment

Textile effluent dilution favoured well both chilli cultivars in the germination experiment. No inhibition on germination by textile effluent was seen on the seeds. Germination percent (GP) of both chilli cultivars was significantly higher after fertilization with textile effluent than control plants (Table 3). Maximum increase in germination percent was seen for the lowest dose of textile effluent (T1), and GP decreased gradually with increasing concentration. Moreover, significantly higher speed of germination (SOG) and peak value (PV) were also observed for textile effluent fertilization with experimental chilli cultivars except for the highest dose of textile effluent fertilization which showed slightly lower SOG than the control. Similarly, germination value (GV) and seed vigor index (SVI) were also higher for textile effluent fertilization with maximum increase under lower dose of textile effluent. However, the delay index (DI) was higher under the higher dose of textile effluent. In general, germination indices of cultivar GVC-101 were more responsive to textile effluent fertilization.

3.3 Plant Growth

Application of textile effluent showed positive response for shoot height and root length in both chilli cultivars (Fig. 1a) at all ages. For shoot height, most suited fertilization dose of textile effluent was T2 at all ages except 30 DAS in GVC-101 which showed maximum growth for T1. Highest shoot lengths of cultivars GVC-101 and GVC-121 were observed at 31.17% and 39.25%, respectively, at 120 DAS for T2 treatment, while root length was highest under T3 treatment at 120 DAS (31.56% and 18.91% for GVC-101 and GVC-121, respectively). Factorial analyses showed significant variation for all factors in both cultivars except interaction of age and treatment for cultivar GVC-101 for root length (Table 4).

Similar to plant height, leaf area was also increased by textile effluent fertilization in both studied cultivars and best result was seen for T2 treatment (Fig. 1b). Increase in leaf area was significant by both factors, i.e., plant age and fertilization dose and its interaction for both experimental cultivars (Table 4). Highest leaf areas, i.e., 26.59 and 21.46 cm² plant⁻¹ for cv. GVC-101 and GVC-121, respectively, were found at 60 DAS for T2 treatment.

Plant biomass was significantly increased at successive growth stages under the lower dose of textile effluent fertilization for both experimental cultivars (Fig. 1c). However, the higher dose of textile effluent (T4) had adverse effect on biomass accumulation for the tested chilli cultivars. Application of textile effluent fertilization with 20% (T2) dilution was found most

Table 3 Effect of textile effluent on Germination percent (GP), Speed of germination (SOG), peak value (PV), germination value (GV), seed vigor index (SVI) and delay index (DI) of chilli (*Capsicum annuum*) cultivars GVC-101 and GVC-121. Rows with different letters indicate statistically significant differences between treatments according to Duncan's multiple range test (at $p < 0.05$)

Parameter	GVC-101 Treatment					GVC-121 Treatment				
	C	T1	T2	T3	T4	C	T1	T2	T3	T4
GP	56.67± 0.04 ^a	85.78 ± 1.03 ^b	77.89 ± 0.11 ^{ab}	74.11 ± 0.05 ^{ab}	62.00 ± 0.04 ^{ab}	68.33± 1.33 ^a	88.00 ± 0.01 ^a	82.22 ± 0.15 ^a	69.00 ± 0.02 ^a	65.11 ± 0.17 ^a
SOG	21.81± 1.42 ^b	30.35 ± 1.09 ^c	26.73 ± 0.01 ^d	25.27 ± 0.08 ^c	21.17 ± 0.03 ^a	22.21± 1.27 ^b	31.33 ± 0.31 ^c	28.54 ± 0.33 ^{bc}	24.84 ± 1.24 ^{ab}	22.29 ± 0.42 ^a
PV	11.33± 0.07 ^a	17.16 ± 0.01 ^c	15.58 ± 0.06 ^d	14.82 ± 0.03 ^c	12.40 ± 0.04 ^b	13.67± 0.01 ^a	17.60 ± 0.04 ^b	16.45 ± 0.11 ^b	13.80 ± 0.14 ^a	13.02 ± 0.08 ^a
GV	642.2±0.1 ^a	1471.6 ± 0.3 ^c	1212.7 ± 0.1 ^d	1098.6 ± 0.2 ^c	768.8 ± 0.1 ^b	933.9± 0.2 ^b	1548.8 ± 0.3 ^d	1352.1 ± 0.4 ^c	952.2 ± 0.2 ^b	847.90 ± 0.1 ^a
SVI	75.75±0.1 ^a	227.06 ± 0.2 ^d	218.88 ± 0.06 ^d	161.14 ± 0.11 ^c	109.81 ± 0.3 ^b	22.13± 0.23 ^a	209.44 ± 0.07 ^d	210.84 ± 0.13 ^c	150.22 ± 0.02 ^c	110.05 ± 0.14 ^b
DI	–	0 ± 0.00 ^a	0 ± 0.00 ^a	0.5 ± 0.002 ^c	1 ± 0.001 ^b	–	0 ± 0.00 ^a	0.2 ± 0.001 ^{ab}	0.5 ± 0.001 ^c	1.5 ± 0.001 ^b

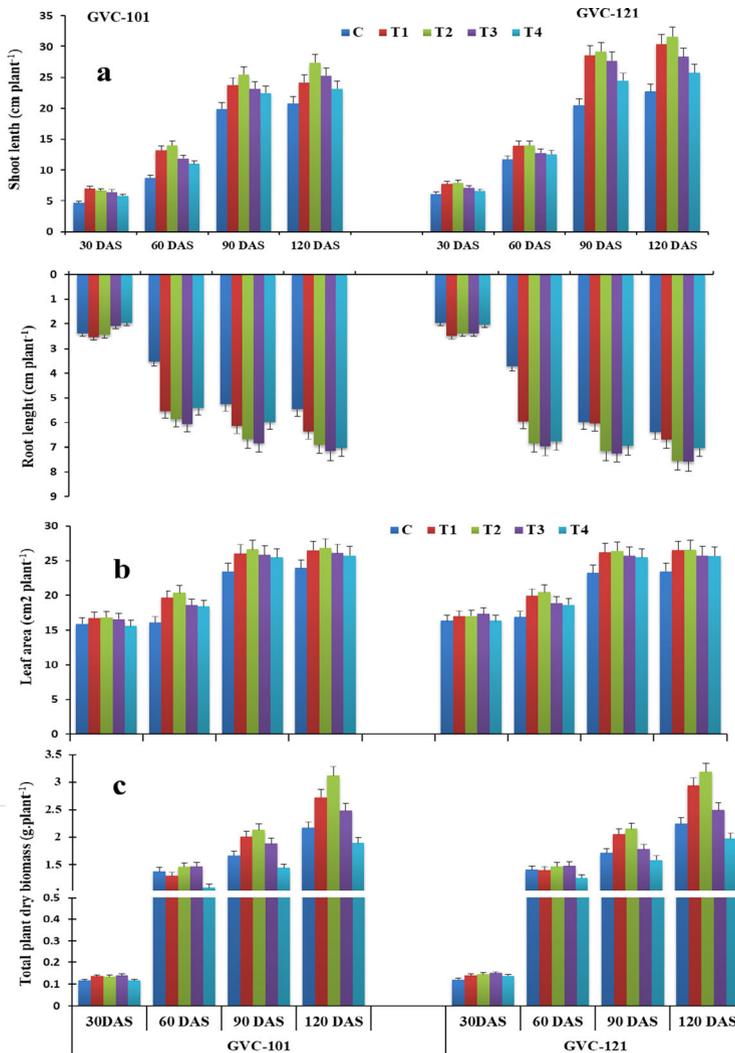


Fig. 1 (a) Shoot height and root length, (b) leaf area, and (c) total dry biomass of chilli (*Capiscum annum*) cultivars (GVC-101 and GVC-121) at 30, 60, 90 and 120 days after sowing (DAS) for the different treatments (different dilution rates of textile effluent fertilization)

suited for total biomass accumulation in both cultivars. Highest increase of total dry biomass was recorded with T2 treatment at 120 DAS for both chilli cultivars, i.e., 43.78% and 42.19% for GVC-101 and GVC-121, respectively, against their respective controls. However, factorial analysis for biomass showed non-significant effect of textile effluent on cultivar GV101 (Table 4).

3.4 Photosynthetic Pigments and Plant Metabolites

Chlorophyll *a* content accelerated significantly from 30 to 120 days in both cultivars at all textile effluent fertilization doses (Fig. 2a). Increase in Chl *a* was found maximum at 120

Table 4 Summary of two-way analysis of variance (ANOVA) at variable levels of significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and NS-not significant)

Parameter	Age		Treatment		Age×Treatment	
	GVC-101	GVC-121	GVC-101	GVC-121	GVC-101	GVC-121
Leaf area (LA)	*	***	**	***	**	***
Root length	***	***	***	***	NS(0.127)	***
Shoot height	***	***	***	***	***	***
RSR	***	***	NS(0.131)	**	NS(0.415)	**
Chlorophyll -a	***	***	***	***	***	***
Chlorophyll -b	***	***	***	***	**	**
Total chlorophyll	***	***	***	***	***	**
Carotenoid	***	***	***	***	***	***
Protein	***	***	***	***	***	***
Total carbohydrate	***	***	***	***	***	***
Proline	***	***	***	***	NS(0.425)	NS(0.560)

DAS (45.25% and 32.66% for GVC101 and GVC-121, respectively) for T2 treatment compared to their respective controls. Similarly to Chl *a*, Chl *b* was also increased in a similar trend (Fig. 2b). Plant fertilized by 40% (T3 treatment) textile effluent exhibited maximum content of total chlorophyll (46.32 and 35.07%, respectively, for cultivar GVC-101 and GVC-121) than control at 120 DAS (Fig. 2c). Analysis of variance showed highly significant effect of both factors and their interactions for chlorophyll content of tested cultivars (Table 4).

Carotenoids content recorded an increase with successive plant age in both tested cultivars (Fig. 2d). Carotenoids content was not much affected by T1 treatment; however, it was found increased with increasing dose. Increase was observed maximum under T3 treatment for both tested cultivars. Highest carotenoids in both cultivars was found at 90 DAS with T3 treatment. Statistically, effect of age, treatment and their interaction was highly significant with both cultivars (Table 4).

Total carbohydrate content was affected positively by textile effluent fertilization in both chilli cultivars of the present experiment (Fig. 3a). Increase in carbohydrate was gradual up to T2 treatment then declined. Highest increase in total carbohydrate content was seen in T2 treatment at 90 DAS for GVC-101 (78%) and in T2 treatment at 120 DAS for GVC-121 (83%). Similar to total carbohydrate, protein content was also increased significantly under textile effluent application in a trend similar to carbohydrate at all ages with all treatments in both tested cultivars (Fig. 3b). Protein content was observed maximum in GVC-101 (72.92%) at T2 at 120 DAS. Order of the increase was T2 > T1 > T3 > T4 in both cultivars compared to their respective control. Variation in total carbohydrate and total protein was statistically significant for both factors viz. age and textile effluent fertilization and their interaction (Table 4).

Contrary to carbohydrate and protein, proline accumulation was reduced at successive ages by textile effluent fertilization in respect to their control except at 30 DAS, where it was found higher in T1 treatment (Fig. 3c). Proline accumulation was least with T2 fertilization in both cultivars. Analysis of variance showed highly significant effect of individual factors, although factor interaction was non-significant (Table 4).

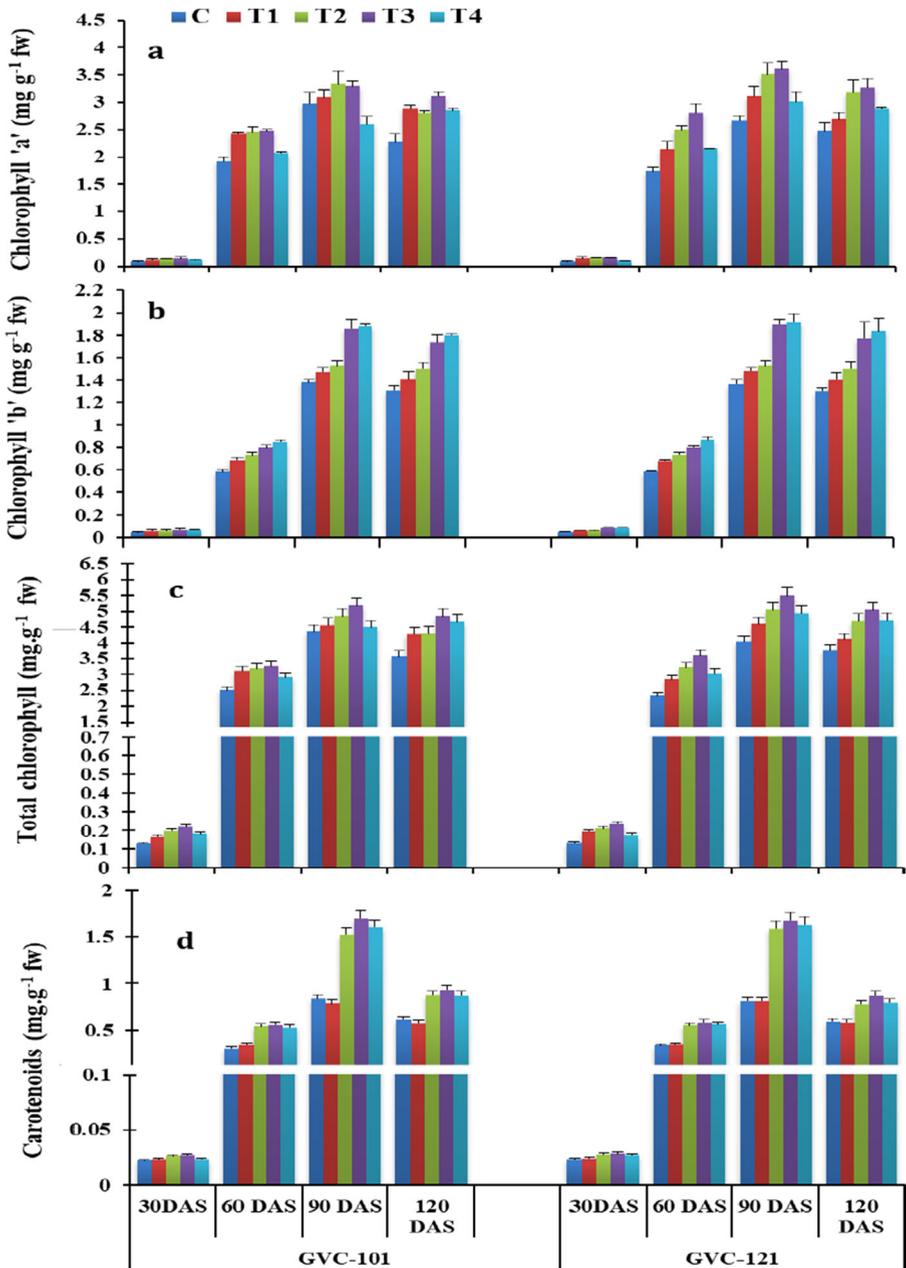


Fig. 2 (a) chlorophyll-a, (b) chlorophyll-b, (c) total chlorophyll, and (d) carotenoids content in leaves of chilli (*Capsicum annum*) cultivars (GVC-101 and GVC-121) at 30, 60, 90 and 120 days after sowing (DAS) for the different treatments (different dilution rates of textile effluent fertilization)

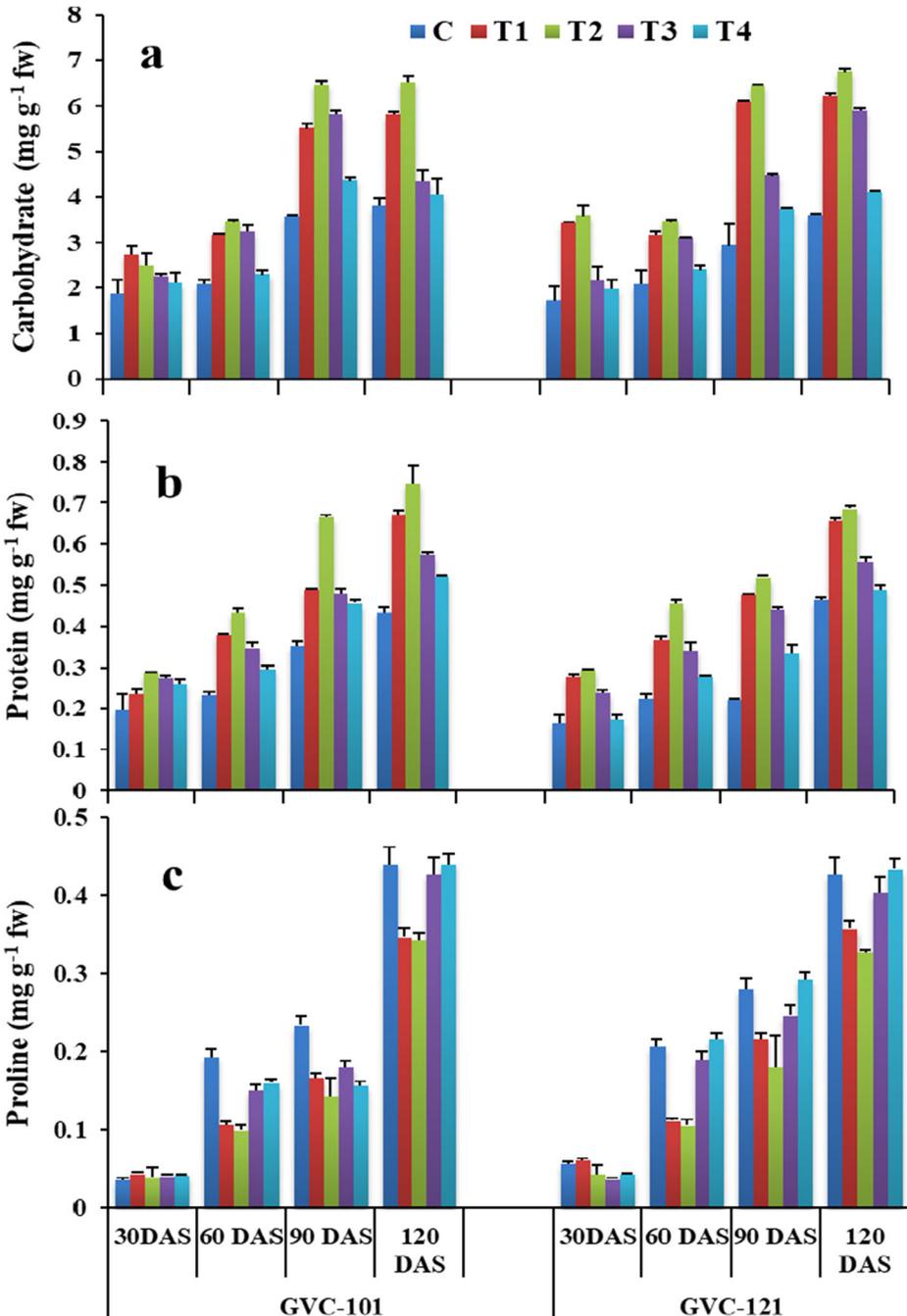


Fig. 3 (a) Carbohydrate content, (b) protein content, and (c) proline accumulation in leaves of chilli (*Capsicum annuum*) cultivars (GVC-101 and GVC-121) at 30, 60, 90 and 120 days after sowing (DAS) for the different treatments (different dilution rates of textile effluent fertilization)

4 Discussion

Textile effluent plays a major role in producing large amounts of water pollution by characteristic toxicity of its effluent, i.e., higher total hardness, TDS, BOD, COD, SO₄, Ca Mg, Pb, Cd etc. However, appreciable amount of mineral nutrients such as Ca, Fe, Mg, Zn, Cu, and Mn are also present in the textile effluent (Kaushik et al. 2005; Singh et al. 2015) making it a possible source of fertilizer. The fertilization property of textile effluent is well discussed in our earlier published report (Singh and Rathore 2018; Singh et al. 2020). Textile effluent used in the present study was found to be rich in various minerals and trace elements required for the plant physiological activity. Used textile effluent helped to increase the plant growth by providing organic matter and micronutrients in the limited range at lower concentration (Yaseen et al. 2017). However, high COD, total solids, total dissolved solids, soaring alkalinity with higher value of chloride and sulphate can make soil alkaline and reduce availability of micronutrients. Results of the present study indicate that fertilization with textile effluent at low dilution increased the germination property, growth and metabolites of chilli cultivars.

The present experiment showed positive response of textile effluent on germination. Kaushik et al. (2005) and Khan et al. (2011) also observed increase in germination of wheat, pea, lentil and gram with low dilution of textile effluent, and a decrease in seed germination with increase in the concentration of the effluent. Results showed higher seed germination compared to control for all used dilutions of textile effluent (up to 60% dilution) in cultivar GVC-101; however, germination of cultivar GVC-121 was increased up to 40% dilution and was reduced with 60% dilution compare to control. Kaushik et al. (2005) and Khan et al. (2011) suggested that the industrial effluent with high osmotic pressure can cause reduction in germination. However, in case of chilli cultivars, the textile effluent was found to break the dormancy as seen in the present experiment. Hassan et al. (2013) studied the growth of country bean (*Lablab niger* cv. typicus Medikus) seeds and seedlings irrigated with textile effluent. They concluded that the neutralized effluent water does not have a negative impact on the germination percentage, germination energy, relative germination rate and relative effluent injury rate of country bean seeds, or its seedling growth. However, the germination percent varied with cultivars of the same species as seen in this experiment. The maximum promoting effect on germination percent was observed with 10% dilution of textile effluent in both cultivars.

In the present study, germination speed, peak value and germination value also followed the same trend as seed germination. Speed of germination is maximum at 10% dilution and was reduced with increase in textile effluent concentration. The reason for the germination inhibition at higher concentration of textile effluent can be explained by the toxic effect of heavy metals and persistent organic compounds present in the composite textile effluent (Singh and Rathore 2019). The Delay index calculated in the present experiment also showed delayed germination at higher dose of textile effluent, while the Vigor index showed vigorous characteristics of used seed at moderate dose of textile effluent. This result is consistent with Ramana et al. (2002) in vegetable crops (tomato, cucumber, bottle gourd etc.) under distillery effluent.

Plant height and leaf area of chilli cultivars (GVC-101 and GVC-121) under textile effluent fertilization were also increased over control plants. However, fertilization at lower dose textile effluent provided more persistent results as compare to high dose. Similarly to the present study, Panda et al. (2016) also demonstrated that the lower concentrations of industrial effluents promote seed germination, seedling growth and dry matter accumulation of *Oryza*

sativa. Ajouri et al. (2004) explained that the lower growth in control plants (without treatment) may be due to nutrient deficiency in soil or unavailability to the plants. Deficiency of nutrients in the soil suppress the growth of plants while accumulation of salts such as Cd that could interfere with the uptake of various nutrient elements, decrease root respiration and inhibit root production (Bhuiyan et al. 2016). Fahim et al. (2019) reported improved nutrient content and soil fertility after addition of pulp and paper mill waste which resulted into increased growth and biomass accumulation of *Abelmoschus esculentus* and *Mentha sachalinensis*, Contrary to the results obtained in the present study, Marwari and Khan (2012) reported reduction in root and shoot length by textile effluent.

Deficiency of Zn causes strong chlorosis and decreases leaf production besides the reduction of crop growth. Increase in leaf area can be correlated with increased photosynthetic surface, and thus, higher production (Noulas et al. 2018). Faster development of leaf size and increase in total photosynthetic rate could lead to a general increase of carbon assimilation as evident in the present experiment by increased plant biomass. Similar results were also obtained by Kaushik et al. (2005) on growth and biomass of wheat cultivars irrigated with textile effluent, and by Araújo et al. (2007) on growth and development of soybean and cowpea treated with textile sludge. However, excess heavy metals developed interferences with synthesis of levulinic acid and protochlorophyllide reductase in tetrapyrrole pathway (Wu et al. 2018), resulting into slow rate of photosynthesis (Shah et al. 2017), which further resulted into less biomass accumulation, as also seen with higher dose of textile effluent fertilization in the present experiment.

Chlorophyll estimation is one of the important plant parameters which are used as an index of production capacity of the plant; carotenoids act as an accessory pigment in photosynthesis. Increase in chlorophyll could be due to addition of nutrient by textile effluent fertilization while high concentration of micronutrient showed synergistic effect on either chlorophyll synthesis pathways or on enzymes used for synthesis. Srivastava and Sahai (1988) supported the view that the increase in carotenoid content at low concentrations of the effluent treatment may be due to the beneficial effect of nitrogen and other inorganic elements present in the textile effluent. Decreased foliar chlorophyll of plants grown without fertilization in the present study was in accordance with Shah et al. (2017) under nutrient deficiency.

Total carbohydrate and protein content showed similar trend as chlorophyll and increased with age and textile effluent fertilization. Increase in carbohydrate with age may be expected, as starch is converted to carbohydrate as the plants mature (Badoni et al. 2016). Although, suppression of carbohydrate content by high concentration of composite textile effluent can be explained by the presence of high amount of metals and the role of carbohydrate in the enzymatic reactions related to the cycles of carbohydrate catabolism during reactive oxygen species (ROS) generation. Similar to our result, Badoni et al. (2016) also reported an increased accumulation of carbohydrate with increasing concentration of Zn compared to the control in *Jatropha curcas*. Amino acid is the basic precursor for proteins taking part in photosynthesis and photosynthetic pigments, and leaf protein content may positively correlated with biomass and total chlorophyll content of plant (Ayyasamy et al. 2008).

Increase in protein under textile effluent fertilization was seen with lower dose. However, gradual decrease of protein content at higher concentration of textile effluent (above 20% to 60%) suggested the breakdown of protein in amino acid due to stress generated in the presence of the toxic concentration of heavy metals in the textile effluent. However, as explained by Rehman and Bhatti (2009), the enhancement in leaf protein exposed to lower concentration of textile effluent is due to synthesis of stress protein gradually from 30 DAS up to 120 DAS.

Increasing chlorophyll and soluble protein content at lower concentration of effluent application agreed with Gupta and Mittal (2017) and is due to the addition of minerals and nutrients.

Proline is a stress amino acid synthesized according to the defensive capability of plants. The accumulation of proline in plant tissue increases due to stress generated during the growth phases (age of the plant) or under different environmental conditions, i.e., heavy metals, UV light, drought, air pollution (Agrawal et al. 2004; Rathore and Chaudhary 2019; Singh and Rathore 2019; Rathore and Chaudhary 2021). Stress generated from nutrient deficiency in the soil can also cause proline accumulation in the plant (Arias-Baldrich et al. 2015) which can be seen at high concentration of proline in control plants (without textile effluent fertilization). In the present study, proline was least under fertilization with lower dose of textile effluent, suggesting low nutrient stress condition; however, increased proline in T4 might be due to the excess heavy metals and salt added into soil at higher textile effluent application.

5 Conclusions

Textile effluent is rich in nutrients for plants, surplus trace elements and high salt content. Positive response of lower dose (with 20% dilution) of textile effluent fertilization on germination, growth and metabolites of chilli cultivars represented mineral utilization from the effluent. Mineral deficiency or excess mineral developed stress symptoms in plants which can be evident by lower biomass development or higher proline accumulation, as seen in the present study with no fertilization or higher textile effluent fertilization. Lower dose of textile effluent fertilization accumulated least proline and was proved as a most suited condition for growth of the two chilli cultivars. Intra-specific variation among the chilli cultivars GVC-101 and GVC-121 to textile effluent was not evident, although slightly higher efficiency was seen in cultivar GVC-101 compared to cultivar GVC-121 in nutrient utilization from textile effluent. The study concluded that the lower dose of textile effluent can be applied as mineral nutrition supplement for plant growth. However, more field studies are needed to scale up the use of textile effluent, dose appropriation and to assess intra-specific variation.

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Author Contributions RS carried out the experiment, recorded the data, interpreted the result, and wrote the final manuscript. DR designed and supervised the experiment, made suitable changes in the manuscript. Both authors read and approved the final manuscript.

Data Availability Not applicable.

Declarations

Ethics Approval and Consent to Participate Not applicable.

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Competing Interests Authors declare no competing interest.

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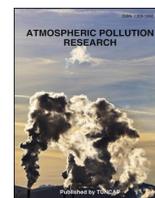
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Effects of tropospheric ozone on groundnut (*Arachis hypogea* L.) cultivars: Role of plant age and antioxidative potential

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ABSTRACT

Ozone (O₃) is a secondary atmospheric pollutant present in the tropospheric environment. Ozone enters in internal tissue of leaf through stomata and produces reactive oxygen species (ROS) which damages cellular membrane, alter gene expression and metabolic activities and ultimately caused death of the plant tissues. Certain defensive mechanism occurs in the internal part of leaves to eliminate generated ROS. However, there is significant change in the levels and kind of defence traits as plants develop from the seedling to juvenile to mature and senescent stages. A field study was conducted for two consecutive seasons with five cultivars of groundnut (*Arachis hypogea* L cv. TG-37A, TPG-41, TAG-24, GG-20, and Dh-86) to assess the intra-specific variation in defensive response against ozone at various plant developmental stage. Results of the study showed that ozone significantly affected biomass and primary metabolites, generate ROS and increases membrane permeability in all the groundnut cultivars. In response to ROS, groundnut cultivars produced non-enzymatic and enzymatic antioxidants. Study found that the severity of the effect varied among the cultivars and by developmental stages. Cultivar Dh-86 showed higher antioxidative activity and least biomass sensitivity to ozone. Although accumulation of non-enzymatic antioxidants was present to provided defence against ozone, activity of enzymatic antioxidants varied at different growth stages and corresponds to the biomass sensitivity. Study concluded that the ozone phytotoxicity is dependent on the antioxidative potential of the cultivars and the growth stage of the plant. Further, the activity of APX played a measure role for age dependent plant sensitivity in groundnut.

1. Introduction

Surface ozone is considered as a toxic pollutant to the environment and has negative influence on the growth and physiology of the plants. This oxidative pollutant enter in to the plant body through stomatal opening and leads a series of reactions in the interior part of the plant cells and generates reactive oxygen species (ROS) (Booker et al., 2009; Peng et al., 2020; Singh et al., 2017). ROS is also generated during natural physiological activities such as, photosynthesis. During photosynthesis, oxygen is generated in chloroplasts and it accepts electrons from passing through the photosystems and takes place the formation of superoxide radicals (Roach and Krieger-Liszkay, 2014; Tripathy and Oelmüller, 2012). Many reviews and reports have confirmed that the ROS and its reaction products damage cell membrane, harm carbohydrates, degrade proteins, lipids, and nucleic acids, and become the cause of cell malfunctioning or death (Anjum et al., 2012; Gill et al., 2012;

Rathore and Chaudhary, 2019; Tuteja, 2010).

Plants develop various chemical defences that include enzymatic and non-enzymatic antioxidant compounds which represent the main trait of the plant natural resistant system (Chen and Gallie, 2005). Under the constant stress condition, various antioxidative defence mechanisms scavenge the reactive oxygen species (Foyer and Noctor, 2005; Singh and Rathore, 2018). The antioxidants are found in almost all cellular compartments and demonstrate the importance of reactive oxygen species detoxification for survival of cell (Podgórska et al., 2017). Stress-induced reactive oxygen species scavenging is occurred by enzymatic antioxidants including various kinds of scavengers, such as SOD, APX, POD, glutathione-S-transferase, and CAT, and non-enzymatic low molecular antioxidative metabolites, like glutathione, ascorbic acid, α -tocopherol, flavonoids and carotenoids (Mittler et al., 2004). Proline is also considered as the best non-enzymatic antioxidant because it reduces the inhibitory effects of reactive oxygen species (Chen and Dickman,

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2005).

During the past two decades, hundreds of species have been reported with significant changes in the levels and kind of defence traits as plants develop from the seedling to juvenile to mature and senescent stages (Barton and Boege, 2017 and references therein). Agrawal (2011) suggested expression of multiple defence strategy of plants at different growth stages during life time due to synergies or trade-offs. However, most of these studies were considered herbivore or pathogen related defence. Information on age dependent plant defence response to ozone stress is scarce. Peng et al. (2020) recently demonstrated that the effect of ozone on water use efficiency, net photosynthesis rate and stomatal conductance is significantly dependent on plants developmental stage. Earlier, Morgan et al. (2003) and Feng et al. (2008) also described the significance of plant developmental stage in relation to ozone sensitivity.

Keeping above points in mind, the study presented here is aimed to assess the intra-specific variation in defence response of groundnut (*Arachis hypogea* L) cultivars under ozone stress at varied growth stages and its correlation with plant tolerance to ozone. We hypothesized that the (1) ozone tolerance is dependent on antioxidative potential of plant cultivars, and (2) develop defence against ozone stress is age dependent and correlated with ozone resistance.

2. Materials and methods

2.1. Plant material and experimental design

Groundnut is an important crop all over the world including India (Akhtar et al., 2010; Alexandratos and Bruinsma, 2012). Van Dingenen et al. (2009) and Ainsworth (2016) predicted 3.7% global and more than 10% regional groundnut yields loss due to ozone pollution. Five cultivars of groundnut (*Arachis hypogea* L) viz. TG-37A, TPG-41, TAG-24, GG-20, and Dh-86 were selected for the present study. Seeds were treated with 0.2% mercuric chloride before sowing. The experiment was conducted using open top chambers (OTCs) of 4X4X3.5 m sized established at the research field of Central University of Gujarat (23.2156° N, 72.6369° E) during February to May of 2017 and 2018. OTCs were made up of multilayered clear polycarbonate sheet (3 mm thick) to provide maximum available sunlight. Plants were grown in the plots of 1 m² sized. The soil of the field was slightly alkaline (pH 7.4) sandy loam of medium fertility. Each plot was mixed with 250g vermicompost during field preparation and was applied with recommended dose of NPK in the ratio of 40:40:20 kg/ha. Plots were regularly irrigated to ensure sufficient water supply and weeds were managed manually. The experiment was set in randomized block design (RBD) with three treatment i.e. enhanced ozone (75 ppb), ambient ozone (control) and EDU supplemented (to minimized ozone effect). Ambient ozone concentration and temperature were monitored with the help of ozone sensor (Ambetric, TC800D) and temperature sensor (HK Tempsensor) during the study period.

During the study period, ambient ozone concentration was varied between 13.9 and 22.4 ppb day⁻¹ in first year of experiment and 26.4–28.1 ppb day⁻¹ in second year experiment while, temperature was varied between 30.9 and 38.5 (Fig. S1). In general, ambient ozone concentration was found lower in February months and higher in April and May months. Ozone concentration for ozone enhancement was fixed and maintained to 75 ppb (varying between 60 and 84 ppb) during peak hours (11 a.m.–3 p.m.) from the germination till harvesting of the crops. Supplemental ozone was provided by ozone generator (Eltech Eng., India). Plots of EDU treatment were supplied with 500 ppm EDU as soil drench at every 10th day from seed germination to maturity. No rain was seen during the study period. EDU dose was decided on the basis of best result of the experiments performed by previous researchers (Agrawal et al., 2005; Manning et al., 2011; Rathore and Chaudhary, 2019).

Plant sample were collected at juvenile (20 days after sowing (DAS)),

vegetative (40 DAS), reproductive (65 DAS) and, maturity stage (100 DAS) for biomass and biochemical characteristics.

2.2. Estimation of plant biomass

Collected plant samples were washed thoroughly by double distilled water to remove the field soil and excess water present on plants was dried by two layers of filter paper. These samples were separated in root, stem and leaves before measuring the weight. Separated plant parts were dried in a hot-air electric oven at 80 °C till a constant weight achieve. Dry weight was measured using single pan balance (Milton-MA224i).

2.3. Determination of relative water content (RWC), photosynthetic pigments and metabolites

Leaf fresh weight was measured and kept in distilled water for 24 h at room temperature followed by drying in hot air oven at 80 °C to measure the turgid weight and dry weight, respectively. The RWC was calculated by formulae of Sangakkara et al. (1996) using these weights.

For photosynthetic pigments, 0.1 g leaf sample was homogenized with 10 mL of 80% acetone solution. The solution was kept overnight at 4 °C and the OD was measured at 663 nm and 645 nm. Chlorophyll and carotenoids contents were measured by the formulae of Machlachlan and Zalik (1963) and Duxbury and Yentsh (1956), respectively.

Foliar sugar contents were determined by the method described by Somogyi (1952). For this, 50 mg leaf sample was crushed in 5 ml of 80% ethanol and centrifuged at 3500x for 15 min. Obtained pellets were washed four-times by 80% ethanol and distilled water. The mixture was centrifuged at each washing. 1 ml of aliquot was added with 1 ml of copper reagent and boiled for 10 min. After boiling, the solution was cooled to room temperature immediately and added with 1 ml of arsenomolybdate. The solution was kept for 30 min to complete the reaction before taking OD at 500 nm to estimate soluble sugars. For determination of reducing sugar, 0.5 ml of diluted aliquot was added with 1 ml of 5% phenol reagent and kept for 10 min to maintain room temperature. This solution was added with 5 ml of H₂SO₄. The solution was shaken well and kept for 10 min in a water bath before measuring the OD at 480 nm. Total soluble sugar and total reducing sugar was calculated by the standard curve obtained by purified glucose. Pellet of the samples was washed twice with 52% (v/v) perchloric acid and distilled water and centrifuged to estimate starch content. The volume of supernatant was made up of to 50 ml with distilled water. 1 ml aliquot of pooled supernatant was taken for determining the starch content.

2.4. Estimation of oxidative stress

Hydrogen peroxide was extracted by 0.25 g of fresh leaf sample homogenized in 5 ml of 0.1% TCA (ice cold) as explained in Velikova et al. (2000). After centrifugation of homogenate, 500 µL of supernatant was added with 500 µL of 10 mM potassium phosphate buffer (7.0 pH). The solution was mixed with 1 mL of 1M potassium iodide (KI) and kept for 20 min at room temperature before taking the OD at 390 nm.

MDA content was measured using the method proposed by Heath and Packer (1968). In brief, 0.25 g fresh leaf of groundnut was homogenized with 5 ml of 5% TCA solution in a pestle mortar and the homogenate was centrifuged. After centrifuged, 500 µL of the supernatant was mixed with 2 mL of 0.5% thiobarbituric acid (TBA). Then the mixtures of the solution were warmed at 95 °C for 50 min in a water bath and cooled immediately with in ice bath. The OD of the solution was taken at 600 nm and 532 nm.

Membrane permeability was measured in the form of electrical conductivity of ions leaked from fresh leaves in deionized water as described in Blum and Ebercon (1981). For this, the leaf samples were cut by punching machine into 1 cm diskettes. 20 diskette of each sample was placed in a glass beaker containing 10 mL deionized water. The

beakers were kept for 3 h at room temperature and the conductivity (mS/cm) of the solution was determined using electrical conductivity meter (Eutech Instruments).

2.5. Estimation of antioxidants

2.5.1. Non enzymatic antioxidants

Flavonoid content was extracted using a mixture of ethanol and acetic acid (99:1, v/v). 100 mg of fresh leaf sample was homogenized in 100 ml of the extraction solution. After extraction, the homogenates were boiled for 2 min and cooled at room temperature. The solution was centrifuged at 8000x for 15 min and the absorbance of the solution was taken from 250 to 350 nm wavelengths. The flavonoid content was presented as proposed by Cameron et al. (1943).

Total phenol content of plant tissue was estimated by the method of Mallick and Singh (1980). For this, 250 mg of leaf sample was homogenized in 5 mL of 70% acetone. The solution was centrifuged at 6000x for 10 min. After centrifuge, 5 mL of 20% sodium carbonate (Na₂CO₃) solution was added into the solution. The final volume of the solution was made to 10 ml using deionized water and absorbance was read at 750 nm.

For ascorbic acid, 500 mg fresh leaf sample was crushed in ice bath in 20 ml of extracting solution of oxalic acid and EDTA. Mixture was centrifuged at 6000x for 15 min and 1 ml of supernatant and was mixed with 5 ml of 2, 6-dichlorophenol-indophenol solution by constant shaking till the mixture develops a pink colour. The optical density of this solution was measured at 520 nm. After reading the optical density, the pink colour was bleached by one drop of purified ascorbic acid and again the OD was measured at the same wavelength. The ascorbic acid content was measured by standard curve of purified ascorbic acid against the blank prepared from DCPIP and extracting solution using formula proposed by Keller and Schwager (1977).

Proline in fresh leaf was estimated by using 3% sulphosalicylic acid solution as explained in Plummer (1979). The solution was filtered and added with 2 ml of ninhydrin reagent and 2 ml of glacial acetic acid. The mixture was kept at 80 °C for 1 h in a water bath and cooled in ice bath immediately. The reaction mixture was extracted in 4 ml toluene and the OD of the solution was measured at 520 nm.

2.5.2. Enzymatic antioxidants

For Antioxidative enzyme activity, 250 mg of fresh leaf sample was crushed in 5 ml (50 mM) of chilled potassium phosphate buffer (7.8 pH). The homogenate was centrifuged at 12,000x for 20 min at 4 °C. Then the supernatant was stored at -20 °C for estimation of following antioxidative enzymes:

Catalase activity was determined by using 100 µL supernatant and 1.9 mL potassium phosphate buffer (50 mM, pH 7.8). 1 mL of 5.9 mM H₂O₂ was also added in the solution and the OD of the solution was measured at 240 nm after every 20 s for 2 min (Chance and Maehly, 1955).

For SOD activity, 50 µL supernatant was taken in a test tube and added with 400 µL distilled water, 250 µL (50 mM) potassium phosphate buffer (pH 7.8), 100 µL L-methionine, 100 µL L-tritron-X, 50 µL nitro blue tetrazolium (NBT) and 50 µL riboflavin. The optical density (OD) of the solution was read at 560 nm (Van Rossum et al., 1997).

For peroxidase activity, 100 µL supernatant mixed with 1.8 mL potassium phosphate buffer (50 mM, 7.8 pH), 100 µL guaiacol (20 mM) and 100 µL H₂O₂ (40 mM) and the absorbance of the solution was measured at 470 nm after every 20 s for 3 min as described in Chance and Maehly (1955).

For the activity of APX, 100 µL supernatant was added with 3 mL mixture containing 100 mM phosphate (pH 7), 0.1 mM EDTA-Na₂, 0.3 mM ascorbic acid and 0.06 mM H₂O₂. The absorbance of the solution was read at 290 nm for 30 s intervals till the ascorbic acid oxidized completely. One unit of APX forms 1 µM of ascorbate oxidized per minute under assay conditions (Nakano and Asada, 1981).

2.6. Statistical analysis

Duncan multiple range test (DMRT) was applied to analyse the significant variation within same set of variables using SPSS software (SPSS Inc., version 17.0). PCA was applied to define the homogenous characteristics of cultivars of groundnut and the correlation among each variable studied under various treatments at all the sampling date using Origin Pro (2019).

3. Results

3.1. Plant biomass

Acute and ambient level of ozone reduces biomass accumulation of all the experimental groundnut cultivars at all growth stages during both the growing season (Fig. 1). However, plant response to acute and ambient level of ozone was variable at different growth stages. Cultivar TG-37A showed highest reduction during vegetative growth stage by acute ozone level than ambient ozone level, while, cultivar TAG-24 showed maximum reduction at vegetative growth stage followed by maturity stage. Cultivar Dh-86 showed almost similar severity in sensitivity to acute ozone at all the growth stages except juvenile stage which showed least affected by acute ozone. Cultivar TPG-41 showed higher sensitivity at juvenile stage followed by maturity stage. Sensitivity to acute ozone in cultivar GG-20 was increased with the age of plant up to reproductive stage and reduced thereafter. This trend of acute ozone sensitivity was confirmed by germplasm which was similar to parent plants. Germplasm of all the cultivar also showed the similar sensitivity trend as their respective parent, however, percent sensitivity to acute ozone was higher. EDU application neutralized the effects of ambient ozone and increases plant biomass in a trend reverse to acute ozone.

3.2. Sugars and relative water content

Soluble and reducing sugars of groundnut cultivars were negatively affected by acute ozone treatment while significantly increased by EDU treatment (Fig. S2). Sensitivity to sugars of all the groundnut cultivars to acute ozone was increased with age of the plants however, cultivar Dh-86 showed least sensitivity at vegetative growth stage. Germplasm of cultivar TAG-24 and Dh-86 showed a sign of resistance for reducing sugar under acute ozone and affected less than primary experiment.

Starch content was less in the germplasm of all the groundnut cultivar than their respective parent plants (Fig. 2). Similar to reducing and soluble sugars, starch was also reduced to acute level of ozone while responded positively under EDU at both the growing season. However, sensitivity of starch to acute level of ozone in germplasm was lower than their respective parent plants while, higher for ambient level of ozone. For starch content, cultivar TG-37A was least sensitive to acute level of ozone while higher sensitive to ambient level of ozone at vegetative growth stage. Cultivar TPG-41 showed highest sensitivity at vegetative growth stage to acute ozone while least sensitive at the same stage by EDU treatment. In cultivar TAG-24, GG-20 and Dh-86, reduction of starch to acute level of ozone was enhances with increasing plant age while, maximum increased under EDU treatment was seen at vegetative stage in TAG-24, at reproductive stage in GG-20 and, at juvenile stage in cultivar Dh-86.

Relative water content of the foliage of groundnut cultivars was significantly reduced under acute and ambient ozone treatment (Fig. 2). Except at some cases, germplasm showed higher reduction of RWC in all the experimental varieties under acute level of ozone. Reduction in RWC due to acute ozone was increased with age in cultivar TG-37A, while decreased in cultivar TPG-41, GG-20 and Dh-86. In cultivar TAG-24, reduction by acute ozone in RWC was not varied much among the plant growth stages.

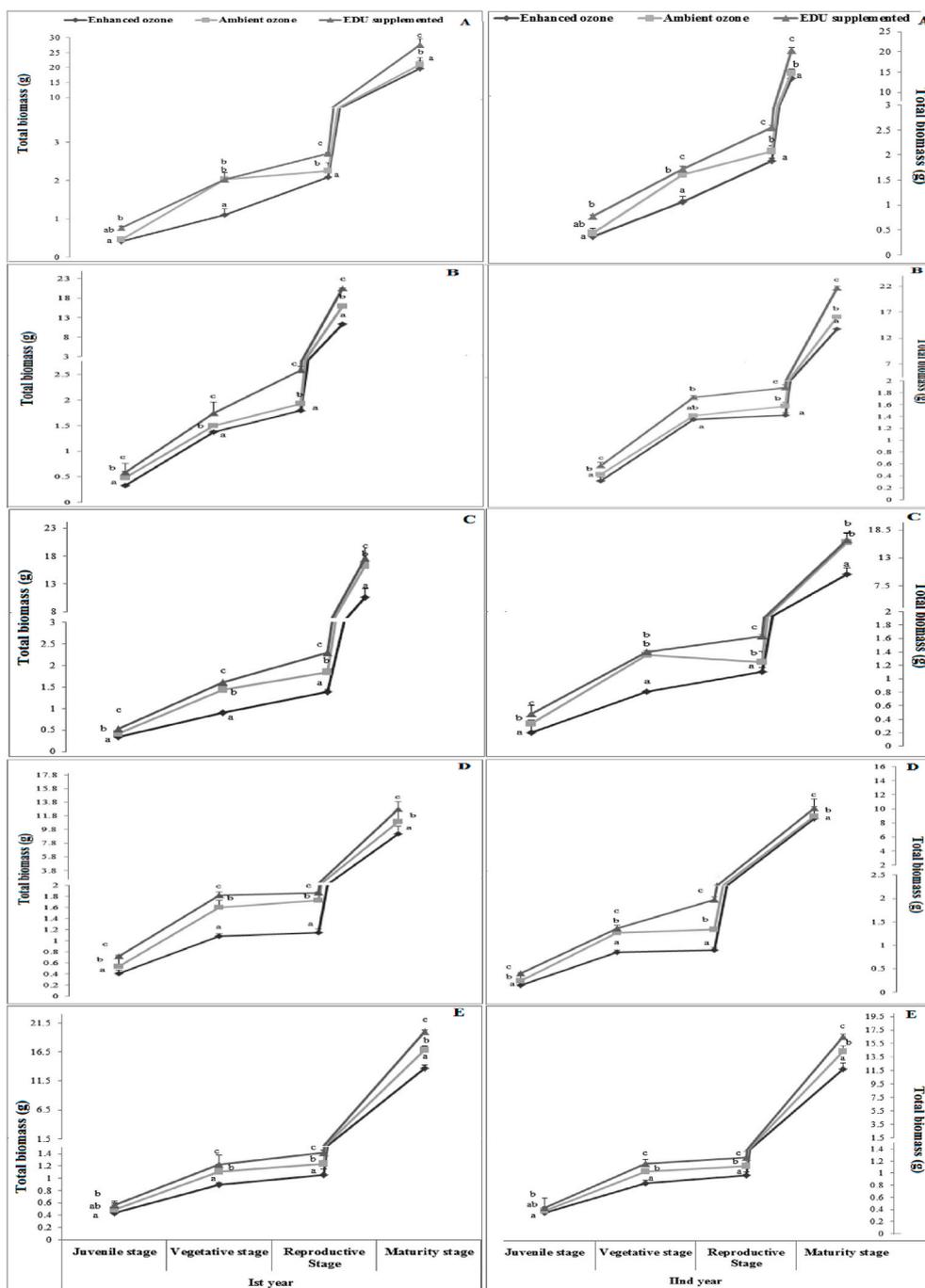


Fig. 1. Effect of ozone on total plant biomass (g plant^{-1}) of groundnut cultivar (A) TG-37A, (B) TPG-41, (C) TAG-24, (D) GG-20 and (E) Dh-86 at different growth stages (Mean \pm standard deviation of three replicates presented by thin vertical bars, Value within each column followed by the same letter are not significantly different ($p < 0.05$) using Duncan's Multiple Range Test).

3.3. Hydrogen peroxide, MDA contents, and membrane permeability

Acute level of ozone significantly increases while EDU reduces hydrogen peroxide and lipid peroxidation in all the experimental cultivars of groundnut (Fig. 3). Except at all the growth stages in cultivar TAG-24 and juvenile and vegetative growth stages of cultivar GG-20, production of hydrogen peroxide was higher in germplasm than their respective parent plants due to acute ozone. Hydrogen peroxide generation increases with increasing age in cultivar TAG-37, TAG-24 and in GG-20. In cultivar TPG-41, increase in hydrogen peroxide was least at vegetative growth stage followed by reproductive stage and was highest at juvenile growth stage while, in cultivar Dh-86, increase in hydrogen

peroxide was least at vegetative stage followed by juvenile stage and was maximum at reproductive stage. Percent variation of MDA content was enhanced due to acute ozone with plant age while, reduced due to EDU treatment in all the tested cultivars. Among all the cultivars at various growth stages, highest variation (63.04%) in MDA was found in germplasm of cultivar TG-37A at reproductive stage.

Electrolyte leakage, a measure of membrane permeability, was significantly increased with the age of plant which further enhances by the acute level of ozone, however, EDU application positively influences membrane permeability in all the experimental cultivar of groundnut at all the growth stages (Fig. 4). Germplasm of cultivar TG-37A and GG-20 have lower percent variation for membrane permeability than the

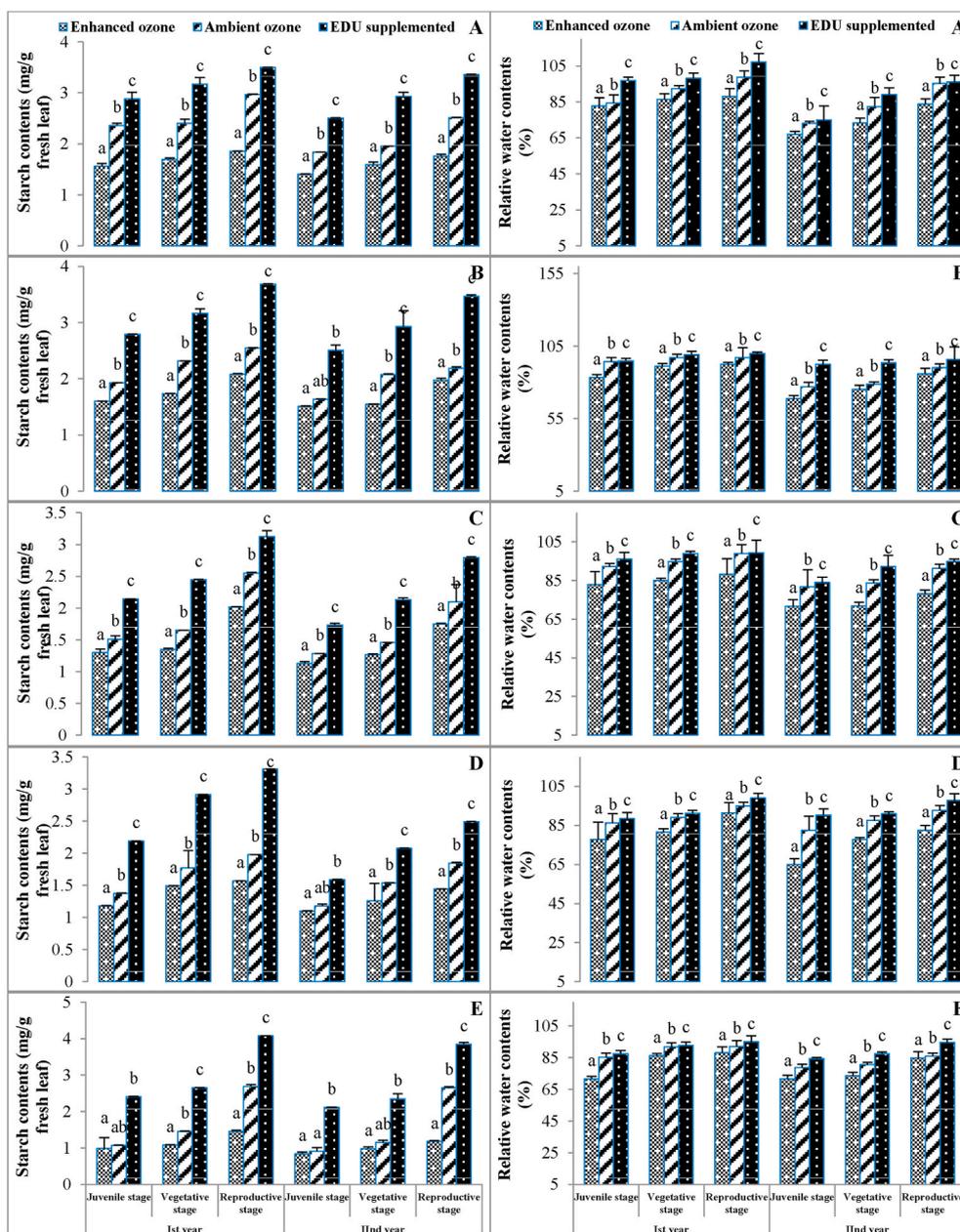


Fig. 2. Effect of ozone on starch contents (mg g^{-1} fresh leaf) and relative water contents of groundnut cultivar (A) TG-37A, (B) TPG-41, (C) TAG-24, (D) GG-20 and (E) Dh-86 at different growth stages (Mean \pm standard deviation of three replicates presented by thin vertical bars, Value within each column followed by the same letter are not significantly different ($p < 0.05$) using Duncan's Multiple Range Test).

parental plants due to acute ozone stress while, germplasm of cultivar TAG-24 have higher percent variation in membrane permeability than the parent plant at all the growth stages. Lower sensitivity for ozone pollution in membrane permeability was found in cultivar TG-37A and TAG-24.

3.4. Antioxidative activity

3.4.1. Non-enzymatic antioxidants

Proline of groundnut cultivars was significantly reduced after exposure to ambient and acute ozone at all the ages (Fig. 5). Least proline accumulation was seen in germplasm of cultivar Dh-86 at juvenile stage (1.38 mg g^{-1}) while the highest proline accumulation was observed in parent plant of cultivar TG-37A at reproductive stage (4.94 mg g^{-1}).

Acute ozone induced total phenol while reduced ascorbic acid in all the experimental cultivars (Figs. 5 and 6). Reduction of ascorbic acid by

acute exposure of ozone was increased with age. EDU treatment increases ascorbic acid of all the groundnut cultivars. Reduction of ascorbic acid by acute ozone dose was higher in germplasm of cultivar TG-37A and TAG-24 while, lower in cultivar TPG-41, GG-20 and Dh-86. Applied EDU enhances ascorbic acid content of parent and germplasm of all groundnut cultivars. Increased ascorbic acid was maximum at vegetative growth stage in TG-37A and TPG-41 while at reproductive stage in remaining cultivars.

Similar to ascorbic acids, flavonoids was also reduced due to acute ozone exposure while increased by EDU treatment in tested cultivars (Fig. 6). Percent decrease under acute ozone exposure was higher in juvenile stage and reduced thereafter in cultivar TG-37A while, least at juvenile stage and increased with age in remaining cultivars. A trend just reverse to acute ozone exposure was seen under EDU treatment. Total phenolics accumulation was increased by age in all the cultivars used in this experiment which was further enhances after exposure of acute

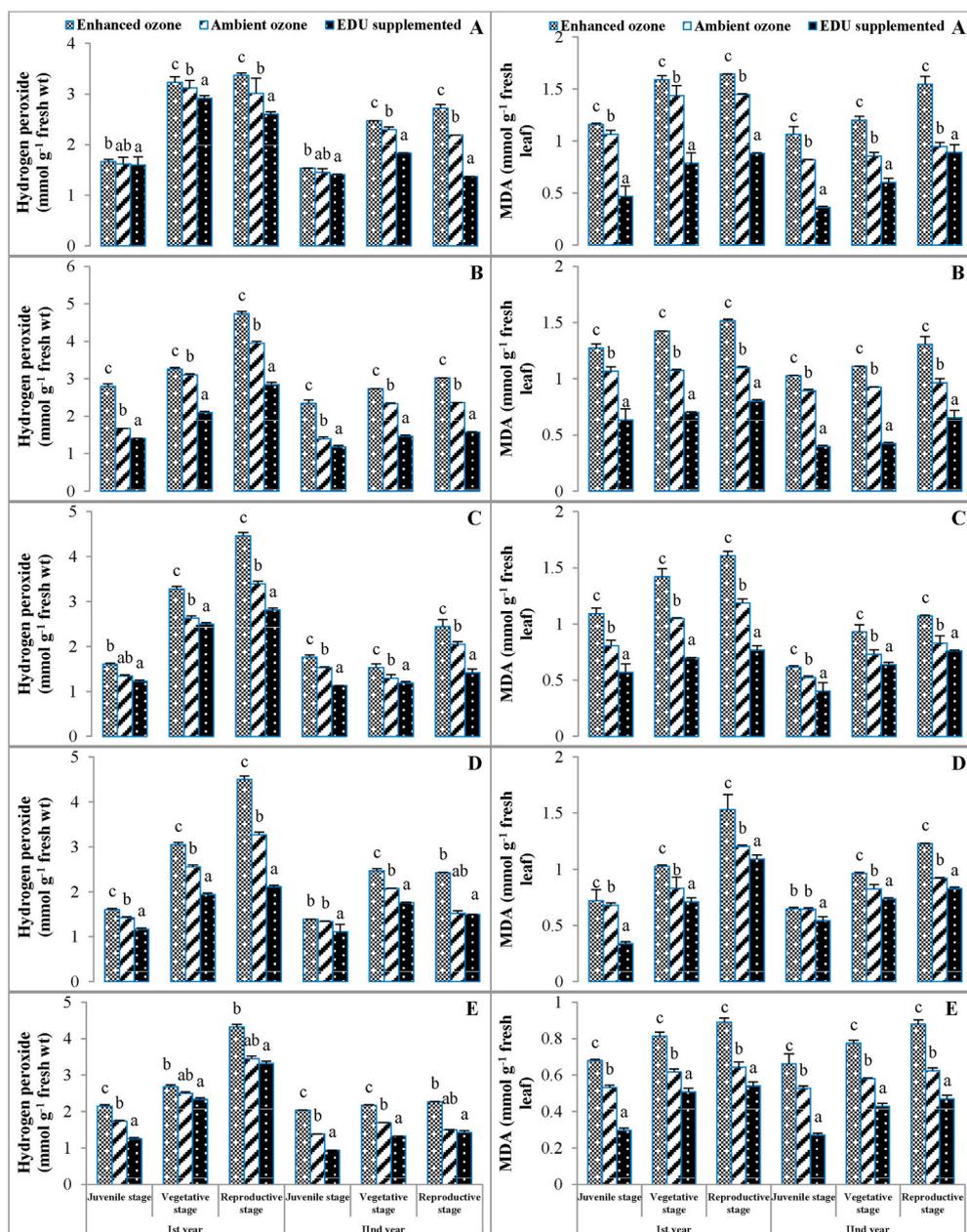


Fig. 3. Effect of ozone on Hydrogen peroxide (mmol g^{-1} fresh leaf) and MDA (mmol g^{-1} fresh leaf) of groundnut cultivar (A) TG-37A, (B) TPG-41, (C) TAG-24, (D) GG-20 and (E) Dh-86 at different growth stages (Mean \pm standard deviation of three replicates presented by thin vertical bars, Value within each column followed by the same letter are not significantly different ($p < 0.05$) using Duncan's Multiple Range Test).

ozone (Fig. 6). Maximum phenolics (14.22 mg g^{-1}) was found in germplasm of cultivar Dh-86 at reproductive stage. Under acute ozone exposure, cultivar TG-37A, TAG-24 and Dh-86 showed least variation at juvenile stage and highest variation at reproductive age while, cultivar TPG-41 showed maximum enhancement at juvenile stage for phenolics. Cultivar GG-20 also showed highest variation at reproductive age although, it's least variation was seen at vegetative growth stage. EDU application reduces total phenolics accumulation of all the experimental cultivars at both the seasons.

3.4.2. Enzymatic antioxidants

Activity of enzymes responsible for ROS scavenging was increased after exposure of acute ozone stress while reduced due to EDU supplementation in all the cultivars tested at all the growth stages.

Activity of SOD was increased with increasing age of the plant and further enhances by ozone exposure at all the growth stages in all the

experimental cultivars (Fig. 7). Activity of SOD was less in germplasm than their respective parents however, percent increase was higher. All the cultivars showed least SOD activity at juvenile stage but maximum ozone exposure enhancement. Cultivar Dh-86 having least SOD activity than other tested cultivar but percent increment was higher. Moreover other cultivars showed reducing trend of ozone induced SOD activity with age of plant, while Dh-86 having maximum activity at vegetative stage followed by reproductive stage.

Correspond to SOD, percent enhancement of catalase activity was increases with the plant age under acute ozone exposure than control in parent and germplasm of cultivar TG-37A, TPG-41 and TAG-24 while, reduced in cultivar GG-20 and Dh-86 (Fig. 7). Percent variation between EDU treatment and ambient ozone exposure was minimize in cultivar TG-37A and Dh-86 with plant age and found least at reproductive stage while for cultivar TPG-41 and TAG-24, it was increased from juvenile to vegetative stage and decreased thereafter. For cultivar GG-20 percent

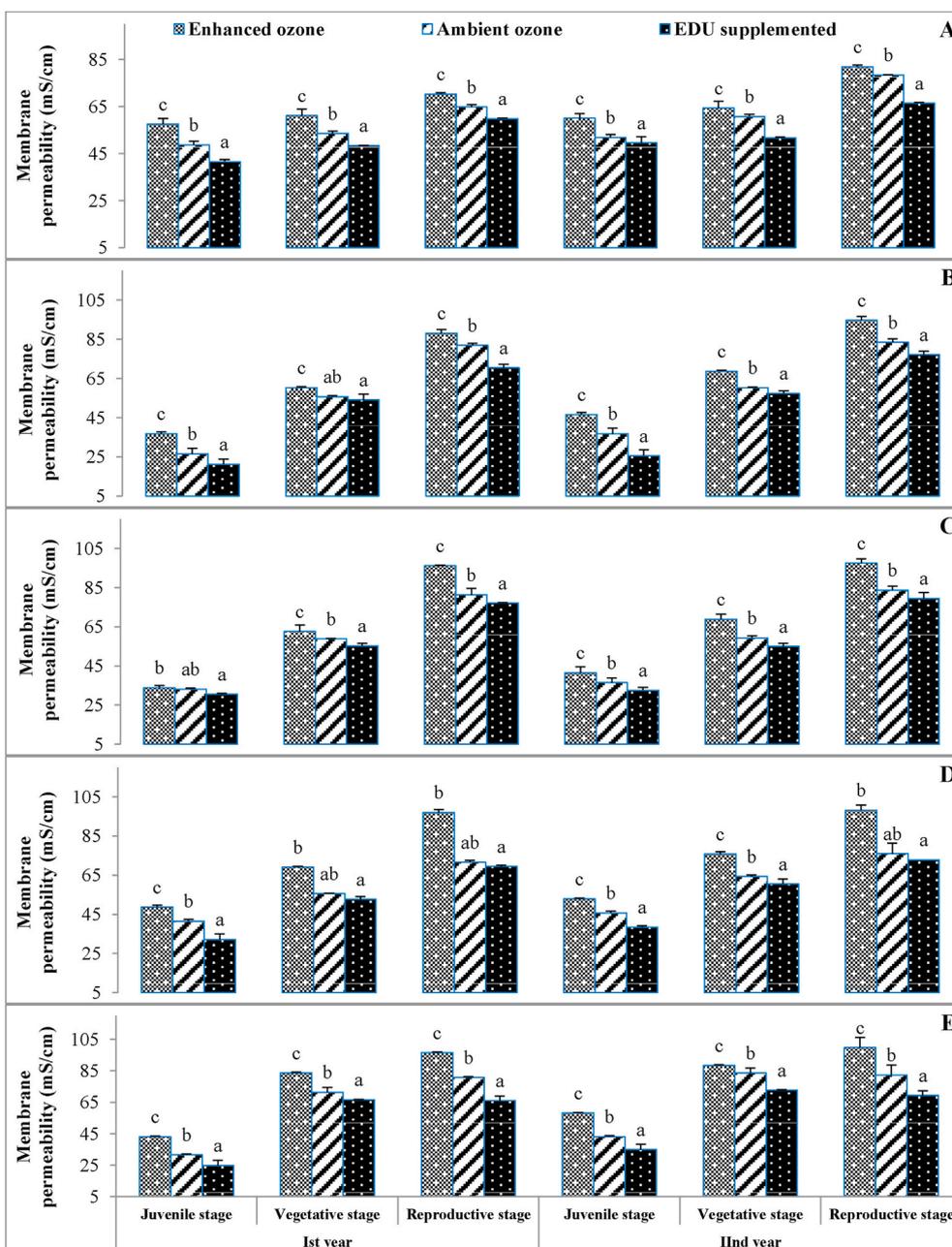


Fig. 4. Effect of ozone on membrane permeability (mS cm^{-1}) of groundnut cultivars (A) TG-37A, (B) TPG-41, (C) TAG-24, (D) GG-20 and (E) Dh-86 at different growth stages (Mean \pm standard deviation of three replicates presented by thin vertical bars, Value within each column followed by the same letter are not significantly different ($p < 0.05$) using Duncan's Multiple Range Test).

variation by EDU decreased from juvenile to vegetative stage and increased at reproductive stage.

Peroxidase activity was seen higher in parent plant than their respective germplasm at the same age (Fig. 8). Peroxidase activity was also increased with plant age in all the tested cultivars which was further enhanced by acute and ambient ozone exposure. Activity of peroxidase was maximum at reproductive stage under acute ozone exposed plants while least at juvenile stage under EDU treatment.

Activity of APX was also followed the trend similar to peroxidase and found maximum in parent under acute ozone exposure at reproductive stage and least in juvenile stage of germplasm under EDU treatment (Fig. 8). Percent variation for APX activity of acute ozone exposed plants than their respective ambient ozone exposed plant was significantly increased with plant age and found maximum at reproductive growth stage for all the experimental cultivars. Similar to catalase and

peroxidase commotion, activity of APX was also reduced under EDU treatment than ambient ozone exposed plants of all the tested groundnut cultivars.

3.5. Principle component analysis (PCA)

PCA analysis of groundnut cultivar TG-37A showed total variability 55.23% (Eigenvalue 8.83) for PC1 and 30.96% (Eigenvalue 4.95) for PC2 (Fig. 9A). These PC's seemed erratic and didn't contribute to any logical interpretation of spatial patterns. Therefore, the biplot graph was built for principal components having maximum variability (PC1 and PC2). Enhanced and ambient ozone at juvenile stage showed negative score value while EDU treated plants at vegetative, and reproductive stages showed positive score value for PC1 and PC2. Biomass, flavonoids and total reducing sugars showed strong relation at PC1. Parameters,

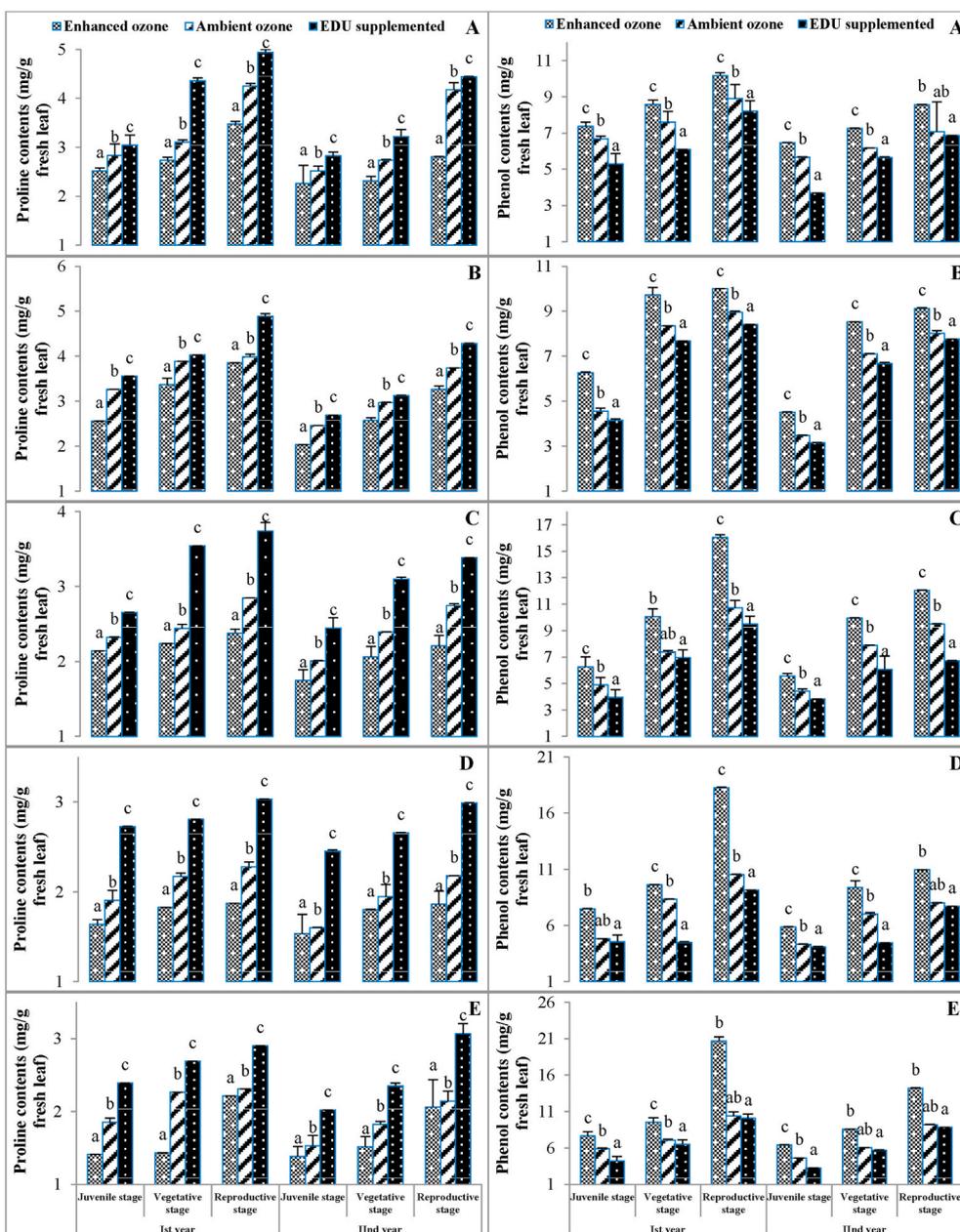


Fig. 5. Effect of ozone on proline (mg g^{-1} fresh leaf) and phenol contents (mg g^{-1} fresh leaf) of different groundnut cultivars (A) TG-37A, (B) TPG-41, (C) TAG-24, (D) GG-20 and (E) Dh-86 at different growth stages (Mean \pm standard deviation of three replicates presented by thin vertical bars, Value within each column followed by the same letter are not significantly different ($p < 0.05$) using Duncan's Multiple Range Test).

such as hydrogen peroxide, MDA, membrane permeability, phenol, CAT, SOD, POD and APX of TG-37A shows positive values at both PC while biomass, relative water contents, flavonoid, starch, proline total soluble sugar and total reducing sugar showed positive values at PC1 and negative values at PC2. Loading value of phenol were 0.26 and 0.25 for PC1 and PC2, respectively. Total variation of PC1 and PC2 in cultivar TPG-41 was 53.79% (Eigenvalue 8.60) and 32.99% (Eigenvalue 5.27) and PCA biplot for this cultivar was visualised somewhat similar to the cultivar TG-37A (Fig. 9B). Loading value for phenol in this cultivar was also same side (0.29 for PC1 and 0.16 for PC2) of selected PCs. The factor affecting the variation of biomass and antioxidative compound shows positive relation in PC1 and negative relation in PC2 while enzymatic activity (CAT, POD, SOD, and APX), phenol and MDA, membrane permeability and hydrogen peroxide shows positive relation at both PC. For cultivar TPG-41, ascorbic acid, TSS and TRS showed strong relation at PC1. MP and proline was also shows strong correlation with each

other at PC1. Per cent variation of PC1 and PC2 for groundnut cultivar TAG-24 was 54.18% (Eigenvalue 8.66) and 34.20% (Eigenvalue 5.47), respectively (Fig. 9C). PC score value for EDU treatment all treatment present at right side of component 1 suggested positive correlation. Most of the data obtained by results were clustered at the positive side of PC1 and PC2. Similar to cultivar TG-37A and TAG-24, loading value of phenol was positively correlated to treatment at both PCs. However, loading value for phenol was positive similar to cultivar TG-37A for PC1 (0.26) and PC2 (0.25). PC1 and PC2 values for Cultivar GG-20 were 49.21% (Eigenvalue 7.87) and 39.57% (Eigenvalue 6.33) (Fig. 9D). In cultivar GG-20 all selected parameters showed positive loading values at PC1 and hydrogen peroxide, MDA, MP, phenol and enzymatic activities (CAT, SOD, POD and APX) showed negative value at PC2. Biomass, MDA and TSS strongly correlated with each other while SOD was strongly correlated with MP. Ascorbic acid, phenol, CAT, POD and hydrogen peroxide was also shows strong correlation with each other. The

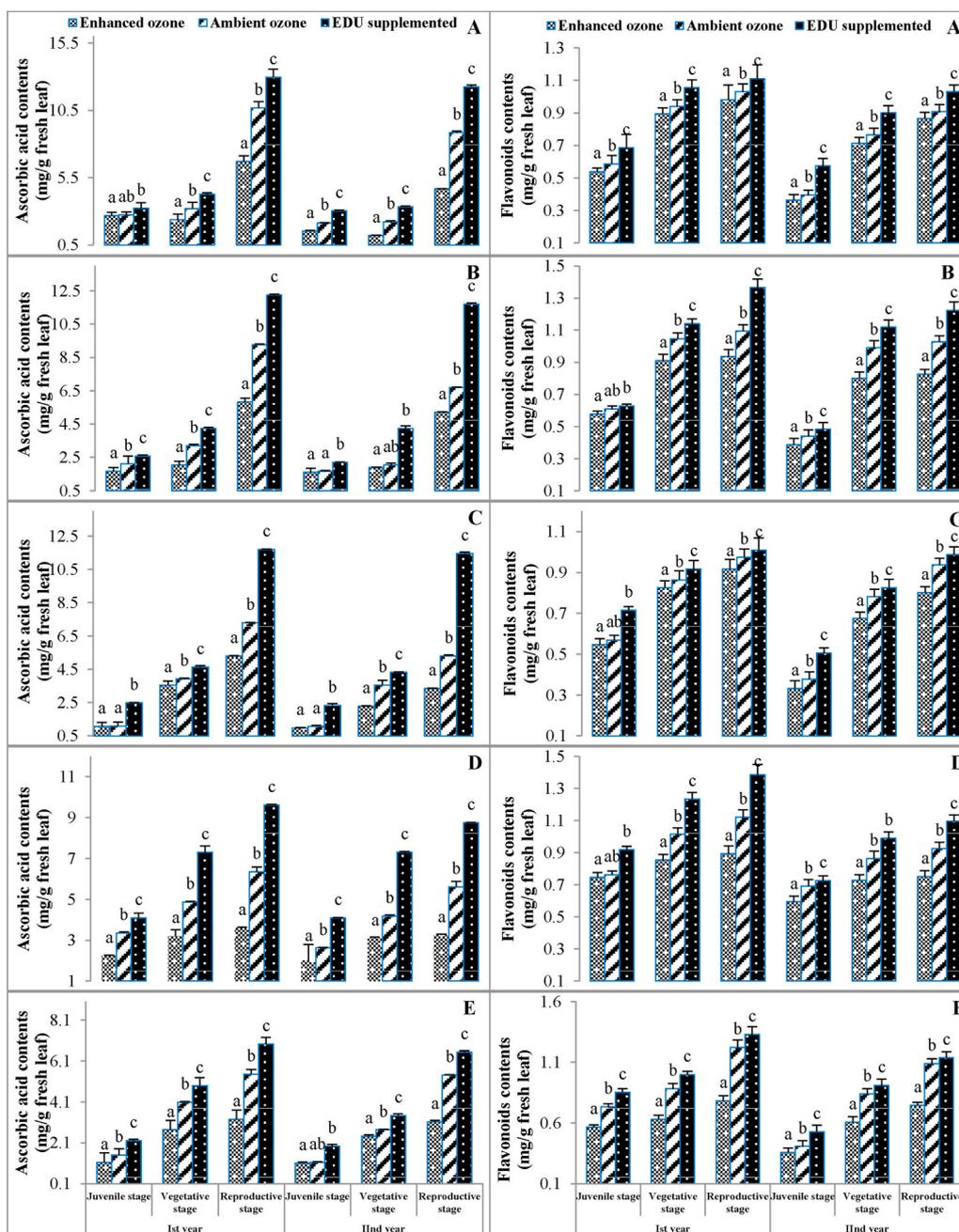


Fig. 6. Effect of ozone on ascorbic acid (mg/g fresh leaf) and flavonoids contents (mg/g fresh leaf) of groundnut cultivar (A) TG-37A, (B) TPG-41, (C) TAG-24, (D) GG-20 and (E) Dh-86 at different growth stages (Mean ± standard deviation of three replicates presented by thin vertical bars, Value within each column followed by the same letter are not significantly different ($p < 0.05$) using Duncan’s Multiple Range Test).

percentage variance of Dh-86 cultivar was (48.40%) at PC1 and (39.57%) at PC2 while eigenvalue was 7.74 and 6.33 at PC1 and PC2, respectively. Selected cultivar Dh-86 shows a positive correlation with enzymatic activity in both PC while PC1 shows a positive relation in all parameters (Fig. 9E). Biomass (loading value 0.31), ascorbic acid (loading value 0.30) and TSS (loading value 0.30) slightly correlated in cultivar Dh-86 at PC1. It means that ascorbic acid shows an active part as a defence against ozone. PC score value of enhanced ozone treatment were present at the negative side of PC1 and PC2 while ambient ozone and EDU treatment score value present at the positive side showed maximum variation or zero correlation except juvenile stages of all treatments. PC score value of ambient ozone at vegetative stages for all the cultivars of this experiment were nearer to the centre of plot suggested highest correlating. PCA analysis correlation biplot of the

treatments showed positive correlation for total biomass, starch, proline, flavonoid, ascorbic acid and relative water contents at reproductive stage in all cultivars (Fig. 9A–E). Overall enhanced ozone increases the enzymatic activity (CAT, SOD, POD, and APX), and phenol content and, also increases the membrane permeability and MDA of all selected cultivars. Therefore enzymatic activity, phenol contents, MP and MDA showed strong correlation with each other. While due to the enhanced level of ozone reduces total plant biomass, relative water contents, flavonoids, ascorbic acid, TSS and TRS and so these parameters showed a strong correlation with each other (Fig. 9A–E).

4. Discussion

Crop biomass is an important factor to estimate the overall growth of

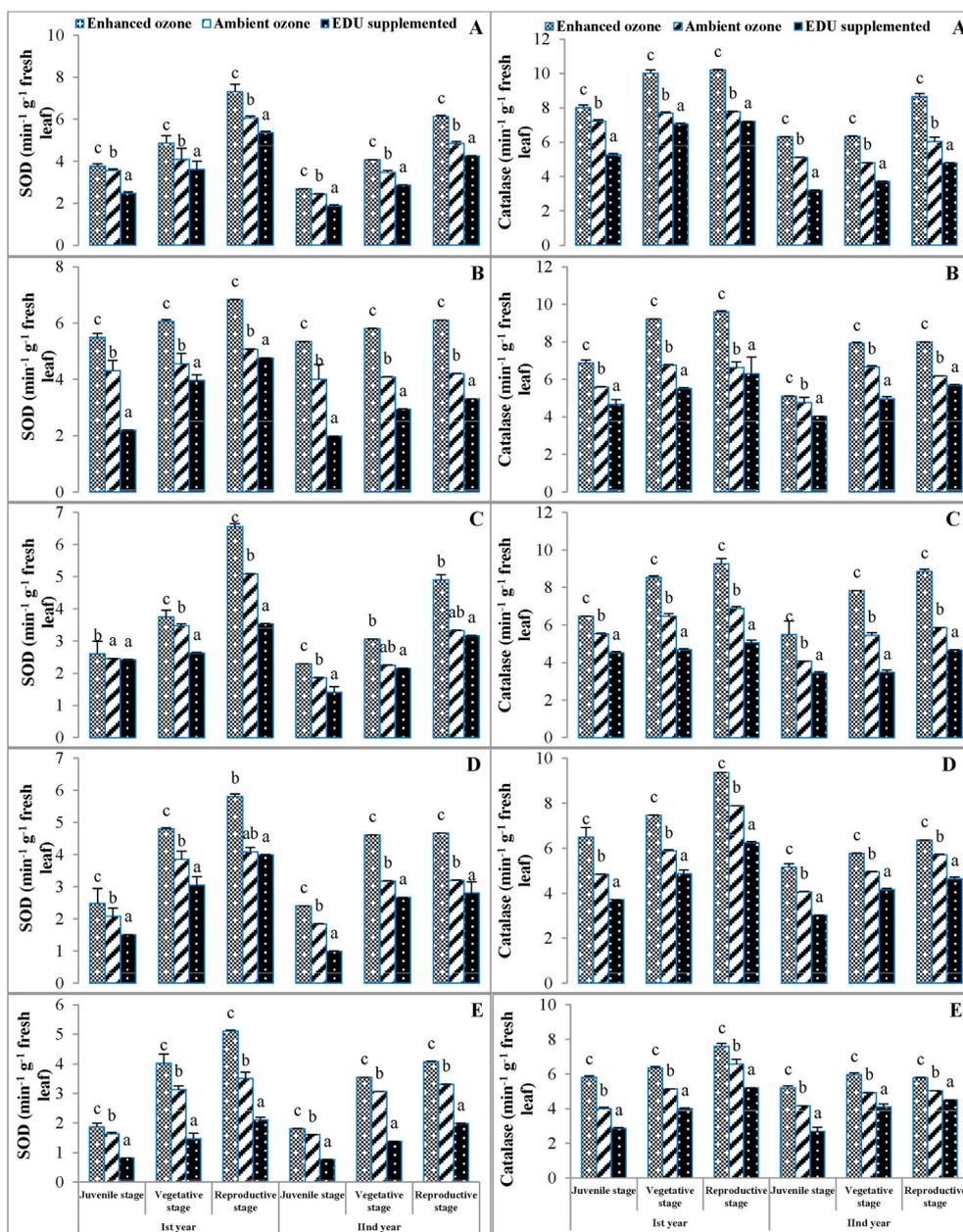


Fig. 7. Effect of ozone on SOD ($\text{min}^{-1} \text{g}^{-1}$ fresh leaf) and catalase ($\text{min}^{-1} \text{g}^{-1}$ fresh leaf) of groundnut cultivar (A) TG-37A, (B) TPG-41, (C) TAG-24, (D) GG-20 and (E) Dh-86 at different growth stages (Mean \pm standard deviation of three replicates presented by thin vertical bars, Value within each column followed by the same letter are not significantly different ($p < 0.05$) using Duncan's Multiple Range Test).

the crop. Despite the availability of literature on biomass reduction in crop of different plant species under ambient and acute ozone exposure, no attention has been paid by the growers due to lack of information and uncertainty in available data. Ozone concentrations of present experiment during both the growing seasons are sufficient to deteriorate crop productivity (Ainsworth, 2016; Ashrafuzzaman et al., 2017). Presented experiment also finds a reduction in biomass of all the groundnut cultivars. However, the level of reduction was cultivar specific and determined by growth stages i.e. the cultivars sensitivity to ozone is based on their growth stage. Frei (2015) explain the higher reduction at reproductive stage in rice as the increased ozone episode during vegetative stage, however, variable sensitivity among the cultivars of present study suggested the sensitivity lies within the genetic makeup based age specific resistance of the cultivar. In general, all of the cultivars showed higher sensitivity of ambient ozone at early growth stage due to lack of inbuilt tolerance and developed certain level of resistance against

moderate (ambient) level of ozone as the plant become strong during vegetative growth. On the basis of obtained results from winter wheat, Feng et al. (2016) also suggested that the effects of ozone depend on the growing stage of plants. Cultivar TPG-41 showed higher level of ambient ozone sensitivity at reproductive stage while, maximum reduction of biomass by acute ozone at maturity stage may be explained as resource allocation for reproductive success rather than biomass accumulation after receiving optimum vegetative growth under acute ozone stress. Rai and Agrawal (2008) demonstrated that the fast growing cultivar showed less reduction in photosynthetic performance and a greater increase in the antioxidative defence system as compared to slow growing cultivar, which favoured biomass translocation toward reproductive parts. Germplasm of all the tested cultivars showed more vulnerability to acute and ambient level of ozone, this might be due to slight higher ambient ozone concentration during the growing period of germplasm (Fig. S1). Moreover, germplasm of groundnut does not showed any sign of

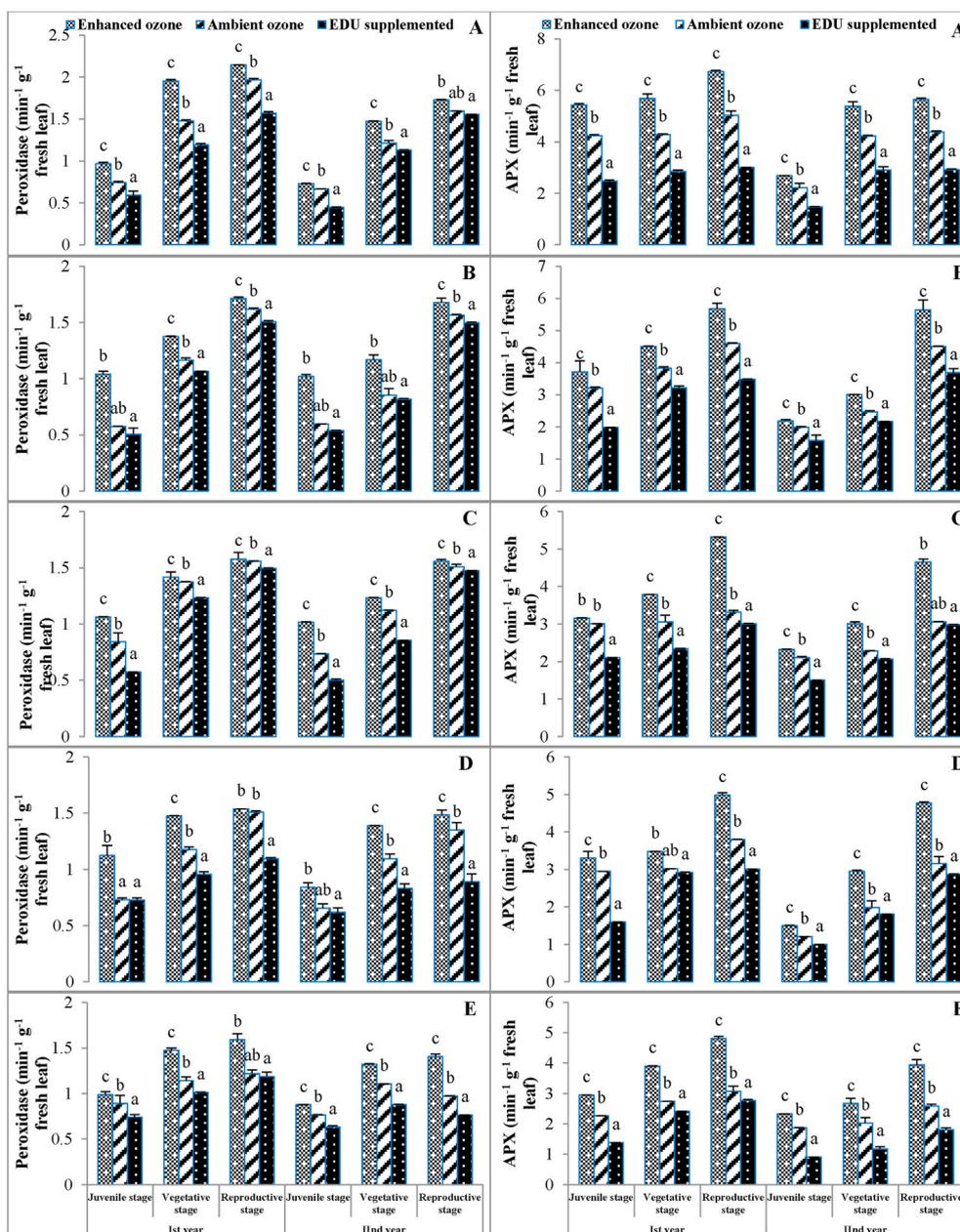


Fig. 8. Effect of ozone on peroxidase ($\text{min}^{-1} \text{g}^{-1}$ fresh leaf) and APX ($\text{min}^{-1} \text{g}^{-1}$ fresh leaf) of groundnut cultivar (A) TG-37A, (B) TPG-41, (C) TAG-24, (D) GG-20 and (E) Dh-86 at different growth stages (Mean \pm standard deviation of three replicates presented by thin vertical bars, Value within each column followed by the same letter are not significantly different ($p < 0.05$) using Duncan's Multiple Range Test).

increased resistance for ambient or acute ozone stress suggested that the acquired resistance is not transferred.

Relative water content in the plant is one of the most important factors which influence air pollution tolerance of plants (Chaudhary and Rathore, 2018). Simultaneously, increased sugar content improve the resistance of the plant and can lessen water loss, preserve turgor, and decrease membrane damage thus improving plant growth under stress conditions (Gallie, 2013; Rodziewicz et al., 2014). However, present study found reduced sugar and relative water content by ozone exposure is well correlated with the reduced capacity at the cellular level for CO_2 fixation as suggested in Ainsworth et al. (2016). Changes in starch content during stress condition are clear indicators of a variety of plant developmental processes which protects the plant growth by synthesizing the sugar and proline (Wei et al., 2015). Cultivar Dh-86 showed maximum percent reduction of foliar starch while lower biomass reduction at all the growth stages by both the level of ozone exposure

indicated higher investment for defence. Similarly, reduction of soluble and reducing sugar under chronic ozone suggested its trade off for defence mechanism. Reduction of reducing and soluble sugars at reproductive phase was almost doubled by acute ozone exposure can be explain as more diversion of sugar to accelerate defensive mechanism. Cao et al. (2017) suggested that the plants with reduced non-structural sugars including soluble sugar and starch having high tolerance to ozone. Similar to this study, Singh et al. (2018) also reported decreased starch, soluble sugar and reducing sugar of maize cultivars under ozone exposure although no correlation with ozone tolerance was shown.

Ozone shows strong oxidative potential and enters through stomata, generates a huge amount of ROS (Tiwari and Agrawal, 2018). Present experiment showed increased hydrogen peroxide and lipid peroxidation of all the experimental cultivars under ambient and enhanced ozone conditions. Excess of hydrogen peroxide in the plant cells caused oxidative stress and deactivate the activity of enzymes by oxidizing thiol

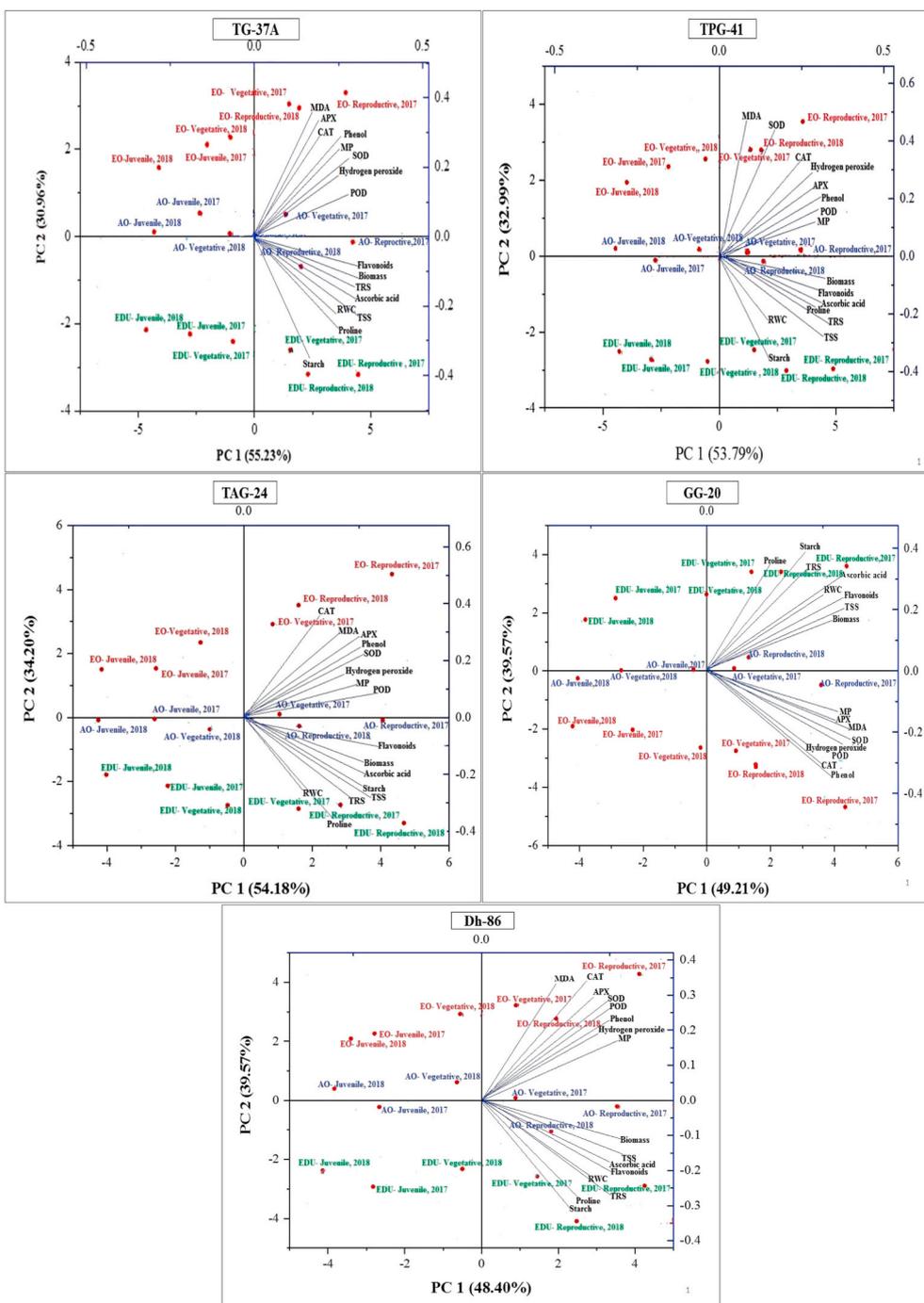


Fig. 9. Principle component analysis (PCA) bi-plot analysis of biomass and defence mechanisms response to ozone exposure. Symbol represent the standardized scores on PC1 (x-axis) and PC2 (y-axis) for the ozone exposure of groundnut cultivars L. (cv. TG-37A, TPG-41, TAG-24, GG-20 and Dh-86). Vector coordinates represent the correlations between standardized variables and principle components (PCs).

groups (Birben et al., 2012; Sharma et al., 2012). Developed hydrogen peroxide was varied among the cultivars and their growth stage. Generation of hydrogen peroxide increased with age by ambient and acute ozone exposure in TG-37, TAG-24 and GG-20 while cultivar TPG-41 and Dh-86 showed maximum hydrogen peroxide at juvenile stage and least at vegetative stage. Least variation at a later growth stage suggested higher reactive oxygen scavenging capability of TPG-41 and Dh-86 at vegetative stage. Accumulation of MDA suggested lipid peroxidation caused by oxidative stress resulted into deteriorated plant growth (Caregnato et al., 2010). Except in cultivar TAG-24, percent variation in MDA accumulation was higher under ambient ozone condition which

was further enhanced marginally by acute level of ozone. This suggested that ozone caused lipid peroxidation even under moderate level. However, percent variation of MDA accumulation decreased with plant age under chronic ozone exposure while, increased with acute ozone exposure suggested that groundnut cultivars tried to cope with moderate ozone exposure. H₂O₂ and lipid peroxidation of germplasm responded variably under ozone exposure than respective parent plants and among the cultivars.

Various researchers demonstrated that both acute and chronic ozone exposure lead to oxidative damage of membrane lipids as measured by increases in malondialdehyde concentration, a product of lipid

peroxidation (Feng et al., 2016; Rai and Agrawal, 2008; Rathore and Chaudhary, 2019; Ueda et al., 2013; Wang et al., 2013, 2014) which changed the integrity of membrane and increased membrane permeability (Chaudhary and Rathore, 2019, 2020). Rathore and Chaudhary (2019) also reported increased membrane permeability and reduced RWC under ozone exposure which is well in line with the presented study. Germplasm of all the groundnut cultivars having slightly higher membrane permeability than their respective parents under all the treatment suggested some factors other than ozone were also activated during growth of germplasm. This expression is further confirmed by less biomass accumulation in germplasm (Fig. 1).

Antioxidative potential of the plant plays an important role by disturbing free radical chain reactions and is able to defuse the destructive effects of reactive oxygen species (Barna et al., 2012). Phenol and flavonoids are secondary metabolite and primary line of defence in plants against environmental stresses (Lin et al., 2016) while, ascorbic acid acts as the most powerful water-soluble antioxidant and protects plants from damage caused by reactive oxygen species (Gallie, 2013). According to Manning et al. (2011), phenolics, ascorbate and many other plant constituents present in the plant apoplast or plasma membrane are in principle attacked by both ozone and singlet oxygen. Reduction in ascorbic acid and flavonoids of groundnut cultivars in presented experiment can be explained as its consumption by either or both of these. Increased reduction with the plant age might be due to cumulative ozone exposure effect than initial plant age. Flavonoids act as neutralizing radicals and scavengers of reactive oxygen species which are damage to plant cells under stress conditions (Løvdaal et al., 2010). Heim et al. (2002) explained the antioxidative potentials of flavonoid and describe it as a compound able to prevent lipid peroxidation, chelate redox-active metals, and lessen other processes linking reactive oxygen species. However in presented study, total phenolics was increased under ozone exposure and the amount of increase was proportionate to decrease in ascorbic acid. Germplasm having less apoplastic ascorbate in the present experiment suggested its higher sensitivity to ozone as seen by higher biomass reduction of germplasm plants than parent plants.

Proline is accumulated in the plant under stress condition and it is an important osmolyte (Anjum et al., 2011; Khedr et al., 2003). Proline is not only considered as an important molecule for redox signaling but also known as an effective ROS scavenger under stress conditions (Liang et al., 2013). The increasing level of proline content in plant cells improve the antioxidant defensive mechanism and reduce oxidative stress from plants and also stabilized the cellular organelles (Demiral and Turkan, 2005; Hayat et al., 2012). Higher accumulation of proline in plants has been associated with improved tolerance against various abiotic stresses (Fan et al., 2012; Kishor et al., 2014). In this study, proline content was also reduced under ozone stress conditions. Reduction of proline content was observed maximum in enhanced ozone > ambient ozone > EDU supplemented plants. The higher concentration of proline content in plants showed tolerance against ozone stress.

Although these non-enzymatic antioxidants played an important role for oxidative detoxification and reduced plant sensitivity to ozone, no correlation of these antioxidants was seen for changing ozone sensitivity to plant age.

SOD, CAT, APX and POD are important antioxidative enzymes to eliminate ROS. SOD plays a key role in plant stress tolerance as it provides the first line of defence against stress (Tripathy and Oelmüller, 2012; You and Chan, 2015) through catalysing the dismutation of superoxide into O₂ and H₂O₂, which consequently removed by CAT and peroxidases (Singh and Rathore, 2018). Catalase has the potential to directly dismutate hydrogen peroxide into water and oxygen (Day, 2009; Weydert and Cullen, 2010). Among all enzymes, catalase enzyme shows highest turnover rate against stress. The conversion rate of one molecule CAT is equivalent to 6 million molecules of H₂O₂ which is broken into H₂O and O₂ per minute (Nicholls et al., 2001; Singh et al., 2017) not requiring a reducing equivalent (Das and Roychoudhury,

2014). SOD activity of the groundnut cultivars of presented experiment was sufficiently higher under ambient ozone which further increases slightly under enhanced ozone suggested activation of plants ROS removal mechanism with lower dose of ozone. However, increase in CAT activity was much higher under enhanced ozone than ambient ozone. Variation in the percent increase of SOD and CAT is correlated with the accumulation of H₂O₂ and MDA at different growth stages suggest age dependent variability of ROS scavenging among the groundnut cultivars and the variable cultivar responded variable at the different growth stages. Rathore and Chaudhary (2019) found higher increase of CAT and POX activity of castor cultivar at flowering stage which is in line for cultivar TAG-24 of present experiment but other cultivars in general having higher activity of CAT at early growth stage.

Catalase is confined to peroxisomes in most cells having prime function is to remove H₂O₂ generated by peroxisomal oxidases (Schrader and Fahimi, 2006). Plants also use heme peroxidases such as APX and thiol-dependent peroxidases (Foyer and Noctor, 2009). Available knowledge described that APX is the major H₂O₂-reducing peroxidase in plants (Ishikawa and Shigeoka, 2008) that use ascorbic acid as a hydrogen donor (Agrawal and Rathore, 2007). APX distributed in at least four separate cell compartments, the stroma (sAPX) and thylakoid membrane (tAPX) in chloroplasts, the microbody (mAPX), and the cytosol (cAPX) (Yadav and Sharma, 2016; Yoshimura et al., 2000). POD activity in groundnut cultivars of present experiment was generally higher at initial growth stages than later growth stages due to either dose of ozone. Although activity of APX was correlated with biomass reduction and found high at juvenile stage than reduced at vegetative stage and then again increase at flowering stage in all the groundnut cultivars except least affected cultivar Dh-86 due to either ozone exposure, suggested potential role of APX in age dependent ROS detoxification and ozone sensitivity.

Although, statistical analysis indicate significant variation for given treatments at different developmental stages (Singh and Rathore, 2018), PCA can be used to elaborate the information such as; correlation, effects and assessment, from large scattered data by reducing the dimensionality (Xu et al., 2016). Biplot of PCA for all the cultivar showed negative effect of ozone exposure and also suggested correlation of antioxidant compounds and enzymes for developed oxidative stress. PCA analysis suggested strong correlation of biomass with flavonoids followed by total soluble sugar in all the cultivars except in case of Dh-86 where it was found highly correlated with total soluble sugar followed by ascorbic acid suggesting potential role of non enzymatic antioxidants for biomass accumulation. Although, higher development of oxidative stress and higher activity of enzymatic antioxidants at the site of ozone exposure suggesting proactive role of enzyme induced defence strategy by groundnut cultivars under ozone exposure. PCA biplot further confirm age dependent regulation of enzymatic defence against ozone in all the groundnut cultivars tested as most of the enzymatic activity was clustered at the site of vegetative and reproductive stage.

5. Conclusion

Biomass accumulation is the measure of overall growth and development of plants which is influenced by various environmental factors including ozone pollution. To deal with the ozone stress, plants developed enzymatic and non enzymatic antioxidative defence mechanism. Result of the present study showed reduced biomass of groundnut cultivar under ambient and enhanced level of ozone suggested that all the cultivars tested were sensitive to ozone. Further, study also found that the biomass sensitivity of the tested cultivars was differing among the cultivars at various growth stages. Among all the tested cultivars, Dh-86 showed higher activity of antioxidative system and found least affected by ozone stress suggested potential role of antioxidative system for ozone sensitivity. Although, activation of the non enzymatic defence was present at all the growth stages its detoxification was not directly correlated with plant age. While, enzymatic defence varied at variable

stages ascribed role of enzymatic antioxidants for age dependent ozone sensitivity. Study concluded that the ground level ozone is detrimental to groundnut cultivars. However, ozone phytotoxicity is dependent on the antioxidative potential of the cultivars and the growth stage of the plant. Further, the activity of APX played a measure role for age dependent plant sensitivity. Although, more studies with different plant group and experimental design is needed for substantial conclusion.

CRedit author statement

Dheeraj Rathore: Conceptualization, Validation, Resources, Writing - original draft, Writing - review & editing, Visualization, Project administration. IndraJeet Chaudhary: Conceptualization, Validation, Resources, Writing - original draft, Writing - review & editing.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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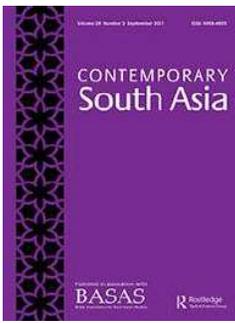
Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.apr.2021.01.005>.

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Embodied spatial practices in the field: critical ethnographies in village studies from India

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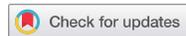
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Embodied spatial practices in the field: critical ethnographies in village studies from India

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ABSTRACT

Postmodern anthropology has used the claims of reflexivity, inter-subjectivity and recognition of differences in individual experiences in its attempt to unsettle the authority of traditional anthropology. This has led anthropologists to assert that ethnography is a cultural construct, whereby 'fieldwork' is conceptualised as 'embodied spatial practice'. Drawing on village studies from India, this paper argues that the recognition of different embodied practices of ethnographers from diverse social locations has not necessarily led to the democratisation of the discipline. Conversely, insufficient engagement with hierarchical, overlapping power relations within the ethnographic field, as well as within the disciplinary establishment, has led to the standardisation of disciplinary articulations of research ethics, the terms of which privilege hegemonic groups within the discipline. The articulation of the concerns of scholars from marginalised social groups often remains difficult within such disciplinary frameworks. This paper argues that establishing a critical tradition in ethnography in the true sense requires the postmodern sensibility of recognition of the differences in experiences, supplemented with feminist and subaltern critical interrogations of power and knowledge.

KEYWORDS

Caste; embodiment; field; gender; space

I. Introduction

The social-anthropological objects of knowledge—the 'field', as well as the fieldwork process, have been the subjects of critical interrogation since the 1960s, initially through the movement of interpretive anthropology, and subsequently in the 1970s with the turn of reflexivity. Apparently, a more self-conscious approach to ethnographic practices emerged in response to the crippling attacks on the anthropological certainty of the field and the anthropologist's ethnographic authority. A broad consensus has developed among social anthropologists that the 'field' is not ontologically given; rather, it is produced through interactive and inter-subjective learning. At the outset, such a conceptualisation of the 'field' seems to be accompanied by a promise of sensitivity to questions of power and the recognition of the multiplicity of individual experiences—a political agenda common to both postmodern anthropologists and feminists. The mapping of the diverse contours of power play, and of negotiations during the fieldwork process, has begged much scholarly attention from both of these theoretical strands. In emphasising the criticality of the process of negotiation in the very constitution of the 'field', postmodern anthropologists such as James Clifford have suggested that fieldwork is an 'embodied spatial practice' (Clifford 1997, 186). Accordingly, the practices of the lived social body of ethnographers and their negotiations in the social space are

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Engaging Diaspora through Foreign Policy: A Study of Indian Health Diplomacy in Mauritius during the COVID-19 Pandemic

Dr. Rajneesh Kumar Gupta*
Banditarani Behera**

Abstract

In the Contemporary world order, Indian Diaspora emerges as an important element of India's foreign policy. These Diasporic communities are playing a major role in the policy formulation and implementation process of host and home countries. They are the key drivers of the development efforts strengthening bilateral relations among the country of origin and destination. Present date health is the most discussed issue in global politics because of the ongoing COVID-19 pandemic. Now securing the standard of health and protecting life has one of the primary concerns of nations. India's engagement in global health diplomacy is not only for the economic benefits but also to create a global political engagement through goodwill gesture. Recent steps of Indian Government have been Diaspora centric and health diplomacy of India during the current pandemic was largely focused on the countries having large presence of Indian communities. India has a strong Diaspora in Mauritius and those constitutes nearly 70% of the total population of the country. This paper mainly focuses on the socio-economic conditions, political status, and cultural practices of Indian Diaspora in Mauritius. It examines Diaspora as a factor in India- Mauritius relations with special attention on COVID-19 Pandemic and emphasize on India's health diplomacy to formulate strategies for enhancing ties of India with her Global Diaspora.

Key Words- Health Diplomacy, Covid-19, Diaspora, Foreign policy and Political engagement, Policymakers
Delineation of the Theme

Hitherto, Diaspora has emerged as an important determinant of foreign policy, especially of India. Diasporas are frequently used as Indian's identity builder and image protector abroad. They are emerged as powerful entities of foreign policy strategy and an agent or catalyst of development of the mother country besides their active role in the host countries. The Diasporas have been widely used as an important device in cultural and public

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Engaging the Diaspora : The Changing Policies of the Indian Government

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Abstract

The earliest migration from India to other parts of the world can be traced to the ancient age to “Gautam Buddha’s disciples who migrated to spread Buddha’s ideas.”¹ In the same age similar developments were seen down south in the Chola Empire. By the medieval age several trading communities from west coast of India migrated to parts of Africa in order to expand their trade. In the imperial age it was pre-dominantly the indentured labour which was taken by the colonial rulers apart from this there were the exiled revolutionaries, employees of the merchant ships and students. However, despite such a long history and tradition of migration and diaspora it took the Indian government a long time to firstly recognize the overseas Indian community as a diaspora and then to consciously develop a policy towards it. The policy which began with capacity or trust building exercises has today reached the stage of taking out obligation from the diaspora.

The paper comprises of three parts. It begins with the history of migration from India and its diaspora. This is followed by the policies of the colonial rulers towards the overseas Indians. The last part of the paper explores and analyses the evolution of the different types of policies of the Indian government towards the Indian diaspora before and after the year 1991. The aim is to analyze the changing nature of the diaspora related policies adopted by the Indian government and how changes in systems led to them.

Keywords: Migration, Diaspora, Colonial Rule, Policy, Systems.

1.1 Introduction

Human migration is a continuous process which has its roots in antiquity. The search for a better life has been one of the key factors motivating people to migrate. The understanding of a better life has altered from time to time. In earlier times it meant easy accessibility to water and food for men and their cattle, in contemporary times the definition has broadened and includes not only a better habitat but also better livelihood opportunities, easy accessibility to technology and so on. Diaspora communities are a direct outcome of this search for a better life. Diaspora as a concept is antediluvian but the strong negative connotations attached to it since long prevented it from evolving as a subject of academic discourse and debate and as a component in government policies. With the strengthening of the concept of nation state after the First World War, definition of citizens and non-citizens became



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Exploring Awareness of Inclusive Education Among Prospective Teachers

*Abhishek Kumar**
*Jayendrakumar N. Amin**

Abstract

The inclusive system provides better quality education for all children and helps change the discriminatory approach. Teachers provide context for the child's first relationship with the world outside their family, helping to develop relationships and social interactions. Respect and understanding increase when students of various abilities and backgrounds play, meet and learn together. This study focused on inclusive education for students with disabilities. The success of inclusive education depends upon the attitude and acceptance of teachers and peer groups. Inclusive education relies on the capacity of teachers in terms of knowing the nature of the disability, selecting appropriate methods and materials for teaching students with disabilities. They play a critical role in creating an inclusive environment that encourages and motivate students with disabilities in active participation in classroom activities. Teachers should be skilled enough to understand the students' diverse needs. The study was an attempt; to explore the awareness of inclusive education for children with special needs among the prospective teachers. A sample size of one hundred sixty prospective teachers as part of the study. To collect the data questionnaire was constructed on the various aspects of disability and inclusive education by the researchers. A quantitative data analysis technique was employed to interpret the result. Together, the results indicate that prospective teachers' level of awareness of inclusive education was low in terms of disability types, learning styles for disabilities, teaching methodology, teaching aids, assistive technology devices, and apps required training imparted to future teachers.

Keywords: Awareness, Inclusive Education, Prospective Teachers

Introduction

The efforts of the Government of India towards the education of children with disabilities over the last five decades have been towards providing a comprehensive range of services. To provide equal opportunities to children with disabilities in schools and facilitate their retention, in 1974 centrally sponsored scheme for Integrated Education for Disabled Children (IEDC) was introduced. Government initiatives in inclusive education date back to the 1986 National Education Policy, which

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recommended, as a goal, 'integrating disabled children with the wider community at all levels as equal partners, preparing them for normal growth and enabling them to face life with courage and confidence. The World Declaration on Education for All adopted in 1990 gave new impetus to the various processes already in place in the country. The Rehabilitation Council of India Act 1992 initiated a training program for the growth and development of professionals to respond to children with special needs. It was the 86th amendment in 2002 to the Indian Constitution where Article 21-A of the constitution guarantees education as a fundamental right and specifies that the state will provide Free and compulsory education for all children in the age group of 6-14 years. It was under fundamental rights and state directive principles until Right to Education RTE 2009 was passed by parliament. The Government of India (GOI) also ratified the United Nations Convention on the Rights of Persons with Disabilities UNCRPD-2007 in the same era. For the first time, the act made it mandatory to include children with disabilities in the mainstream educational system. It applies to students with disabilities also. Subsequently, the Rights of Persons with Disabilities RPWD Act, 2016 came into force, replacing the Persons with Disabilities (Equal Opportunities, Protection of Rights and Full Participation) Act (PWD Act), 1995. According to RPWD Act 2016, it is a system of education wherein students with and without disabilities learn together. The teaching and learning system has adapted to meet the learning needs of students with diverse types of disabilities. The paper attempt to explore the awareness of prospective teachers on disability/divyang and inclusive education to ascertain the domains as a level of awareness, types of disabilities, learning styles, teaching methodologies assistive technologies and government schemes. National Education Policy (NEP) 2020 aims to ensure equity and inclusion in and through education by addressing all forms of exclusion and marginalization, disparity, vulnerability, and inequality in education access, participation, retention, and completion and in learning outcomes. Education needs a greater focus on accessibility, equity, and quality. The National Policy for Persons with Disability, 2006, attempts to clarify the framework under which the state, civil society, and private sector must operate to ensure a dignified life for persons with disabilities and support for their caretakers. The most recent advancement is the Right of Children for Free and Compulsory Education (2009) which guarantees the right to free and compulsory education to all children between ages six to fourteenth act read in conjunction with Chapter V of the Persons with Disability Act, 1995. Chapter V of the PWD Act ensures that every child with a disability is entitled to free education up to 18 years. Keeping in view, Govt. of India had accelerated the new scheme of Inclusive Education to achieve the target of Education for All (EFA) by 2010. Teachers from various states highlighted the challenges facing the nation and the way forward for teacher education. A majority of stand-alone TEIs - over 10,000 in number are not even attempting serious teacher education but are selling degrees for a price. Regulatory bodies have neither been able to curb the malpractices in the system nor enforce basic standards for quality. Have had a negative effect in curbing the growth of excellence and innovation in the sector. Hence, in urgent need of revitalization through radical action; Restoring integrity, reliability, efficiency, and high quality of teacher education system and raising standards. In such situations, the essential goal of providing education to all under one roof is compromised and is also necessary to achieve an inclusive and

equitable society in which all citizens can dream, prosper, and contribute to the nation. Inscribed in the Universal Declaration on Human Rights (1948), 'education is a basic right a range of declarations and conventions highlight the importance of education for a person with disabilities: the Salamanca Statement on education and special needs in 1994. Article 24 of the United Nations Convention on the Rights of Persons with Disabilities (CRPD (Convention on the Rights of Persons with Disabilities) (Convention on the Rights of Persons with Disabilities)) was adopted in 2006. The importance of 'education for all' is also included in the Convention against Discrimination in Education 1960. "The Incheon Declaration recalled the importance of inclusive education for all targets 4.5 and 4.a or access to education and the construction of adapted facilities for children with disabilities." (Education 2030). All over the countries, people with disabilities have lower literacy rates than people without disabilities. (United Nations, 2018). In many countries around the globe, teachers do not have the confidence and skills to deliver inclusive education. There is also a difference based on the nature of the disability; illiteracy is higher in children with visual impairments, multiple or mental disorders compared to children with motor disabilities. Education for the all-in-one umbrella is only a small component of the teacher training programs that teachers receive but are always not evaluated. Awareness of inclusive education and effective skills related to mastery so that teachers can become more empathetic, have a positive attitude, and have a strong desire to participate in professional training programs. It should be organized from time to time. Emphasizing the analysis of inclusive education experience of some countries, which have quite sophisticated inclusion, indicates that the satisfactory results of inclusion turned out only after serious and energy-consuming reforms and innovations. Diniz(2017) mentioned that according to the 2011 Census, 2.70 crore people with disabilities live in India. Minimum 15 lakh special educators are required to address the needs of the disabled population, but currently, 90,000 to 1 lakh rehabilitation professionals get registered with the Rehabilitation Council of India RCI. This statistic shows the importance and the role of the general teacher in inclusive education. A successful inclusive education program depends on well-trained teachers. One of the key issues in inclusive education is teachers training on curricular adaptation and evaluation methods to handle students with disabilities (SSA, Confluence report, 2016). It shows that teachers should be skilled enough to understand the students' diverse needs. They should have some knowledge about the different disability conditions and teaching methods to handle students with disabilities in the classroom environment Times of India (2018) reported that in Chandigarh, school-going students with disabilities face tough times due to not having enough educators to handle them. As per the academic session 2017 – 2018, around 4,418 students with disabilities enrolled in the different schools with only 26 special educators. It shows that one educator on every 196 students with special needs. As per RPWD Act 2016, the number of disabilities increased from seven to twenty-one. Teacher educators and special educators should be developed skills and knowledge to handle students with special disabilities. Accordingly, the curriculum of pre-service and in-service teacher training programs need revision to provide comprehensive training to their trainees to cater to the needs of disabled students. Learning Curve (2019) collaboration is the cornerstone of Inclusive Education. It is a process and not a product of a series of interventions. Dialogue and self-reflection

within the school community will lead to a better understanding of the implementation process. Children with special needs have the same need to belong as all other students do. However, contradictions arise owing to inadequate self-reflection over attitudes and insufficient opportunity to collaborate among teachers. NEP 2020 talks about equitable access to quality education for all students with a Special emphasis on SDGs. Entry into quality higher education can open a vast array of possibilities that can lift both individuals and communities out of the cycles of disadvantage. For this reason, making quality higher education opportunities available to all individuals must be among the highest priorities.

Significance of the Study

The NEP 2020 addresses several aspects of teacher education, preparation, and service conditions viz: relevant for children with disabilities includes short-term specialization courses to instruct children with disabilities and modules on teaching children with disabilities within existing programs teacher education programme contains content regarding inclusive education and disability for the prospective teachers to equip them for inclusive education of children with special needs. Hence, the study is needed to assess the awareness of teachers' trainees. Inclusive education in India has been described exclusively to children with disabilities. Disabled children rarely progress beyond primary school, and only 9% complete secondary education. Around 45% of disabled people are illiterate, and only 62.9% of disabled people between the ages of 3 and 35 have ever attended regular schools. Specific disability categories and genders are affected disproportionately. For instance, children with autism and cerebral palsy and girls with disabilities are least likely to be enrolled in schools. Disability is most likely to inhibit a child's access to home-based education and primary education. Less than 40% of school buildings have ramps, and around 17% of schools have accessible toilets. While technology is an important part of the NEP goal, only 59% of schools across the country have access to electricity. Moreover, teachers will be provided greater autonomy in selecting pedagogical tools relevant to their classroom contexts and will no longer be required to perform non-teaching tasks.

Objectives of the Study

The main objective of the study is to assess the existing knowledge of prospective teachers about inclusive education in the context of recognition and identification of disability types, learning styles for disabilities, teaching methodology, teaching aids, assistive technology devices, and apps required training imparted to future teachers.

Research Questions (RQ)

To seek pertinent information under the stated objectives, the following research questions were framed.

- RQ1 What is the extent of understanding of prospective teachers about inclusive education?
- RQ2 What is the extent of understanding of prospective teachers about types of disabilities in students?

- RQ3 What is the extent of understanding of prospective teachers about the teaching methodology used in the teaching-learning process?
- RQ4 What is the extent of understanding of prospective teachers about assistive technology and teaching aids used in teaching?
- RQ5 What is the extent of understanding of prospective teachers about govt schemes introduced for children with special needs?

Methodology

Research Design

The survey method was adopted for the study to evaluate awareness towards inclusive education among prospective teachers holistically because surveys are a flexible method of data collection that can be used in many diverse types of research especially with a questionnaire.

Participants

The population of five B.Ed. colleges participants were 480 prospective teachers. Participant colleges were selected through a simple random sampling technique. The ideal sample size was determined of one hundred sixty 160 prospective teachers. The participants were selected through a simple random sampling method. Participant colleges were NCTE recognized institutions and affiliated with Binod Bihari Mahto Koylanchal University, Dhanbad, Jharkhand

Data Collection tool

The data is quantitative and gathered through a self-constructed questionnaire based on findings of the literature on google form before administration. The first item analysis was for 50 items. A pilot study on identical college participants was done to determine the modalities of the final survey. The tool standardized of 35 items after item revision presented under five domains: Inclusive education, Types of disabilities, Teaching methodology, Assistive technology and Teaching aids, Government schemes were ready and distributed through proper medium.

Procedures

The data is acquired only after taking the necessary approvals or permissions of the institutions. The nature of the response option was multiple choice where the respondent is presented with a set of responses and asked to choose one or more. Acceptance of responses was locked after 45 min and google links were terminated so that no later chance to modify online assistance of teacher educators of same institutions was also provided. Later results were analyzed and interpreted.

Operational definitions of the variables

Awareness

Awareness is the state of being conscious of something. More specifically, prospective teachers can directly know, or be cognizant of the constructs.

Prospective Teachers

Prospective teachers imply students who are enrolled in a B.Ed. the program and they are in their final year of education.

Inclusive education:

Inclusive education can be determined from the integration of students with special educational needs in a regular classroom. Inclusive education requires a commitment on the part of educators at all levels of the system to its underlying philosophy and a willingness to implement. The paper includes the historical perspective, meaning, characteristics, needs, objectives, types of disabilities associated, adapted teaching methodologies, assistive technologies, teaching aids, and government schemes for children with disabilities.

Types of disabilities

According to the Rights of Persons with Disabilities (RPwD) Act, 2016, enacted on 28.12.2016 and came into force from 19.04.2017, the person with a disability means a person with long-term physical, mental, intellectual, or sensory impairment which, in interaction with barriers, hinders his full and effective participation in society equally with others. Types of disabilities include in this study are visual impairment, hearing impairment, locomotor impairment; cerebral palsy, children with learning disabilities (dysgraphia, dyscalculia, autism spectrum disorders, intellectual disability), characteristics, symptoms, causes of disabilities, identification approaches, and techniques.

Teaching methodology

The child learns best through one or more learning channels mentioned below, and the teacher can help him to become a successful learner by teaching the child through his primary learning style(s). When programs say they are "multi-sensory", this means the instruction utilizes all, or most, of VAKT channels in each lesson, and multisensory teaching is of benefit to all children. Teaching methodology includes the learning style such as Visual, Auditory, Kinesthetic and Tactile (VAKT), to achieve a teaching goal for children with several types of disabilities.

Assistive technology & Teaching aids:

Teaching aids are used by teachers to make teaching more interesting. Teaching aids play a vital role in a classroom for children with disabilities. Assistive technology and Teaching aids such as typo scope, light filtering lenses, talking calculators & electronic braille notetaker include the use of assistive devices, apps for the teaching-learning process symbol talk-acc talker, tap tap see, word magic, ginger, and any material used by a teacher to enhance or stimulate interest during classroom instruction.

Government schemes:

There are several government schemes and programmes that can be availed by a person with a disability in India. Government schemes include The National Trust for Welfare of Persons with Autism, Cerebral Palsy, Mental Retardation and Multiple Disabilities, Samarth Scheme, GHARAUNDA (Group Home and Rehabilitation Activities for Disabled Adults), Niramaya (Health Insurance Scheme), Rehabilitation Council of India, legal provisions, fellowship schemes for children with special needs, Govt organizations, and NGOs working to achieve the aims of inclusive education.

Results

The results of the study areas shown through graphical representation herein. Inclusive education settings bring all students together in one classroom which makes the inclusive environment wherein students with and without disabilities learn together. In this context, a question for ascertaining the awareness towards inclusive education among the participants was asked and the answer obtained is as presented in figure -I.

Figure I Awareness on Inclusive Education



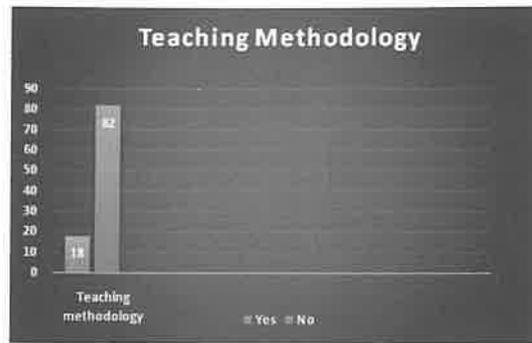
The results reveal that 66% of prospective teachers were aware of the meaning and ideas of inclusive education. They mentioned that each child is valued, accepted, and respected, each child acquires academic and social skills with a positive attitude, every child gets the equal the opportunity of participation.

Figure II Awareness on types of disabilities



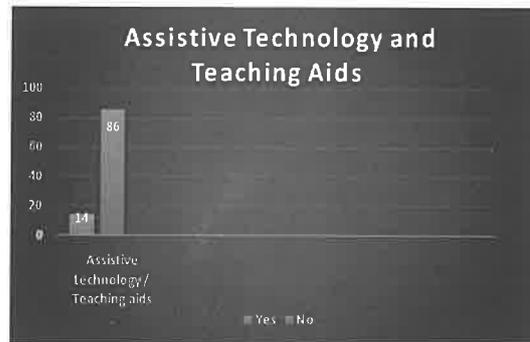
Disability has been defined based on an evolving and dynamic concept. RPWD act mentions 21 types of disabilities: They are blindness, low vision, individuals recovering from leprosy, locomotor disability, dwarfism, intellectual disability, mental illness, cerebral palsy, specific learning disability, speech, and language disability, hearing loss, muscular dystrophy, acid attack sufferers, Parkinson's disease, including sclerosis, thalassemia, haemophilia, sickle cell disease, autism spectrum disorder, chronic neurological conditions, and deaf-blindness. To the participants based on the types of disability (Figure- II), Questions were asked 62% of the prospective teachers were not aware of the RPWD Act 2016. Only 38% of participants were correct of a few types of disabilities such as visual impairment, hearing impairment, and intellectual disabilities.

Figure III Awareness of teaching methodologies



Selection of appropriate teaching methodology, learning styles are crucial in the teaching-learning process. Teacher trainees must have some knowledge about the different methods used for teaching students with disabilities. Questions to the participants related to teaching methodology (Figure -III) shows that very few 18 % of prospective teachers knew. The 82 % remaining reported that they are not aware of the methods and materials to handle students with disabilities.

Figure IV Awareness on Assistive Technology & Teaching Aids



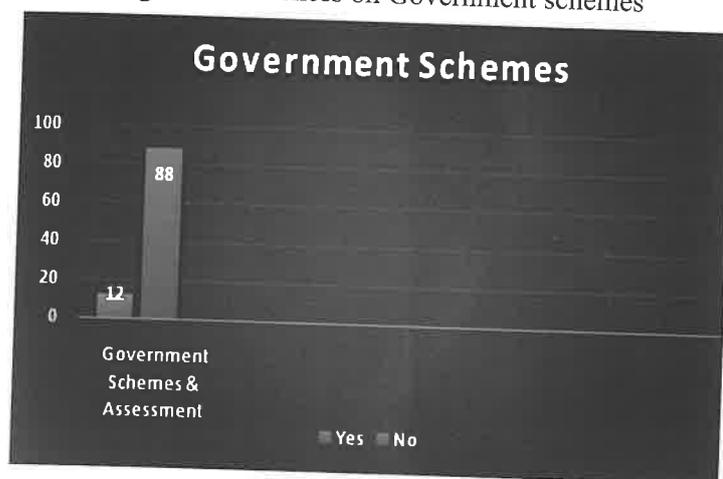
Assistive technology and teaching aids are any tool or device that helps students with disabilities to perform activities more quickly, easily, or independently. Assistive technology devices, apps, and teaching aids enhance the participation of students with disabilities in the home, school, and community. The teacher should have basic knowledge about assistive devices for different disabilities based on their needs and abilities. Questions related to assistive technology and teaching aids to the prospective teachers. The results are shown in (Figure-IV) indicate that only 14 % were aware of the assistive technology and its uses. It implies that Prospective teachers should know more about the devices used for students with disabilities.

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Figure V Awareness on Government schemes



The government of India announced various facilities and schemes for students with disabilities. A teacher should know some of the Schemes and facilities available for special children like scholarships, reservation policy, Legal provisions, involvement of Government organizations, and NGOs working to achieve goals of inclusive education, etc. The result showed in (Figure- V) that only 12 % of prospective teachers were aware of Government schemes remaining 88 % of them were unaware of the facilities and Schemes meant for students with disabilities.

Conclusion

Inclusive education means that all types of students can receive education in regular and same classrooms. Through this, children with special needs can participate in community activities. It also provides better learning opportunities. It helps them to set up independent from all of their activities. Inclusive education is possible only because of the teacher's attitude and knowledge of the disability. The National Educational Policy 2020, under inclusive education, mentioned that inclusive education is an integral part of both pre-service teacher education. As well as In-service professional development, including for Anganwadi workers, preschool and schoolteachers, school leaders, and other education functionaries. These programs will ensure that all teachers are continuously sensitized about different learners and hence will be able to cater to the educational needs of all learners. Based on the recommendations given by the various acts, the study results conclude that there is a significant need for providing content and training on disability in the curriculum of B.Ed.

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Financial Inclusion of Trafficking women through Social Entrepreneurship: A Case Study of PRAJWALA (Anti-trafficking Organisation)

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Abstract

This paper intends to analyze how the entrepreneur skills provide economic rehabilitation to the largely marginalized and alienated victims of trafficking women for their social sustainable development. Prajwala emerged as an anti-trafficking organization in Hyderabad, which believes in preventing women and children from entering prostitution, which is the worst form of sexual slavery. Its evolution of other interventions such as rescue, rehabilitation, reintegration and community based prevention. This Anti-trafficking Organisation developed the Rescue and Restoration Program (RRP) in order to break the cycle of exploitation, and end the torment faced by the victims of trafficking. The RRP team at Prajwala fulfils the four-fold objectives of undertaking rescue missions, providing crisis counseling at police stations, conducting home investigations, and supporting the judicial process. The team is comprised of a director, coordinators and barefoot counselors, many of whom are survivors of sex trafficking themselves. Apart from RRP, it has Anti Human Trafficking Units and Crisis counseling centres. The other dynamic aspect of Prajwala is in the process of social rehabilitation, in which the victims get institutional care and protection which involves counseling in an ambience of healing and building capacities of self-esteem and self-confidence through Life Skills Education.

Its uniqueness involved five basic component models such as Identifying need-based, Aptitude based, Market assessed, Viable and sustainable economic options, and livelihood training. The researchers would like to explore the role of Prajwala in fostering entrepreneurial skills for long term social reintegration and become an entrepreneur in corporate and professional environment. The research design of the study will be based on an Empirical work, with semi-structured interview schedules are used consists of 100 respondents from Prajwala Enterprises. The outcome of the research will be helpful for the development strategies in rising future innovative entrepreneurial skills and inculcate the spirit of social entrepreneurship (A modern approach to social value creation) through new voices of alienated.

Introduction

In the wake of globalization and the resultant marginalization and alienation of large sections of humanity, sex trafficking of women and girls has become a matter of urgent concern today

worldwide. The report of CRIME IN INDIA 2011 Statistics by National Crime Records Bureau, Ministry of Home Affairs, enumerates the crime against women in India is very high in general and it is more among the Schedule Caste, Schedule tribe and Minority women and girls in particular. This report explains the incidence of IPC crimes against body during 2011, Andhra Pradesh stood first with 14.67% among the Indian states followed by Maharashtra and Madhya Pradesh with 9.59% and 9.54% respectively (Report page:48). In India alone, over 200,000 women and children are inducted into the flesh trade every year. The State of Andhra Pradesh is one of the largest suppliers of women and children for the purpose of commercial sexual exploitation. The reasons are many in developing nation India to enter into the flesh trade namely, poverty, rape, kidnapping and abduction, cheating in the name of love, crazy about to enter into media and cinema, auto drives cheating and role of pimps have had reported as remarkable reasons in trafficking women and girls. Economic hardships coupled with the prevailing status of women in society, and changing public attitudes towards sex and morality creates the context for the flourishing of this modern-day form of slavery. An incredibly disturbing fact is that the age of the children is progressively declining to meet the male demand for younger prostitutes. There is a widely held belief that sex with children, especially virgins, will cure sexually transmitted diseases and prevent one from contracting HIV/AIDS. One of every four victims rescued from prostitution is a child, and 60% of these children are HIV positive.

Sex trafficking not only results in a severe violation of human rights but also causes adverse physical, psychological and moral consequences for the victims. All hopes and dreams of a better life are shattered and over time the girls become penniless, mentally broken and affected with serious or life-threatening illnesses such as HIV/AIDS. The journey of sex trafficking destroys the body, mind and soul of a victim, and fundamentally takes away her capacity to trust herself or anyone around her. The damage done is deep rooted and often irreversible, as the sense of rejection, betrayal and numbness that a trafficked woman or girl goes through makes her lose faith in humanity. Skewed identity, poor self-worth and learnt helplessness also make her believe there is no hope for her in the outside world and her destiny is to sell her body. Prajwala holds the conviction that prevention is better than cure, and that addressing the root cause of sex trafficking is the most effective means of eradicating it. As a result, the organization has developed two interventions that strive to eliminate the sources of trafficking. The first is providing education to equip vulnerable children with skills to ensure a bright future, and the second is targeting vulnerable women to make them aware of the dangers of commercial sexual exploitation by the means of Rehabilitation through entrepreneur skills which ultimately provides victim a source of livelihood, sustainable development and dignity of life.

The Prevention Through Education Program (PTEP) aims to provide children of women in prostitution with the capacities needed to live life with dignity and access meaningful, viable career opportunities for their futures. Prajwala runs 7 transition centers in vulnerable areas throughout Hyderabad where children are at-risk of being trafficked for the purposes of forced prostitution. The centers function as educational environments which not only foster the overall development of the children, but also equip them with the necessary psychosocial and scholastic skills to ensure a bright future that breaks the cycles of poverty and social exclusion. After completing 7th class from the Prajwala schools, children subsequently enroll in local private or government schools to complete their 10th standard and beyond. Student committees as well as

mothers groups are actively involved in the center's functioning in order to promote a sense of community ownership and responsibility for the education of their children.

Secondly, Prajwala believes that rehabilitation is long-term holistic process, with interconnected approaches that cannot be stand-alone interventions. The first step involves psychological rehabilitation of victims, to ensure that the damage caused by being trafficked for a prolonged period of time is undone, and that their emotional stability and self-confidence is restored. The next stage of providing economic options ensures that the victim has access to livelihood opportunities that reduce the chances of re-trafficking. Finally, civic support provides the survivors with an identity, financial stability and security in the form of financial aid, subsidized housing, assets etc. that further reduces the risk of re-entry into prostitution. Essentially, the three pillars of Prajwala's rehabilitation strategy include: Psychological rehabilitation in Therapeutic Shelter Homes, Economic rehabilitation through Prajwala Enterprises and Employability Training Unit and Civic rehabilitation which supports the victims with identity cards, immediate relief funds and access to government welfare benefits.

This research paper gave more emphasis on Economic Rehabilitation of trafficking women of Prajwala. For this reason, Prajwala created its own production cum training unit in 2004 called Prajwala Enterprises (PE), which began with support from the International Organization for Migration and Catholic Relief Services. In the process of economic rehabilitation, the Prajwala Enterprise and the Employability Training Unit has two interventions which includes the livelihood skills and occupational training needed to face the world with a proud smile and confidence. Prajwala Enterprise aims at a key aspect of preparedness for social reintegration is the employability factor. Hence, it is necessary to select the kind of livelihood training based on interest and aptitude by assessing the victim as well as market viability of the trade. A potential of the victim that is effectively tapped at Prajwala is her extraordinary inner strength and lack of awkwardness in a male-dominated trade. It has been observed that survivors excel in trades that are non-conventional and mostly from a male bastion. Livelihood training opportunities in cab driving, security guard, masonry, etc. Depending on the aptitude, health and legal status of survivors, the trades available for on-the-job training at Prajwala Enterprise include welding/fabrication, screen printing, lamination, carpentry and book-binding. During orientation, each trainee spends 1 full day in each of the four available trades to see which was most appropriate for their interests and skill-sets. Then, they spend 3 months gaining real-time work experience in the pre-designated department of their choice. Apart from this, the 10-day security guard training was undertaken in collaboration with Balaram Security Services, Bangalore. Two retired army officers—Major Prashanth Rai and Major Bhawana Chiranjay conducted the certificate course, which was intended to train the candidates on basic skills required to enter in to security sector and who gained employment in 44 Kasturba Gandhi Balika Vidyalaya (KGBV) schools throughout 20 districts in Andhra Pradesh. With the Prajwala's inception over 5081 women were reintegrated with their families and total of 37 victims have been repatriated to their respective countries.

Objectives of the Study

- ❖ To Study the Socio-economic conditions and social categories of the respondents
- ❖ To Examine the role of Prajwala Enterprise in rehabilitation of Trafficking women
- ❖ To Know how the PE and ETU are imparting Entrepreneur skills through Social Entrepreneurship
- ❖ To Study the challenges which impedes the innovative strategies of entrepreneurship development

Review of Literature

Johanna (2010) in her article 'Social Entrepreneurship: Taking Stock and Looking Ahead' explains the existing endeavors to conceptualize Social entrepreneurship and illustrate the context-specific nature of the phenomenon and derive implications for fostering social entrepreneurship as a positive force for social and economic development. She gave much focus on the productive agenda and the real insights for advances in both theory and practice of social entrepreneurship. According to her, social entrepreneurship is not viewed as an isolated phenomenon but an integral part of a social system. Thus, the role, nature and score of social entrepreneurship cannot be discussed without considering the complex set of institutional, social, economic and political factors, in which they are interlinked. For research it represents an exciting opportunity to unpack mechanisms which drives social and economic development. Finally she concluded that, the potential of social entrepreneurship research to bridge disciplinary divides and inspires the broader fields of entrepreneurship to take advantage of building on theories from both the disciplines of sociology and economics.

J. Gregory Dees (2005) in his commentary on 'Social Entrepreneurs and Education' talks about that; Social entrepreneurship is a very new to the academic world, especially outside of business and public policy. Academic attention to this "field" is only about a decade old. Only recently this topic has been attracting the attention of scholars from the disciplines of education, public health, and social work. He explains about the term "social entrepreneur" is not meant to convey the mindset common to large business corporations. Entrepreneurs, even in business, are the subversives, those who are "reforming or revolutionizing the pattern of production," as economist Joseph Schumpeter put it, they are the agents of "creative destruction"-out with the old and in with the new. He also added Howard Stevenson's, vision a professor of entrepreneurship at Harvard, explicitly contrasts them with "administrators" who preserve the status quo. Entrepreneurs may be part of what Habermas calls "the system," as Humphries and Grant suggest, but they are not corporate or bureaucratic. They promote change within the system, and some of them actively work to change the system. Yes, most business entrepreneurs are concerned about profit and without it they cannot survive. But for many entrepreneurs this is not the primary motivation. By marrying the concept of entrepreneurship with the word "social," he hopes to describe someone who focuses on social change instead of profit as the goal, and approaches this goal with an entrepreneurial spirit, as one of determination, innovation, and resourcefulness. He concluded that social entrepreneurship construct has a great potential for helping us mobilize resources for the social good and for stimulating new thinking about economic and social institutions.

Srivastava (2007) in his article 'Benefiting from Social Entrepreneurship and Social Businesses in India', talks about that he believes that India needs extensive application of multidisciplinary approaches and entrepreneurial energy in social and environmental sectors. The challenge is: How to have more number and better quality of social entrepreneurs in the country? Social entrepreneurship, although an old field of practice, is a new and emerging field of systematic knowledge and learning. The growth in social entrepreneurship should not be left to chance. He also emphasizes Peter Drucker's book 'Innovation and Entrepreneurship' that everyone who can face up to decision making can learn to be an entrepreneur and behave entrepreneurially. Entrepreneurship, then, is behavior rather than personality trait. And its foundation lies in concept and theory rather than in intuition. Even if we concede some role for personality, factors as some other experts have suggested, a large number of selected people (after initial screening) can be trained to be entrepreneurs. Extending this logic further, he thinks that most people who can combine a spirit of social service and entrepreneurial behavior can learn and develop themselves as social entrepreneurs. Furthermore, when social entrepreneurial education and training is spreading in other countries (e.g., USA and UK), there is growing need to promote such education in India also. The social entrepreneurship, as a special type of leadership, can flourish here only if its value is recognized by a significant section of Indian society, especially the policy makers, educationists, media and the other government bodies. If this happens, it can create new waves of opportunities, strategies, approaches, and impacts in the direction of sustainable development.

Johanna & Ignasi (2004) in their working paper 'Social Entrepreneurship and Research: A source of Explanation, Prediction and Delight', they explain that social entrepreneurship, as a practice and a field for scholarly investigation, it provides unique opportunity to challenge, question and rethink concepts and assumptions from different fields of management and business research. They talk about the view of social entrepreneurship as a process that catalyses social change and important social needs in a way that is not dominated by the direct financial benefits for the entrepreneurs. It is seen as differing from other forms of entrepreneurship in the relatively higher priority given to promoting social value and development versus capturing economic value. They have concluded in this paper by trying to identify the distinctive domain of social entrepreneurship and argued that social entrepreneurship differs from other forms of entrepreneurship in that it gives high priority to social value creation.

UNICEF (2007) in its report entitled 'Adolescents and Civil Engagement: Social Entrepreneurship and Young People' enumerates about the children and young people have a vision of themselves in this world and they have a role to play in their own development as well as their own development of their communities. Taking initiative, creating a project, conceptualizing and launching one's own venture are exceptions, not the rule in most young people's experience. Every child is good at something. Encouraging and giving that child the opportunity to succeed is extremely important in shaping their personality and tolerance for risk. Young people are of the world's human capital and they are increasingly being as key participants in decision making and development. Finally this report aims to show that the role that social entrepreneurship can play in the achievement of holistic development in young people while also allowing young people to contribute to the development of their own

communities and the achievement of the MDGs. It will provide an overview of social entrepreneurship as it currently exists, outline the ways in which young people are involved, demonstrate the ways in which young people are contributing as social entrepreneurs to UNICEF's goals, and provide guidelines to support and scale-up youth social entrepreneurship interventions.

Suresh & Sudesh (2011) in their article 'Social Entrepreneurship: A Growing trend in Indian Business' enumerates about the organizations of social Enterprises and towards improving the general welfare of the society and they apply market-based strategies to achieve a social purpose. The social entrepreneurship movement includes both non-profit and for-profit organizations, the non-profit organizations use to adopt the business models to pursue their social goals and the for-profit organizations incorporating a social agenda into their business model. The focus of the article addressing the challenges of lacks appropriate sources of financing, proper regulations, societal recognition and suitable information system. The authors concluded that it is the right time for the non-governmental organizations (NGOs), governmental organizations and social entrepreneurs to come forward to encourage further development of social entrepreneurship and in building a healthy platform for social enterprise development in India.

Brooks (2006) in his chapter 'Social Entrepreneurship-A modern approach to social value creation' explains about the definition of social entrepreneurship and how it differs from commercial entrepreneurship. As he cited the example definitions of major writers of the concerned discipline as Social Entrepreneurship address social problems or needs that are unmet by private markets or governments. It is motivated primarily by social benefit and lastly it generally works with-not against-market forces. In fact one of the common claims about social entrepreneurs is that they adopt a business-like approach to social innovation. Lastly he cites the Dees definition of social entrepreneurs as 'change agents in the social sector' who adopt a mission to create and sustain social value. The article concluded with the successful characteristic of multiple stimuli of social entrepreneurship process is environmental factors, resource availability and his or her awareness and preparedness to see and explore the social opportunities and personality traits which contributes for the development of social entrepreneurship in the world. The personality traits which include innovativeness, achievement orientation, independence, a sense of control over one's destiny, relatively low risk aversion, and a tolerance for ambiguity and it also requires a sense of community or social need.

Methodology

This study deals with the Prajwala (Non-Government Organization), Hyderabad, of Andhra Pradesh. The Prajwala was emerged as an anti-trafficking organization, which believes in preventing women and children from entering into prostitution, which is the worst form of sexual slavery. Since its inception of 1996 till date there are 9000 women and children got rescued. Among these 5000 children and adolescent girls and 4000 were women of middle age. The researchers have taken the sample size of 100 respondents from different streams of Prajwala's Enterprise, those who are actively engaged in vocational training and entrepreneur skills. In this study, we used a combination of observation, interview and document materials. We have used random Sampling technique to select the respondents and the research carried out

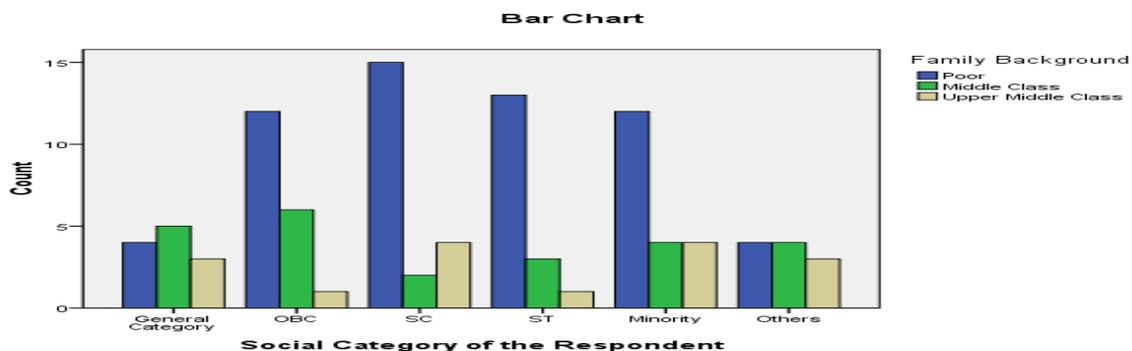
by personal interviews of respondents, trainee and with rescue team as well as shelter team members. The data collected were duly tabulated and analyzed by using SPSS16 software. The field work had been conducted from 5th December 2018 to 9th January 2019. Secondary information has been collected from various documents such as books, articles, newsletter, reports, magazines, daily newspapers, as well as from the existing literature to understand the role of Prajwala’s vision and the practice of social entrepreneurship in promoting the marginalized voiceless women as empowered social entrepreneurs. The concept of social entrepreneurship is emerged as a research area in the social sciences and in management field, which aims to create an impression of a modern approach to social value creation.

Data Analysis

Table: 1

Social Category of the Respondent * Family Background

Social Category of the Respondent	Family Background of the Respondent			Total
	Poor	Middle Class	Upper Middle Class	
General Category	4 33.3%	5 41.7%	3 25.0%	12 100.0%
OBC	12 63.2%	6 31.6%	1 5.3%	19 100.0%
SC	15 71.4%	2 9.5%	4 19.0%	21 100.0%
ST	13 76.5%	3 17.6%	1 5.9%	17 100.0%
Minority	12 60.0%	4 20.0%	4 20.0%	20 100.0%
Others	4 36.4%	4 36.4%	3 27.3%	11 100.0%
Total	60 60.0%	24 24.0%	16 16.0%	100 100.0%



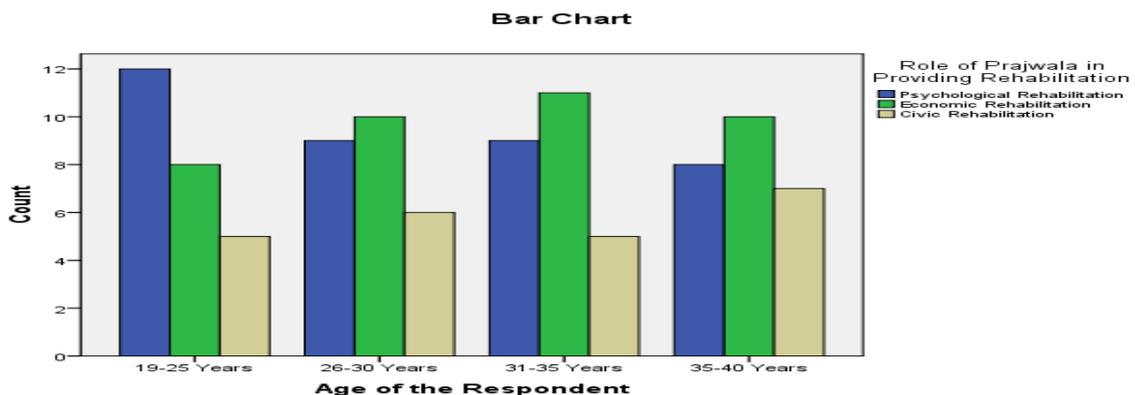
The above table enumerates about the social category of the respondents with respect their family background from Prajwala Enterprise. Majority of the 60% victims are from the poor background and prone to be victimized as victims of sex trafficking and rescued by the

Prajwala’s powerful rescue team and some are joined by the crisis counseling of police and law enforcement agencies, followed by 24% middle class family background and the remaining 16% are from rich family background. The social category of the respondents reflects that more vulnerable are from Schedule caste and Minority background women with 21% and 20% respectively, followed by Other Backward Class women and Schedule Tribe women with 36 percent. Lastly, the general category and other category women with 23% with the various reasons they have been trapped into sex trafficking. The reasons behind this trafficking are most of them are cheated by their close relatives and friends, some are by middlemen, poverty, crazy towards the movie industry.

Table: 2

Age of the Respondent * Role of Prajwala in Providing Rehabilitation

Age of the Respondent	Role of Prajwala in providing Rehabilitation			Total
	Psychological	Economic	Civic	
19-25 Years	12 48.0%	8 32.0%	5 20.0%	25 100.0%
26-30 Years	9 36.0%	10 40.0%	6 24.0%	25 100.0%
31-35 Years	9 36.0%	11 44.0%	5 20.0%	25 100.0%
35-40 Years	8 32.0%	10 40.0%	7 28.0%	25 100.0%
Total	38 38.0%	39 39.0%	23 23.0%	100 100.0%



The above table enumerates about the age of the respondents and the role of Prajwala in providing Rehabilitation to the victims. The multiple traumas faced by girls who are sex trafficked encompass the beginning of a life struggle which is marked by ill-treatment, oppression and indebtedness. Victims of sex trafficking are sold and resold over and over again in a continuous cycle of exploitation. Once a trafficked girl eventually succumbs to her circumstances within prostitution, she is closely watched and her movements and interactions are monitored and restricted. A constant struggle for existence in an exploitative structure of pimps, goondas and brothel-madams makes any escape attempt virtually impossible. The

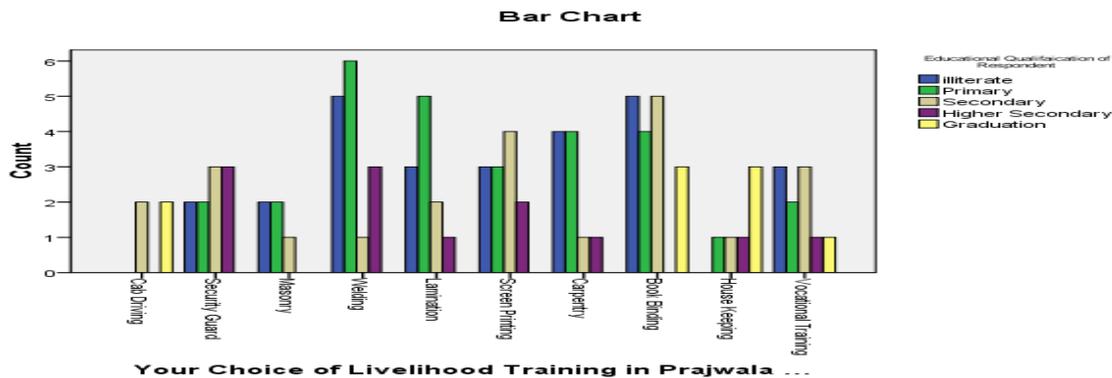
respondents from different age groups are undergoing the psychological and economic rehabilitation with almost an equal percent 39% and 38% followed by 23% by civic rehabilitation.

Before introducing the rehabilitation process, Prajwala started the rescue and restoration program (RRP) team which fulfils the four fold objectives undertaking powerful rescue mission, crisis counseling at police station, through conducting home investigations, law enforcement agencies and other capacity building programs. This will provide the paramount to effectively fight human trafficking.

Table: 3

Respondent's Choice of Livelihood Training in Prajwala Enterprise * Educational Qualification

Livelihood Training in Prajwala Enterprise	Educational Qualification of Respondent					Total
	illiterate	Primary	Secondary	Higher Secondary	Graduation	
Cab Driving	0 .0%	0 .0%	2 50.0%	0 .0%	2 50.0%	4 100.0%
Security Guard	2 20.0%	2 20.0%	3 30.0%	3 30.0%	0 .0%	10 100.0%
Beautician Course	2 40.0%	2 40.0%	1 20.0%	0 .0%	0 .0%	5 100.0%
Welding	5 33.3%	6 40.0%	1 6.7%	3 20.0%	0 .0%	15 100.0%
Lamination	3 27.3%	5 45.5%	2 18.2%	1 9.1%	0 .0%	11 100.0%
Screen Printing	3 25.0%	3 25.0%	4 33.3%	2 16.7%	0 .0%	12 100.0%
Carpentry	4 40.0%	4 40.0%	1 10.0%	1 10.0%	0 .0%	10 100.0%
Book Binding	5 29.4%	4 23.5%	5 29.4%	0 .0%	3 17.6%	17 100.0%
House Keeping	0 .0%	1 16.7%	1 16.7%	1 16.7%	3 50.0%	6 100.0%
Voc. Training	3 30.0%	2 20.0%	3 30.0%	1 10.0%	1 10.0%	10 100.0%
Total	27 27.0%	29 29.0%	23 23.0%	12 12.0%	9 9.0%	100 100.0%

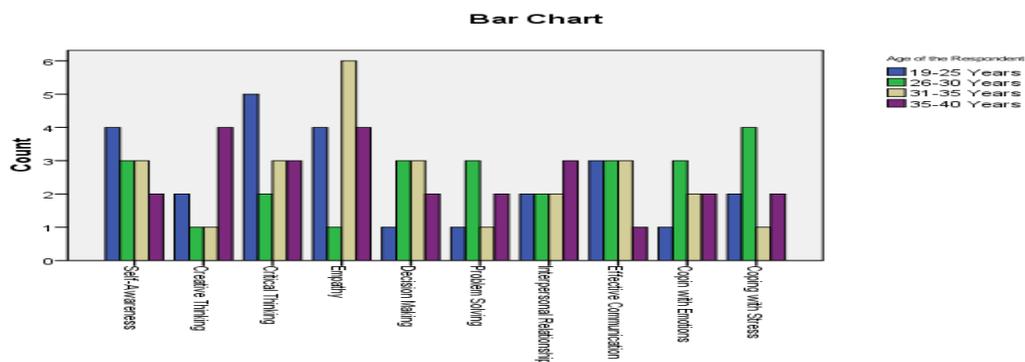


The table explains above is the respondents choice of livelihood training with respect to their educational qualification. Prajwala’s uniqueness involved in its five basic component models such as Identifying need-based, Aptitude based, Market assessed, Viable and sustainable economic options, and livelihood training. According to this model, as majority of them were from very low educational background such as illiterate, primary and secondary education level with 79% and the remaining 21% have higher secondary and graduation level education. Out of 100 respondents 62% have opted hard jobs (Cab driving, Security guard, welding, Carpentry and House Keeping) and 38% have opted soft jobs such as beautician course, lamination, screen printing, vocational training. Prajwala support for these victims is incredible in imparting the entrepreneur skills and to protect their self-esteem and confidence, which in turn led to their all round development.

Table: 4
Life Skills Education Adopted * Age of the Respondent

Type of Life Skills Education Adopted	Age of the Respondent				Total
	19-25 Years	26-30 Years	31-35 Years	35-40 Years	
Self-Awareness	4 33.3%	3 25.0%	3 25.0%	2 16.7%	12 100.0%
Creative Thinking	2 25.0%	1 12.5%	1 12.5%	4 50.0%	8 100.0%
Critical Thinking	5 38.5%	2 15.4%	3 23.1%	3 23.1%	13 100.0%
Empathy	4 26.7%	1 6.7%	6 40.0%	4 26.7%	15 100.0%
Decision Making	1 11.1%	3 33.3%	3 33.3%	2 22.2%	9 100.0%
Problem Solving	1 14.3%	3 42.9%	1 14.3%	2 28.6%	7 100.0%
Interpersonal Relationship	2 22.2%	2 22.2%	2 22.2%	3 33.3%	9 100.0%

Effective Communication	3 30.0%	3 30.0%	3 30.0%	1 10.0%	10 100.0%
Coping with Emotions	1 12.5%	3 37.5%	2 25.0%	2 25.0%	8 100.0%
Coping with Stress	2 22.2%	4 44.4%	1 11.1%	2 22.2%	9 100.0%
Total	25 25.0%	25 25.0%	25 25.0%	25 25.0%	100 100.0%



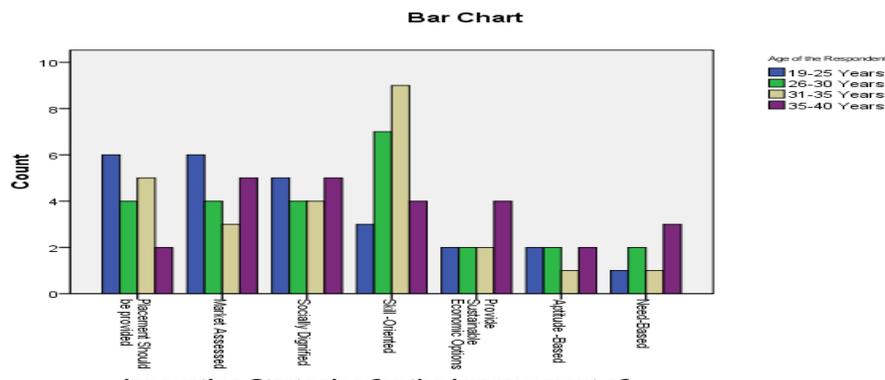
Prajwala being an Anti-Trafficking Organisation introduced a dynamic aspect of learning of Life Skills Education as well as training in safe and dignified activities helps the victims build self-confidence and feelings of self-worth to eventually empower them to unlearn negative thought patterns and begin the healing process from within. The changes in respondents life reflects the positive impact of LSE which they have adopted not only helps to increase their employability but also help reduce the risk of being re-trafficked into prostitution. Half of the respondents are started thinking critically and creatively, aware about themselves and practicing and learning effective communication skills, the remaining 50% respondents are in the process of decision making, developing interpersonal relationships and empathy towards others as well as problem solving techniques.

Table: 5

Innovative Strategies for the improvement of Entrepreneur Skills for marginalized * Age

Innovative Strategies for Entrepreneur Skills	Age of the Respondent				Total
	19-25 Years	26-30 Years	31-35 Years	35-40 Years	
Placement	6 35.3%	4 23.5%	5 29.4%	2 11.8%	17 100.0%
Market Assessed	6 33.3%	4 22.2%	3 16.7%	5 27.8%	18 100.0%

Socially Dignified	5 27.8%	4 22.2%	4 22.2%	5 27.8%	18 100.0%
Skill -Oriented	3 13.0%	7 30.4%	9 39.1%	4 17.4%	23 100.0%
Economic Options	2 20.0%	2 20.0%	2 20.0%	4 40.0%	10 100.0%
Aptitude -Based	2 28.6%	2 28.6%	1 14.3%	2 28.6%	7 100.0%
Need-Based	1 14.3%	2 28.6%	1 14.3%	3 42.9%	7 100.0%
Total	25 25.0%	25 25.0%	25 25.0%	25 25.0%	100 100.0%



The above table enumerates the innovative strategies for the improvement of entrepreneur skills for marginalized and the age of the respondent. The stakeholders have suggested the innovative strategies for the improvement of entrepreneur skills such as 36% have suggested market assessed and socially dignified jobs, followed by 23% have responded skill-oriented training should be given, provision of placement facilities, and 14% suggested aptitude and need-based training and lastly, 10% suggested entrepreneurs should have viable economic options.

Conclusion

The study concludes that most of the victims of trafficking for commercial sexual exploitation have gone through a journey of betrayal, abuse and pain. From the initial stage of being trafficked to years of leading live as a slave, and finally of being rescued and subjected a different sort of pain, these victims see it all. Psychosocial interventions thus pose a significant challenge, as on one hand is the healing of pain and the other is undoing all the damages done by stakeholders who were custodians of protection. Throughout the entire rescue-restoration process, the custodial and judicial procedures immediately post-rescue are usually not victim-friendly and make the survivor believe that her journey of victimization is never-ending.

The organizations' efforts have been fruitful as today the society is much more aware about the crime of human trafficking. Structural changes have also been implemented and executed both

within the state and nationally to ensure successful rehabilitation and reintegration of survivors. However, many more laws must be passed and others enforced to protect victims and punish perpetrators. Thus advocacy is one of the most important tasks undertaken in Prajwala. Prajwala's motivation and initiation for long-term systemic change to happen in the anti-trafficking sector, through social entrepreneurship and by imparting entrepreneur skills for the victims livelihood and sustainable economic growth which in turn led to produce a more number of entrepreneurs for the welfare of the society.

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The recurrence of aberrations in the form of illiberalism in liberal democracies is not a 'by-chance phenomenon', but exists due to the presence of intrinsic structural linkages that herald a return of protracted anti-people rhetoric and practices time. The satyagraha discourse attempts to offer an alternative paradigm in which democracy itself contains the notion of satyagraha for the arrival of swaraj. A thorough musing over 'truth as a vantage point', 'life as satyagraha', 'moral-ethical parameters of truth', 'civilisational discourse', 'nature of education', 'location of truth in global philosophical traditions', 'relation between religion and politics', 'complexity of information age' and 'life as satyagraha' becomes a sine qua non for the effective unravelling of substantive nuances of satyagraha for global transformation.

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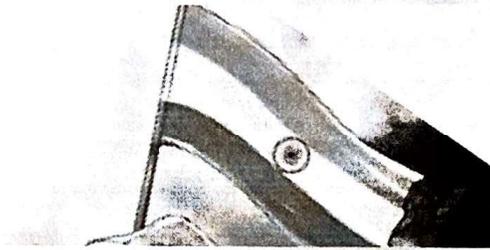
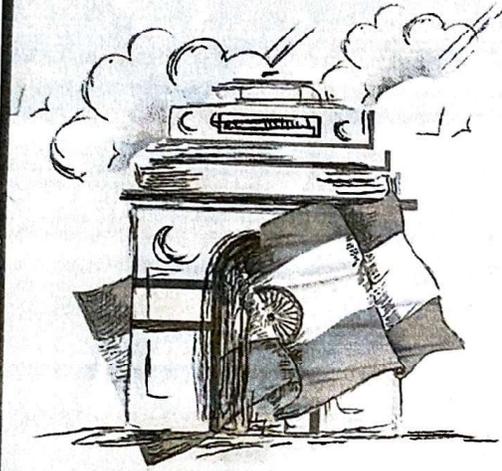
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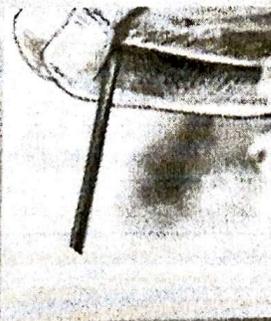
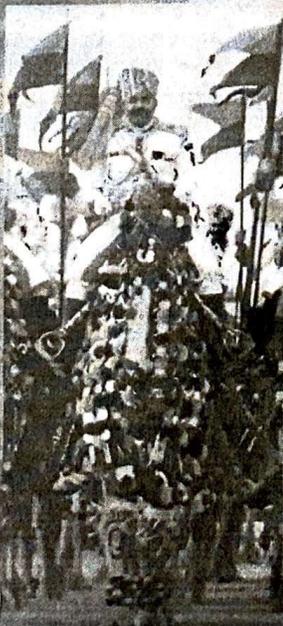
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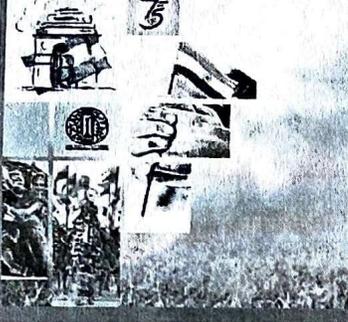
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डॉ. आशीष कंधवे

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राष्ट्रगौरव और कृष्ण करुणा के कवि- मैथिलीशरण गुप्त

डॉ. संजीव दुबे

महावीर प्रसाद द्विवेदी को मैथिलीशरण गुप्त ने अपना काव्य मार्गदर्शक माना था। उनकी काव्य रचना में महावीर प्रसाद द्विवेदी की प्रेरणा को आलोचकों ने बखूबी चिन्हित किया है। महावीर प्रसाद द्विवेदी ने 'संपत्ति शास्त्र' नामक ग्रन्थ की रचना की थी एवं सरस्वती पत्रिका के माध्यम से तत्कालीन हिन्दी साहित्य चेतना का नेतृत्व किया था। 'संपत्ति शास्त्र' की भूमिका का आरंभ तत्कालीन भारत की दरिद्रता के कारुणिक उल्लेख से होता है। देश की दरिद्रता का बड़ा कारण वे किसानों की उपेक्षा को मानते हैं। 'महावीर प्रसाद द्विवेदी और हिन्दी नवजागरण' पुस्तक में रामविलास शर्मा ने 'देश की बात' शीर्षक से सरस्वती में प्रकाशित द्विवेदी जी की टिप्पणी उद्धृत की है, जिसमें द्विवेदी जी सुधारवादियों की देश भक्ति की संकीर्णता को रेखांकित करते हुए देश भक्ति का मतलब किसानों की सेवा सिद्ध करते हैं। विचारणीय है कि हिन्दी नव जागरण में महावीर प्रसाद द्विवेदी के प्रदेय का मूल्यांकन करनेवाली इस पुस्तक में मैथिलीशरण गुप्त की चर्चा से जैसे बचने की कोशिश की गयी है।

मैथिलीशरण गुप्त का काव्य जीवन लगभग छः दशकों में विस्तृत है। इन छः दशकों के काव्य जीवन में भाषा एवं शिल्पगत परिवर्तनों के साथ विचारों की समवेशिकता हमें देखने को मिलती है। वे हठधर्मी कवि नहीं थे। वे एक ऐतिहासिक दायित्व और भूमिका का निर्वहन करने के लिए काव्य के क्षेत्र में उतरे थे। विचारणीय है कि वह ऐतिहासिक दायित्व क्या था? वह कौन सी भूमिका उन्होंने स्वीकार की थी? उनके अवदान का ऐतिहासिक महत्त्व है। उनकी काव्य रचना का तात्कालिक उद्देश्य भी कम महत्त्वपूर्ण नहीं है। रचनाकार की ऐतिहासिक भूमिका और तात्कालिक उद्देश्य को नजरअंदाज करनेवाली आलोचना दृष्टि को कवि की काव्य सृष्टि में अनेक न्यूनताएँ दिख सकती हैं। उन न्यूनताओं को कवि की हेठी नहीं माननी

चाहिए, बल्कि उसके अंतर्विरोधों को समझने की कोशिश करनी चाहिए। गुप्त जी अपने काव्य जीवन की प्रस्तावना व्यक्त करते हुए अपनी अक्षय कीर्ति की आधार कृति 'भारत-भारती' में लिखते हैं "सिर्फ मनोरंजन न कवि का कर्म होना चाहिए, उसमें निहित उपदेश का भी मर्म होना चाहिए"।

सर सैयद अहमद के अनुरोध पर अल्लाफ हुसैन हाली ने 'मुसद्दस' लिखा था। राजा रामपाल सिंह के 'मुसद्दस' के ढंग की एक कविता पुस्तक हिन्दुओं के लिए लिखने के अनुरोध पर 'भारत-भारती' (1912) की रचना करते हुए गुप्त जी भूमिका में अपने दायित्व का उल्लेख करते हैं "संसार में ऐसा काम नहीं जो सचमुच उद्योग से सिद्ध न हो सके। परन्तु उद्योग के लिए उत्साह कहीं आवश्यकता है। बिना उत्साह के उद्योग नहीं हो सकता। इसी उत्साह को, इसी मानसिक वेग को उत्तेजित करने के लिए कविता उत्तम साधन है।" इस प्रकार कविता उनके लिए एक साधन है, साध्य नहीं है। 'भारत-भारती' की रचना गुलाम भारत को जगाने का एक साधन थी, जिसके माध्यम से वे सिर्फ अतीत का गौरव गान करने के लिए ही काव्य क्षेत्र में नहीं उतरे थे, अपितु वर्तमान की सामाजिक, सांस्कृतिक एवं आर्थिक दुरवस्था को कड़ी आलोचना करना उनके काव्य का प्रमुख हेतु था। जो रचनाकार अपने वर्तमान की आलोचना का साहस करता है, वही भविष्य के लिए हमें सन्देश भी दे सकता है।

उनकी रचनाओं से गुजरते हुए सहज ही अनुभूति होती है कि जैसे वेगौरवशाली भारतीय संस्कृति का महाकाव्यात्मक इतिहास प्रस्तुत कर रहे हैं। अपनी काव्य चेतना के सहारे उन्होंने अतीत के साथ एक संवाद स्थापित किया है। अतीत के साथ उनका यह संवाद एकतरफा नहीं, दो तरफा है, जिसे समझने की जरूरत है। उनके युग बोध में प्राचीनता और नवीनता का अद्भुत सामंजस्य है। उनकी कविता में देश के गौरव, अतीत की महिमा, ब्रिटिश राज्य की प्रशंसा, अंग्रेजी दासता की दोनता, स्वातंत्र्य आन्दोलन, किसानों की दयनीय दशा, जातिवाद का दंश, क्षेत्रीयतावाद, हरिजन समस्या, पारचात्य प्रभाव, समाज सुधार, नारी उद्धार, राज भक्ति आदि के जो संदर्भ आते हैं, वे एक बड़े कवि के विजन की दशाते हैं। ध्यान देने योग्य है कि उनकी कविताएँ नवजागरण की

कैम्ब्रिज में जन्म। बीएचयू से एमए तथा जेएनयू से एमफिल एवं पीएचडी। कविता संग्रह 'रिग्वेदी मर समय', सम्पादित पुस्तक 'भाषा और ह्रस्व' प्रकाशित। नवोदित लेखक सम्मान, डॉ. अजना खलबाला कविता सम्मान-2012 से पुरस्कृत। संप्रति- गुजरात केंद्रीय विश्वविद्यालय, गांधीनगर में ऑनरिस्ट प्रोफेसर। सम्पर्क : pranavod@vng.ac.in



डॉ. प्रमोद कुमार तिवारी

स्मरण

जयंती पर विशेष

भारत भूषण मदन मोहन मालवीय

काशी हिन्दू विश्वविद्यालय में प्रत्येक रविवार को पं. मदन मोहन मालवीय गीता प्रवचन का एक कार्यक्रम रखावे थे और नियमित रूप से स्वयं भी उसमें शामिल होते थे। 18 अगस्त 1940 को उनकी तबीयत ठीक नहीं थी। लगभग 80 वर्ष की उम्र और ठीक पिछले दिन के कार्यों के कारण पेट और जंघ में तेज दर्द भी था पर वह गीता प्रवचन में जाने को तैयार हो गए। उनके सहयोगी पंडित राधाकान्त ने कहा आज मत जाइए छुट्टी का दिन है चार ही लड़के तो आए होंगे इस पर मालवीय जी ने जरा तीब्र स्वर में कहा "तो पंचवा में ही जाऊंगा।"



बहुत ही भावुक और विनम्र माने जाने वाले मालवीय जी की दृढ़ता किस स्तर की थी यह उपरोक्त घटना से पता चलता है। साथ ही यह घटना इस बात की ओर भी संकेत करती है कि उस समय के देश के कई बड़े नेताओं के लिए गीता किन्तनी महत्वपूर्ण थी। गांधीजी भी गीता को महत्वपूर्ण ग्रंथ मानते थे और बाल गंगाधर तिलक ने तो गीता रहस्य नामक पुस्तक ही लिखी थी। इसके साथ ही यह घटना एक और महत्वपूर्ण बात की ओर संकेत करती है कि हमें धर्म और संप्रदाय में फर्क करना चाहिए। पश्चिम की आधुनिकता चर्च और रिलिजन के विरोध में पैदा हुई थी, चर्च के कारण वहां अधिकार गुप्त चल रहा था जिससे मुक्ति के लिए रेनेसां और जागरण की बात शुरू हुई थी, जबकि उसी समय भारत के महान कवि मक्ति कविता लिख रहे थे और सांस्कृतिक उत्कृष्टता के प्रतिमान रच रहे थे। जिस भारत को टुकड़ों में बंटा हुआ सैकड़ों राजाओं का देश कहा जाता था, वहां अखिल भारतीय स्तर पर मक्ति आंदोलन को तरह चल रहा था और हर क्षेत्र में, हर भाषा में नये तरह की कविताएँ लिखी जा रही थीं। पश्चिम की तरह वह समय भारत का अधिकार गुप्त नहीं था, बल्कि

10वीं सदी के बाद से ही सिद्ध कवि, जैन कवि और अहमदाबाद जैसे कवि भाव, भाषा और विचार के धरातल पर नये भावबोध की कविताएँ रच रहे थे। विद्यापति भी नये तैवर की कविताएँ लिख रहे थे।

इसी सांस्कृतिक समझ को लेकर महात्मा मदन मोहन मालवीय ने दशा आरंभ की और लंबे समय तक भारतीय समाज की दशा और दिशा को निर्धारित करने में महत्वपूर्ण भूमिका निभायी। 25 दिसंबर, 1861 को प्रधानराज (इलाहाबाद) में पंडित वृजनाथ और पुना देवी के घर मदन मोहन मालवीय का जन्म हुआ था। मालवीय जी के पूर्वज मध्यप्रदेश के मालवा क्षेत्र के निवासी थे। माना जाता है कि पंद्रहवीं शताब्दी के मध्य में वे उत्तर प्रदेश चले आए। मालवा का होने के कारण वे लोग 'मल्लई' कहलाते थे, जो बाद में 'मालवीय' हो गया। परिवार में धर्म और संस्कृत भाषा का अच्छा माहौल था। उनके चाचा पं. गदाधर मालवीय संस्कृत के भाषी विद्वान थे। मदन मोहन मालवीय ने पांच वर्ष की उम्र से ही संस्कृत की शिक्षा ग्रहण करनी शुरू कर दी थी। प्राथमिक शिक्षा जहां शुरू हुई उस विद्यालय का नाम था 'धर्मज्ञानोपदेश पाठशाला'। इस विद्यालय में संस्कृत ज्ञान और शारीरिक संवर्धन पर बल दिया जाता था। विद्यालय प्रमुख पं. हरदेव जी संगीत प्रेमी थे और पाठशाला में कुस्ती भी लड़वाते थे। आगे चलकर मालवीय जी ने कारागी हिन्दू, विश्वविद्यालय में भी इन बातों का ध्यान रखा, संगीत और व्यायाम को प्रमुखता दी। सुगठित शरीर वाले छात्रों को देखकर वे बहुत प्रसन्न होते थे। बहुत कम उम्र में मालवीय जी ने संस्कृत और अंग्रेजी पर अच्छा अधिकार हासिल कर लिया। हिन्दी के प्रति उनका लगाव इतना अधिक था कि उसके लिए अनेक स्तरों पर आंदोलन का नेतृत्व किया। मात्र 16 वर्ष की आयु में भिर्नापुर में आयोजित एक 'धर्मसभा' में इन्होंने भाषणा देने का अवसर मिला और पहला ही

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Gauging the Social Media Attention of COVID-19 Articles

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A B S T R A C T

Introduction: Altmetrics or alternative metrics has become a novel technique for measuring the impact of the research. It considers social web as a major source of data and measures how a piece of literature has discussed in various social platforms in terms of shares, mentions, bookmarks, tweets, saves, etc. Rather than measuring the scientific impact, it gauges the social impact of the research which can not be done by the conventional way which primarily consisted of counting the citations bagged by an article.

Importance: The deadly pandemic COVID-19 has travelled in social media very fast and subsequently fake information has become a headache. The main impetus for conducting the present study is to know how well articles on COVID-19 have propagated and discussed in social platforms since the disease is unknown to many and social media has become a major carrier of fake information which tempted many to make wrong decisions. We decided to conduct the present study to know how well COVID-19 articles are diffused in social platforms and to find out the hot platform for the discussion. The result of the study can be used for taking proper decisions regarding the management of various social platforms amid this kind of epizootic pandemic.

Objectives: The main objective of the study is to measure the social media attention/ altmetrics of COVID-19 articles.

Methodology: The data for the study would be collected from Dimension database which is a dedicated database for altmetric studies. A search by using the keyword "COVID-19" would be carried out in the database to retrieve articles on the pandemic as on 01 Dec 2020. The articles would be sorted according to the number of social media attention received from highest to lowest. A total of 25 articles with the highest social media attention would be selected and their major metrics from Facebook, Twitter, Mendeley, Blogs, News outlets would be measured and tabled using Excel for the subsequent analysis. The data would be subjected to correlation to know the associations between citations and altmetric attention score in the case of COVID-19 articles.

Keywords: Altmetrics, Tweets, Citations, Social Media, COVID-19, Corona

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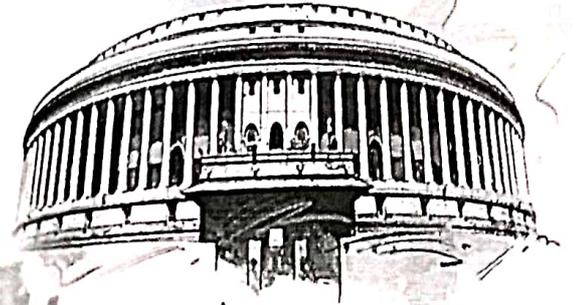
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विधान सभा



ग्राम सभा

पंचायत से पार्लियामेण्ट तक



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गुजरात में पंचायती राज की शुरुआत से वर्तमान तक की व्यापक समीक्षा



प्रो. राजेश मकवाना

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Literature, Marginal Literature, Folklore.

भूमिका

पंचायतीराज यानी राजकीय सत्ता का जनमत के अनुसार विकेंद्रीकरण करती व्यवस्था है। उसका प्रारूप त्रिस्तरीय है। ग्राग पंचायत, तालुका पंचायत और जिला पंचायत इन तीनों व्यवस्था में ग्राम पंचायत उनके मूल में है। मध्य में तालुका पंचायत और उच्च स्थान पर जिला पंचायत है।

भारत देश गांव से बना है एवं भारत की आत्मा गांवों में बसती है। यदि देश का विकास करना है तो गांवों को समृद्ध बनाना ही पड़ेगा। इसलिए गांधीजी जी ने ग्रामोद्धार के लिए विशेष ध्यान केंद्रित किया था।

गुजरात में सर्वप्रथम सन् 1963 में पंचायतीराज का पदार्पण हुआ। उसके बाद अनेक समितियों के सुझावों पर अमल किया गया। गुजरात में पंचायतीराज की शुरुआत बलवंतराय मेहता के अध्यक्ष समूह की लोकशाही विकेंद्रीकरण अर्थात् पंचायतीराज के सुझावों से अमल में है। तत्पश्चात् गुजरात में पंचायतीराज की व्यवस्था निरंतर सुदृढ़ होती गई है। भारतीय संविधान में 73 वें संशोधन के बाद गुजरात में पंचायत अधिनियम 1993 अमल में आया। गुजरात राज्य में राज्य सरकार द्वारा पंचायतीराज में ग्राम विकास एवं लोक कल्याणकारी योजनाओं का शुभारंभ हुआ। इसमें गैर-सरकारी संगठनों का सहयोग भी प्रशंसनीय है।

पंचायतीराज की परिभाषा और इसका इतिहास

पंचायत शब्द को दो भागों में बांटा जा सकता है। पंच और आयत। पंच शब्द का प्रयोग संस्कृत और गुजराती में भी संख्या पंच के अर्थ में किया जाता है 'पंच वहाँ परमेश्वर' की पौराणिक कहावत में आधार है। वैदिक काल से गांव को एक बुनियादी इकाई के रूप में माना गया है। इसका उल्लेख अथर्ववेद और ऋग्वेद में भी मिलता है। माना जाता है कि गंगा और यमुना नदियों के बीच लोक बस्ती के दौरान पृथु राजा ने पंचायत प्रणाली की शुरुआत की थी। ब्रिटिश शासन के दौरान पंचायतों की स्थापना के लिए आधे-अधूरे प्रयास किए गए। 1908 में नियुक्त रॉयल कमिशन ऑन विकेंद्रीकरण ने अपनी रिपोर्ट में कहा कि गांवों को पंचायतों द्वारा स्वायत्तता प्राप्त थी।



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बलवंतराय मेहता समिति

ग्रामीण विकास को गति देने और इसमें लोगों की भागीदारी सुनिश्चित करने के लिए 1952 में सामुदायिक विकास कार्यक्रम की शुरुआत की गई। इस कार्यक्रम के सहयोग के लिए 1953 में राष्ट्रीय विस्तार सेवा की शुरुआत की गई। जनवरी 1957 में भारत सरकार ने इसी सामुदायिक विकास कार्यक्रम 1952 तथा राष्ट्रीय विस्तार सेवा 1953 द्वारा किए गए कार्यों की जांच और उनके बेहतर ढंग से कार्य करने के लिए उपाय सुझाने के लिए एक समिति का गठन किया। इस समिति के अध्यक्ष बलवंत राय मेहता थे। समिति ने नवम्बर 1957 को अपनी रिपोर्ट सौंपी और लोकतांत्रिक विकेंद्रीकरण की योजना की सिफारिश की जो की अंतिम रूप से पंचायतीराज के रूप में जाना गया।

समिति द्वारा दी गई विशिष्ट सिफारिशें

1. तीन स्तरीय पंचायतीराज पद्धति की स्थापना-गांव स्तर पर ग्राम पंचायत, ब्लॉक स्तर पर पंचायत समिति और जिला स्तर पर जिला परिषद।
2. ग्राम पंचायत की स्थापना प्रत्यक्ष रूप से चुने प्रतिनिधियों द्वारा होना चाहिए, जबकि पंचायत समिति और जिला परिषद का गठन अप्रत्यक्ष रूप से चुने सदस्यों द्वारा होनी चाहिए।
3. सभी योजना और विकास के कार्य इन निकायों को सौंपे जाने चाहिए तथा इन निकायों को पर्याप्त स्रोत मिलने चाहिए ताकि ये अपने कार्यों और जिम्मेदारियों को संपादित करने में समर्थ हो सकें।
4. पंचायत समिति कार्यकारी निकाय तथा जिला परिषद सलाहकारी, समन्वयकारी और पर्यवेक्षण निकाय होना चाहिए।
5. जिला परिषद का अध्यक्ष, जिलाधिकारी होना चाहिए।

गुजरात के पंचायतीराज का संक्षिप्त इतिहास

सन् 1 मई 1960 को एक अलग राज्य के रूप में स्थापित किया गया था,

तब पंचायतीराज के कार्यान्वयन के लिए एक महत्वपूर्ण पृष्ठभूमि का गठन किया गया था। गुजरात राज्य के स्वतंत्र अस्तित्व में आने के दो महीने के भीतर एक लोकतांत्रिक विकेंद्रीकरण समिति द्वारा पंचायतीराज की व्यवस्था या संरचना की सिफारिश की गई और इस तरह से पूरे राज्य में पंचायतीराज की शुरुआत 1 अप्रैल 1963 से की गई। गुजरात में पंचायतीराज के लागू होने से पहले, जिला स्थायी बोर्ड, जिला स्कूल बोर्ड और धातक सलाहकार समितियां ब्रिटिश शासन के पुराने क्षेत्रों और कुछ देशी राज्यों में काम कर रही थीं। इसके इलाके अपने राज्य बड़ोदरा में 'प्रातीय पंचायतें' थीं। पुराने स्थानीय बोर्ड में भी ग्रामलों में मौजूदा जिला पंचायतें थीं जिनमें उस समय और भी विभिन्न शक्ति निहित थी। मनरेगा के अंतर्गत अपने गांव के समीप ही रोजगार प्राप्त हो रहा है। जिससे ग्राम जनों को रोजगार के लिए दूर शहरों में पंचायत नहीं करना पड़ रहा है। गांव में ही रोजगार उपलब्ध होने से गांवों में गांधीजी जीका गांवों को सक्षम करने का स्वप्न साकार होगा। इससे गांवों में ग्रामीण गरीब प्रजा में नए प्राणों का संचार कर उन्हें नई चेतना प्रदान की है। राष्ट्रपिता गांधीजी सदा 'ग्राम स्वराज' की बात करते थे और मनरेगा भी महात्मा गांधीजी के स्वप्न को साकार करती योजना होने के कारण 'ग्राम स्वराज' योजना है इसलिए अब 'मनरेगा' के नाम से पहचानी जाती है।

छोटे व्यवसायों को विकसित करने के लिए सभी जिलों में एक कदम उठाना जिला पंचायत का कर्तव्य है। विशेष रूप से प्राथमिक आपदाओं जैसे सूखा, बाढ़, आपदा, भूकंप आदि के समय जिला पंचायतों को यह दायित्व है कि वह आपदा पीड़ितों को सहायता प्रदान करे, उनके जीवन के राहत कोष के माध्यम से सुरक्षा प्रदान करे और गांवों का पुनर्निर्माण कराये। इसके अलावा, वर्तमान में केंद्र सरकार की पंचवर्षीय योजना के लगभग सभी कार्यों को गुजरात राज्य में पंचायतों को सौंप दिया गया है। साथ ही साथ, वार्षिक बजट बनाना, संस्कृति पर आकड़े और विभिन्न कार्यक्रम प्रस्तुत करना। एक महत्वपूर्ण कार्य पंचायतों के कौशल को बढ़ाने का है। राधा राभव कोशिश करना भी जिला पंचायत के अधिकार क्षेत्र में है। जिला पंचायत सेवा के कर्मचारियों के अधिकारों और हितों को बनाए रखना और उन्हें कर्तव्यों का पालन करने और उन्हें विनियमित करने के लिए जिला पंचायत जिम्मेदार है। गुजरात देश का इकलौता राज्य है, जहां नियमित रूप से राज्यव्यापी पंचायत चुनाव होते हैं। पंचायत की अवधि 5 साल की है। असाधारण परिस्थितियों (जैसे सूखे या अन्य प्रतिकूल परिस्थितियों) में एक वर्ष के लिए बढ़ा दी जाती है क्योंकि यह राज्य सरकार का प्राथमिक अधिकार है। गुजरात राज्य में दलित समाज और कमजोर वर्गों के हितों के लिये सामाजिक न्याय समितियों का गठन किया गया है। गुजरात राज्य में सामाजिक न्याय समितियों को बड़ी शक्तियां दी गई हैं।

गुजरात में पंचायतीराज और ग्राम स्वराज

भारत दुनिया का सबसे बड़ा लोकतंत्र है। दुनिया के सभी देशों में सबसे बड़ी आबादी और विविधता के एक बड़े क्षेत्र के साथ लोकतंत्र की शक्ति के लिए पिछड़े लोगों के सामने आने वाली समस्याओं को समाधान निकालना और महसूस करना और निदान कर पाना काफी मुश्किल है।

हमारा देश गांवों में बसता है, राष्ट्रपिता महात्मा गांधीजी ने कहा करते थे कि 'देश के गांवों के लिए खतरा भारत के लिए खतरा है'। उन्होंने मजबूत गांवों का सपना देखा था जो भविष्य के भारत की रीढ़ बन सकेंगे। उन्होंने स्वराज गांव का विचार देते हुए कहा कि 'पंचायतीराज' का अर्थ अधिकार होने चाहिए। गांधीजी के इस सपने को पूरा करने के लिए लोकतंत्र की शक्ति का विकेंद्रीकरण करने का निर्णय लिया गया।

1960 में गुजरात राज्य की स्थापना के बाद, पंचायतीराज को सरकारी आधार पर लागू किया गया था। इसलिए पंचायतीराज के इस व्यवस्था के चलते वर्तमान में ग्रामपंचायतों से कार्यपालिका तक सभी स्तरों में पहुंच रहा है। हालांकि, ग्रामीण क्षेत्रों को शासन में शामिल करने के लिए भागीदारी बनाने की पारंपरिक प्रणाली भी बहुत महत्वपूर्ण है। पंचायत न

केवल भारत में बल्कि पाकिस्तान, बांग्लादेश, नेपाल और श्रीलंका जैसे दक्षिण एशियाई देशों में भी विद्यमान हैं।

1947 में देश के स्वतंत्रता के पश्चात, जवाहरलाल नेहरू ने भी एक लोकतांत्रिक देश में शासन के विकेंद्रीकरण के लिए पंचायतीराज के महत्व पर जोर दिया था। भारत में पहली पंचायतीराज व्यवस्था 2 अक्टूबर 1959 को राजस्थान के नागौर जिले में लागू की गई थी। इसमें पंचायतीराज व्यवस्था पर बलवंतराय मेहता समिति द्वारा की गई अधिकांश सिफारिशें शामिल थीं। हालांकि, राजस्थान के बाद गुजरात, महाराष्ट्र और आंध्र प्रदेश में पंचायतीराज को धीरे-धीरे लागू किया गया।

समय-समय पर विशेषज्ञों की समितियां भी गठित की गईं ताकि पंचायतीराज में सुधार कर उसे मजबूत किया जा सके और सरकार को इसकी सिफारिशें दी जा सकें। अप्रैल 1993 को संविधान में 73वें संशोधन के तहत पंचायतीराज संस्थाओं को संवैधानिक दर्जा दिया गया। हमारी सामाजिक और आर्थिक गतिविधियों में गांवों का हमेशा से ही महत्व रहा है। गांव प्राचीन वक्त से एक इकाई रहा है। आज भी देश की अधिकांश जनसंख्या ग्रामीण क्षेत्रों में निवास करती है। महात्मा गांधीजी ने गांव को ग्रामस्वराज की एक इकाई के रूप में बताते हुए कहा था कि ग्रामस्वराज एक ऐसा गणतंत्र है जो अपने पड़ोसियों से पूरी तरह स्वतंत्र है लेकिन अपनी विशाल सामंजस्यताओं के लिए एक-दूसरे पर निर्भर है।

पंचायतीराज एवं लोकतांत्रिक विकेंद्रीकरण

गुजरात में पंचायतीराज और लोकतांत्रिक विकेंद्रीकरण की मूल बातें लागू होने के बाद पंचायतीराज को ग्रामीण विकास का एक महत्वपूर्ण साधन बनाने के लिए लगातार प्रयास किये जा रहे हैं। ग्राम विकास कार्यक्रम के कार्यान्वयन में प्रागिन तंत्र को विकासोन्मुख प्रशासन के रूप में जाना जाता है। विकास के क्रियान्वयन के साथ-साथ सरकार द्वारा चलाए जा रहे सामाजिक, आर्थिक परिवर्तन कार्यक्रम में विकासोन्मुख प्रशासन में पंचायतीराज का महत्वपूर्ण योगदान है।

गुजरात में त्रिस्तरीय पंचायतीराज संस्थाओं का कार्यान्वयन 01/04/12 से जारी है। गुजरात सरकार ने 18वें अधिनियम के माध्यम से इसे लागू करना शुरू कर दिया है। निम्नकी विशिष्ट विशेषताएं इस प्रकार हैं

1. गांव/तालुका स्तर पर स्वायत्त वैधानिक निकायों के निर्माण के समर्थन लागू।
2. पंचायतों के निर्वाचन चुनाव और पांच साल की अवधि के बाद अनिवार्य चुनाव। निर्वाचन प्रणाली चुने हुए प्रतिनिध की मृत्यु हो जाती है या वह पदमुक्त हो जाता है एवम स्थिति में चुनाव 6 महीने के अंदर करा लेने चाहिए (इसके अलावा राष्ट्रीय चुनाव आयोग की होगी)।
3. पंचायतों के परामर्श वित्तीय पबंधन के लिए वित्त आयोग का गठन।
4. पंचायत संस्थाओं एवं सरपंच/अध्यक्षों की कुल सीटों का महिलाओं के लिए आरक्षण अधि का प्रावधान और साथ-साथ आवंटन।
5. पंचायतों में कुल संख्या के अनुपात के आधार पर अनुसूचित जाति और अनुसूचित जाति समुदायों के लिए सीटें आरक्षित हैं और अन्य पिछड़ा वर्ग (अन्य पिछड़ा वर्ग) के लिए 10% सीटें आरक्षित करने का प्रावधान भी किया गया है।

गुजरात के पंचायतीराज की विशिष्ट विशेषताएं

1. जिला स्तर पर ग्राम कार्यकारी निकाय का गठन।
2. जिला स्तर पर शिक्षा समितियों को विशेष दर्जा देना तथा पिछड़े वर्गों के गठन के लिए ग्राम/तालुका/जिला स्तर पर विशेष दर्जा सामाजिक न्याय समितियों का गठन करना।

3. पंचायतों को प्रभाविता करने वाली नीति से संबंधित विभिन्न भागलों पर सरकार को सलाह देने के लिए राज्य पंचायत परिषद के संवैध में वैधानिक प्रावधान।
4. जिले के भीतर विकास के क्षेत्र में शक्तियों, कार्यों और कर्तव्यों का उचित हस्तांतरण।
5. गतिविधियों के हस्तांतरण के साथ-साथ पंचायतों को विवेकाधीन गतिविधियों के लिए कर-शुल्क और भू-राजस्व पर योगदान लगाने की शक्ति।
6. संपूर्ण विकास प्रणाली को ग्राम स्तर से जिला स्तर तक पंचायतीराज संस्थाओं को हस्तांतरित करना।
7. सत्ता के आगे हस्तांतरण और प्राधिकरण के संवैधानिक रूपों के लिए एक आंतरिक योजना एवं कुछ नियंत्रित राजस्व कार्यों को पंचायतीराज संस्थाओं को हस्तांतरित करना और इसके लिए आवश्यक शक्तियों का हस्तांतरण भी करना।
8. जिला पंचायतों के संबंध में मुख्य कार्यकारी अधिकारियों के रूप में कार्य करने के लिए कम्प्यूटर के पद के साथ जिला विकास अधिकारियों की सेवाएं प्रदान करने के लिए, तालुका विकास अधिकारियों को तालुका पंचायत सचिव के रूप में होना।
9. राज्य स्तर पर सेवा चयन बोर्ड और जिला स्तर पर जिला चयन समिति का गठन शर्ती एवं नौकरी सलाह के लिए राज्य राजस्व के संग्रहण।
10. पंचायतीराज संस्थाओं को शत-प्रतिशत भू-राजस्व के साथ एव कार्यों का साविधिक आवंटन।
11. कमजोर क्षेत्रों की मदद करने, कर चोरी के प्रयासों को रोकित विकास गतिविधियों को बढ़ावा देने के लिए एक गैर-रक्षीय प्रणाली का सुझाव।

पंचायतीराज व्यवस्था में प्रतियोगी आयोजन

गुजरात राज्य में सर्वश्रेष्ठ ग्राम पंचायतों के लिए प्रतियोगी प्रणाली आयोजन इस दृष्टि से किया जा रहा है कि पंचायतीराज व्यवस्था को प्रभावी रूप से चले और पंचायतों स्वस्थ रूप से प्रतिस्पर्धा करें और एकात्मिक विकास की पूरक बनें, पंचायतों का प्रशासन बेहतर हो। गुजरात राज्य के पंचायतों को भी सर्वश्रेष्ठ जिला पंचायतों के रूप में सम्मानित किया जाये। गुजरात में जिला पंचायतों के अलावा, गुजरात राज्य को जिला स्तर पर एक अकादमी द्वारा गठित गुजरात पंचायत परिषद जैसे संगठनों द्वारा प्रयोग करके का सम्मान दिया जाता है। इस प्रकार गुजरात में पंचायतीराज से जुड़े पदाधिकारियों के जिम्मे को संगठित रूप पंचायतीराज से जुड़े मुद्दों को लेकर गुजरात में एक विशेष सर्कुलर बनाया गया है। जिले के लेखक और प्रशासनिक समस्याओं को सुलझाने के साथ-साथ सरकार का ध्यान केंद्रित करना और उनका निवारण करना प्रमुख प्राथमिकता

निष्कर्ष

इस प्रकार हम कह सकते हैं कि गुजरात राज्य में पंचायतीराज महत्व रावसे अधिक है। पंचायतीराज को दी गई शक्तियों, संसाधनों और प्रशासना के कारण, पंचायतों में जनश्रद्धा आत्मनिर्वास विकसित हुआ है। ग्राम



पंचायतों के अलावा, गुजरात में तालुका स्तर पर जिला पंचायतों के महत्व को स्वीकार किया गया है। गुजरात में पंचायतीराज को पूरी राशि पंचायतों को दी जाती है। पंचायतीराज की प्रतिस्पर्धा के लिए अस्तित्व को पंचायत में पहले ही प्रयोग किया जा चुका है। गुजरात में आने वाले गुजरात के दूसरे बार को के रूप में, गुजरात में महता ने कार्य किया था। लोकमान्य तिलक के विचारों को प्रयोग करने के लिए उन्हें 'पंचायतीराज के शिल्प' के माना जाता है। अक्सर कहा जाता है कि महता एवं गांधीजी के सपनों को साकार करके गुजरात में पंचायतीराज व्यवस्था निरंतर वारतंत्रिता को प्रयोग कर सका है।

संदर्भ सूची

- तालुका स्तर पर 2015 में आयोजित 'गोल्ड इनबॉक्स' प्रकाशन, गुजरात सरकार।
- अकादमी पंचायतीराज, गुजरात, 'पंचायतीराज', युवा उपनिषद प्रकाशन, अहमदाबाद।
- 'गोल्ड इनबॉक्स' प्रकाशन, 'गुजरात का पंचायतीराज वारसो' भाविक मारु प्रकाशन, अहमदाबाद।
- 'गोल्ड इनबॉक्स' प्रकाशन, 'गोल्ड इनबॉक्स' अने प्रशासन', अक्षर प्रकाशन, अहमदाबाद।

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વિશેષ

ગુજરાતી કવિતાની તાસકમાં બનારસી પાનનું બીડું : બનારસ ડાયરી

અજયસિંહ ચૌહાણ

ગુજરાતના સાહિત્ય જગતમાં જે સમાચારની ઘણા વર્ષોથી રાહ જોવાતી હતી એ આખરે હમણાં મળ્યા. સાહિત્ય અકાદમી, દિલ્હીનો બે હજાર વીશનો વાર્ષિક પુરસ્કાર આપણી ભાષાના વિશિષ્ટ કવિ હરીશ મીનાશ્રુના બનારસ ડાયરી (પ્ર.આ. ૨૦૧૬, પ્રકાશક : ગૂર્જર સાહિત્ય પ્રકાશન, અમદાવાદ)ને જાહેર થયો. હરીશ મીનાશ્રુ અનુઆધુનિક સમયના મહત્વના કવિ તો છે સાથેસાથે ગુજરાતી કવિતા પ્રવાહના વિશિષ્ટ રમણીયતા આપનાર સંપ્રજ્ઞ કવિ છે. તેમને ગુજરાતના મહત્વના પુરસ્કારો, જેવા કે નરસિંહ મહેતા એવોર્ડ, વલી ગુજરાતી ગઝલ એવોર્ડ, નિરંજન ભગત મેમોરિયલ એવોર્ડ, ભારતીય કક્ષાએ મહત્વ ધરાવતું કુસુમાંજલિ સાહિત્ય સન્માન (ગુજરાતી) ઉપરાંત સાહિત્ય અકાદમીનું અનુવાદ પ્રાઈઝ જેવાં અનેક મહત્વનાં સન્માન પ્રાપ્ત થયાં છે. તા. ૦૩/૦૧/૧૯૫૩ના રોજ આણંદ મુકામે જન્મેલા હરીશ કૃષ્ણારામ દવે એટલે કે હરીશ મીનાશ્રુ પાસેથી, ઈ.સ. ૧૯૮૮માં પ્રકટ થયેલાં 'ધ્રિભાંગસુંદર એણીપેર ડોલ્યા'-થી શરૂ કરી 'સુનો ભાઈ સાધો', 'તાંબૂલ', 'તાંદુલ', 'પર્જન્યસૂક્ત', 'પદપ્રાંજલિ', 'શબદમાં જિનકું ખાસ ખબરાં પડી', 'પંખીપદારથ', 'નાચિકેતસૂત્ર' અને 'બનારસ ડાયરી' એમ દસકાવ્યસંગ્રહો મળે છે. એ દસે કાવ્યસંગ્રહોમાં કવિ નોખી નોખી રીતેભાતે વ્યક્ત થાય છે. પ્રબળ કવિ પ્રતિભા વગર આવું રસનિષ્ઠ સાતત્ય જાળવવું શક્ય નથી. ગીત, ગઝલ, અછાંદસ, ગદ્યકાવ્ય, છંદોબદ્ધ રચનાઓ એમ કવિતાનાં અનેક સ્વરૂપોમાં એક સમાન સામર્થ્યથી વિહરતા આ કવિનું પ્રદાન કાવ્યભાવકો અને કાવ્ય મર્મજ્ઞો-ઉભયને પ્રસન્નકર રહ્યું છે. હરીશ મીનાશ્રુ કાવ્યસર્જન ક્ષેત્રે પ્રવૃત્ત થાય છે એ સમય આધુનિકતાનો હતો. આધુનિક કવિઓની વિદ્રોહભરી ભાષા-અભિવ્યક્તિ રીતિઓની સર્વત્ર ચર્ચાઓ હતી. એ બધાની વચ્ચે 'ધ્રિભાંગ સુંદર...'થી એમણે પોતાની એક નોખી મુદ્રા અંકિત કરી અને પછી સતત નિજલીલાપૂર્વક એમનો શબ્દ વિલસતો ગયો. એમની કવિતામાંથી પસાર થતાં ત્રણ બાબતો તારવી શકાય.

૧. શરૂઆતની 'ધ્રિભાંગ સુંદર...' જેવી રચનાઓમાં વ્યક્તિની અભ્યંતર રહેલા વિભાજિત વ્યક્તિત્વ અને સમકાલીન સાહિત્ય પરિવેશ સહિત સ્વયંનું વિડંબન કરતી કવિતા.

૨. 'સુનો ભાઈ સાધો', 'તાંદુલ', 'તાંબુલ', 'પદપ્રાંજલિ', 'શબદમાં જિનકું ખાસ ખબરાં પડી', ને 'બનારસ ડાયરી' જેવા સંચયોમાં સંતમત અને મધ્યપૂર્વના ફકીરોની દાર્શનિકતા, કબીરની પ્રેમ લક્ષણાથી સભર જ્ઞાનમીમાંસા અને સાંસારિક અનુભવોથી ચૈતન્ય તરફની અંતરમુખ ગતિ આદિની કવિતા.

૩. પંખીપદારથમાં જીવન, જગત, ગાર્હસ્થ્ય આદિની કલ્પનોથ અભિવ્યક્તિ સમાનરચનાઓ ઉપરાંત સામાજિક સંદર્ભ, સામાજિક નિસબતને સંકેતતી કવિતા.

અભ્યાસની સરળતા માટે ભલે આપણે આ કવિતાઓનું આવું વર્ગીકરણ કરીએ, પણ આ રચનાઓ એકબીજાથી પૃથક અને સ્વ-તંત્ર હોવાની સાથેસાથે પ્રચ્છન્ન પ્રકારનું દાર્શનિક અનુસંધાન પણ રચતી રહે છે એ નોંધવું રહ્યું.

સાહિત્ય અકાદમી પુરસ્કાર સર્જકના એક પુસ્તકને કેન્દ્રમાં રાખી આપવામાં આવે છે પણ એની પાછળનો આશય તો (જૂજ અપવાદોને બાદ કરતાં) મહદંશે પુસ્તકને નિમિત્ત બનાવીને સર્જકની સર્જનશીલતાને પોંખવાનો હોય છે. એ રીતે ‘બનારસ ડાયરી’ એ નિમિત્ત છે હરીશ મીનાશ્રુના સમગ્ર સર્જનને પોંખવાનું. ‘બનારસ ડાયરી’માં એ પ્રાચીન નગરીના સ્થળલક્ષી વિશેષની સાથે સ્થલાતીત બનારસનો ગૂઢ સંદર્ભ ગૂથાતો રહે છે, એની મિથીકલ પ્રાચીન પવિત્રતાની સાથેસાથે અર્વાચીનનાં સુંદર અસુંદર અંશો પણ વણાતા જાય છે. ડાયરી શબ્દથી જાત સાથેના અંતર્સંવાદનું ઈંગિત પણ રચાય છે. સંગ્રહના પ્રથમ કાવ્ય ‘બનારસ ડાયરી-૧’માં રતિકીડા અને કાવ્યકીડાના સંકેતોના અપૂર્વ સાહચર્યથી કવિ ‘સુબહે બનારસ’નું સંકુલ પણ વિસ્મયકારી ચિત્ર આપે છે.

‘કવિના ખડિયાની ગહેરાઈમાં ઘૂસે છે અનંગ/એક કિત્તો તંગ/ ચિત્તની છીપોલીમાં જરામરણનાં ઝેરકોચલા જેવું વાંગમય/ લહીને લોહીનો લય/ બની જાય છે ટઠડઠણ સરીખું કઠણ/તથદધન/ જેવું કામુક મન/ ઈશારા કરે છે મોઘમ, જાણે પફબભમ/ યરલવશ હણહણીને ઉછાળે છે/ આગળના બે પગ કામવશ’

‘બનારસ ડાયરી’ ગુચ્છની રચનાઓમાં કબીર એક પાત્ર લેખે પણ હાજરાહજૂર છે. ગુચ્છની ચોથી રચનાઓ જુઓ :

“એ દિવસે મારો જનમ દિન હતો/ એટલે મારા ચહેરા પર/ વિષાદની વ્યંજનામાં પરિપક્વ બનેલો આનંદ હતો/ મને એટલી તો ખબર હતી કે/ એમનું જનમ વર્ષ ઈસવીસન તેરસો નવ્વાણુ/એટલે સમજોને, લગભગ છસો પ્રકાશવર્ષનું અંતર કાપીને/કબીર ખાસ મને વધામણી ખાવા આવ્યા.”

અહીં છસો વર્ષ પહેલાં થઈ ગયેલા કબીર આજની ક્ષણે પધારે છે તો એ છસો પ્રકાશવર્ષ કાપીને આવે છે. આ વિજ્ઞાનપરક શબ્દથી કવિ રાધાસ્વામી સંતમત નિર્દેશિત અંતરતમ ચૈતન્યદેશ કે જેમાં સત્પુરુષનો વાસ છે, એની ગહનતાનો, એના ચૈતસિક અંતરનો સહજ સંકેત તો કરી જ આપે છે પણ વર્ષ સાથે પ્રકાશ શબ્દ સાંકળીને એને જયોતિર્મયતા સાથે પણ જોડી આપે છે. આ કાવ્યગુચ્છની અગિયારમી રચનામાં મણિકર્ણિકા ઘાટના સ્થળરૂપના વર્ણનની સમાંતરે જીવનમરણના પ્રતીકો સમેટતું મણિકર્ણિકાનું સૂક્ષ્મ રૂપ રચાતું જાય છે. આ લયાન્વિત રચનામાં કર્ણિકા શબ્દની એકાધિક અર્થચ્છાયાઓનો કાવ્યાત્મક વિનિયોગ થયો છે. ‘બનારસ ડાયરી’નાં કાવ્યો જ નહીં, પણ કવિની સમગ્ર કવિતામાંથી પસાર થતાં જે પહેલું પાસું તરત નજરે પડે છે એ એની ભાષા. વાચકના ચિત્તમાં પહેલાં સ્પંદિત થાય છે કવિની ભાષાની રમણીયતા. કેટલાક કવિઓની કવિતામાં પ્રથમ સંવેદન સ્પર્શતું હોય છે અથવા રચનાપ્રયુક્તિ પર પહેલી

નજર ઠરતી હોય છે. પણ હરીશ મીનાશુની કવિતામાં ભાષાક્રીડાનાં રહસ્યો ઉકેલ્યા બાદ સંવેદન અને વ્યંજનાના તંતુઓ ખૂલતા હોય છે. એમની કવિતામાં ભાષાનાં એવાં વિભિન્ન સ્તરો એક કાવ્યમાં આવીને બેસી જતાં હોય છે, છતાં નહીં સાંધો નહીં રેણની જેમ અખિલાઈનો અનુભવ કરાવે છે. એમના 'તાંબુલ' સંગ્રહની પંક્તિઓ જુઓ :

‘અમે ઉઘાડા તાસક જેવા મધ્ય તિક્ત તાંબુલ
ઝડપ બીડું અધિ બલમ પિંજરે મૈના અતિવ્યાકુલ’

અહીં શરૂઆતની અડધી પંક્તિમાં ગુજરાતી પદાવલી પછી સંસ્કૃત તત્સમ શબ્દ પ્રયોગો અને બીજી પંક્તિમાં વ્રજ-હિન્દી એમ ભાષાના ત્રિવિધ સ્તરો હોવા છતાં ક્યાંય કોઈ પદ આગંતુક લાગતું નથી. એજ રીતે બનારસ ગુચ્છના તેરમાં કાવ્યમાં ભાષા-લય, કાવ્ય સ્વરૂપોના અનેક સ્તરો છે ને બનારસના અનેક સંદર્ભો.

સંગ્રહના બીજાં બે કાવ્યગુચ્છો છે ‘ચન્દ્ર વિષે ચાટૂક્તિઓ’ અને ‘કવિતા વિષે ચાટૂક્તિઓ’. ચન્દ્ર વિષે ચાટૂક્તિઓમાં પ્રતિપદાથી પૂનમના ચન્દ્રની વિવિધ કળાઓ સાથે જીવનનાં અનેક રૂપો છે. અહીં ‘કોઈ શિશુના કુમળા રુદનની ઓકળીઓ છે’ તો નરસિંહ અને બળવંતરાય ઠાકોર જેવા પૂર્વસૂરિઓની રચનાઓનાં અવનવાં આલંબનો પણ છે.

‘ન માંગવાનું માગી બેસે છે/ક્યારેક કોઈ કવિ/ ભાષા પહેલાં તો મુંઝાઈ મરે છે/
પછી તરત એને જડી આવે છે/ એ ચન્દ્રપટ્ટને પટાવવાનો એક મિથિકલ નુસખો/ જળ ભરેલી થાળીમાં ઊતરી આવે છે/ ચળકતું ચાંદરણું/ કવિ બની જાય છે પ્રસન્ન/ ને ભાષા ધન્ન’

બાળ રામની ચાંદો માગવાની જીદ અને એ પ્રસંગને વર્ણવતી પૂર્વસૂરિઓની રચનાઓના આલંબને અહીં કવિ અને ભાષાની વચ્ચે નિરંતર ચાલ્યા કરતી મથામણનો સંદર્ભ છે. એજ રીતે ‘બેઠી ખાટે/ ફરિ વણિ બધે/ (ઓહ થેન્ક ગોડ, અહીં નથી પાળવાના લઘુ-ગુરુના કશાય મલાજા) મેડિયો ઓરડામાં/ ને છેલ્લે/ ધબધબ કરતી બબ્બે પગથિયાં સાથે ચડતીકને/ પહોંચી ગઈ અગાસીમાં/ કે અચાનક જડી આવ્યો/ કેટલાય દહાડાથી/ ખોઈ નાખેલો/ ચન્દ્ર/ પડોશનો’

આ કાવ્યમાં બળવંતરાય ઠાકોરના ‘જૂનું પિયરઘર’ના આલંબને એક નવો સંદર્ભ કવિ રચે છે. લઘુગુરુની છૂટ લેતા બ.ક.ઠા. જેવા દિગ્ગજ પૂર્વજની મીઠી મશ્કરી પણ કરી લે છે.

‘કવિતા વિષે ચાટૂક્તિઓ’ની બાર રચનાઓમાં કવિતાકળા, કવિતાની સમજ, કવિતાના કાર્યને કવિ અનેક સંદર્ભોથી વળ ચડાવે છે. ‘કરુણાભર્યા/હાડકાના દાગતરની જેમ/ કવિતા સર્જરી કરે છે/ ને કાળજીપૂર્વક/ બદલે છે દુખિયારી કીડીના ઘૂંટણના સાંધા’ છેલ્લી ચાટૂક્તિમાં ‘તોપના નાળયામાં કવિતા સેવે છે એકાકી ઈંડું’ જેવી પંક્તિ દ્વારા યુદ્ધના કલ્પનની સામે કવિ અને ભંગુર ઈંડાનું કલ્પન મૂકીને જીવસટોસટ વિદ્રોહની ભૂમિકા રચી આપે છે.

ઉપરક્ત કાવ્યગુચ્છો ઉપરાંત ‘બનારસ ડાયરી’માં ‘માણસો : અતડા-મળતાવડા’, ‘પેઢીનામું’, ‘પુત્રવધુને’, ‘ઉડવા વિષે’, ‘ઊઘડવા વિષે’, ‘ફોબિયા’,

પરબ ❖ એપ્રિલ, 2021

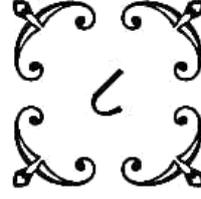
‘વાંધાઅરજી’, ‘શ્રાદ્ધ’ જેવી રચનાઓ છે. જેમાં ‘માણસો : અતડા-મળતાવડા’ને ‘વાંધાઅરજી’ જેવાં કાવ્યોની કથનશૈલી ધ્યાનપાત્ર છે. એજ રીતે ‘પંખીપદારથ’ના ‘ગૃહસ્થસંહિતા’નું અનુસંધાન અહીં ‘પેઢીનામું’ અને ‘પુત્રવધૂને’એ રચનાઓમાં છે. આ બંને રચનાઓમાં કવિ પોતાના કુળ-મૂળ અને પુત્રવધૂ દ્વારા થતાં એના વિસ્તારને કથનની અવનવી શૈલીથી વ્યંજિત કરે છે. ‘પાછોતરા વરસાદ’માં તેમની અગાઉની પર્જન્યસૂક્તની રચનાઓથી ભિન્ન એવું વરસાદનું એક રસાર્દ ચિત્ર ઊભું થાય છે. ‘બનારસ ડાયરી’ની અંતિમ રચના ‘શ્રાદ્ધ’ એ મૂળે તો કવિની સર્જનાધીન મહત્વાકાંક્ષી રચના ‘રત્નાકરભૂષણ પ્રબંધ : એક જાતક-કથા’ નામની સુ-દીર્ઘ રચનાનો એક સ્વતઃસંભવા ખંડ છે, જે સ્વતંત્ર અભ્યાસ ખમે તેવી રચના છે. ‘ત્રિભાંગ સુંદર...’માં જેમ સાહિત્યિક પરિવેશનું વિ-રૂપણ અને વિડંબન છે, એમ આ રચનામાં ધાર્મિક વિધિવિધાનોનું વિ-રૂપણ અને વિડંબન છે. શ્રાદ્ધના ચાલી રહેલાં વિધિવિધાનો અને કવિના ચિત્તમાં ચાલતા ભૂત અને વર્તમાનના સંચલનોની વચ્ચે અનેક સંદર્ભોથી કાવ્ય કવિના અંગત અનુભવને બિનઅંગત બનાવી વિશેષ અર્થ પ્રગટાવે છે. આવી અરુઢ રચનાઓ દ્વારા કવિએ ગુજરાતી કવિતાની તાસકમાં બનારસી પાનનું બીડું ‘બનારસ ડાયરી’ રૂપે આપ્યું છે ને એ પુરસ્કૃત થયું એ કવિનું તો સન્માન છે જ એક રીતે ગુજરાતી કવિતાનું પણ સન્માન છે. કવિને ખૂબ અભિનંદન !



સાભાર સ્વીકાર

પ્રકીર્ણ

સિંહશાસ્ત્ર : ડૉ. સંદીપકુમાર/ડૉ. નિવેદીતા ગાંગુલી, ૨૦૧૯, આર.આર. શેઠ એન્ડ કંપની પ્રા.લિ., મુંબઈ/અમદાવાદ, પૃ. ૨૪૮, રૂ. ૨૯૯. કામલતા : મુનિ શીલગુણવિજય, ૨૦૧૯, આર.આર. શેઠ એન્ડ કંપની પ્રા.લિ. મુંબઈ/અમદાવાદ, પૃ. ૧૬૭, રૂ. ૧૭૫. સમય સાથે Selfie : અંકીત ત્રિવેદી, ૨૦૧૯, આર.આર. શેઠ એન્ડ કંપની પ્રા.લિ. મુંબઈ/અમદાવાદ, પૃ. ૧૧૨, રૂ. ૧૫૦. ઈશ્વરે આપેલી ભેટ : અંકીત ત્રિવેદી, ૨૦૧૯, આર.આર. શેઠ એન્ડ કંપની પ્રા.લિ. મુંબઈ/અમદાવાદ, પૃ. ૧૧૬, રૂ. ૧૫૦. અંતરદીપ : ગણપત ઉપાધ્યાય, ૨૦૧૯, ગરવી વિઝન, અમરેલી, પૃ. ૧૨૮, રૂ. ૧૫૦.



ગુજરાતમાં ગોદડિયા પંથ : પરિચય અને સાહિત્ય

ડૉ. રાજેશ મકવાણા

ભારતમાં ગોદડિયા પરંપરા ઘણી પ્રાચીન છે. ભારત સરકાર દ્વારા તૈયાર થયેલા ગેઝેટિયરોમાં પણ ગોદડિયા સંતોની જીવનપરંપરાના નિર્દેશો પ્રાપ્ત થાય છે. પાટણની સરસ્વતી નદીના કાંઠે એક હજાર વર્ષ પહેલાં થઈ ગયેલા ગોદડિયા સંતોનો મહિમા આજે પણ લોકપરંપરામાં જાણીતો છે.

ભૂપેન્દ્ર ધોળકિયા નોંધે છે, એ અનુસાર ‘ચાલુક્ય વંશના સ્થાપક મૂળરાજદેવ પ્રથમના સમયમાં સરસ્વતી નદીને તીરે એક ગોદડિયા મહારાજ ધૂણો ધખાવીને તપ કરતા હતા. મૂળરાજદેવ ગુજરાતની રાજધાની અણહિલવાડ પાટણના સૌથી મોટા શિવાલયના મહંત તરીકે જવાબદારી સ્વીકારવા વિનંતી કરે છે. ગોદડિયા બાપુ આ પ્રસ્તાવનો અસ્વીકાર કરે છે. ગોદડિયા બાપુને જ પાટણના મુખ્ય શિવાલયના મહંત બનાવવા માટે મૂળરાજદેવે ઘણા પ્રયત્નો કર્યા, એક દિવસ એમને રાજમહેલમાં યુક્તિ કરીને માધુકરી માટે આમંત્રણ આપ્યું, તેઓ જેવા આવ્યા કે તરત જ એમની ઝોળીમાં ભાખરીની એક મોટી થપ્પી પધરાવી દીધી. બાપુએ પોતાના ધુણે જઈને ભાખરીની વચ્ચે જોયું તો એમાં એક ત્રાંબાનું પતરું હતું. જેમાં રાજાએ પાટણના શિવાલયનો વહીવટ ગોદડિયા બાપુને સોંપેલ હતો, એ વિશેનું લખાણ હતું. રાજા મૂળરાજે આ રીતે એમને મનાવવાનો પ્રયત્ન કર્યો હતો. એથી ગોદડિયા બાપુએ કહ્યું કે ‘તમારી ભિક્ષા મેં જમણે હાથે લીધી છે એથી

પરપ્રાંતથી ભ્રમણ કરતા ગુજરાતમાં આવ્યા હતા. મનુજ યોગીની નોંધ મુજબ 'આબુ પર્વત પર બ્રહ્માકુમારી કેન્દ્રથી નજીકમાં જ વિશ્વવિખ્યાત પરમ વિદુષી બ્રહ્મલીન પૂ. વિમલાતાર્થ કકારનો જે આશ્રમ છે તે મૂળ આ ગોદરિયા સંતનો હતો એમ કહેવાય છે. શક્યતઃ આ જગ્યા શિહોરી(રાજસ્થાન)નાં મહારાણીશ્રીએ (જે ગુજરાતના જામનગર રાજ્યનાં રાજપુત્રી હતાં) પોતાના ગુરુજી પૂ. પ્રકાશાનંદજી ગોદરિયાને અર્પણ કરી હતી. આ આશ્રમમાં ઘોડા સમય પ્રકાશાનંદજીએ નિવાસ કર્યો હતો પછી ગુજરાતમાં વિચરી ગયા હતા.'

હરિરામ બાપાના પરમ શિષ્ય સમર્થ શ્રી રામદાસ બાપા ગોદરિયાનું જીવન પણ નિસ્પૃહી, અકિંચન અને અપરિગ્રહી હતું. રામદાસ બાપા સૌરાષ્ટ્રના મહુવા - રાજુલા પાસેના દાવિયા ગામના પ્રજાપતિ હતા. ગોદરિયા પરંપરાની સમાંતર રહેલા તથા રામદાસ બાપાના ખાસ સ્નેહપદને પામેલા દશનામી સંતશિરોમણિ સ્વામી શ્રી બ્રહ્મપુરી બાપુ 'ગોદરી વિનાના ગોદરિયા' સંત તરીકે ખ્યાત થયા છે.

ઉત્તર ગુજરાતના વઢિયાર પંથકમાં તેમજ પાટણવાડા વિસ્તારમાં ગોદરિયા પરંપરાને વિસ્તારવાનું શ્રેય પ્રેમદાસ બાપાને ફાળે જાય છે.

'ગજામેં ગોદરી અંગ તો ઉઘાડા,
તરસ છિપાવી બાપુ તુંબડીયે,
મેં'બી આજસુ, સૂઈ રહીયા,
જ્ઞાન બતાવ્યું, ગુરુ ગોદરિયે.'

તેઓ સમગ્ર ગુજરાતમાં વિહાર કરતા હતા. એથી ચરોતર, ભાલ જેવા પંથકમાં પણ એમની શ્રદ્ધા ભક્તિ પ્રસરેલી છે. તેઓ સાચા અર્થમાં પરિવ્રાજક હતા. પ્રેમદાસ બાપાનો જન્મ અમરેલી જિલ્લાના દામનગરના મેથળી ગામમાં થયો હતો. એમનું પૂર્વાશ્રમનું નામ આવરદાન હતું. પ્રેમદાસ બાપાનાં ૪૪ ભજનનું સંકલન પણ થયું છે. 'ગોદરિયા લીલામૃત'માં પણ સમાવેશ પામેલાં આ ભજન વેદાંતની ભાવ કોટીએ પહોંચે એ પ્રકારનાં છે. અનુયાયીઓ સત્સંગ વખતે સમૂહમાં એનું ગાન કરે છે. એક ઉદાહરણ જોઈએ.

અખંડ પુરુષ અવિનાશી ગુરુરામ

અખંડ પુરુષ અવિનાશી

સત્ ચિત આનંદ રૂપ તુમારો, મુક્તિ બની છે દાસી.

અક્ષરાતીતથી આપ પપાર્યા, વૈકુંઠ પામના વાસી.

સર્વદેવોમાં દેવ ગુરુરામ આદિ પુરુષ અવિનાશી,
નિર્ગુણ છતાં સગુણ વપુધારી સદ્ગુરુ સોહં પ્રકાશી;
અષ્ટમા સિદ્ધિ આપને ચરણો, નવનિધિ છે દાસી
જલચર, થલચર, નભચર ગામી સચરાચરના વાસી,
જડ ચેતનમાં વાસ આપનો, અંતર ઘટ જ્યોતિ પ્રકાશી;
તતપદ ત્વંપદ અળગાં કીધાં, અસીપદના છો ઉપાસી,
પ્રેમદાસ કહે પુરણ બ્રહ્મગુરુ સત્ ચિત આનંદ રાસી.

વગેરે ભજનો એમના કંઠે અવિરત વહેતાં રહેતાં. મનુજ યોગીસંપાદિત 'ગુરુગમ વાણી' પ્રેમદાસ બાપારચિત પદ, ભજન, કુંડળિયા, છપ્પય, છંદ વગેરે પ્રકારની રચનાઓનો સંગ્રહ છે. આ રચનાઓ સગુણ અને નિર્ગુણ બંને પ્રકારની છે.

ગોદરિયા પરંપરાના સંત બ્રહ્મપુરીજી બાળપણથી જ સેવાભાવી પ્રકૃતિ ધરાવતા હતા. મૂળ વિસનગરના અને નાગર બ્રાહ્મણ હતા. ભાવનગર દેશી રાજ્યમાં ન્યાયાધીશના પદ પર હતા. મહારાજ કૃષ્ણકુમારસિંહજી સાથે સારા સંબંધ હતા. કોઈ કેસમાં સગાં-સંબંધી તરફથી પોતાની તરફેણમાં ચુકાદો આપવા દબાણ થયું. એ જ રાત્રે મનોમંથન કરતાં જાતે જ મધરાતે પર છોડી દીધું. શબ્દના કોથળાને કાપી પહેરી લીધો અને સંન્યાસી બની ગયા. 'ગોદરિયા લીલામૃત'માં એમનો ઉલ્લેખ છે. શ્રીરામ બાપાના સ્નેહનું પદ પામેલા આ સંતે દીક્ષા લીધા પછી ગુજરાતના વિવિધ પ્રાંતો ઉપરાંત ઉત્તર ભારતમાં પણ ભ્રમણ કર્યું હતું. એમની સરળ શબ્દ સરવાણી જોઈએ :

મન વિહંગને વારો, ભાઈ જપો અજંપા જાપ
ભૂત ભવિષ્યને સોચો નહિ, તો તળે ત્રિવિધિ તાપ... મન.
સતગુરુને શરણે જાઓ, દિલની બૂઝે આગ
આશા તૃષ્ણા બાળી દીધો, તો પોવાશે સર્વે દાગ... મન.
વર્તમાનમાં રહેવું સદા, શુભ વિચારો સાથ,
વિશ્વાસીના શિર વસે, એ છે અનાથોનો નાથ... મન.
સુરતાને શબ્દે જોડો, તજી દયો મન વિકાર,
શ્વાસે શ્વાસે શબ્દ ભાળો, નામ નિશાન છે સાર... મન.
પાંચ પચીસની આપદા ઠાળો, નીરખો સુંદર શ્યામ
માયા તો અનાદિ શાત છે રે ચૈતન્ય હરીનામ... મન.

એ ફોક નહીં જાય, હું આ શિવાલાયનો વહીવટ તો નહીં કરું પણ એ માટેની વ્યવસ્થા કરીશ'. ત્યાર બાદ પોતાના શિષ્યને એ શિવાલયનો વહીવટ સોંપેલો હતો.'

અધ્યાત્મદષ્ટિએ ગોદડીનો ઇતિહાસ ઘણો લાંબો છે. કબીર સંપ્રદાયમાં પણ ગોદડી હતી, પરંતુ તેનો મહિમા થોડો ઓછો હતો. સમગ્ર ગુજરાતમાં ગોદડીને ગાજતી રાખવાનું શ્રેય હરિરામ બાપા, રામ બાપા અને પ્રેમદાસ બાપાને ફાળે જાય છે. ગોદડી શબ્દમાં આવતા અક્ષરોમાં 'ગો'નો અર્થ સંસ્કૃતમાં ઈન્દ્રીય, ગાય, પૃથ્વી, આકાશ અને વાણી થાય છે. જ્યારે 'દડી' શબ્દ 'દડીન' સંસ્કૃત શબ્દમાંથી ઊતરી આવ્યો છે, જેનો અર્થ 'દંડ' પારણ કરનાર સાધુ કે સંન્યાસી એવો થાય છે. તેનો અર્થ 'જેમણે ઈન્દ્રિયોને સંયમમાં રાખી છે તેવો સંન્યાસી' થાય છે. આવા સંતો જ્યાંથી મળે ત્યાંથી રોટલાનો ટુકડો ખાઈને દેહમાં આત્મા ટકાવી રાખતા. જ્યારે પોશાક માટે તો તેઓ ફક્ત ચીથરાની ગોદડી જ રાખતા. આધ્યાત્મિક વિકાસને વરેલા આ સંતો હંમેશાં પ્રસિદ્ધિથી દૂર રહે છે. એમના વિશે ખૂબ અલ્પ પ્રમાણમાં સાહિત્ય ઉપલબ્ધ થાય છે. 'ચપટ પંજિરિકાસ્તોત્ર'ના સોળમા સ્લોકની પ્રથમ પંક્તિમાં ગોદડિયા પરંપરાના બાહ્ય તેમજ આંતરિક દર્શનને રજૂ કરતી વિગત મળે છે. જેમ કે, જેણે શેરીઓમાં રહેલી ચીથરાઓની ગોદડી બનાવી છે અને જેનો માર્ગ પાપથી રહિત છે તેવા સંન્યાસી ગોદડિયા કહેવાય.

ગોદડિયા પરંપરાના પારિભાષિક શબ્દો પણ સરળ છે. જેમ કે હરિહર એટલે જમવું. વિશાળ અર્થમાં 'હરિહર' એટલે પોતાની આસપાસ રહેલા તમામ ભૂખ્યાજનોની સાથે બેસીને જમવું. એમ 'પૂણો' એટલે શિવરાત્રિ ઉપર ભવનાથમાં વિશેષ પૂજા પ્રગટાવવામાં આવે છે. અગ્નિની શોષ પાણી યજ્ઞપ્રધાન વૈદિક ધર્મ શરૂ થયો. 'ટુકડારામ' એટલે જે જગ્યામાં રોટલો કઢાપિ આખો પીરસવામાં આવતો નથી પણ ટુકડા કરીને પીરસાય છે. હરિનું નામ ભજવું અને ખૂબ જ ઓછામાં ઓછી જરૂરિયાત સાથે જીવન જીવવું તેમ જ અન્યને ઉપયોગી થવું એ ગોદડિયા પરંપરાનો મુખ્ય હેતુ છે. આ પ્રકારની વિગતો વિસનગરની એમ.એન. સરકારી કોલેજના ઇતિહાસ વિભાગના નિવૃત્ત અધ્યાપક ડૉ. ઈશ્વરલાલ ઓઝાએ પોતાના 'ગુજરાતમાં ગોદડિયા પરંપરા' ગ્રંથમાં નોંધી છે.

પ્રાચીન કાળમાં કંથડીનાથ, ભર્તૃહરિ, માણેકનાથનો સેતુ ગોદડિયા પરંપરા સાથે જોડાયેલો હતો, એ વિશે વિવિધ પ્રમાણો 'શ્રી ગોદડિયા લીલામૃત' ગ્રંથમાં એના લેખક મનુજ યોગીએ આપ્યાં છે. નાથ સંન્યાસી પરંપરાના આદ્યગુરુ મત્સ્યેન્દ્રનાથજી દ્વારા નાથ પરંપરાના સાધુ-સંતોને જ્ઞાનમાર્ગનો ઉપદેશ આપવા

રચાયેલી કાવ્યકૃતિ 'જ્ઞાન ગોદડી'માં કાયા, માયા અને ઈશ્વરે રચેલી પ્રકૃતિનું જ્ઞાનમાર્ગી વર્ણન છે. ગોદડિયા પરંપરા પણ મૂળે નાથ પરંપરામાંથી જ ઉદ્ભવ પામેલ માનવામાં આવે છે. જેમાં 'જ્ઞાન ગોદડી' નાથ સંન્યાસીઓ ઉપરાંત કાપડી સંપ્રદાય, કબીર સંપ્રદાયમાં પણ ગવાય છે. શીખ સંપ્રદાયમાં પણ 'જ્ઞાન ગોદડી'નો ઉલ્લેખ છે.

'નાથ કહે દોઉં કર જોરી, યહ સંશય મેટો પ્રભુ મોરી.
કાયા ગોદડીકા ચિસ્તારા, તાં સે હો જીવન નિસ્તારા.'

ગુજરાતમાં ગોદડિયા સંત-પંથની પૂણી પખાવનારા સમર્થ સંતો વિશે અને એમની વાણી વિશે વિગતે જોઈએ.

ગુજરાતમાં ગોદડિયા પરંપરાના આઠ સમર્થ સંત હરિરામ બાપા છે. મહુવાના ધોરાળા ગામે એમનો જન્મ થયો હતો. એમના વિશે વધુ માહિતી મળતી નથી, કારણ કે સામાન્ય રીતે તેઓ એકાંતવાસી હતા અને સતત વિચરણ કરતા. એક દિવસ સૂફી સંત સત્તારશાહ બાપુને ત્યાં ગયા. સત્તાર બાપુના ખોરડે સ્વર, શબ્દ, લય અને તાલની એવી તો પાબંધી રચાઈ છે કે શ્રોતાજનો રસપ્રવાહમાં તલ્લીન બની ગયા છે.

'પહેરી કફની બન્યો યોગી, હવે દુનિયા અકારી છે,
નથી પરવા અમીરીની, ફકીરી સૌથી સારી છે.'

એવામાં સત્તારશાહની નજર સમર્થ શ્રી હરિરામ બાપા ગોદડિયા પર પડી અને ગઝલ પરથી સંતને ગમે, સંતને ભાવે એવું ભજન ગવાઈ ગયું.

એવા સદ્ગુરુ જેના સાચા રે, તે તો સૌને સરખા ગણે.
સાચી જેની વાચા રે, મુખથી એ જુઠું ના ભણે
એવા સદ્ગુરુ જેના સાચા
કોઈ બ્રાહ્મણ, કોઈ વાણિયા, કોઈ સૈયદ ને શેખ:
જ્ઞાન કરીને જોઈ લ્યો, આત્મા સૌનો એક,
એમ ન્યાત પડી છે જુદી રે, માતા સૌને સરખા જણે
એવા સદ્ગુરુ જેના સાચા

ગોદડિયા સંતો નાત, જાત, સંપ્રદાયની કોઈ વાડાબંધીમાં માનતા નથી. માનવમાત્ર ઈશ્વરનું સંતાન છે, અને સત્તારશાહ એ જ વાત ભજનમાં ગાઈ રહ્યા છે. ('શ્રી ગોદડિયા લીલામૃત' પૃ.૫૬).

ગોદડિયા પરંપરાના બહુ જાણીતા નહિ એવા સંત પ્રકાશાનંદજી ગોદડિયા

અનામીની ઓળખાઈ ભાઈ, કરાવે કોઈ સંત
નિરાશી થઈને ફરતા રહે, તોડીને સર્વે તંત... મન.
બ્રહ્મપુરી કહે ગુરુ ચરણે, રાખો નિરંતર વાસ
એક હરિનું નામ ભજી લ્યો, રાખીને વિશ્વાસ... મન.

પ્રાગદાસ બાપા ગોદડિયાનો જન્મ વિ.સ. ૧૯૭૫ ના કારતક સુદ પૂર્ણિમાએ હારીજ તાલુકાના જૂના માંકા ગામે થયો હતો. આ વિસ્તારમાં આવતા સંત-મહંત એમના ઘેર જ રહેતા. એમનું જીવન સંપૂર્ણ ભૌતિક હતું. એમની પાસે કાંકરેજ તાલુકાના જામપુર ગામની સીમમાં છસો વીધાં જમીન માત્ર નેનું રૂપિયામાં રાખી હતી. આ વિસ્તારના સમૃદ્ધ જમીનદારની જેમ તેઓ પણ ખેતી કરાવતા. અધિકારીઓ ઉપર પણ એમનો ગજબનો પ્રભાવ હતો. દેશની સાંપ્રત રાજકીય બાબતોથી પણ તેઓ વાકેફ રહેતા. એક સ્વાતંત્ર્યસૈનિક તરીકે પણ એમણે પ્રશંસનીય કામગીરી કરી હતી.

વર્ષ ૧૯૩૬માં ઉત્તર ગુજરાતના એક નાનકડા ગામના મોટા સમૃદ્ધ જમીનદારનો પુત્ર ઓલ એસ.એસ.સી.ની પ્રથમ ભેચમાં ઉત્તીર્ણ થઈ અનોખી સિદ્ધિ પ્રાપ્ત કરે છે. એ સમયે એસ.એસ.સી.નું મહત્ત્વ આજના આઈ.એ.એસ. કરતાં પણ વિશેષ હતું. સમૃદ્ધિની છાંયો વચ્ચે ઊછરેલ આ યુવાન એ સમયે સરકારી અમલદાર બનવાને બદલે અલબની આરાધનાની રાહ પર ચાલે છે. આ ગામ એટલે પાટણના હારીજ તાલુકાનું જૂના માંકા અને એ યુવાન એટલે ગોદડિયા પરંપરાના સંત પ્રાગદાસ બાપુ. ગુરુ પ્રેમદાસ બાપુ પાસેથી દીક્ષા મેળવી એમના વિલય બાદ પ્રાગદાસ બાપુને ગુરુ રામ બાપા તરફથી બે ગોદડી મળી હતી. જેમાંની એક ગોદડી જૂના માંકાની રામમઢીમાં અને બીજી ગોદડી જૂનાગઢની રામવાડીમાં આજે પણ મોજૂદ છે. જૂનાગઢમાં એમની રાહબરી હેઠળ રામવાડીનો વિકાસ થાય છે. વિ.સ. ૨૦૩૨માં રામવાડી ખાતે ગોદડિયા બાપાનું ભવ્ય મંદિર ઊભું થાય છે. પ્રાગદાસ બાપુએ ઉચ્ચશિક્ષણ મેળવેલું હોવાથી શિક્ષણના પ્રચાર-પ્રસાર સાથે પણ સક્રિય રીતે જોડાય છે. પ્રાગદાસ બાપુ શિષ્યપરંપરાના કટ્ટર વિરોધી હતા. એમણે પોતાના જીવનકાળ દરમિયાન કોઈને કંઠી બાંધી ન હતી. એમની સેવાકીય પ્રવૃત્તિ પુત્ર નારાયણભાઈ પટેલ દ્વારા વિકસે છે. આ પરિવાર તરફથી જૂના માંકાના રામમઢી ખાતે રામરોટીની વ્યવસ્થા સહિતનાં સેવાકાર્યો આજે પણ ચાલે છે. એમણે આ વિસ્તારમાં પ્રશંસનીય કામગીરી કરી હતી. પ્રેમદાસ બાપા સાથે સંપર્ક થતાં એમના કદવનો પલટો થયેલો. રામમઢીની સ્થાપના કરવા પાછળ પણ એ સત્સંગ જવાબદાર રહ્યો છે.

ગોદડિયા પરંપરાના મુળભૂત સિદ્ધાંતોમાં કોઈ પણ પ્રકારની બાધછોડ ક્યાં વગર એમણે સદ્કાર્યોની સુવાસ ફેલાવી છે. એથી જ તેઓ ગોદડિયા પરંપરાના સુકાની બની રહે છે. શ્રી પ્રાગદાસબાપા ગોદડિયાની જગ્યા રામમઢી, જૂના માંકા, તા. હારીજ આજે પણ ઈશ્વર નામસ્મરણ કરતાં કરતાં, બીજા માટે થોડો ત્યાગ કરીને સાદું તથા નીતિપરાયણ જીવન જીવવાનો મંત્ર શીખવે છે. આનર્ત પ્રદેશમાં ગોદડિયા સંતોની પરંપરાને વિકસિત કરવામાં પાનભાઈ ભીલ (રાધનપુર), મલ્લિરામ ઠક્કર (હારીજ), કુંવરબેન (સમી), સેવાદાસ બાપુ (તલોદ) અને મોહનરામ (વારાહી)નું પણ મહત્ત્વનું યોગદાન છે.

ગોદડિયા પરંપરાના અનન્ય ભક્ત હરિશંકર બાપા, સુખ્યાત ભજનિક નારાયણ સ્વામી, જૂનાગઢના સેવાદાસજી ગોદડિયા, હારીજના મલ્લિરામદાસ ગોદડિયા, વધાણ, વિરમગામના બટુકદાસજી ગોદડિયા, અસા - નર્મદાકિનારે કુટીરમાં રહેતા ભગવાનદાસજી ગોદડિયા વગેરે સંતોએ પરંપરાનો વિસ્તાર કર્યો છે. જાતિ, ધર્મ, સ્ત્રી-પુરુષના કોઈ પણ ભેદભાવ વગર ગોદડિયા પરંપરાના સંતો લોકસેવાનાં કાર્યોમાં પણ અગ્રેસર છે, આ પરંપરામાં માન-સન્માન કે કીર્તિની કોઈ અભિલાષા કે આસક્તિ નથી હોતી. ઈશ્વર નામસ્મરણ સાથે ઓછામાં ઓછી જરૂરિયાત સાથે અત્યંત સાદું સરળ જીવન જીવવું અને અન્યને ઉપયોગી થવું એ ગોદડિયા પરંપરાનો મુખ્ય હેતુ છે.

સંદર્ભગ્રંથ :

૧. 'સંદેશ' અર્ધસાપ્તાહિક પૂર્તિ, તા.૧૭-૦૧-૨૦૦૭, લેખ 'ગુજરાતમાં ગોદડિયા પરંપરા', ભૂષેન્દ્ર ધોળકિયા
૨. 'ગુજરાતમાં ગોદડિયા પરંપરા', ડૉ. ઈશ્વરલાલ ઓઝા, રામમઢી, જૂના માંકા, હારીજ, જિ. પાટણ, બીજી આવૃત્તિ-૨૦૦૬
૩. 'શ્રી ગોદડિયા લીલામૃત', શ્રી મનુજ યોગી, શ્રી માગધ પ્રકાશક ગૃહ, વાંસા, જિ. પાટણ, પ્રથમ આવૃત્તિ-૨૦૧૯
૪. 'ગુરુગમવાસી', સં. શ્રી મનુજ યોગી, શ્રી માગધ પ્રકાશક ગૃહ, વાંસા, જિ. પાટણ, પ્રથમ આવૃત્તિ-૨૦૧૯

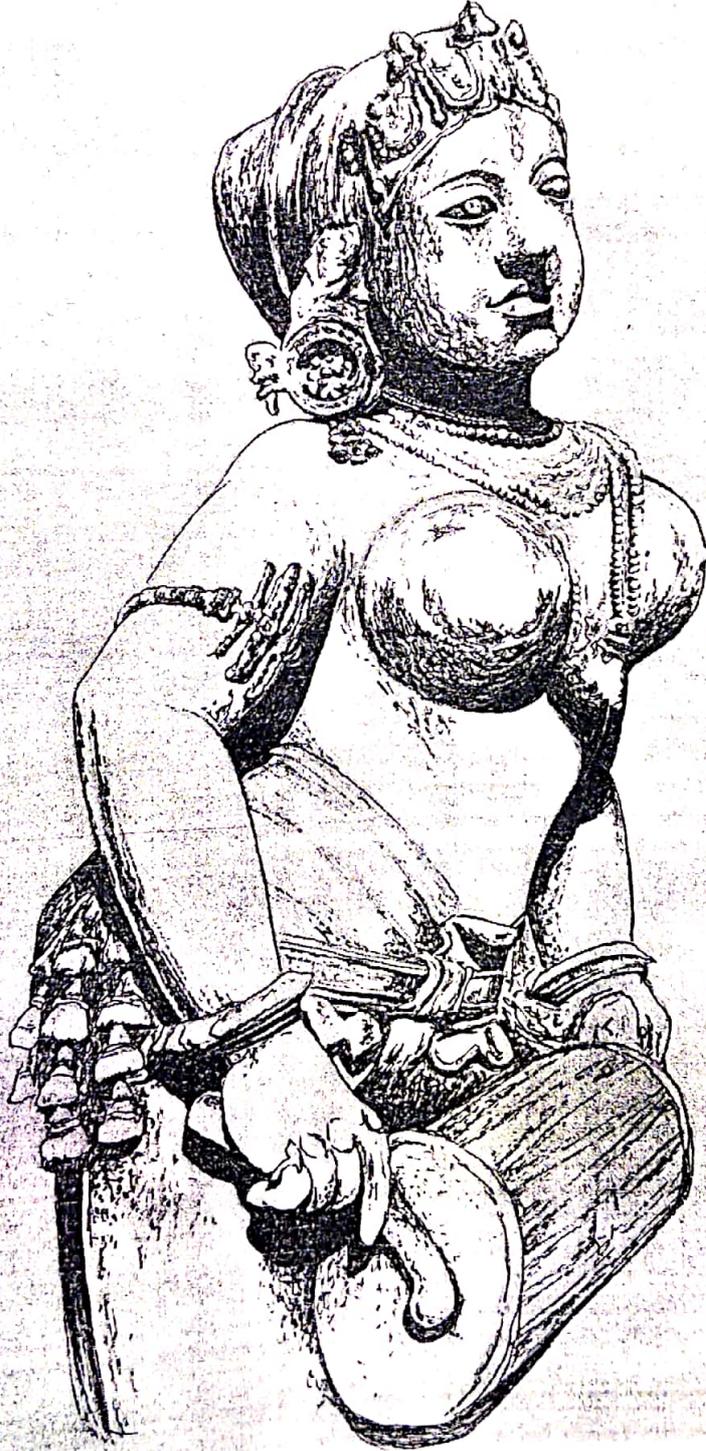


શિક્ષણ

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જ્ઞાન અને વાત્સલ્યનો ઘેઘૂર વડલો : હસુ યાજ્ઞિક

અજયસિંહ ચૌહાણ

વર્ષ બે હજાર બારની વાસંતી બપોરે અમે (પ્રશાંત પટેલ, યોગેશ પટેલ) થલતેજ ગામમાં પન્નાવતી બંગલોઝ શોધતા હતા. જે વ્યક્તિને મળવાનું હતું એ વ્યક્તિના કાર્ય સાથે રૂપાવતી, કામાવતી જેવી મધ્યકાલીન વારતાઓનાં નામો જોડાયેલાં એટલે પન્નાવતી બંગલોઝ શોધતી વખતે સહજ મનમાં મધ્યકાલીન, કથાઓના વિચાર ચાલતા હતા. અંતે અમે ૧, પન્નાવતી બંગલોઝના દરવાજે આવીને ઊભા રહ્યા. ડોરબેલ વગાડ્યો તો દરવાજો ખૂલતાં અમારી સામે ઊભા હતા હસમુખરાય યાજ્ઞિક એટલે કે ગુજરાતી સાહિત્યજગતના હસુ યાજ્ઞિક અને હવે અમારા હસુદાદા. ઊંચો પાતળો દેહ, ગૌરવર્ણ, ઈન્ડો આર્યન યોદ્ધા જેવો તીક્ષ્ણ નાક વાળો ચહેરો. આ પહેલાં એમને જોયા નો'તા એવું નહીં પણ, આટલા નજીકથી પહેલી વાર જોયા. અમને પ્રેમભર્યો આવકાર આપ્યો. મળવાનું કારણ હતું અમારો ઈનોવેટિવ અભ્યાસક્રમ. એન.એસ. પટેલ આર્ટ્સ કોલેજમાં અમે યુજીસીમાં દરખાસ્ત કરવા માટે એક ઈનોવેટિવ અભ્યાસક્રમ વિચારતા હતા. જેના કેન્દ્રમાં ગુજરાતનું મધ્યકાલીન સાહિત્ય, લોકસાહિત્ય અને આદિવાસી સાહિત્ય હતું. એ સંદર્ભે હસુદાદાને ફોન કરી માર્ગદર્શન માંગ્યું અને બહુ જ ઉમળકાથી એમણે ઘરે આવવા નિમંત્રણ આપ્યું. અમે ઘરે પહોંચ્યા તો એમણે અભ્યાસક્રમનું પ્રારૂપ લેખિતમાં તૈયાર કરીને રાખ્યું હતું. હસુદાદા અને હસુબહેન બંનેનો સહજ-સરળ સ્વભાવ કે એ દિવસથી એમની સાથે સ્નેહનો તંતુ જોડાઈ ગયો. એમનો આશુરોષ અને આશુતોષ જેવો સ્વભાવ સહેજ પણ શુષ્ક નહીં. એની પ્રતીતિ તો એમના પીએચ.ડી.ના વિષય પરથી જ થાય. મધ્યકાલીન ગુજરાતી પ્રેમકથાઓ પર એમણે શોધનિબંધ લખેલો. જીવ સંશોધનનો પણ લોકપ્રિય સાહિત્ય ખૂબ લખ્યું. એમની વર્તમાનપત્રોમાં પ્રગટ થતી ધારાવાહી નવલકથાઓ ચપોચપ વેચાતી. 'હાઈવે પર એક રાત', 'મોટુ હટારી' જેવી પાંત્રીસ એક નવલકથાઓ અને ટૂંકી વાર્તાના સંગ્રહો એમની પાસેથી મળ્યાં. આ લખાણોથી એમને પૈસો અને પ્રસિદ્ધિ બંને મળ્યાં. પણ એમાં મોટો વળાંક આવ્યો ગુરુ હરિવલ્લભ ભાયાણીના કહેણથી. ભાયાણીસાહેબે કહ્યું કે આ બહુ લખ્યું. તમે મૂળે સંશોધનના માણસ છો તો મધ્યકાલીન અને લોકસાહિત્યના ક્ષેત્રમાં કામ કરો. અને આપણને મળ્યા સંશોધક હસુ યાજ્ઞિક. અવસાનના પંદર દિવસ પહેલાં સુધી એ સતત લખતા રહ્યા. એમનાં કુલ એકસો સિત્તેર પુસ્તકોમાંથી પચાસ સંપાદનો બાદ કરીએ તો પણ એમણે સોથી વધુ પુસ્તકો લખ્યાં. કોઈ પણ પરિસંવાદમાં વક્તવ્ય આપવા જવાનું હોય

તો એ વાત ભલે મૌખિક કરે પણ એ વક્તવ્ય અગાઉથી જ લખીને તૈયાર હોય. આપણા મોટા ભાગના સારસ્વતો કરતાં લખવા-વાંચવાના સમય બાબતે એમનો ક્રમ ઊલટો. મોડી રાતના બે વાગ્યા સુધી એ લખતા હોય કે વાયોલિન વગાડતા હોય. સવારે દસ પછી ઊઠીને બાર વાગ્યા સુધીમાં પરવારવાનું. એટલે એમને ફોન કરવો હોય તો બપોરે બારથી રાતના બાર સુધી ગમે ત્યારે કરી શકાય. હસુબહેન હતાં ત્યાં સુધી તો ફોન એ જ ઉપાડે. સંગીતના રસને માત્ર એમણે રસ પૂરતો જ મર્યાદિત ન રહેવા દીધો પણ એમાં એટલા ઊંડા ઊતર્યા કે સંગીત વિશેનાં પુસ્તકો લખ્યાં. આધિભૌતિક તત્ત્વોમાં રસ અને શ્રદ્ધાને કારણે 'તિબેટની તંત્રસાધના', 'વિશ્વના ધર્મો અને ગુપ્તસાધના' જેવાં પુસ્તકો પણ એમણે લખ્યાં. મોડી રાત્રે માલકૌંશ ન વગાડવો એવું એ કહેતા. ઢાંકીસાહેબની જેમ હસુદાદા પણ મોડી રાત્રે માલકૌંશ વગાડવાથી થયેલા આધિભૌતિક અનુભવોની વાત કરતા. એમના સંગીત વિષેનાં પુસ્તકો વાંચીને ગુજરાતમાં અનેક લોકો સંગીતમાં અને સંગીતનાં જુદાં જુદાં વાજિંત્રોમાં રસ લેતાં થયા હોય અને વગાડતા થયા હોય એના અનેક દાખલા છે. આમ છતાં આપણે ત્યાં એમનાં સંગીત વિશેનાં પુસ્તકોની ભાગ્યે જ ચર્ચા થઈ છે. એમનાં કેટલાંક ઓછાં જાણીતાં કામોમાં એક મહત્ત્વનું કામ એટલે ગૂર્જર પ્રકાશનની વિશ્વ સાહિત્યની શ્રેણી. એની પ્રસ્તાવનાઓ વાંચતાં આપણને ખ્યાલ આવે કે કામૂ અને માર્કવેજ જેવા અનેક આધુનિક સર્જકોનો એમને એવો જ પરિચય હતો અને એમના સાહિત્યની મૂળગામી સમજ હતી. એમની એ પ્રસ્તાવનાઓનું એક પુસ્તક કરવું એવી મારે ઘણી વાર એમની સાથે ચર્ચા થતી. એમણે જીવનના છેલ્લા દસકામાં લોકસાહિત્ય અને આદિવાસી સાહિત્ય ક્ષેત્રે અનેક મૂલ્યવાન ગ્રંથો આપી પોતાના અભ્યાસનો અનેક ગણો હિસાબ આપ્યો. 'ગુજરાતની લોકવિદ્યા', 'લોકવિદ્યા વિજ્ઞાન', 'લોકસાહિત્ય વિજ્ઞાન', 'લોકસાહિત્ય સંશોધન પદ્ધતિ', 'લોકસાહિત્ય : નૂતન દિશાઓ', 'ગુજરાતના આદિવાસી લોકસાહિત્યનો ઇતિહાસ', 'લોકમહાકાવ્ય અને બીજા લેખો' જેવાં પુસ્તકોમાં લોકસાહિત્યને જોવાનો આખો નવો દૃષ્ટિકોણ એમણે આપ્યો. લોકસાહિત્ય અને આદિવાસી લોકસાહિત્ય બે અલગ છે અને એ બંને વચ્ચેનો સ્પષ્ટ તફાવત એમણે ચીંધી બતાવ્યો. આ ઉપરાંત ઘોસ્ટલોર, સર્પન્ટલોર, લોકવિદ્યા અને જાતિગત મનોવિજ્ઞાન જેવા લેખો દ્વારા લોકસાહિત્યને માત્ર સાહિત્યિક આસ્વાદની ભૂમિકાએથી બહાર લાવ્યા. લોકસાહિત્યને માત્ર સાહિત્યિક માપદંડોથી તપાસવાને સ્થાને એને મનોવિજ્ઞાન, નૃવંશશાસ્ત્ર, ભાષાશાસ્ત્ર, ઇતિહાસ, સંસ્કૃતિ, મિથકશાસ્ત્ર, સમાજશાસ્ત્ર, સંગીત આદિથી એનો અવબોધ અને આસ્વાદ થાય એના પર સતત ભાર મૂકતા રહ્યા. એમના આ બધાં શાસ્ત્રોના અભ્યાસના પરિપાક જેવું 'લોકકથા અર્થદર્શન અને અભ્યાસ' પુસ્તક હજુ થોડા

સમય પહેલાં જ પ્રગટ થયું છે. જેમાં સ્ટીવ થોમસન, એલન દંડીસ જેવા વિદ્વાનોએ કઈ રીતે લોકકથાઓની ચર્ચા કરી છે એ અભ્યાસનું માળખું આપ્યું છે. લોકકથા અભ્યાસની ફિનિશ સ્કૂલ, જર્મન સ્કૂલ, રશિયન સ્કૂલ અને અમેરિકન સ્કૂલ વિષે એમણે વિગતે વાત કરી છે. છેલ્લાં વર્ષોમાં એમનું બીજું મહત્વનું કામ એટલે લોકમહાકાવ્યોનો અભ્યાસ અને એના સ્વરૂપની ચર્ચા. ભારતના જુદા જુદા પ્રદેશોના 'ચનૈની', 'બાપુજી', 'ગલાલેંગ', 'બગડાવત કથા' જેવાં લોકમહાકાવ્યોનો અભ્યાસ કરી એક છેડે વીરગાથા અને બીજે છેડે લોકમહાકાવ્ય એનું આખું સ્વરૂપ સ્પષ્ટ કરી આપ્યું છે. એ જ રીતે ભગવાનદાસ પટેલ સંપાદિત 'ભીલોનું ભારથ', 'રોમ સીતમાની વારતા', 'રાઠોરવારતા', 'ગુજરાંનો અરેલો' જેવાં આદિવાસી લોકમહાકાવ્યોનો અભ્યાસ કરી આદિવાસી લોકમહાકાવ્યનું સ્વરૂપ સ્થાપી આપ્યું. આ બધા અભ્યાસોનું ખરું મૂલ્ય તો એ બીજી ભાષાઓમાં જશે ત્યારે ગુજરાતના સાહિત્યજગતને થશે.

એમની સાથેની પ્રથમ મુલાકાત પછી તો અનેક વાર એ અમારી કોલેજમાં, વિદ્યાર્થી શિબિરોમાં આવતા. ખરા અર્થમાં બહુશ્રુત એટલે અમારી અનોપચારિક બેઠકોમાં માત્ર સાહિત્ય જ નહીં પણ અનેક વિષયો પર ચર્ચા થાય. દાદા થોડી થોડી વારે સૂડીથી ઝીણી ઝીણી સોપારી કાતરતા જાય. પોતે ચરોતરી તમાકુ સાથે એનો સ્વાદ માણે અને અમને સોપારી આપતા જાય. મને તો એમના બાળપણ સાથે એ સમયમાં પણ રસ પડે. એમના પિતા મોરબી રાજ્યના ચીફ સર્વેયર. એટલે બાળપણની જાહોજલાલીની દમામથી વાતો કરે. મોરબી ઠાકોરસાહેબ લખધીરસિંહના ખોળામાં રમેલા. માતાને રણવાસમાં જવાનું હોય તો પડદાવાળી મોટર લેવા આવે. આવી અનેક વાતોથી અમારી રાતો સભર છે. સોળ વર્ષ ગુજરાત સાહિત્ય અકાદમીના મહામાત્ર રહ્યા એટલે અનેક સર્જકોના માંઘલા ગુણ-અવગુણની ગઠરિયાં ખોલે. જાણે બધું છતાં જાહેરમાં કોઈની ટીકા ન કરે. આણંદ આવવાનો એમને ઉત્સાહ હોય. હું, પ્રશાંત, ભરત ખેની, જે.એમ. ચંદ્રવાડિયા, રાજેશ મકવાણા, ભરત પંડ્યા બધા સાથે હોઈએ ત્યારે રાત્રિ બેઠકોમાં સાથે બેસે. ઉંમરનો છોછ રાખ્યા વગર અમારી વાતોમાં સામેલ થાય એટલું જ નહીં એમના સમયના ટુચકાઓ-વાતોનો અમને લાભ આપે. ખાવા-પીવા માટેના કોઈ આગ્રહો નહીં. એમની ઉંમરની મોટા ભાગના વ્યક્તિઓ કરતાં દાદાનો રાત્રે મોડા સુધી જાગવાનો અને સવારે મોડા ઊઠવાનો નિત્યક્રમ ઊલટો એમ મોટી ઉંમરે થતી શરીરની તકલીફો પણ ઊલટી. મોટે ભાગે બધાને આ ઉંમરે ખાંડ અને મીઠું ઓછું ખાવાનું હોય પણ એમને ખાંડ અને મીઠું વધારે પ્રમાણમાં જોઈએ. જો એ વધારે ન લે તો શરીરમાં સોડિયમ અને સુગર ઓછી થઈ જાય. એટલે અમે મોડી રાત્રે આઈસક્રીમ ખાવા નીકળીએ. અંતે સૂતાં પહેલાં સરસ મજાના પાનનું

જમણ થાય. યુવા અધ્યાપકો માટેનો એક શિબિર આબુ રાખ્યો ત્યારે દાદા અમારી સાથે આવ્યા હતા. રાત્રે અમે ફરવા નીકળીએ તો એ પણ બાબાગાડીમાં બેસીને સાથે ફરવા નીકળે. મારી સાથે એ પણ બુઢીના બાલ ખૂબ જ આનંદથી ખાય. ગયા વર્ષે જામનગરમાં ડોલરરાય માંકડ વિશેના કાર્યક્રમમાં હું અને અજય રાવલ જવાના હતા. દાદા જામનગર અમારી સાથે આવ્યા. કારણ માત્ર એક જ, એમના મિત્ર શાંતિભાઈ આચાર્યને મળવા. અમે પહોંચ્યા ત્યારે શાંતિભાઈ તૈયાર થઈને છેક સવારથી પોતાના મિત્ર હસુ યાજ્ઞિકની રાહ જોતા હતા. એ બંને વર્ષો પછી એકબીજાને જે ઉત્કટતાથી મળ્યા એ દશ્ય મારી અને અજય રાવલ માટે જીવનનું એક સંભારણું છે.

મને જ્યારે મહામાત્રની જવાબદારી મળી ત્યારે એમના વર્ષોના એ અનુભવનો લાભ તો મળ્યો જ, સાથે સાથે અશોકભાઈ પટેલ જેવા અકાદમીના જૂના કર્મચારી પાસેથી દાદાની કામ કરવાની લાક્ષણિકતાઓ પણ જાણવા મળી. એ કહેતા ‘કોઈ પણ પત્ર લખવાનો હોય કે કોઈ દરખાસ્તનો મુસદ્દો તૈયાર કરવાનો હોય યાજ્ઞિકસાહેબે મોઢે બોલે અને હું કે મીતાબહેન લખતાં હોઈએ. કોઈ બીજો ડ્રાફ્ટ નહીં.’ એવું જ એમના સાહિત્યિક લખાણનું. રાત્રે કોઈ લેખ લખવા બેસે તો ચાર-પાંચ કલાકે એ પૂરો કરીને જ ઊભા થાય. છેલ્લે છેલ્લે તો એમનાં પુસ્તકો પણ રસ-રુચિ અનુસાર અમને બધાને આપી દીધેલાં. એ કહેતા, ‘મારે હવે કંઈ કામ નથી. મેં જીવતે જગતિયું કરી દીધું છે.’ એ પછી પણ એમનાં પાંચ પુસ્તકો અને અનેક લેખો પ્રગટ થયાં. એ બધાંના સંદર્ભો એમને યાદ. મૂળ પુસ્તક જોવું ન પડે એવી સ્મૃતિ.

અમારા ગુજરાતી ભાષા-સાહિત્ય કેન્દ્રના સેમિનારમાં યુવાબહેનના ઘરેથી ઓનલાઈન વ્યાખ્યાન આપવાના આગ્રહ છતાં યુનિવર્સિટીમાં આવ્યા. એમણે કહ્યું કે ‘હું તો માણસોનો માણસ છું. સામે બેઠેલા શ્રોતાઓની આંખોમાં જોઈને વાત કરવાનો જે આનંદ આવે છે એ લેપટોપની સ્ક્રીનમાં નથી.’ લોકડાઉન પછીના પાંચ મહિના પછી નિરાંતે આખો દિવસ અમારી સાથે રહ્યા. એ એમના જીવનનું છેલ્લું વ્યાખ્યાન. હજી તો કેટકેટલાં આયોજનો વિચાર્યાં હતાં. એન.એસ. પટેલ આર્ટ્સ કોલેજના રેકોર્ડિંગ સ્ટુડિયોમાં એમની લાંબી મુલાકાત લેવાની હતી. એ માટે વિધાનગર મારે ત્યાં ત્રણ દિવસ રોકાવાનું હતું. પણ..., આમ અચાનક એમનું ચાલ્યાં જવું સ્વીકારી નથી શકાતું. એ જીવનને ઉત્સવની જેમ જીવ્યા. આ પૃથ્વી પર જન્મ્યા-જીવ્યાનો પૂરેપૂરો હિસાબ એમણે પોતાના કાર્ય દ્વારા આપ્યો. મધ્યકાલ અને લોકસાહિત્યના અગાધ સાગરમાં ડૂબકી મારવા ઈચ્છતા સંશોધકોને એમનું લેખન-સંશોધન દીવાદાંડી બની પ્રકાશ પાથરતું રહેશે.

काशी और किष्कणोई

प्रमोद कुमार तिवारी

परग

कहा जाता है कि काशी इस धरती पर नहीं है वह शिव के विश्रुत पर

टिकी हुई है और धरती पर होकर भी धरती से ऊपर है. काशी के आस-पास के इलाके के बहुत सारे पुराने लोग जब काशी जाते हैं तो सीमा प्रारंभ होते ही जुते-चप्पल उतारकर झोले में रख लेते हैं कि काशी विश्वनाथ की नगरी में जुते पहनकर कैसे प्रवेश किया जा सकता है. जाहिर है यह कथन और लोगों का यह व्यावहार आस्था और धर्म आदि का मामला है परंतु भरे लिए काशी का विश्रुत पर होना ठेठ यथार्थ का मामला है. इसके यथार्थ के मूल में कौन-सी परिस्थितियां रही होंगी इसकी चर्चा करना तो विषयान्वित होना होगा. पर संक्षेप में

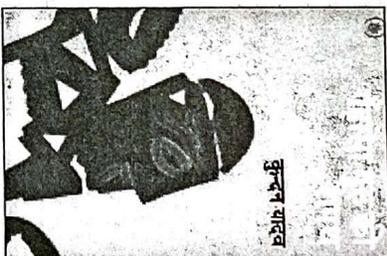
यही कि संस्कृत और संकृति का पालना होने की ठसक ने, शिव जैसे औषड देता के सर्पक ने, मृत्यु का योग साक्षात्कार कराने वाले मणिकर्णिका और हरिश्चंद्र जैसे पाटों ने और भोजपुरिया इलाके की थोड़ी पिछड़ी, थोड़ी सामंती पर रिश्ते निर्माने वाली दुर्लभ आत्मीयता ने इस गांव जैसे शहर का एक अलग ही चरित्र निर्मित किया है जिसके लिए कभी कवि केदारनाथ सिंह ने कहा था कि 'यह आधा

जल में है, आधा मंत्र में, आधा फूल में है आधा शव में, यह आधा है और आधा नहीं भी है.'

काशी के इस संत ढंग को भारतेंदु हरिश्चंद्र, मेरा जली तेरा से लेकर जयशंकर प्रसाद और काशीनाथ सिंह तक के लेखन में देखा-महरसा जा सकता है. काशी की इसी मस्ती और आत्मीयता का प्रमाण

युद्धन यादव के ताला कलानी संग्रह 'गंडासा गुरु की श्राप्य' में नजर आता है. इस संग्रह की ज्यादातर कहानियां बनारस के आस-पास के परिवेश में रची-बसी हैं और जो नहीं भी हैं उनमें काशी की अंतर्लय नजर आती है.

घाट-घाट के पानी का स्वाद लेने वाले ठेठ बनारसी युद्धन यादव पर काशी के घाटों का रंग ऐसा चढ़ा हुआ है कि शेष सारे रंग फीके पड़ जाते हैं. इन कहानियों की सबसे खास बात है कहानीकार की किस्सागोई और पात्रों के निर्माण में उसकी जबरदस्त निरीक्षण क्षमता, जो पाठकों को बांध के रखती है. इन कहानियों में मौजूद खिलंदड़ापन, भोजपुरी संवादों की ठोंक और बाहर से



कुलन्दन यादव

नजर न आने वाले पर भीतर ही भीतर समाज के छल-छद्म को स्पष्ट ढंग से उजागर करने वाले संकेत इन कहानियों को विशेष बनाते हैं. शायद यही कारण है कि ये कहानियां एक बैठक में खुद को पाठक ढागा पढ़वा ले जाती हैं.

कुल 12 कहानियों के इस संग्रह की ज्यादातर कहानियां काशी केंद्रित हैं और रिसों की उस संवेदना को उकरोती हैं जो धीरे-धीरे हमारे हाथों से फिसलती जा रही है. निश्चित रूप से रिसों की सीमाएं हैं, अपने घात-प्रतिघात हैं, एक-दूसरे को नीचा दिखाने की भरपूर कोशिश भी है पर कुछ ऐसा भी है जो लगातार ऊर्जा देता है और पाठक को आत्मीय ढंग से न केवल बांधे रखता है बल्कि यह सोचने पर मजबूर भी करता है कि इस भौतिक चकाचौंध से आखिर क्या मिला. खासतौर से 'फूलचंद का स्कूटर' और 'राजा काशी हल' कहानियां इस दृष्टि से बेहतरीन बन पड़ी हैं.

संग्रह की ज्यादातर कहानियों का परिवेश भोजपुरी इलाके का है जहां आज भी भोजन केंद्रीय भूमिका निभाता है.



લોકગુર્જરી



સળંગ અંક : બાસઠ



(ત્રૈમાસિક : દસમું વર્ષ, ત્રીજો અંક, ડિસેમ્બર-૨૦૨૧)

સંપાદક : ડૉ. બળવંત જાની



શ્રી ઝવેરચંદ મેઘાણી લોકસાહિત્ય કેન્દ્ર
સૌરાષ્ટ્ર યુનિવર્સિટી, રાજકોટ-૫

અનુક્રમણિકા

વિભાગ - ૧ : લોકશાસ્ત્ર

૧. 'વોરાદાદા ઉવાચ' : લોકશાસ્ત્રનાં પાયાનાં
ઘટકોને પ્રગટાવતો ગ્રંથ ડૉ. બળવંત જાની ૩
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૩. ડાક-ડમરુ, વેરાડી અને લોકદેવતાઓ :
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૫. સંતકવિ રજજબ : જીવન અને કાર્ય ડૉ. હસમુખ વ્યાસ ૫૨
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૭. 'હરિરસ'માં રામકથા : સ્વરૂપ અને સંદર્ભ ડૉ. બળદેવ પ્રજાપતિ ૭૮
૮. ગુજરાતમાં ગોદડિયા પંથ :
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૧૦. લોકગીતનાં સંશોધન-સંપાદનક્ષેત્રે
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૧૨. 'કાગ'ની કવિતામાં કર્મની ફિલસૂફી ડૉ. વિનોદ જોશી ૧૩૫



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ડૉ. બળદેવ પ્રજાપતિ

સંસ્કૃત ભાષામાંથી પ્રાકૃત અને એ જ માર્ગે ગુજરાતી ભાષાનું અવતરણ થયું. આ ગુર્જરગિરાને અનેક સાહિત્યસાધકોએ પોષી છે, પોષી છે. સાહિત્યના સાધકોએ પોતાની વાણી લોકજ્ઞાન માટે સતત વહાવી છે. એટલે જ ગુર્જર ભાષામાં ભક્તિ અને સમર્પણની સુવાસ પ્રસરે છે. ગુજરાતી ભાષાના સંત પ્રકૃત ચારણી સાહિત્યના ઉપાસક કવિ મહાત્મા ઈસરદાસજીએ અનેક ગ્રંથો ગુર્જરગિરાને ચરણે અર્પણ કર્યા છે. ચિરકાલીન પટનાઓ જોઈએ તો, દરેક વ્યક્તિનું અંતિમ ધ્યેય હરિસ્મરણ કરી મુક્તિ પામવી. પણ નરસિંહ મહેતા આ વાતને જુદી રીતે વણવે છે કે - ‘હરિના જન તો મુક્તિ ન માંગે’. અહીં વાત વિરોધાભાસ લાગે, પણ મનુષ્ય અવતાર શ્રેષ્ઠ એ સાબિત કર્યું. એમાં મનુષ્ય નર મટીને નારાયણ બની શકે, પણ ત્યાં સુધી પહોંચવાનો રસ્તો કપરો છે. છતાં આપણા અનેક સંતોના દાખલા આપણી સામે છે. ગુજરાતી ભાષામાં એક દુહો છે કે -

“રાજ, ચારણ ને વાણિયો, ચોથી નાનકડી નાર;
એને ભક્તિ ન નીપજે, નીપજે તો બેગ્રો પાર.”

વાત યોગી અટપટી, ઝટપટ ના સમજાય. સંપત્તિ અને સત્તાના મદમાં જીવતો રાજા, રાજયાશ્રય અને કવિત્વથી જીવતો ચારણ, પીકતો વ્યાપાર અને વૈભવમાં જીવતો વણિક અને સુંદર નારી - આ ચારેય અનુક્રમે સત્તા-સંપત્તિ,

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કવિત્વ, બુદ્ધિ અને રૂપ-ગુણના ગર્વથી રંગાયેલાં હોય છે. એટલે એમના ઉરમાં ભક્તિ નીપજતી નથી. પણ ઉપરોક્ત દુહામાં કહ્યું તેમ નીપજી જાય તો, પછી બેગ્રો પાર થઈ જાય છે. જેમાં ભરથરી(ભર્તુહરિ) જેવા રાજા, મહાત્મા ઈસરદાસજી જેવા ચારણ, રવિસાહેબ જેવા વણિક અને મીરાંબાઈ જેવી નારીનાં દૃષ્ટાંતથી આપણે જ્ઞાત છીએ. આ સર્વેએ ભવસાગર તરી બીજાને તાર્યા છે. સંસારનો નિયમ છે કે, જેણે સમાજ માટે કંઈક સમર્પણ કર્યું, એનાં નામ રહ્યાં છે. બાકી પરમેશ્વરા’ તરીકે પણ જાણીએ છીએ. તેમનો જન્મ રાજસ્થાનના ‘માડમેર જિલ્લાના ભાદરેશ ગામમાં પિતા સૂરાજી રોહડિયા અને માતા અમરબાની કૂખે સંવત-૧૫૧૫ શ્રાવણ સુદ બીજના રોજ થયો હતો. તે સમયે કોઈ કવિનો રચેલો દુહો જોઈએ તો-

“સંવત ૫૧૨ ૫૧૩૦તરે, જનમ્યો ઈસરદાસ;
ચારણ વરણ ચકારમાં, ઈણ દિન હુઓ ઉજાસ” ૧

‘પુત્રનાં લક્ષણ પારણામાં’ ન્યાયે માતા-પિતાના સંસ્કાર અને સંતોના આશીર્વાદ એમના જીવનમાં ઉતાર્યા હતા. બાળપણથી જ ગંભીરપણું અને દિવ્ય મુખાકૃતિ વર્તાતી હતી. એક દંતકથા મુજબ હિમાલયના મહંત સમાધીગિરજી સુરાજીના પરિવારમાં સમાધાન માટે જાય, તેના ભાઈઓ સંતનું અપમાન કરે, પણ સુરાજી જમાડી પ્રસન્ન કરે છે. એટલે પોતે સુરાજીને ત્યાં જન્મ લેવાનું વચન આપે છે. આ સંદર્ભે ચારણ પીંગળશીભાઈ જણાવે છે કે - “સમાધીગિરજી બોલ્યા કે, ‘અબ એ ગામકા પાની મેં નહીં પિયુંગા; ફેર તેરે ઘર આઉગા જબ પિયુંગા.’ એટલું કહી ચાલી નીકળ્યા. તે પછી ચાર મહિને સૂજા બારોટની સ્ત્રીને ઓધાન રહ્યું અને નવ માસે પુત્રનો જન્મ થયો. ગામનાં સર્વે મનુષ્ય કહેવા લાગ્યાં કે નિશ્ચય સૂજા બારોટને ઘરે સમાધીગિરજીનો જન્મ થયો.” ૨ નાનપણમાં જ પોતાનાં માતા-પિતાનું અવસાન થાય છે. એમની શિક્ષા-દીક્ષા કાકા આશાજી બારોટ પૂરી કરે છે. ચૌદ વર્ષની ઉંમરે તે કવિતા કરવા લાગે છે. સમયાંતરે દેવલભાઈ સાથે લગ્ન થાય છે, પણ થોડાં વર્ષમાં વીંછીના ઉખથી દેવલભાઈનું મૃત્યુ થાય છે. તે સમયે તેઓ કહીને જાય છે કે - દેવલભાઈ બોલ્યાં કે - “ભક્તરાજ, તમારી સેવા હુંથી પૂર્ણ બની નહીં, એટલામાં તો દેહાંતનો સમય આવ્યો, મારો જીવ આપમાં છે, માટે હું સૌરાષ્ટ્ર દેશમાં જામનગર છે ત્યાં તુંબેલ ચારણને ત્યાં જન્મ લઈશ. આપ ત્યાં આવશો, હું તમને વરીશ” ૩ શીલવાન સ્ત્રીના વિયોગથી મનમાં દુઃખ થયું. પણ બીજાં લગ્ન કરતા નથી. સૌરાષ્ટ્રના ગિરનારમાં સાપુદર્શન માટે કાકા આશાજી અને ઈશ્વરદાસ આવે ત્યાં સંતદર્શનથી ઈસરદાસને બ્રહ્મજ્ઞાન થાય છે. ત્યાં નગરની

ગાદી ઉપર જામ રાવળનું રાજ્ય હતું. સાંજના સમયે કાકી-ભત્રીજો રાજાને કવિતા સંભળાવવા હાજર થાય છે. ઈસર બારોટ પોતાની રચેલ કવિતા સંભળાવે છે.

કે-

“તેરી તીવ્ર તડિગા તેગ, દેખી અન્ન પૂરન દેગ,
કિના કવિજન કા કામ, જુગ જુગ જીયો રાવળ જામ,
નીકા બિછાયા તે નગ્ર, ઓપે ઈન્દ્ર પુરસે અગ્ર,
વસિયો આર જુગા વાસ, બારટ જપે ઈસરદાસ.”

આ છંદ સાંભળી જામ રાવળ પ્રસન્ન થાય છે, પણ રાજ્યના વિદ્વાન એવા પીતાંબર ભટ્ટ સામું જોયું તો તેમને કાવ્યમાં શંકા ગઈ, કારણ પીતાંબર ભટ્ટ યોગ્ય માથું ડોલવતા નથી. એટલે સભા બરખાસ્ત થાય છે. કાકા-ભત્રીજાની નગરમાં યોગ્ય વ્યવસ્થા થતી નથી એટલે ઈસર બારોટને પીતાંબર ભટ્ટ પ્રત્યે કુભાવ પ્રગટે છે. તેને મારી નાખવાની યોજનાના ભાગ રૂપે, તલવાર લઈને ભટ્ટના ઘરમાં જઈ, તુલસીના ક્યારા પાછળ સંતાઈ જાય છે. એ જ સમયે પીતાંબરના ઘરમાં ઈસર બારોટનાં વખાણ થાય છે. પુત્રી તેમની ચિંતા જોઈ પ્રશ્ન કરે છે. તેના જવાબ રૂપે પીતાંબર ભટ્ટ કહે કે- “બેટા, આજ તો મારવાડના બે કવિઓ કચેરીમાં આવ્યા હતા. તેણે જામસાહેબની કવિતા ઘણી સારી રચેલી હતી. તેમાં ઈસરદાસ નામના યુવાન કવિની ઉત્તમ રચના તથા કહેવાની છટા જોઈ આખી કચેરીનાં મનુષ્ય છક બની ગયાં. ત્યારે મારા મનમાં એવો વિચાર આવ્યો કે આવો ઉત્તમ કવિ તે જોઈશ્વરના ગુણ ગાતો હોય તો સારું; મનુષ્યના ગુણ તો ઉદર નિમિત્તે ગાવાના છે. એમ ધારી મેં મારું માથું ડોલાવ્યું તેથી જામસાહેબને કાંઈ કવિતા વિશે શંકા રહી હશે એમ અનુમાન થાય છે.” આ વાક્ય સાંભળતાં જ ઈસરદાસ પીતાંબર ભટ્ટના પગમાં પડી, ગુરુ માની માફી માગે છે. એ વાતનો ‘હરિરસ’ કૃતિની શરૂઆતમાં ગુરુવંદના કરે ત્યાં ઉલ્લેખ થયો છે :

“લાગાં હોં પહળો લળે, પીતાંબર ગુરુ પાય;
ભેદ મહારસ ભાગવત, પાયો જેણ પસાય.”

અર્થાત્ — “જેમની કૃપા-પ્રસાદી થકી, શ્રીમદ્ ભાગવત ગ્રંથના મર્મરૂપી મહારસને હું પામી શક્યો છું, તે ગુરુવર્ષ શ્રી પીતાંબર દાસજીનાં ચરણે હું સર્વ-પ્રથમ નમીને વંદન કરું છું.” ત્યાર પછી જામનગરમાં રોકાઈને ‘હરિરસ’ કૃતિ લખી; દારિકામાં ભગવાન સમક્ષ રજૂ કરી; અર્પણ કરી; એવા સંદર્ભો પણ પ્રાપ્ત થાય છે. સાથે સાથે જામનગરમાં પૂર્વપત્ની દેવલબાઈનાં વચન પ્રમાણે, રાજા જામ રાવળ તુંબેલ ચારણની દીકરી સાથે લગ્ન કરાવી બે ગામ દત્તક આપ્યાં. એમાં

‘સયાણા’ વધારે સમય રોકાયા. અત્યારે પણ ‘સયાણા’ અને રાજસ્થાનનું ‘ભાદરેશ’ ગામમાં આશ્રમ છે. એમની જન્મજયંતી ખૂબ મોટા કાર્યક્રમો સાથે ધામધૂમથી ઊજવાય છે. એમણે ચારણી સાહિત્યના જે ગ્રંથો રચ્યા એમાં ‘દેવીયાણ’, ‘ગુણ-રાસ લીલા’, ‘ગુણ પ્રમાણ’, ‘ગુણ વૈરાટ’, ‘ગુણ નિદાસ્તુતિ’, ‘ગુરુગુપ્તરાણ’, ‘ભજન’ જેવા અનેક ગ્રંથો છે. તેમાં સૌથી શિરમોર ગ્રંથ એટલે ‘હરિરસ’. આ ગ્રંથ સંદર્ભે હરસુરભાઈ ગઢવી પ્રસ્તાવનામાં લખે છે કે — “ ‘હરિરસ’ કાવ્યની રચના કર્યા પછી ઈસરદાસજીએ આ કાવ્ય દારકાપીશને અર્પણ કર્યાની માન્યતા છે. આમ તો ઈસરદાસજી ઘણી વખત દારકા ગયા હોવાનું મનાય છે, પરંતુ સહુપ્રથમ સને ૧૫૪૦ (સંવત-૧૫૯૬)માં દારકા ગયાની અને ‘હરિરસ’ કાવ્ય દારકાપીશને અર્પણ કર્યા અંગેની કેટલીક માહિતી ઉપલબ્ધ થાય છે. જે ઉપરથી સંવત-૧૫૯૬માં જામનગરમાં આ કાવ્યની રચના કરીને, દારકાપીશની સન્મુખ પઠન કરી અર્પણ કર્યા પછીથી જન્મતાના લાભાર્થે મુક્ત કર્યું હોવાનું અનુમાન કરી શકાય છે.”

આ સિવાય જીવનની અનેક ઘટનાઓ, યમત્કારિક બનાવો તેમની સાથે જોડાયેલાં છે. જ્યારે તેમનો અંત સમય આવતાં, ગામનાં સર્વે મનુષ્યોને ભેગાં કરી, ઘોડા ઉપર સવાર થઈ, સમુદ્રમાં ચાલ્યા ગયા, ભગવાનનાં ચરણોમાં સમર્પિત થયા. એ સમયના તેમના દુહાઓ મળે છે જેમ કે —

“સંવત સોળ બાવીશ, સુદ નૌમી ચઈતર માસ;
ઈસાણંદ કવિ ઉધરે, વિશ્વ કરો વિશ્વાસ.
ઈસર ઘોડા ઝોકીયા, ભવસાગર કે માંચ;
તારણહારા તારશે, સાચાં પકડી બાંધ.”

આમ મહાત્મા ઈસરદાસજી તેમની કૃતિ ‘હરિરસ’થી સાક્ષરદેહે તો ઠીક, અક્ષરદેહે અમર થયા છે. આ સંદર્ભે આચાર્ય બદરીપ્રસાદ સાકરિયા નોંધે છે કે — “ઈસરદાસજી ને અપની આયુ કા અધિક ભાગ સૌરાષ્ટ્ર મેં હી બિતાયા, કિન્તુ શેષાવસ્થા મેં વે અપની માતૃભૂમિ મરવાડ મેં આ ગયે થે ઓર અપને ગૌવ માદરેશ ઓર ગુઢા કે બીચ લૂણી નદી કે કિનારે જંગલ મેં જોંપડી બના કર વિરક્ત કી ધૌંતિ રહતે હુએ શ્રી રણછોડરાય કી ધક્તિ ઓર ધક્તિ-પરક સાહિત્ય નિર્વાળ મેં લગે રહે ઓર અનેકો કુટિયા કો તીર્યરૂપ દેકર સં. ૧૬૭૫ મેં આયુ કે ૮૦ વર્ષ સમાપ્ત કર અપને નશર શરીર કી આત્મા કો યગવાન શ્રી રણછોડરાય કી પરમજ્યોતિ મેં લીન કર દિયા.” મતલબ એવું કહી શકાય કે “દિવ્ય પુરુષ ભવસાગર તરી અને મીરાંની જેમ પ્રભુમય બની જાય છે.” એમની કૃતિનું નામ ‘હરિરસ’ કેવી રીતે પડ્યું એના વિશે

પુસ્તકની શરૂઆતમાં હરીન્દ્ર દવે લખે છે કે - 'હરિરસ' ગ્રંથ લખાતો હતો ત્યાં સુધી રોષે ભરાયેલી પત્ની મીઠા વગરનું ભોજન પીરસે એ ખાઈ જનારા ઈસરદાનજી ગ્રંથ પૂરો કર્યા પછી જમવા બેઠા. ત્યારે શાક અને દાળ મોંમાં મૂકતાં જ બોલી ઊઠ્યા, "અરે, આમાં તો મીઠું નથી." પત્નીએ કહ્યું : "છેલ્લા મહિના દિવસથી ઊંડું રસોઈમાં મીઠું નાખતી નહોતી. તમને આજે ખ્યાલ આવ્યો?" ઈસરદાનજીએ કહ્યું - "હરિરસ ખૂટ્યો એટલે રામરસ યાદ આવ્યો." તો ગુજરતી ડાયરામાં રાજભા ગઢવીએ ઉપરોક્ત ઘટનાને ઈસરદાસ અને દીકરીની ઘટના તરીકે રજૂ કરી છે. જોકે ઘટના એક જ જેવી એટલે પાત્રકેર થાય પણ વસ્તુકેર થતું નથી. જ્યારે હરિરસમાં મન પરોવાય, હરિરસની ભૂખ જાગે પછી કોઈ રસની જરૂર પડતી નથી. એવો ચારણી સાહિત્યનો દિવ્ય ગ્રંથ 'હરિરસ' જેમાં માત્ર ને માત્ર ઈશ્વરના જ ગુણગાન છે. ભાગ્યશાળી જ આ જ્ઞાનનો લાભ લઈ શકે ! મને મળ્યો એનો આનંદ છે. આ કૃતિનાં અનેક સંપાદનો થયાં તેમાં છેલ્લે હરસુરભાઈ ગઢવી દ્વારા પ્રવીણ પુસ્તક ભંડારમાં પ્રકાશન થયું છે. આ પુસ્તક ૩૬૦ કંડિકાથી ગ્રંથન થયું, તેમ ૩૮ કંડિકામાં ભગવાન શ્રીરામની વાત કરી છે. બીજું એ પણ સમજાવ્યું કે ભગવાનનાં સર્વે સ્વરૂપો એક જ છે. પણ ઈસરદાસજીએ ભગવાન રામના જીવનને વર્ણવ્યું, તેના આધારે 'હરિરસમાં રામકથા' જોવાનો ઉપક્રમ છે.

ચારણી સાહિત્યની વિશેષતા એ કે એને વાંચવાથી ખબર ન પડે. આ સાહિત્ય લય, તાલ સાથે જોડાયેલું છે. એટલે એને સાંભળી તો જ ખબર પડે. એના સર્જકો અને સાધકો પાસેથી લય-તાલમાં સાંભળી અર્થ સમજી ત્યારે આનંદ આવે. બાકી શબ્દોના અર્થની ખબર ન પડે. એટલે આવા ચારણ વિદ્વાન ઈસરદાસજીએ પોતાની કવિત્વશક્તિનો, ઉત્તમ કલાકીય સાધનાનો પરિચય કરાવ્યો છે. જેમાં રામનામમહિમા, રામજીવન, રામનામપ્રતાપ, રામવંદના વગેરેને જોઈએ. રામનામમહિમા :

કવિએ આ 'હરિરસ'માં ભગવાન રામના નામસ્મરણના મહિમાને પ્રગટ કર્યો છે. 'કલયુગ કેવલ નામ આધારા' નામસ્મરણનો વિશેષ મહિમા જે કવિએ કાવ્યમય રજૂ કર્યો છે. જેમ કે, સંસારના સર્વે રસોમાં ઉત્તમ એવા રામરસમાં મગ્ન બન, કેમ કે એ જ મહાધર્મ, એ જ તીર્થ-તપ છે. માટે હે જીવત્મા ! જાગતાં તો ટીક, પણ નિદ્રામાં, ઊઠતાં, બેસતાં, ચાલતાં પણ રામને સંભાળજે. રામનું રટણ કરતાં આળસ ન કર, જે ગુણ તું જાણતો નથી તે વેદપુરાણમાં છે. તારું ભાગ્ય સાડું હોય તો રામનું ભજન કર, સમય સારો હોય તો દાન-પુણ્ય કર, બુદ્ધિ હોય તો પરોપકાર કર, એમાં જ જીવનની સાર્થકતા છે.

રહે વિલંબો રામરસ, અનરસ ગણી અલભ;
એક મહા-ધ્રમ આતમા, એ તીરથ એ તપ્પ. (હરિરસ કંડિકા-૧૫)

જદ જાગે તદ રામ જપ, સૂતાં રામ સંભાર;
ઉઠત બેઠત આતમાં, ચાલતા ચીતાર. (હરિરસ કંડિકા-૧૬)

રામ જપંતા રે હિદ્રા, આળસ મકર અજાણ;
જે તું ગુણ જાણે નહીં, પૂછવ વેદ પુરાણ. (હરિરસ કંડિકા-૧૮)

ભાગ્ય બડો તો રામ ભજ, બખત બડો કુછ દેહ;
અકલ બડી ઉપકાર કર, દેહ ધર્યો ફળ એહ. (હરિરસ કંડિકા-૨૦)

તો આગળ કવિ કહે છે કે - તમારી ઉપર છત્ર ઢોળાય એવી સમૃદ્ધિ હોય તોપણ રામનામ ભૂલીશ નહીં, કારણ હાથ, જીભ, આંખ, કાન જેવી કર્મેન્દ્રિયો ભગવાન સિવાય કોઈ આપતું નથી. માટે સૌ ઠેકાણે વસેલા અંતર્યામી પ્રભુશ્રી રામની હૈયામાંથી ભક્તિ છોડીશ નહીં, અને જીભથી રામનું સ્મરણ છોડીશ નહીં. હે હૃદય ! રામનામનો ઉચ્ચાર કરતા રહેવાથી કેટલા લાભ થાય છે. જેમ કે - પ્રભુને પ્રિય થવાય; જગત માન આપે; વળી કોઈ શત્રુ કાંઈ હાનિ પણ પહોંચાડી શકતો નથી. નવ ખંડ ધરતીમાં ભગવાન શ્રીરામના નામનું સ્મરણ કરવા જેવો વ્યવસાય કોઈ નથી, માટે આઠ પ્રહર ભગવાનના નામનું અખંડ રટણ કરવું જોઈએ. હંમેશાં, સમયે-સમયે, તું શ્રીરામના નામનું રટણ કરતો જ રહેજે; કારણ કે શ્વાસોચ્છવાસ બંધ થઈ ગયા પછી મુખમાંથી શ્રીરામના નામનો ધ્વનિ ફરી વખત આવવાનો નથી.

રામ ન ભૂલો બખ્પડાં, જે શિરછત્ર પ્રણોય;
કર, જીહા, લોચણ, શ્રવણ લિયો ન આપે કોય.

(હરિરસ કંડિકા-૨૧)

હિયા મ છડે હરિ ભગતિ, રસણા, મ છડે રામ;
અંતરજામી આપણો, ઠાકર હું તહ કામ. (હરિરસ કંડિકા-૨૨)

રામ ભણંતા રિક્ષ, કહ ક્રતાં ગુણ લીય; (હરિસ ક્રિકા-૨૭)
કાકર માને, જગ નમે, પ્રિસણ ન ગિજે ક્રીય. (હરિસ ક્રિકા-૨૭)

રામ નામ રટતે રહૈ, આહં પહીર અખંડ; (હરિસ ક્રિકા-૨૯)
શુભરણ શા સોદા નહિ, નિરખ દેખ નવખંડ. (હરિસ ક્રિકા-૨૯)

રામ રામ રસણા રટ, વાસર ભૈર-અભૈર; (હરિસ ક્રિકા-૩૦)
આટકયા પછં નહિ આવસી, રામ તણી મુખ રેર. (હરિસ ક્રિકા-૩૦)

ઈસરદાસજી સ્મરણ વિશે જણાવતાં કહે કે - હું મૂર્ખ, ગર્વથી છઠ્ઠી જઈને, રામને કેમ ભૂલી જાય છું? જે દિવસે રામનું સ્મરણ ભુલાઈ જાય, તે દિવસ વર્ષ છે એમ હું માનજે. શ્રીરામચંદ્રજી સર્વ પ્રકારનું શ્રેય કરે છે. ભક્તોની સહાય કરવા માટે સદાય તત્પર જ રહે છે. પરમાત્માનું નામ મહાન તીર્થ, વ્રત, લાભદાયક કાર્ય સમાન છે, પરમાત્માના નામનો એક જ અક્ષર સર્વ તત્ત્વોને ફળદાયક છે, માટે હંમેશાં જાણ્ય શ્રીરામના નામનું રટણ કરો. ભગવાન શ્રીરામના નામનો જપ કરતા રહેવાથી રાજ્ય, લક્ષ્મી, સિદ્ધિ-સિદ્ધિ તેમજ નવ નિષિઓ પણ પ્રાપ્ત થાય છે. શ્રીરામના નામનો પ્રભાવ તો જુઓ ! વિભીષણે સૈન્ય એકઠું કર્યા વગર જ કરતા શ્રીરામના નામનું રટણ કરતાં કરતાં જ લંકાનું રાજ્ય પ્રાપ્ત કરી લીધું હતું.

રામ વિસારી ક્યું રિયો, રે મૂરખ મદ-અંધ;

જિય દી રામ ન સંભરૈ, ઉ દી અંધાધૂંધ. (હરિસ ક્રિકા-૩૬)

રૂડી કરશી રામજી, સહ બાતાં શીરંગા; (હરિસ ક્રિકા-૪૦)
ભગતાં પર ભૂધર ધણી, ચાઠણ નીર સુરંગ. (હરિસ ક્રિકા-૪૦)

નામ સુતીરથ, નામવ્રત, નામ સલભનો કામ; (હરિસ ક્રિકા-૫૧)
એકો અક્ષર તત્વફળ, જપ જહા શ્રીરામ. (હરિસ ક્રિકા-૫૧)

રામ જપતાં રાજશ્રી, રામ ભણંતાં રિક્ષ; (હરિસ ક્રિકા-૫૩)
રામ નામ સંભારતૈ, પામીજૈ, નવ નિહ. (હરિસ ક્રિકા-૫૩)

હરિસ'માં રામકથા : સ્વરૂપ અને સંદર્ભ

દાખૈ ઈસરદાસ યું, કટક ન લીણાં ક્રીય; (હરિસ ક્રિકા-૫૬)
રામ, રામ, રટતાં થકાં, લંક બલ્લીખણ લીય. (હરિસ ક્રિકા-૫૬)

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ભગવાન શ્રીરામના નામનું રટણ વારંવાર કરવું અને અન્ય મનુષ્યો પાસે લીઠાનું તાણું લગાવી દેવા યોગ્ય સમજવું. હે જીવ ! તું શ્રીરામનામરૂપી સજીવન-શ્રીહરિનાં ગુણગાન સાંભળતો રહેજે અને નેત્રો દ્વારા ભગવાન શ્રીરામનાં દર્શન કરતો રહેજે. જો તારું (આત્માનું) ભલું ઈચ્છતો હોય, સર્વ સુખ ચાહતો હોય તો ભગવાન શ્રીરામનું રટણ કર. જ્યાં સિંહનો વાસ હોય ત્યાંથી હરણાં નાસી જાય, તેમ હે રાધવ, મારા હૃદયમાં વાસ કરી અંદર રહેલી વાસનાને દૂર કરો. શ્રીરામ, કૃષ્ણ, નારાયણ, સચ્ચિદાનંદ, ગોવિંદ, વાસુદેવ, વિહલ, નરસિંહ તેમ ગોકુળમાં ચક્ર રૂપે પ્રકાશિત થયેલા એવા સર્વત્ર વ્યાપક, પરબ્રહ્મ પરમાત્માનું સદૈવ સ્મરણ કરતો રહેજે.

રામ ભણી ભણ રામ ભણ, અવસં રામ ભણાય; (હરિસ ક્રિકા-૫૯)
જિસ મુખ રામ ન ઉચ્ચરૈ, તા મુખ લીહ જડાય. (હરિસ ક્રિકા-૫૯)

રામ સજીવણ મંગ રટ, બધણાં રામ બિચાર; (હરિસ ક્રિકા-૬૦)
શ્રવણાં હર ગુણ સંભળી, નૈણાં રામ નિહાર. (હરિસ ક્રિકા-૬૦)

રસણાં રટૈ તો રામરટ, આમય લગી ન અંગ; (હરિસ ક્રિકા-૬૧)
જે સુખ ચાહૈ જીવરો, સુમિર, સુમિર, શીરંગ. (હરિસ ક્રિકા-૬૧)

મનણા ડાકણ માહરૈ, રાધવ કાઢ્ય રૂદાહ; (હરિસ ક્રિકા-૭૭)
જિસ, વનમં કેહર વસૈ, ચાટૈ મૂળલાં તાહ. (હરિસ ક્રિકા-૭૭)

રામ, ક્રિસન, નારાયણા, સચ્ચિદાનંદ, ગોવિંદ; (હરિસ ક્રિકા-૮૪)
વાસુદેવ, વિહલ, વિભુ, નરહર, ગોકુળ ચંદ. (હરિસ ક્રિકા-૮૪)

રામજીવન :

રામના જીવનની કથાને કવિએ ખૂબ સરસ રીતે વર્ણવી છે. અધીયાનગરીમાં આપે દશરથરાજને ત્યાં રામ રૂપે અવતાર લઈ, રામ-લક્ષ્મણ

રૂપે વિશ્વામિત્ર ઋષિના યજ્ઞનું રક્ષણ કરેલું. ત્યાર પછી જનકરાજાને ત્યાં યજ્ઞમાં માતા સીતાજીને વ્યાપેલા વિષાદને દૂર કરવા, ભગવાન શિવના ધનુષ્યના ત્રણ ટુકડા કરી નાખેલા અને રાજા દશરથના મહાબળવાન પુત્ર રૂપે પ્રસિદ્ધ થયા. એ સમયે પરશુરામ ઋષિ ક્રોધાગ્નિ વરસાવતા ત્યાં આવે, પણ રામના સત્ય દર્શનથી બળનું ગુમાન ત્યજીને અત્યંત સરળ બની રહેલા. ભગવાન રામે વનવાસી ગુહ-રાજાને મિત્ર બનાવી કુટુંબ સહિત તેમનો ઉદ્ધાર કરેલો. વનવાસ દરમિયાન શૂર્પણખાનાં નાક-કાન લક્ષ્મણજી કાપી નાખે ત્યારે ખરદૂષણ જેવા દૈત્યોનો સંહાર કરેલો, હનુમાન સાથે મૈત્રી બાંધી વાલિનો સંહાર કરી, સુગ્રીવને રાજ્ય આપ્યું. વળી લંકા ઉપર ચઢાઈ કરવા, સમુદ્રસેતુ બાંધવા નલ-નીલ જેવા વાનરોની આગેવાની હેઠળ બે કરોડ વાનરોને કામે લગાડ્યા. આ વાનરયોદ્ધાઓ રાવણ ઉપર ક્રોધિત થઈ તાડ તેમજ પર્વતની ટોચો પાડવા લાગ્યા. આપે, પહાડોના પથ્થરોથી સમુદ્રસેતુ બાંધી અઢાર પદ્મ સૈન્યને સામે પાર ઉતાર્યું, ત્યારે વિભીષણ આવી આપનાં ચરણોમાં પડતાં, હે પ્રભુ શ્રીરામ, આપે તેને ગળે લગાડ્યો હતો.

ધરૈ, નરદેહ, અજોધ્યા ધામ, રાજા દસરત્ય, તણૌ ઘર, રામ;
બિહું, રઘુ-લખ્મણ, પુત્ર બુલાય, સઝૈ, જાગ, વિસ્વામિત્ર સહાય.

(હરિરસ કંડિકા-૧૯૦)

જનકતણો, વણિ, જોયૌ જાગ, ભાંગૌ ધનુ, કટ્ટણ, સિય-વિસાગ;
કિયો, ત્રય ખંડ, ભૂતેસ કો દંડ, બેટો દસરત્ય તણો, બળ ચંડ.

(હરિરસ કંડિકા-૧૯૧)

અયો, રૂષિ, ક્રોપ સ્ત્રીવંત અંગાર, તજ્યૌ, બળ-ચાપ, હુઓ, દુજતાર;
મહાદિય માન, કરી ગુહ મીત, તારે, સહ કીર, કટ્ટમ્બ સહિત.

(હરિરસ કંડિકા-૧૯૨)

વિરૂપ કિધી, સુપણખાય વત્ર, નદી, ખર, દુખ, વહોડિય તત્ર;
કિધૌ, કપિમિત્ર, સુગ્રીવ સુકાજ, રહંસિય વાણિ, દિયો, ઉણ રાજ.

(હરિરસ કંડિકા-૧૯૩)

ઉપાડ, બંધાડ, સમંદર ઓડ, કપી, સમ-નીલ, જકે, દુ કરોડ;
જ્યો અગણંત, મિલે, કપિ જોધ, છેદે, સર, તાડ, પહાડ, સકીધ.

(હરિરસ કંડિકા-૧૯૪)

ધરી, દધિપાજ, પાહાડાંયધાર, પદમ્મ અઢાર, ઉત્તારિય પાર;
પડ્યૌ, તદ, આય, વિભીષણ પાય, લિયો, જદ, રાધવ કંઠ લગાવ.

(હરિરસ કંડિકા-૧૯૫)

આપે વિભીષણને અભય બનાવી રક્ષણ આપ્યું, તેમજ તેને દાન રૂપે લંકા આપી દીધી હતી. આપે કુંભકર્ણ જેવા યોદ્ધાનો સંહાર કરીને, લંકાનગરીને પણ જીતી લીધેલી. આપે ઈંદ્રજિત, તેમજ દૈત્યોનો સંહાર કરીને સીતાજીને પાછાં મેળવ્યાં, દેવતાઓનાં બંધનો છોડાવવા માટે આપે સમુદ્રસેતુ બાંધેલાં છે અને રાવણનો નાશ કરેલો. આપે ભક્ત વિભીષણને લંકાની ગાદીએ બેસાડેલા છે; સીતાજીને કલંકરહિત કરીને પાછાં લઈ આવેલાં છે, તેમજ દુર્વાસા ઋષિના ભયંકર શાપમાંથી ભક્ત રાજવી અંબરીષનું રક્ષણ કરીને, આપના એ ભક્તને આપના સમાન પદ આપેલું છે.

ઉગાર વભિષણ, કીધ અભીત, દિધી તૈ, લંક, અલીધી, દત્ત;
લિયૌ, તૈ, વાર કિતાં ગઢ લંક, સંધારિય કુંભ, મનાડૈ સંક.

(હરિરસ કંડિકા-૧૯૬)

જુડૈ, તૈ વાર કિતાં ઈંદ્રજીત, સંહરિય દઈતાં, વાળીય સીત;
દળૈ, તુમિ વાર કિતાં દશકંધ, બંધ્યૌદધિ, દેવ, ધુડાવણ બંધ.

(હરિરસ કંડિકા-૧૯૭)

વિભીષણ, લંકા, પાટ બેસાર, આણી ધર સીય, કલંક ઉતાર;
ઉબારિયા શ્રાપ અગાં, અખરિખ, સેવગ્ગ કિયૌ તૈ, આપ સરિયા.

(હરિરસ કંડિકા-૨૪૪)

રામનામપ્રતાપ :

‘રામ સે બડા રામ કા નામ’ — નામનો મહિમા મોટો, પણ રામનામ-સ્મરણનું કેવું ફળ પ્રાપ્ત થાય તેની વાત કવિએ કરી છે. જે રામચંદ્ર, લક્ષ્મણના નામની વાંછના કરનારા મનુષ્યને જળની ઘાત નડતી નથી; રામનામના એવા બે અક્ષરોને જે જપતો રહે છે, તેની સમીપે પ્રાણપ્રિય પ્રભુ સદૈવ વાસ કરે છે. ભગવાન રામના નામનું રટણ સ્વયંભૂ રીતે થતું રહેતું હોય, તેના શરીરમાં સંસારના પાંચ વિષયો વ્યાપી શકતા નથી. હે ભગવાન, આપના નામના ગુણોનું ગાન કરનારાનાં પાપ, તૃષ્ણા તેમજ માન-અપમાનાદિ વગેરેનો નાશ થઈ જાય છે. હે પ્રભુ, આપ ચારેય પ્રકારના મોક્ષને આપવાવાળા દાતાર તેમજ કળી શકાય નહિ

એવા અકળ પુરુષ પણ છે. આપ આનંદસ્વરૂપ છો. હે રઘુપતિ, આપનાં હજારો નામો, જેનો ઉચ્ચાર કરવાથી અમૃત સમાન રસની પ્રાપ્તિ થાય છે. ઈસરદાસજી કહે છે કે શ્રીરામનામના પ્રતાપથી હનુમાનજી દ્રોણાગિરિ પર્વતને ઉપાડીને લઈ આવ્યા, ઈંદ્રે ઈન્દ્રાસનની પ્રાપ્તિ કરી, ધ્રુવને અવિચળ સ્થાનની પ્રાપ્તિ થઈ, પાંડવકુળ નિષ્કલંક કહેવાયું. એવા શ્રીરામનામને રસણા દ્વારા રટતાં રટતાં, અનેક ભક્તજનોનો ઉદ્ધાર થયો છે. હે જીવ, પરભવના ડરને સમજી, એનું ધ્યાન કરવાનું, તેમજ મુખેથી રામના નામનો ઉચ્ચાર કરવાનું શરૂ કરી દે. આગળ ઈસરદાસ કહે છે કે ભગવાન રામના નામનો પ્રતાપ પ્રગટ જ છે. જેણે યમદૂતોને ત્રાસ પમાડીને (દુષ્ટ અજ્ઞામિલને) વૈકુંઠમાં વસાવ્યો છે, જેનાથી લક્ષ્યોપશિનીના કેરાઓ મટી જાય છે, જેનાથી હૃદયમાં સહેજ પણ સંતાપ રહી શકતો નથી, રામના નામના પ્રતાપથી જળ ઉપર પથ્થરો પણ તરે. હે પ્રાણી, એ પરમાત્માનું ધ્યાન ધરી તે અને મુખથી રામનામનો ઉચ્ચાર કરતો રહે.

વાંચે, તોં નામ લખમણ-વીર, નરાં, પિંડ-ઘાત, હુવૈ, નહ નીર;
દાખે, તૂઝ નામ, સુઅકબર દોય, વૈડો, રહ પ્રાણ નિયારો ન હોય.
(હરિરસ કંડિકા-૨૫૧)

ઓળગે, રામજ, આપહિ આપ, લિખે, તન, પંચ, સકૈ, નહં વ્યાપ;
ભણે, ગુણ, તૂઝ તણા, ભગવાન, ખયે, તિહિ, પાપ, તૃષ્ણા, ખૈમાન.-
(હરિરસ કંડિકા-૨૫૬)

દાતાર, મુગત, અણકળ દેવ, સાલોક, સામીપ, સાયુજય, સાવેવ;
સદાણંદ, દાતાહ, નામ સહસ્સ, રઘુપતિ, ઉચ્ચિત, અમ્રત રસ્સ.-
(હરિરસ કંડિકા-૨૫૯)

રામનામ પરતાપ, હણૂ, દ્રોણાગિરિ લાયો,
રામનામ પરતાપ, ઈંદ્ર ઈંદ્રાસણ પાયો;
રામનામ પરતાપ, ધુરૂ, અવચળ હુઈ, રહિયો,
રામનામ પરતાપ, પાંડુકુળ, નકળાંક કહ્યો;
સોઈ રામનામ, રટાં રસણ, અનંત ભક્ત જન ઉદ્ધરૈ,
ધર ધ્યાન, 'ઈસરા' સંકધર, અજુ, રામ, મુખ ઉચ્ચરૈ.-
(હરિરસ કંડિકા-૩૫૨)

પ્રગટનામ પરતાપ, વાસ વૈકુંઠ, વસાયો,
પ્રગટનામ પરતાપ, દુત-જમ, ત્રાસ દિખાયો;
પ્રગટનામ પરતાપ, ચંડ ભાગે, ચૌરાસી,
પ્રગટનામ પરતાપ, ઊંર નવ રહે ઉદાસી;
રામરો નામ, પ્રાણી રટૈ, તાણું, જળ, પાયર તટૈ,
ધર ધ્યાન, 'ઈસરા' સંકધર, અજુ, રામ, મુખ ઉચ્ચરૈ.

(હરિરસ કંડિકા-૩૫૩)

રામવંદના :

મનુષ્ય રૂપે અવતાર ધારણ કરનાર મર્યાદાપુરુષોત્તમ ભગવાન શ્રીરામની ક્વિએ વંદના કરી છે. પૃથ્વીને એકવીસ વાર નક્ષત્રી કરનારા, જમદગ્નિ ઋષિના પુત્ર, પરશુરામને નમસ્કાર. યુદ્ધમાં રાવણનર મારી, ભક્ત વિભીષણનું કાર્ય સિદ્ધ કરવાવાળા ભગવાન રામચંદ્રજીને નમસ્કાર કરું છું. હે પુરુષોત્તમ, હે પૂર્ણબ્રહ્મ, વેદોની મર્યાદાને અખંડિત રાખવાવાળા, ભરત તથા શત્રુઘ્ન વચ્ચે સ્નેહ-સ્વરૂપ; ભક્તોને ક્યારેય છેલ નહિ આપવાવાળા, હે ભગવાન શ્રીરામ આપને નમસ્કાર છો. ગુરુધ્વજધારી શ્રીવિષ્ણુ ભગવાનને નમસ્કાર; ગુણ તથા દોષથી પર એવા, લક્ષ્મણજીના વડીલ બંધુ ભગવાન રામચંદ્રને નમસ્કાર; સાગરસેતુ બાંધનારા તથા યુદ્ધમાં રાવણ અને તેના રાજ્યરૂપી લંકાનગરીનો નાશ કરનારા પ્રભુ રામચંદ્રને નમસ્કાર. કુંભકર્ણની દીર્ઘ ભુજાઓના કાળસ્વરૂપ, દુષ્ટ રાક્ષસકુળોનો નાશ કરનારા, લંકાનગરીનો વિધ્વંસ કરનારા, રઘુવંશના સૂર્યરૂપ એવા અતિસુંદર - સર્વશ્રેષ્ઠ ભગવાન રામચંદ્રને વારંવાર નમસ્કાર.

નમો, રિખ, જામદગન્ન, સુરીસ, નમો, કિય, વાર, નછત્રિ ઈકીસ;
નમો, રણ રાવણ, મારણ રામ, નમો કિય, સિદ્ધ, વિભીષણ કામ.

(હરિરસ કંડિકા-૨૧૩)

નમો, પુરુષોત્તમ, પૂરણ બ્રહ્મ, નમો, મરજાદ, અખંડ-નિગમ્મ;
નમો, સતરૂઘણ, ભરત, સનેહ, નમો, અવઅત, ભગત અછેહ.

(હરિરસ કંડિકા-૨૭૯)

નમો, ધક-પંખ, સરોવર-ધજજ, ગુણાદિ-અતીત, લખન-અમ્રજજ;
નમો, પ્રભુ, સાયર-બાંધણ-પાજ, નમો, રણ - રાવણ - રોણ - રાજ.

(હરિરસ કંડિકા-૨૮૦)

નમો, કુંભેણ તણાં, ભુજ, કાળ, નમો, ખળ, રાખસ, વંસ, ખેંગાળ;
નમો, રધુવંસ તણાં, રિવ, રામ, વિઘ્નસણ, લંક, બડા, બરિયામ.

(હરિરસ કંડિકા-૨૮૧)

રામસ્વરૂપદર્શન :

ભગવાન રામની સર્વવ્યાપકતાનો અનુભવ કવિએ ખૂબ સરસ રીતે રજૂ કર્યો છે. હે રામ, સર્વ સ્થળે જુદાં જુદાં સ્વરૂપે આપ જ રમણ કરી રહ્યા છો. સમુદ્ર સ્વરૂપે, તેની લહેરો રૂપે પણ આપ જ છો. પ્રત્યેક પદાર્થમાં આપનો અંશ વિદ્યમાન છે. હંસસ્વરૂપ પ્રભુ, આપનું આવું યથાર્થ સ્વરૂપ પ્રગટ કર્યા પછી હવે મારાથી છુપાશો નહિ. હે રામ, કલ્પના દ્વારા આપને, જેવા સ્વરૂપે મેં ચિંતવ્યા, બરાબર એવા જ સ્વરૂપે, મેં મારા દેહમાં આપનાં દર્શન કર્યાં. મારી તમામ શંકાઓ તેમજ મારા મનની તમામ ગ્રંથિઓ છૂટી ગઈ છે. માટે, હે હરિ, મને ગળે લગાડીને વાતો કરો.

રમે, તૂં રામ, જુવા ધરી, રંગ, તુંહીજ સમંદ, તુંહીજ તરંગ;
અનોઅન માંય, તૂંહીળો અંસ, હિવે, મ સંતાય, છતો થઈ હંસ.

(હરિરસ કંડિકા-૩૧૦)

જોયો મેં રામ, વિમાસિય જેમ, તનાં, ઘટ ભીતર, દીકઉ, તેમ;
ગળે ગો, ભ્રમ્મ, બિહૂટી ગંઠ, કરો, હરિ વાત, લગાડિય કંઠ.

(હરિરસ કંડિકા-૩૧૪)

‘હરિરસ’માં મહાત્મા ઈસરદાસજીએ પરમકૃપાળુ પરમેશ્વરની આરાધના કરી છે. પરમતત્ત્વ એક જ છે, અલગ નથી. પણ એમાંથી રામને અભ્યાસનો વિષય બનાવ્યો છે અને એના રસને પામવાનો પ્રયાસ છે.

સંદર્ભ સૂચિ

૧. ‘હરિરસ’ ગ્રંથ પ્રસિદ્ધકર્તા : પિંગળશી પાતભાઈ (ભાવનગર રાજકવિ), સંવત ૧૯૮૦, આવૃત્તિ ૨, ધી આનંદ પ્રિન્ટિંગ પ્રેસ, ભાવનગર, પૃષ્ઠ ૧૧
૨. એજન, પૃ. ૧૪
૩. એજન, પૃ. ૧૫
૪. એજન, પૃ. ૧૭
૫. એજન, પૃ. ૧૮
૬. ‘હરિરસ’ - સંપાદક : હરસુરભાઈ ગઢવી, પ્રવીણ પુસ્તક ભંડાર, આવૃત્તિ ચોથી, વર્ષ ૨૦૧૮, પૃ. ૩૩

‘હરિરસ’માં રામકથા : સ્વરૂપ અને સંદર્ભ

૭. એજન, પૃ. ૨૯
૮. ‘હરિરસ’ ગ્રંથ, પ્રસિદ્ધકર્તા : પિંગળશી પાતભાઈ (ભાવનગર રાજકવિ), સંવત ૧૯૮૦, આવૃત્તિ ૨, ધી આનંદ પ્રિન્ટિંગ પ્રેસ, ભાવનગર, પૃષ્ઠ ૩૦
૯. ‘હરિરસ’ - સંપાદક : આ. વદરીપ્રસાદ સાકરિયા, પ્રકાશક : સાદૂલ રાજસ્થાની રિસર્ચ ઇન્સ્ટિટ્યૂટ-વીકાનેર, વર્ષ ૧૯૬૦, પૃ. પ્રસ્તાવના ૧૪
૧૦. ‘હરિરસ’ - સંપાદક : હરસુરભાઈ ગઢવી, પ્રવીણ પુસ્તક ભંડાર, આવૃત્તિ ચોથી, વર્ષ ૨૦૧૮, પૃ. પ્રથમ.



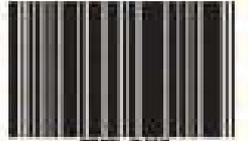
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Heritage as a Tool of Electoral Power Politics in India: A Critical Discourse

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ABSTRACT

India is a land of ancient cultures and civilizations. It has a rich history and material culture. India's material culture in the form of heritage was enriched in successive phases of its history. Each phase of Indian history has its share of material culture in the form of art and architecture; many times, juxtaposed or super imposed on the previous phase. Depending on the sway of power politics, heritage structures in general and faith-based heritage structures in particular had been destroyed, added, altered, partially disfigured, vandalized and even razed. With the independence of India in 1947 and the advent of electoral politics, heritage became a tool of power politics in India. The present paper tries to explore phase wise, various nuances of electoral power politics in India centring round the built heritage. The paper will also try to highlight the ramifications and future course of heritage based electoral politics in India. The methodology of the study is based on the scientific study and documentation of events and incidents related to heritage based electoral power politics from primary sources like interviews of political leaders and other secondary sources on the subject.

Keywords: Heritage, Power Politics, Material Culture, Discourse

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HUMAN RIGHTS EDUCATION AND INCLUSIVE PERSPECTIVES: RELEVANCE IN THE CONTEMPORARY TIMES

Mr. Jagannatham Begari

Abstract

Human rights and inclusive education are interrelated and interdependent. Human rights education is the important means to educate the people on human rights issues as the society at present witnesses diverse socio-cultural; economic, political conflicts and structural inequalities. Human rights education attempts to resolve them and educate all the sections including state, non-state actors and others stakeholders. In view of this, this paper is an attempt study the evolution and importance of human rights, various inclusive perspectives, teaching methods and their relevance in the contemporary times.

Keywords: Human rights Education, humanistic approach, transformative, egalitarianism, United Nations of Organisations, inclusive perspectives and criticality

1.1 Human Rights Education and Its Importance

HRE is the most important tool in upholding human rights and strengthen the universal and egalitarian values: democracy, socialism and secularism and strive for the freedom, justice and peace in the world. The UN and its affiliated agencies have not only taken initiatives but also have been implementing various programmes and actions for making human rights education a great success. There has been a tremendous impact of Human Rights Education (HRE) on the people. HRE intends to educate students, teachers, social activists and policymakers and implementers through both the conventional and unconventional modes to build the culture of human rights by the imparting knowledge, skills and mould the people to protect and promote human rights. Thereby uphold fundamental freedoms, dignity, tolerance, gender equality and racial, ethnic and religious tolerance. This belief certainly enables individuals to act and participate in public domain freely. The objective Human rights education is to develop the human rights knowledge, skills, and values of human rights. In other words, the objective of HRE is to develop critical faculty and empower a person to discover his/her inner abilities and critical thinking on socio-political and economic-cultural aspects. (Stone, Adam, 2002).

1.2. United Nations of Organization and Human Rights Education:

Ile209 of *Leishmania donovani* xanthine phosphoribosyltransferase plays a key role in determining its purine base specificity

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Xanthine phosphoribosyltransferase (XPRT) and hypoxanthine-guanine phosphoribosyltransferase (HGPRT) are purine salvaging enzymes of *Leishmania donovani* with distinct 6-oxopurine specificities. LdXPRT phosphoribosylates xanthine, hypoxanthine, and guanine, with preference toward xanthine, whereas LdHGPRT phosphoribosylates only hypoxanthine and guanine. In our study, LdXPRT was used as a model to understand these purine base specificities. Mutating I209 to V, the conserved residue found in HGPRTs, reduced the affinity of LdXPRT for xanthine, converting it to an HGXPRT-like enzyme. The Y208F mutation in the active site indicated that aromatic residue interactions with the purine ring are limited to pi-pi binding forces and do not impart purine base specificity. Deleting the unique motif (L55-Y82) of LdXPRT affected enzyme activity. Our studies established I209 as a key residue determining the 6-oxopurine specificity of LdXPRT.

Keywords: guanine; hypoxanthine; *Leishmania*; phosphoribosyltransferases; purine salvage; purine specificity; xanthine; xanthine phosphoribosyltransferase

Leishmania donovani, the protozoan parasite causing visceral leishmaniasis lacks the metabolic machinery required for the *de novo* synthesis of purine nucleotides. Hence, it salvages purine bases from the human host [1]. Due to the critical dependence of *L. donovani* on the purine salvage pathway, enzymes acting in this pathway could serve as a suitable target for antileishmanial chemotherapy [2]. *Leishmania* has three phosphoribosyl transferases that convert dephosphorylated purines to nucleosides monophosphates: hypoxanthine-guanine phosphoribosyltransferase (HGPRT), xanthine phosphoribosyltransferase (XPRT), and adenine phosphoribosyltransferase (APRT) [3]. XPRT and HGPRT

are specific for 6-oxopurines. Of the two, the enzyme XPRT of *L. donovani* has been a focus of attention due to its notable absence in mammals. Our earlier studies [4] have shown that there are also significant structural differences between LdXPRT and its closest human counterpart, that is, HGPRT, therefore making it an attractive target for selective inhibition. Null mutational studies have also shown that the XPRT activity alone is sufficient to salvage adenine, hypoxanthine, and guanine in *L. donovani* promastigotes [5,6].

LdXPRT in addition to converting xanthine to xanthine monophosphate can also convert hypoxanthine to inosine monophosphate and guanine to guanine

Abbreviations

APRT, Adenine phosphoribosyltransferase; CD, Circular dichroism; GMP, Guanosine 5'-monophosphate; GPRT, guanine phosphoribosyltransferase; HGXPRT, hypoxanthine-guanine-xanthine phosphoribosyltransferase; IMP, Inosine 5'-monophosphate; LdHGPRT, *Leishmania donovani* hypoxanthine-guanine phosphoribosyltransferase; LdXPRT, *Leishmania donovani*-Xanthine phosphoribosyltransferase; PRPP, 5-Phospho- α -D-ribose 1-diphosphate; PRT, Phosphoribosyltransferase; XMP, Xanthosine 5'-monophosphate.

monophosphate, albeit with a lesser affinity toward hypoxanthine and guanine compared to xanthine, whereas the LdHGRPT can phosphoribosylate only hypoxanthine and guanine but not xanthine. A unique XPRT gene is present only in few organisms such as *Leishmania*, certain fungi, and bacteria [4]. Xanthine phosphoribosylating enzymes in other organisms like *P. falciparum* [7], *T. gondii* [8], and *T. foetus* [9] is the hypoxanthine-guanine-xanthine PRT (HGXPRT) which prefers hypoxanthine and guanine as a substrate over xanthine. However, the LdXPRT substrate profile is different from the HGXPRT enzymes, as it favors xanthine over hypoxanthine and guanine. This unique substrate specificity of LdXPRT compared to other purine PRTs as well as its intriguing presence in *Leishmania* along with a separate HGPRT makes it an ideal model to investigate the molecular determinants of the unique purine base specificity of PRTs.

Crystal structures of purine PRTs show a conserved PRPP-binding domain and a less conserved purine-binding domain. The latter domain shows variations in amino acid residues among different purine PRTs [9] which might play a crucial role in the purine base specificity of these enzymes. There are earlier reports of alteration of the PRT substrate specificity by single amino acid substitutions [9,10]. Since the 6-oxopurines (xanthine, hypoxanthine, and guanine) differ only by the substituents on the C-2 atom of their purine ring, we hypothesized that the residues interacting specifically with these atoms may be critical in determining the purine base specificity of PRT enzymes.

Based on sequence and structure comparisons with other PRTs, especially with *Leishmania terantolae* HGPRT-GMP (PDB ID: 1PZM) structure, we identified certain residues and a domain that may be important for this specificity in LdXPRT [4]. In the present study, we investigated the amino acids interacting with the substituent on C-2 atom of purine ring (Ile 209 and Glu 215), an aromatic residue forming stacking interactions with the purine ring (Tyr 208) as well as a unique motif (Leu 55 to Tyr 82) found in leishmanial XPRTs through mutational studies. Previous reports on the mutational study of E215D showed that Glu 215 alone cannot be the determinant for purine base specificity of LdXPRT [11]. Hence in the present study, we mutated E215D together with the I209V. Based on our detailed sequence and structure analysis, we generated four mutants of LdXPRT (ldxpY208F, ldxprtI209V, ldxprtI209V;E215D, and ldxprtL55_Y82del). Characterization of the mutant proteins through enzyme assays and biophysical methods could evince the role of these residues in governing the purine base specificity of LdXPRT.

Materials and methods

Materials

All the chemicals used in the present study were molecular biology grade. The chemicals for enzyme kinetic assays including hypoxanthine, guanine, xanthine, and PRPP were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). High-fidelity DNA polymerase (iProof™) and other enzymes were procured from Bio-Rad (Hercules, CA, USA) and Thermo Fisher Scientific (Waltham, MA, USA), respectively.

Structural modeling and sequence comparison

The LdXPRT sequence (UniProtKB-Q9U6Y2) was retrieved from the Universal protein resource database (<http://www.uniprot.org/>). The LdXPRT model was generated by the homology modeling program SWISS-MODEL [12] using *T. brucei* HGXPRT (PDB ID: 6MXC) structure [13] as a template. The model was minimized using the ModRefiner program [14], and the stereo-chemical properties of the energy minimized model were evaluated by Ramachandran statistics using the program RAMPAGE [15]. The quality of the minimization model was also checked using the Verify 3DE [16] and ERRAT plot [17]. The refined LdXPRT model was superimposed over the crystal structure of the *L. tarentolae* HGPRT-GMP complex (PDB ID: 1PZM) to compare the active-site residues. The structures were superimposed using PYMOL [18]. Multiple sequence alignment of LdXPRT with other 6-oxopurine PRTs was carried out using the ClustalW program [19]. The PRT enzyme sequences were downloaded from NCBI (<https://www.ncbi.nlm.nih.gov/>) and UniProt (<https://www.uniprot.org/>) databases.

Site-directed mutagenesis

Site-directed mutations were introduced in the LdXPRT gene by polymerase chain reaction using our previously generated pET 15b-LdXPRT construct, as template [4]. Primers were designed and synthesized with additional restriction sites to allow easy selection of mutants (Table S1). Restriction sites were introduced by modifying nucleotides in such a way that their amino acid codon remains the same. Primers of deletion mutant were synthesized with phosphorylated 5' end for the ligation. Following the polymerase chain reaction, mutant plasmids were transformed and amplified into *E. coli* strain DH5 α and were sequenced to verify mutations.

Expression and purification of LdXPRT mutants

The mutant plasmids were transformed into the Rosetta (DE3) cells for expression. The cells were grown on LB agar media supplemented with ampicillin (50 $\mu\text{g}\cdot\text{mL}^{-1}$) and

chloramphenicol (34 $\mu\text{g}\cdot\text{mL}^{-1}$), and the expression was induced by adding 0.1 mM IPTG at 18 °C for 16 h. The cells were harvested by centrifugation at 8000 *g* at 4 °C for 10 min and were suspended in the lysis buffer containing 50 mM Tris/HCl, pH 7, 10 mM MgCl_2 , 200 mM NaCl, 10% glycerol, 0.1% Triton X-100, and 1 mM PMSF. The cells were lysed in a sonicator followed by centrifugation at 12 000 *g* for 20 min at 4 °C, and the lysate was loaded on a HIS-Select HF Nickel Affinity Gel column, pre-equilibrated with equilibration buffer (50 Tris/HCl buffer, pH 7, 10 mM MgCl_2 , and 200 mM NaCl). The protein was eluted by 300 mM Imidazole and was analyzed on SDS/PAGE as per the method of Laemmli [20], and the concentration of purified proteins was determined by the Bradford method. The purified proteins were dialyzed in 50 mM Tris/HCl buffer, pH 7, 10 mM MgCl_2 , and 200 mM NaCl.

Spectral analysis

The CD spectral measurements were performed on JASCO-J815 spectropolarimeter at 20 °C. The mutant proteins were dialyzed in the buffer containing 20 mM sodium phosphate, pH 6.5, and 10 mM MgCl_2 . The far-UV CD spectra were collected at 190 to 250 nm using a 0.5 mm path length cuvette at 0.3 $\text{mg}\cdot\text{mL}^{-1}$ of protein concentration, and the near-UV CD spectra were collected at 250–350 nm using a 2 mm path length cuvette at 1.3 $\text{mg}\cdot\text{mL}^{-1}$ of protein concentration. To record the spectra of mutant proteins in the presence of ligands (XMP, GMP, and IMP), the proteins were incubated for 2 h with 500 μM of each ligand prior to the CD experiment. Data were calculated by subtracting the spectra of ligands and medium from the corresponding spectra of proteins. UV absorbance spectra were recorded on Eppendorf BioSpectrometer kinetic from 240 to 330 nm using a protein concentration of 0.8 $\text{mg}\cdot\text{mL}^{-1}$ protein in 50 mM sodium phosphate buffer, pH 6.5, and 10 mM MgCl_2 .

Fluorescence quenching analysis

Fluorescence measurements were carried out on a Cary Eclipse fluorescence spectrophotometer-G9800A with a slit width of 5 nm for both the monochromators and scan speed of 600 $\text{nm}\cdot\text{min}^{-1}$. Mutant proteins were diluted up to 3.9 μM in the buffer containing 50 mM Tris/HCl, pH 7, 200 mM NaCl, and 10 mM MgCl_2 . The proteins were titrated by adding 10–12 aliquots of ligands (XMP, GMP, and IMP) from 1 mM stocks, and the samples were excited at 280 nm. The emission spectra were recorded at 27 °C, from 285 to 370 or 450 nm depending on the protein. Each titration experiment was performed in triplicates. The effective concentrations of ligands were corrected to compensate dilution effect upon the addition of each aliquot of ligand. The dissociation constant (K_d) was calculated according to the method described in reference [4].

Steady-state kinetic analysis of LdXPRT mutants

Kinetic parameters of LdXPRT mutants for nucleobase substrates xanthine, hypoxanthine, and guanine were determined by measuring the formation of their respective products, XMP at 250 nm ($\Delta\epsilon$ 3900 $\text{M}^{-1}\text{cm}^{-1}$), IMP at 243 nm ($\Delta\epsilon$ 2200 $\text{M}^{-1}\text{cm}^{-1}$), and GMP at 257 nm ($\Delta\epsilon$ 4200 $\text{M}^{-1}\text{cm}^{-1}$), respectively. The assay was performed in 100 mM Tris/HCl buffer, pH 7, and 10 mM MgCl_2 using 1.0 cm path length on Eppendorf BioSpectrometer® kinetic equipped with a kinetic software package. Kinetic parameters were calculated by using the software GRAPHPAD PRISM (San Diego, CA, USA). The K_m and k_{cat} values for nucleobase substrates were determined at 1.0 mM PRPP and either 10–250 μM xanthine, 10–250 μM guanine, or 10–1000 μM hypoxanthine. The K_m and k_{cat} values of PRPP were determined at 1.0 mM xanthine or hypoxanthine by ranging the PRPP concentrations between 10 and 1000 μM , respectively.

Results

Structural modeling and sequence comparison

The LdXPRT model was constructed using *T. brucei* HGXPRT crystal structure (PDB ID: 6MXB) as a template [13]. The LdXPRT model superposed over the *T. brucei* HGXPRT crystal structure with an RMSD of 0.394 Å. The model so generated was subjected to energy minimization for improving the overall model quality. The energy minimized model showed acceptable Ramachandran plot statistics with 100% residues in the allowed region and acceptable ERRAT plot and Verify 3D scores (Table S2). The model was superimposed over LtHGPRT-GMP (PDB ID: 1PZM) structure to compare the active sites. The active site of LtHGPRT-GMP structure shows three interactions with the guanine ring. The first interaction was between Lys 157 and 6-oxo group, and N7 atoms of the guanine ring (Fig. 1). This lysine residue (Lys 186) was conserved in LdXPRT (Fig. 2) and probably involved in binding to the 6-oxo group of xanthine. The second interaction was an aromatic stacking interaction between Phe 178 and the guanine ring. The third interaction in LdHGPRT-GMP was between Val 179 and the 2-amino, 6-oxo, and N1 group of guanine ring. The fourth interaction was between Asp 185 and the 2-amino group of guanine ring. Structural comparisons with other HGPRTs with bound substrates showed that these interacting residues and these interactions were mostly conserved across the HGPRT family. LdXPRT has a Tyr (Tyr 208) corresponding to Phe 178, Ile (Ile 209) corresponding to Val 179, and Glu (Glu 215) corresponding to Asp 185 of

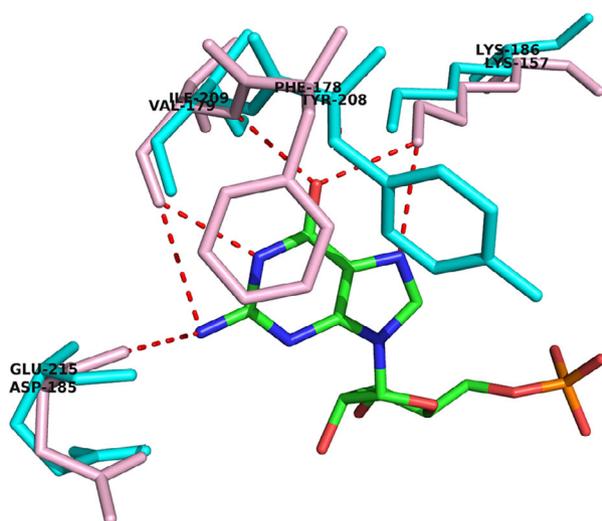


Fig. 1. Comparison of the active site of *L. tarentolae* HGPRT with LdXPRT. Residues of *L. tarentolae* HGPRT-GMP complex (PDB ID: 1PZM) are indicated by pink color. Corresponding residues in LdXPRT are indicated by cyan color.

LdHGPRT. The sequence alignment of PRTs (Fig. 2) showed that these residues (Ile 209, Glu 215, and Tyr 208) were rarely found in HGPRTs. Hence, these three amino acids were mutated to investigate their role in the substrate specificity of LdXPRT.

From our earlier studies, we had identified a 28 residue long unique motif (Leu 55 to Tyr 82) in LdXPRT which was absent in other PRT enzymes. Molecular dynamic studies indicated that this region might be having a long-range interaction with the active site [4].

Site-Directed Mutagenesis and enzyme purification

Based on *in silico* analysis, four mutants of LdXPRT were generated by polymerase chain reactions. We mutated Ile 209 to a Val, the conserved residue found in place of Ile in most HGPRTs (ldxpI209V). Since previous reports on the mutational study of E215D in LdXPRT showed that mutating Glu 215 alone to an Asp does not alter purine base specificity of LdXPRT [11], in our study, we generated a double mutant—ldxpI209V;E215D in which the Glu 215 was mutated to Asp and Ile 209 was mutated to Val. Tyr 208 in LdXPRT was mutated to Phe (ldxpIY208F). A deletion mutant of LdXPRT where residues from Leu 55 to Tyr 82 were deleted was generated to check the role of this motif in the enzyme activity. All the PCR amplified mutant genes were transformed and amplified into *E. coli* DH5 α cells. The mutants were

screened by digestion with specific restriction endonucleases and verified by sequencing. The expression of LdXPRT mutants was obtained in *E. coli* Rosetta (DE3) cells, and the proteins were purified by Ni-NTA affinity chromatography (Fig. 3).

Structural analysis of LdXPRT mutants by CD spectroscopy

The far-UV CD spectra showed that all the LdXPRT mutants were well folded (Fig. 4A). The mutants showed slight variations in the secondary structure content compared to the wild type.

The CD difference spectra generated by the subtraction of the far-UV CD spectrum of the LdXPRT from the ldxpI209V showed an increase in the minima between 200 and 210 nm (Fig. 5A), indicating an alteration in the environment of the phenylalanine side chains [11,21]. This was further supported by the increase in UV absorbance of ldxpI209V at ~ 250 nm (Fig. 5B) [11].

Similarly, a negative peak of ldxpIY208F and ldxpI209V;E215D at ~ 227 nm in the CD difference spectra and an increase in the UV absorbance at ~ 280 nm indicate an alteration in the local environment of one or more tyrosine residues [11,22].

The effect of nucleotide substrates, XMP, GMP, and IMP on the secondary and tertiary structure pattern of LdXPRT mutants was determined by far and near-UV CD spectroscopy. The addition of XMP to LdXPRT and ldxpIY208F induces a negative shift in the far-UV CD spectra (Fig. 4B,C) indicating that the protein folds upon binding with XMP. The addition of XMP to ldxpI209V, ldxpI209V;E215D, and ldxpL55_Y82del causes positive shift in the spectra. Further, this XMP-induced shift is higher than IMP and GMP (Fig. 4D–F) indicating that the higher structural change was required to accommodate XMP compared to IMP and GMP.

The addition of IMP to all the proteins causes increased ellipticity with no significant change in the pattern of near-UV CD spectra (Fig. S1). The addition of GMP to LdXPRT, ldxpIY208F, and ldxpL55_Y82del gives a sharp negative peak at 257 nm; however, the binding of GMP to ldxpI209V and ldxpI209V;E215D showed a positive peak. Interestingly, LdXPRT, ldxpIY208F, and ldxpL55_Y82del have Ile 209 in the purine-binding pocket; however, ldxpI209V and ldxpI209V;E215D have Val in place of Ile hence suggesting that the interaction of Ile 209 with the 2-amino group of guanine might cause the alteration in the environments of aromatic side chains of the protein.

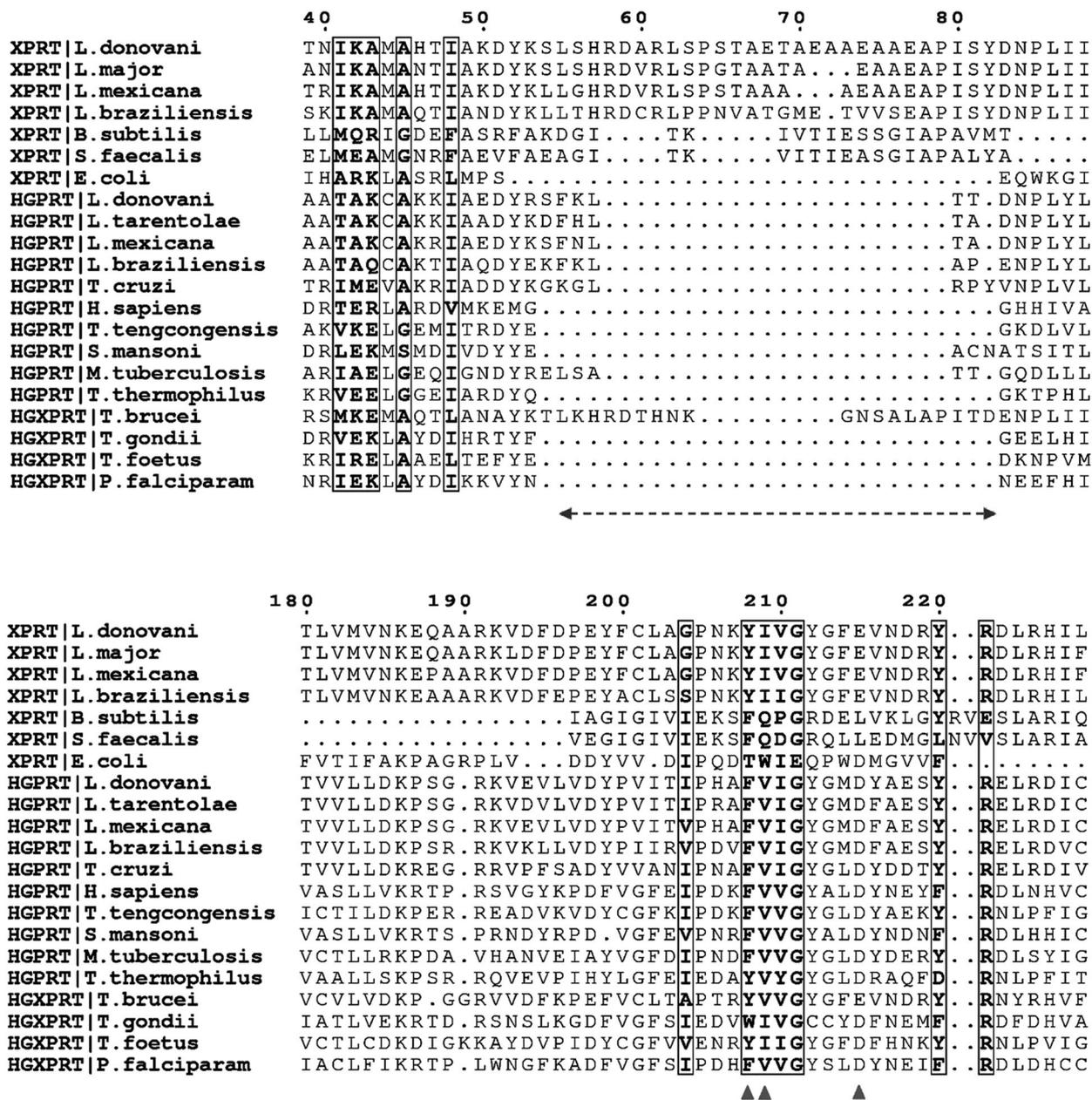


Fig. 2. Multiple sequence alignment of LdXPRT with PRT enzymes. The residues which were mutated are highlighted as (▲). The unique region (55–82) is highlighted by a dotted line.

Fluorescence spectroscopy for binding affinities of mutants with different ligands

The fluorescence emission spectra of ldxprtY208F and ldxprtI209V;E215D showed a similar emission maximum (305 nm) as wild-type protein (Fig. 6); however, λ_{max} of ldxprtI209V and ldxprtL55_Y82del shifts from 305 nm to 337 nm. Since the LdXPRT sequence does not contain tryptophan, this shift in the fluorescence spectra is likely due to the ionized form of tyrosine, that

is, tyrosinate which is produced by the proton transfer to the carboxylate anion of the nearby glutamyl residues [23]. The I209V mutation might be modifying the microenvironment of the nearby glutamate residues, that is, Glu 215 to facilitate the ionization of the surrounding tyrosine (Tyr 208) to tyrosinate which gives rise to abnormal emission at around 340 nm. Similarly, the structural changes occurring due to the absence of the motif in the ldxprtL55_Y82del might be creating a

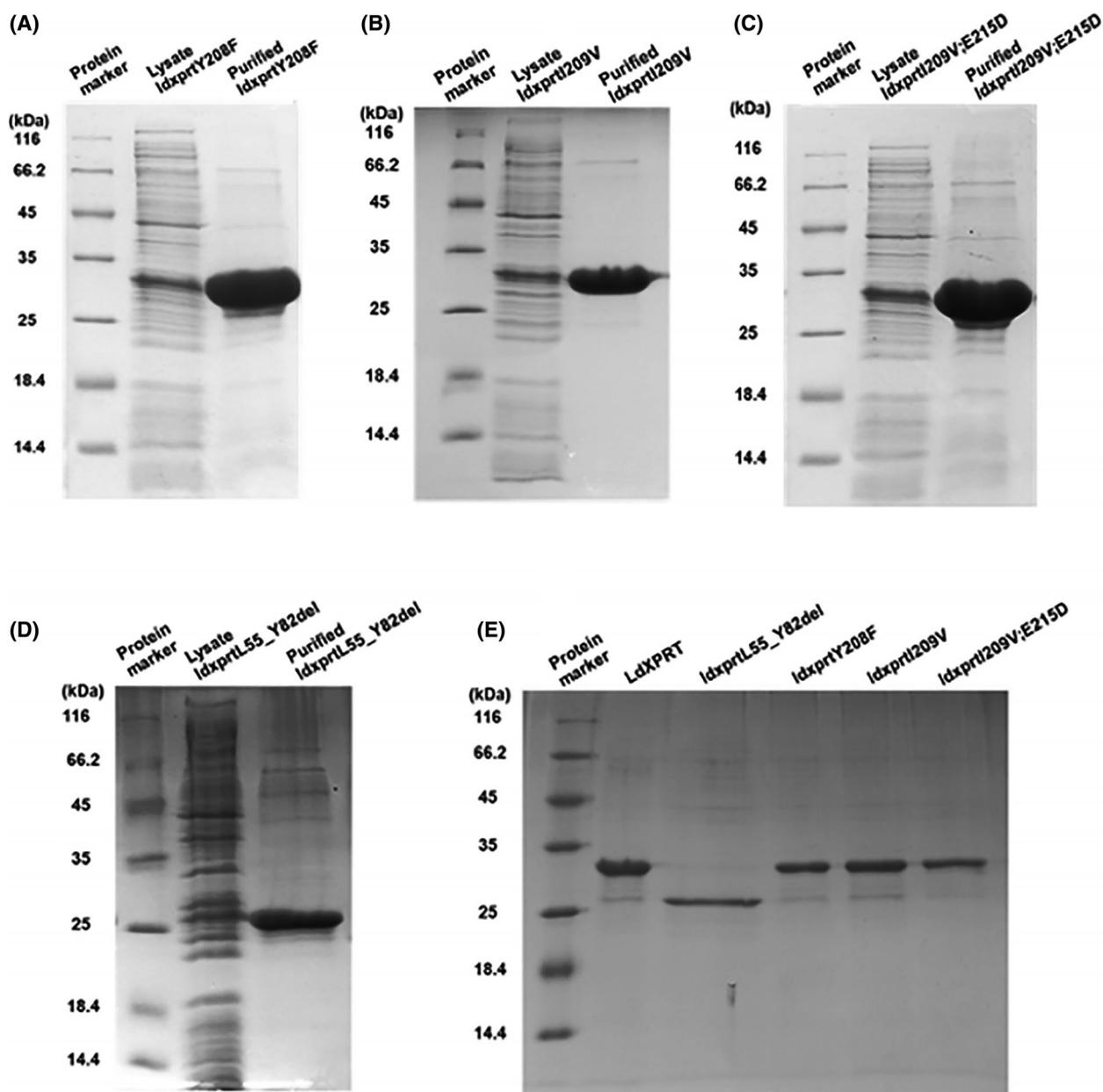


Fig. 3. SDS/PAGE analysis of the purified LdXPRT mutants. (A) LdXPRT Y208F; (B) LdXPRT I209V; (C) LdXPRT I209V;E215D; (D) LdXPRT L55_Y82del; and (E) Comparison of LdXPRT with mutant proteins.

similar microenvironment surrounding the tyrosine residue. This is not the case in LdXPRT I209V/E215D, since the position of glutamate which provides a carbonyl acceptor group to tyrosine for the conversion of tyrosinate is mutated to aspartate.

The binding affinities of mutants with XMP, IMP, and GMP were determined in terms of the dissociation constant (K_d), measured from the fluorescence quenching spectra. Fluorescence quenching was carried out by titrating the protein samples against ligands

(Fig. 7). Wild-type enzyme showed K_d values of 5.16×10^{-5} , 10.28×10^{-5} , and 6.35×10^{-5} Mol for XMP, IMP, and GMP, respectively, indicating that it prefers XMP over IMP and GMP. The K_d values of LdXPRT I209V and LdXPRT I209V;E215D revealed that these two proteins have higher affinities for IMP and GMP than XMP (Table 1). LdXPRT Y208F showed a very minor shift in K_d of XMP and GMP with a notable decrease in K_d of IMP; however, its specificity remained the same as xanthine was a more favored

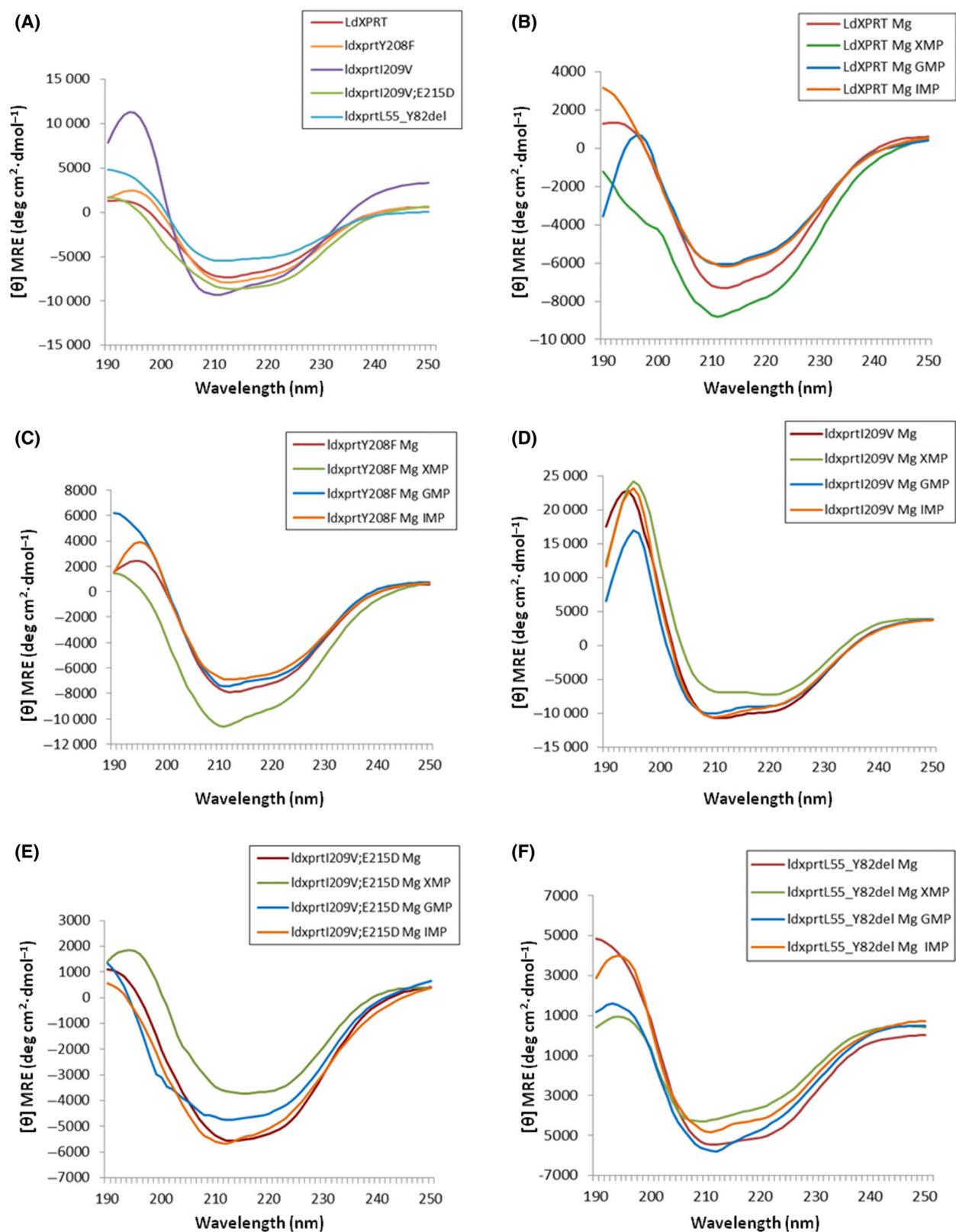


Fig. 4. Far-UV CD spectral analysis of LdXPRT mutants. (A) Far-UV CD spectra of wild-type LdXPRT and mutants; (B) LdXPRT with ligands; (C) IdxpY208F with ligands; (D) IdxpI209V with ligands; (E) IdxpI209V;E215D with ligands; and (F) IdxpL55_Y82del with ligands.

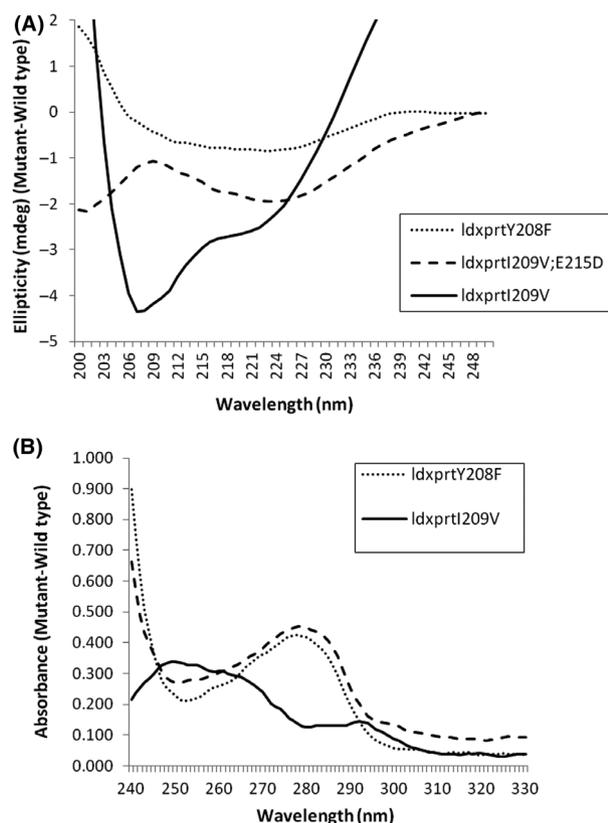


Fig. 5. Spectral analysis of LdXPRT mutants. (A) CD difference spectra were generated by subtracting the far-UV CD spectra of LdXPRT from the spectra of ldxprtY208F, ldxprtI209V, and ldxprtI209V;E215D. The spectra were recorded from 190 to 250 nm. (B) UV absorbance difference spectra were generated by subtracting the LdXPRT spectra from the spectra of ldxprtY208F, ldxprtI209V, and ldxprtI209V;E215D. The spectra were recorded from 240 to 330 nm.

substrate than hypoxanthine and guanine. The ldxprtL55_Y82del showed a significant increase in K_d for all three ligands indicating a decreased affinity for all.

Kinetic analysis of LdXPRT mutants

Kinetic analysis demonstrated that LdXPRT favors xanthine over hypoxanthine and guanine (Table 2). The K_m values of LdXPRT for xanthine, hypoxanthine, and guanine in Tris/HCl buffer, pH 7 were 8.9 ± 1.5 , 204.2 ± 14.67 , and 103.7 ± 15.24 μM , and the k_{cat} values were 1.8 ± 0.041 , 1.1 ± 0.093 , and 0.455 ± 0.042 s^{-1} , respectively. The ldxprtY208F showed a slight increase in K_m for xanthine (25.54 ± 2.9 μM) and a notable decrease in K_m values of hypoxanthine (82.21 ± 15.2 μM) and guanine (57.03 ± 17.87 μM). However, its specificity for nucleobases remains the same as that of LdXPRT (xanthine>guanine>hypoxanthine). The

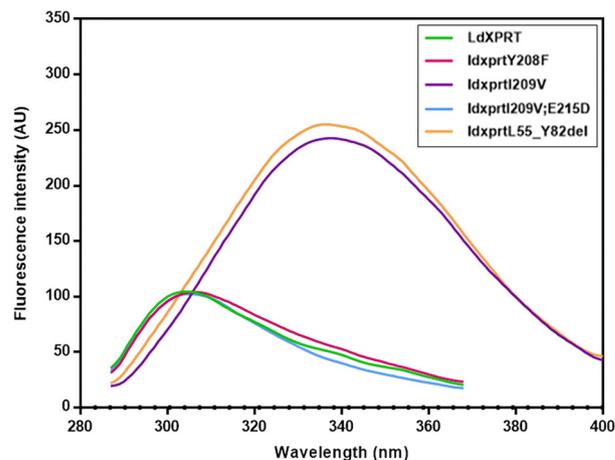


Fig. 6. Fluorescence emission spectra of mutants and wild-type LdXPRT.

ldxpirtI209V showed a sevenfold-eightfold increase in K_m for xanthine with a fourfold-eightfold decrease in K_m for hypoxanthine and twofold-sevenfold decrease in K_m for guanine. The K_m value of ldxprtI209V;E215D for xanthine was almost similar to that of ldxprtI209V with an additional decrease in K_m of hypoxanthine and guanine indicating that these mutations change the specificity of the enzyme for nucleobases. Surprisingly, ldxprtI209V;E215D showed a marked increase in k_{cat} values of all purine bases and PRPP; however, ldxprtI209V does not cause any significant change in k_{cat} values indicating that the Asp 215 is responsible for higher turnover rates. The ldxprtL55_Y82del did not show activity for any of the nucleobases suggesting the importance of this region in enzyme activity as the mutant protein in CD studies was found to be stable and in folded form.

Discussion

XPRT and HGPRT are the main enzymes for purine salvage in *L. donovani* and potential targets for designing antileishmanial agents [6,24]. LdXPRT is the focus of attention since long as it is absent from humans. The mutational studies of LdXPRT reported earlier were focused only on acidic residues of the purine-binding domain [11]. In the present study, we mutated all the differing active-site residues of LdXPRT from the purine-binding residues of HGPRT to identify the molecular determinants of its unique 6-oxopurine specificity. We also generated a mutant where the unique motif that was identified in leishmanial XPRT enzymes was deleted to elucidate its effect on the structure and activity of the enzyme. From sequence and structure comparison of LdXPRT with the crystal structure of *L. tarentolae* HGPRT, four purine-

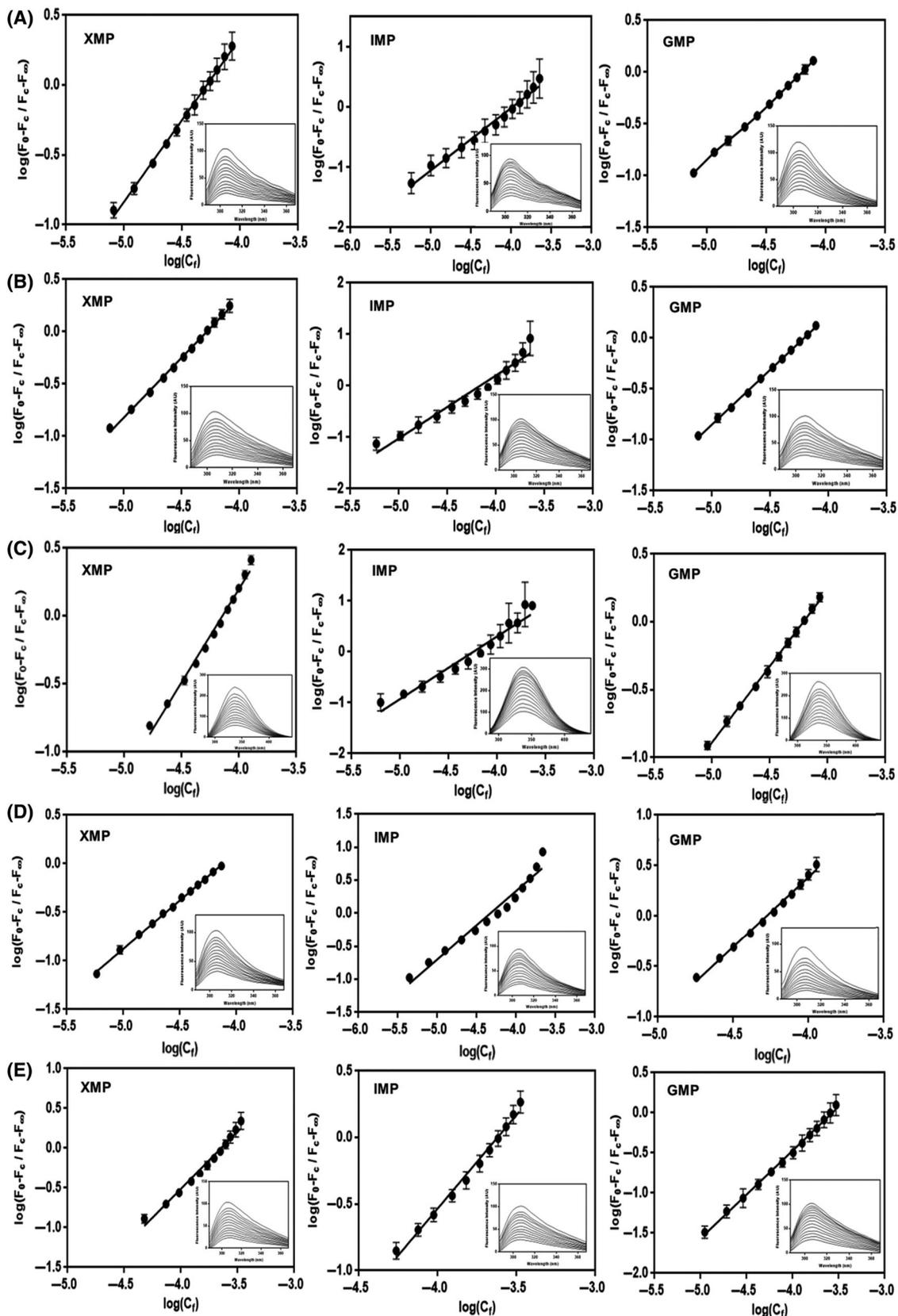


Fig. 7. Plots of fluorescence quenching for mutants and wild-type LdXPRT with ligands. The inset figures represent the progressive emission spectra of each enzyme upon addition of ligand. (A) LdXPRT; (B) ldxprtY208F; (C) ldxprtI209V; (D) ldxprtI209V;E215D; (E) ldxprtL55_Y82del. F_0 , F_c , and F_∞ are the relative fluorescence emission intensity of the enzyme alone, the enzyme in the presence of a concentration (C_f) of ligand, and the intensity of enzyme saturated with ligand, respectively. C_f is the free ligand concentration in molarity. The dissociation constant (K_d) for each ligand was determined from the plot of $\log(C_f)$ versus $\log(F_0 - F_c / F_c - F_\infty)$ using antilog of X intercept when $Y = 0$, through the relation $K_d = 1/K_a$; where $K_a = \text{antilog}(-pK_a)$.

Table 1. Dissociation constants of mutant–ligand interactions determined by fluorescence spectroscopy.

Enzymes	XMP K_d (Mol)	IMP K_d (Mol)	GMP K_d (Mol)
LdXPRT	$5.16 \times 10^{-5} \pm 0.123$	$10.28 \times 10^{-5} \pm 0.402$	$6.35 \times 10^{-5} \pm 0.167$
ldxprtY208F	$5.19 \times 10^{-5} \pm 0.063$	$6.8 \times 10^{-5} \pm 1.220$	$6.13 \times 10^{-5} \pm 0.016$
ldxprtI209V	$7.21 \times 10^{-5} \pm 1.040$	$5.63 \times 10^{-5} \pm 0.830$	$6.16 \times 10^{-5} \pm 0.085$
ldxprtI209V;E215D	$7.58 \times 10^{-5} \pm 0.824$	$4.7 \times 10^{-5} \pm 0.391$	$5.24 \times 10^{-5} \pm 0.454$
ldxprtL55_Y82del	$22.59 \times 10^{-5} \pm 2.13$	$26.85 \times 10^{-5} \pm 1.55$	$23.87 \times 10^{-5} \pm 1.45$

binding residues (Lys 186, Tyr 208, Ile 209, and Glu 215) were identified in the binding site of LdXPRT. The Lysine residue (Lys 186) was conserved in all PRTs whereas Tyr 208, Ile 209, and Glu 215 were replaced by Phe, Val, and Asp, respectively, in most of the HGPRT enzymes (Fig. 2 & Table 3).

The substitution of Tyr 208 by Phe did not cause any significant change in the purine base specificity of the LdXPRT. A previous report on mutation study of *T. foetus* HGXPRT [9] also showed that Y156W and Y156F do not cause any significant change in the nucleobase specificity of the enzyme, hence suggesting that the interactions of the hydroxyl group of Tyr 208 with the exocyclic C-2 substituent of the purine ring might be minimal and the interactions between aromatic amino acids and the purine ring might be limited to the pi-pi binding forces. The ldxprtI209V and ldxprtI209V;E215D showed a significant decrease in affinity for xanthine and increased affinity for hypoxanthine and guanine. Similar substrate specificities were also observed in most of the PRTs (Table 3) having Val at the corresponding position; they show negligible or no activity for xanthine. The *G. lamblia* GPRT [25] also has Val at the corresponding position, and it also does not have specificity for xanthine. Enzymes having Gln (*B. subtilis* [26] and *S. faecalis* XPRT [27]) in place of Ile 209 of LdXPRT have a strong affinity for xanthine but a negligible affinity for hypoxanthine and guanine whereas those PRTs (*T. gondii* and *T. foetus* HGXPRT, LdXPRT) having Ile in this position can recognize all three purine bases (Table 3). Hence, it suggests that the Ile 209 in LdXPRT might be responsible for the affinity of LdXPRT for all three purine bases with good affinity toward xanthine whereas Val at the corresponding position might reduce the affinity for xanthine. The only exception in this pattern is the *T. brucei* HGXPRT

(Table 3) which despite having Val at this position showed a higher affinity for xanthine. The authors have speculated that this may be due to the formation of the hydrogen bond between the hydroxyl group of Tyr 201 and the main-chain carbonyl oxygen of Glu 208 which assisted the binding of xanthine/XMP by holding the base in place [28].

Previous mutational studies of E215D [11] and D163E [9] in LdXPRT and *T. foetus* HGXPRT, suggested that these single point mutations do not give any significant change in the purine base specificity of the enzyme. However, in the present study the double mutant ldxprtI209V;E215D showed a ~ twofold-threefold decrease in K_m for hypoxanthine and guanine without alteration in the K_m of xanthine compared to ldxprtI209V (Table 2). Hence, the I209V and I209V;E215D mutations converted LdXPRT to a HGXPRT-like enzyme which exhibits nucleobase specificity comparable to *T. gondii* [8], *T. foetus* [9], and *P. falciparum* [29] HGXPRTs. Surprisingly, ldxprtI209V;E215D gives a marked increase in turnover rates with all purine bases. Such increased catalytic efficiency was also observed in the single mutant E215D [11] indicating that the presence of Asp might be responsible for the increased turnover rates of the enzyme. In many HGPRTs, this Asp in addition to direct interactions with the purine ring is also involved in binding to an Mg atom that is linked through a water molecule to the purine substrate and helps to orient the purine base for catalysis [30–32].

CD spectroscopic studies revealed that the ldxprtI209V causes alteration in the phenylalanine side chains of the protein whereas the ldxprtY208F and the ldxprtI209V;E215D cause an alteration in the local environment of the tyrosine residues. CD analysis also suggested that the binding of XMP to ldxprtI209V,

Table 2. Kinetic parameters of enzyme assay for mutants and wild-type LdXPRT. NA represents no enzyme activity.

Enzymes	Xanthine			Hypoxanthine			Guanine			PRPP (Xanthine)			PRPP (Hypoxanthine)		
	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($\text{s}^{-1} \mu\text{M}^{-1}$)	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($\text{s}^{-1} \mu\text{M}^{-1}$)	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($\text{s}^{-1} \mu\text{M}^{-1}$)	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($\text{s}^{-1} \mu\text{M}^{-1}$)	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($\text{s}^{-1} \mu\text{M}^{-1}$)
LdXPRT	8.9 ± 1.5	1.8 ± 0.041	0.2022	204.2 ± 54.67	1.1 ± 0.093	0.0053	103.7 ± 15.24	0.455 ± 0.042	0.0043	18.85 ± 4.5	2 ± 0.054	0.106	41.76 ± 12.41	1.3 ± 0.056	0.0311
ldxprrtY208F	25.54 ± 2.9	1.126 ± 0.029	0.044	82.21 ± 15.2	0.577 ± 0.04	0.0070	57.03 ± 17.87	0.308 ± 0.03	0.0054	19.54 ± 6.4	0.81 ± 0.03	0.0414	32.53 ± 9.1	1.1 ± 0.036	0.0338
ldxprrtI209V	66.62 ± 8.3	4.19 ± 0.18	0.0628	48.62 ± 14.27	1.23 ± 0.067	0.0252	37.30 ± 4.16	0.518 ± 0.016	0.0138	29.9 ± 4.75	4.38 ± 0.09	0.146	35.76 ± 6.84	1.21 ± 0.029	0.0338
ldxprrtI209V;E215D	60.98 ± 6.73	15.92 ± 0.59	0.2610	26.49 ± 8.83	9.89 ± 0.41	0.3733	14.41 ± 2.52	8.68 ± 0.24	0.6023	36.69 ± 7.06	13.29 ± 0.36	0.3622	22.57 ± 4.67	7.99 ± 0.27	0.3540
ldxprrtL55_Y82del	NA	NA	NA												

ldxprrtI209V;E215D, and ldxprtL55_Y82del requires higher structural change than the IMP and GMP (Fig. 4D–F). It was also observed that the binding of GMP to the proteins having Ile 209 (LdXPRT, ldxprtY208F, and ldxprtL55_Y82del) in the purine-binding pocket causes the alteration in the environments of aromatic side chains of the proteins.

In our previous report, we identified a unique motif (Leu 55 to Tyr 82) in the LdXPRT sequence which was absent in most of the PRTs including leishmanial, human, and trypanosomal HGPRs. The region was predicted to be involved in stabilizing the interaction of the enzyme with the substrate during the enzyme reaction [4]. The deletion of this region from the enzyme caused the increase in K_d (Table 1) for all the three nucleotides and loss of activity in enzyme assay (Table 2) indicating its importance in enzyme activity since the protein was stable and in folded form.

LdXPRT is a protein with the remarkable ability to recognize more than one substrate albeit with different catalytic efficiencies. Our biochemical investigations with the mutants of LdXPRT have unraveled key residues that allow LdXPRT to act as an XPRT and not as an HGXPRT. A single residue mutation of isoleucine that interacts with the C-2 substituent group of the purine ring to a valine could modulate LdXPRT specificity for binding to xanthine and make it behave more like an HGXPRT. Understanding the active-site residues which determine the structural discrimination of different purine substrates will be helpful to design specific inhibitors against this potential antileishmanial drug target. The present study can be further enhanced by crystal structures of LdXPRT and its mutants with different substrates which can throw more light on how precisely these binding site residues assemble in order to achieve nucleobase specificity.

Acknowledgements

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Table 3. List of 6-oxopurine PRTs showing their active-site residues and K_m values for nucleobase substrates. The active-site residues were determined from multiple sequence alignment and structure superposition (<https://www.rcsb.org/>). NA represents No activity.

Active site residues	Source organisms	Enzymes	K_m (μM)			References
			Xanthine	Hypoxanthine	Guanine	
Y_I_E	<i>L. donovani</i>	XPRT	7.1	204	103	Present study
F_Q_L	<i>B. subtilis</i>	XPRT	2.2	1250	281	[26]
F_Q_L	<i>S. faecalis</i>	XPRT	20	-	NA	[27]
T_W_D	<i>E. coli</i>	XGPRT	30.5	90.8	4.3	[33]
W_I_D	<i>T. gondii</i>	HGXPRT	14.4	1.6	2.1	[8]
Y_I_D	<i>T. foetus</i>	HGXPRT	6.1	3	2.4	[9]
F_V_D	<i>P. falciparum</i>	HGXPRT	420	0.9	1.4	[7]
Y_V_E	<i>T. brucei</i>	HGXPRT	2.8	17.3	13.0	[13]
F_V_D	<i>T. brucei</i>	HGPRT	221	5.5	2.3	[28]
F_V_D	<i>T. cruzi</i>	HGPRT	NA	8.63	12.4	[34]
F_V_D	<i>L. donovani</i>	HGPRT	NA	6.1	5	[35]
F_V_D	<i>L. tarentolae</i>	HGPRT	NA	4.4	2.8	[36]
F_V_D	<i>H. sapiens</i>	HGPRT	NA	1.4	4.5	[37]
F_V_D	<i>T. tengcongensis</i>	HGPRT	> 200	2.4	3.6	[38]
F_V_D	<i>S. mansoni</i>	HGPRT	NA	3.7	2.1	[39]
F_V_D	<i>M. tuberculosis</i>	HGPRT	NA	26	10	[40]
Y_V_D	<i>T. thermophilous</i>	HGPRT	NA	3.9	7.4	[41]
Y_V_E	<i>G. lamblia</i>	GPRT	NA	> 200	16.4	[25]

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Data accessibility

Research data pertaining to this article are located at figshare.com.

The data that support the findings of this study are available from the corresponding author [anju.p@cu-g.ac.in] upon reasonable request.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig S1. Near-UV CD spectral analysis of LdXPRT mutants with ligands.

Table S1. The list of oligos for the PCR amplification of LdXPRT mutants.

Table S2. Ramachandran plot, Verify 3D, and ERRAT plot scores of LdXPRT model before and after energy minimization.

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India-Bangladesh Energy Relations and the Northeast of India

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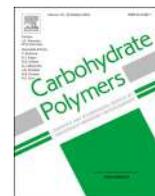
Abstract

Energy has been the lifeline of India – a welfare state with a national policy committed towards poverty reduction, economic growth, rural development, basic education and adequate health facilities. Given that the population of India is growing fast and is expected to reach at 1.47 billion by 2031-32 and the fact that the sizeable population of the country, today is living below the poverty line; rapid growth at around 8% per annum over the next 25 years has become essential for attaining these goals. Therefore, India needs the energy to fuel this economic growth at a sustained rate. As of 2015, India imports about 70 per cent of oil. By 2020 India is expected to import 80 per cent of its energy needs. Domestic energy sources of India though huge are inadequate for fueling the sustained economic growth in the long term and insufficient in meeting the development goals of the country. Therefore, the import of energy has always been one of the supreme foreign policy objectives of India in recent times. Amongst other options, India has been looking eastwards to the extensive natural gas reserves of Bangladesh, which have become vital for India's economic growth. The geographic proximity of Bangladesh to India makes the import of gas, not just convenient, but an economically attractive proposition as well. In addition, the energy needs of eastern India, particularly the north-eastern states, would be better served by gas from Bangladesh rather than from reserves elsewhere in the world. It is in this context, this article makes a modest attempt to venture into the newly emerging area of energy relations between India and Bangladesh. With this premise, this article attempts to know the role and position of Northeast of India in establishing and expediting energy cooperation between India and Bangladesh.

Keywords: *India, Bangladesh, Northeast, Energy Security, Gas, Hydroelectricity, Myanmar-Bangladesh-India Pipeline, etc.*

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Inulin-pluronic-stearic acid based double folded nanomicelles for pH-responsive delivery of resveratrol

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ABSTRACT

Herein, we introduce a novel amphiphilic bioconjugate (INU-F68-SA), synthesized by functionalization of pluronic F68 with a polysaccharide inulin (INU) and a lipid stearic acid (SA). The synthesis of INU-F68-SA was confirmed by FTIR and ¹H-NMR analysis. INU-F68-SA can self-assemble into nanomicelles and therefore, its application in delivering of hydrophobic resveratrol (RSV) was investigated. The RSV-loaded INU-F68-SA nanomicelles (RSNM) had about 172 nm size, spherical shape, 0.237 polydispersity index, and -18 mV zeta potential. More importantly, the RSNM showed high drug entrapment efficiency, controlled drug release and protection of drug during storage. The RSNM significantly enhanced the cytotoxicity of RSV against colorectal cancer cells by inducing apoptosis and changing mitochondrial membrane potential. Further, *in-vivo* pharmacokinetic experiment indicated an improvement in pharmacokinetics of RSV after administering as RSNM. Thus, the use of self-assembled nanomicelles of amphiphilic INU-F68-SA bioconjugate could be a better alternative to overcome the poor *in-vitro* and *in-vivo* performance of RSV.

1. Introduction

Polysaccharides are well-known glycans that play important roles in energy storage and structural support in the living organism (Lopes et al., 2017; Mirzadeh, Arianejad, & Khedmat, 2020; Ratajczak & Stobiecka, 2020). Polysaccharides are mainly originated naturally from plant, animals and microbial organisms. Because of their natural origins, polysaccharides are highly biocompatible and biodegradable. Therefore, polysaccharides are used in various fields including biomedical sciences. Currently, polysaccharide or their derivatives are potential biomaterials in area of some low-fat food, cosmetics, tissue-engineering and drug delivery applications (Xie et al., 2020). Many polysaccharides have also been investigated for their applications in the field of nanomedicine like nano bioimaging, green synthesis of nanoparticles, surface modification of nanoparticles and designing amphiphilic nano delivery systems (Pooja, Panyaram, Kulhari, Rachamalla, & Sistla, 2014, 2015; Rasoulzadeh & Namazi, 2017).

Recently, in 2018, FDA has approved inulin (INU) as food additives *i.e.* dietary fibres in food products (Gupta, Jangid, Pooja, & Kulhari, 2019). INU is a type of plant polysaccharide and is found in wide variety of fruits and vegetables. It is a hydrophilic, biopolymer

composed of β -(2 \rightarrow 1) fructofuranosyl units, and a terminal α -glycopyranose unit (1 \rightarrow 2) (Li, Gunenc, & Hosseinian, 2020). Interestingly, INU is not digested by the enzymes present in upper gastrointestinal tract. This unique physicochemical property of INU makes it attractive for the pH-responsive delivering of the drugs in the lower GIT in case of amoebiasis, inflammatory bowel disease, colorectal cancer, colitis *etc* (Mensink, Frijlink, van der Voort Maarschalk, & Hinrichs, 2015). Licciardi et al. conjugated INU to poly ethylene glycol 2000 and ceramide to deliver doxorubicin to the colon (Licciardi, Scialabba, Sardo, Cavallaro, & Giammona, 2014).

In this research work, we have designed INU and stearic acid (SA) functionalized pluronic-based nanomicelles for the oral delivery of resveratrol (RSV). Pluronic F68 is a well-known copolymer and has been extensively investigated for preparation of various drug delivery system because of low cost, high biocompatibility and biodegradation in biological fluids. The unique properties of pluronic F68 make it an excellent material for improving solubility and oral bioavailability of hydrophobic drugs. However, pluronic F68 polymer also has its own limitation like high critical micelle concentration (CMC), low drug encapsulation efficiency, and short PPO segment which results in instability after the dilution. Therefore, to design a safe and pH-

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responsive drug delivery vehicle based on pluronic F68, we have modified it with SA and INU to overcome its drawbacks and to make it a pH-responsive drug releasing system. The stearic acid was conjugated at one terminal of pluronic F68 to increase hydrophobicity segment and decrease the CMC of the conjugate. At another terminal of pluronic F68, we conjugated INU to protect the drug loaded nanomicelles at the stomach pH and to maximize the drug release in lower segment of GIT.

RSV is a natural polyphenol found in many plant species and is reported to possess several biological activities *i.e.* anticancer, antioxidant, antiviral and anti-inflammatory agent. RSV is a potential anticancer molecule and suppresses proliferation of various cancer cells such as breast, lung, liver, pancreas, prostate, colon, skin and stomach (Chikara et al., 2018; Thapa, Pandey, Park, & Kyung Sohng, 2019). However, because of quick metabolism and low aqueous solubility RSV shows poor oral bioavailability (Summerlin et al., 2015). RSV is also highly sensitive drug molecule and is unstable in the presence of UV light, certain pH conditions and temperature which create the several challenges in its formulation development (Xiong, Ren, Li, & Li, 2018; Zupančič, Lavrič, & Kristl, 2015).

To achieve effective delivery of RSV to colon, a drug-loaded formulation needs to be protected from degradation and absorption of the upper gastrointestinal tract (pH 1.2) and should be able to release the drug molecule in the colon. Considering the highly biocompatibility and unique features of inulin. This work was to evaluate inulin modified nanomicelles as a vehicle for the delivery of RSV in colon cancer.

2. Experimental section

2.1. Materials

Trans-Resveratrol (RSV) was purchased from TCI, Tokyo, Japan. Stearic acid (SA), N-(3-(dimethylamino)propyl)-N'-ethylcarbodiimide (EDC), inulin (INU) from chicory (molecular weight ~6173 g/mol), hydroxybenzotriazole (HOBt), 4-Dimethylaminopyridine (DMAP), succinic anhydride, CDCl₃, dimethyl sulfoxide (DMSO), dialysis tubing (molecular weight cut off 12,000–14,000), acridine orange, ethidium bromide and hoechst 33,342 were obtained from Sigma-Aldrich (St. Louis, MO, USA). Dichloromethane (DCM), diethyl ether and triethylamine (TEA) were purchased from Loba Chemie, (Mumbai, India). Pluronic F68 was received from BASF Corporation (New Jersey, USA) as a gift sample and used without further purification. DMEM high glucose, RPMI-1640 high glucose cell culture medium, penicillin-streptomycin-amphotericin B (PSA) cocktail, and 0.25 % trypsin-EDTA, fetal bovine serum (FBS) were purchased from Gibco, Thermo-fisher scientific (Waltham, MA, USA). 3-(4, 5 Dimethylthiaziazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was purchased from Himedia laboratories (Mumbai, India).

2.2. Synthesis of stearic acid-pluronic F68 conjugate

SA (0.088 mmol, 1.0 eq.), EDC (0.105 mmol, 1.2 eq.) and HOBt (0.105 mmol, 1.2 eq.) were dissolved in anhydrous DCM and stirred at room temperature for 3 h under N₂ condition for the activation of carboxylic group of SA. After that, F68 (0.097 mmol, 1.0 eq.) and DMAP (0.088 mmol, 1.0 eq.) were slowly added to the reaction mixture and kept the reaction at room temperature for 24 h under N₂ protection. After completion of reaction, cold anhydrous diethyl ether was added and kept overnight in refrigerator at -4 °C. The white precipitate was collected and redispersed in anhydrous DCM and again precipitated by cold anhydrous diethyl ether. This process was repeated three times to remove unconjugated SA and other activators. Finally, the precipitate was dried by vacuum drying for 12 h.

2.3. Synthesis and characterization of stearic acid-pluronic F68-inulin (INU-F68-SA) bioconjugate

The hydroxyl (-OH) group of synthesized SA-F68 was converted into succinoyl SA-F68 via a ring-opening mechanism. Briefly, SA-F68 conjugate (0.012 mmol, 1.0 eq.), succinic anhydride (0.012 mmol, 1.0 eq.) and DAMP (0.014 mmol, 1.2 eq.) were mixed in anhydrous DMSO and stirred at room temperature for 12 h under inert condition. The succinoyl SA-F68 was obtained by dialysis of the mixture for two days in water, lyophilized the sample and used for further conjugation with INU.

Succinoyl SA-F68 (1.0 eq., 0.015 mmol) conjugate was activated by the EDC (0.105 mmol, 1.2 eq.) and HOBt (0.105 mmol, 1.2 eq.) in anhydrous DMSO at room temperature for 12 h under inert condition. After that, INU (1.0 eq., 0.016 mmol) and TEA (0.3 eq.) were slowly added to activated succinoyl SA-F68 solution. The reaction was maintained at room temperature for 24 h under inert condition. After the completion of the reaction, the reaction mixture was dialysis (molecular weight cut off 12–14 kD) in water for three days to remove EDC, HOBt, TEA and unconjugated INU, and lyophilized the product *i.e.* INU-F68-SA for further use. The synthesized bioconjugate was characterized by the ¹H-NMR and FTIR techniques. For ¹H-NMR analysis, samples were dissolved in deuterated NMR solvent and scanned with Bruker 500-MHz Ultra shield plus NMR instrument. For FTIR analysis, samples were mixed with KBr, palletised and scanned for % transmittance using FTIR (PerkinElmer Spectrum 65 series) in the range of wavenumber 4000 to 400 cm⁻¹.

2.4. Determination of CMC of INU-F68-SA

CMC of synthesized bioconjugate was determined according to our previously reported method (Jangid et al., 2019). Briefly, pyrene was dissolved in acetone for making stock solution (6 × 10⁻⁷ M). A series of concentrations from 50 to 500 µg/mL of the bioconjugate (INU-F68-SA) were prepared. After that, 25 µL of pyrene stock solution was added to each sample of bioconjugate and incubated in dark for 1 h. At the fix excitation wavelength (339 nm) and emission wavelengths (373 and 383 nm), the fluorescence intensities were measured using a Microplate reader (Synergy H1 Hybrid Reader, Biotek; Winooski, VT, USA). The ratio of I₃₈₃/I₃₇₃ vs Log C (µg/mL) was plotted for the determination of CMC value.

2.5. Preparation of RSV loaded INU-F68-SA nanomicelles (RSNM)

RSV loaded nanomicelles (RSNM) were prepared by the solvent evaporation method as reported previously (Kulhari et al., 2015). Briefly, an excess amount of RSV (2 mg) was dissolved in 1 mL of acetone in an amber colour vial and 10 mg of bioconjugate was dissolved in 5 mL milliQ water in another vial. The RSV solution was slowly added to aqueous bioconjugate solution and kept for magnetic stirring (1000 rpm) at room temperature for 4 h in dark for complete removal of acetone. After that, the formed RSNM were centrifuged at 10,000 rpm and then filtered through 0.22 µm nylon syringe filter to remove free drug and were analysed for RSV content by HPLC analysis. The RSV loaded SA-F68 nanomicelles (FNM) were also prepared similar to RSNM preparation method. The percentage encapsulation efficiency (%EE) was calculated as follow:

$$\%EE = \left[\frac{\text{Amount of drug encapsulated in NM}}{\text{Total amount drug added into NM}} \right] \times 100$$

2.6. HPLC analysis of RSV

An analytical method was validated for RSV as per previous reports (Das, Lin, Ho, & Ng, 2008; Vasconcelos et al., 2019). The RSV content

was analysed using HPLC system (Water, USA) equipped with a photodiode array detector and a C18 column (Water, 250 × 4.6 mm, 5 mm) at 307 nm. The HPLC column temperature was maintained at 35 ± 5 °C. The mobile phase consisted of acetonitrile (30%) and 30 mM phosphate buffer saline, pH 7.0 (70%), in isocratic mode, and the flow rate was 1.0 mL/min. Calibration curve was prepared by measuring peak areas of known RSV in the concentration range from 0.1 to 10 µg/mL with r^2 value of 0.999.

2.7. Physicochemical characterization of nanomicelles

2.7.1. Dynamic light scattering method

The particle size, polydispersity index and zeta potential of RSV loaded SA-F68 nanomicelles (FNM), blank INU-F68-SA bioconjugate nanomicelles (BNM), RSV loaded INU-F68-SA bioconjugate nanomicelles (RSNM) were determined by DLS using a Zetasizer Nano ZS 90 (Malvern Instruments, UK).

2.7.2. Transmittance electron microscopic analysis

The morphological characterization of the prepared RSNM was observed by the TEM (JEOL JEM-2100 TEM, Tokyo, Japan). A drop of RSNM was placed on the carbon coated copper grid, vacuum dried and used for TEM analysis.

2.7.3. Proton NMR and FTIR analysis

The chemical interactions between RSV and INU-F68-SA bioconjugate were analysed by the ¹H-NMR and FTIR analysis. For the ¹H-NMR analysis, RSV and lyophilized BNM, or lyophilized RSNM were dissolved in DMSO-*d*⁶ and scanned with Bruker 500-MHz Ultra shield plus NMR instrument. For the FTIR analysis, pure RSV or lyophilized BNM or RSNM were mixed with KBr, pelletized and scanned for % transmittance with FTIR (PerkinElmer Spectrum 65 series) in the range of wavenumber 4000 to 400 cm⁻¹.

2.7.4. Powder X-ray diffraction

PXRD patterns of RSV, BNM and RSNM were obtained using an X-ray diffractometer (D8 Advance, Bruker, Germany) equipped with a Cu-Kα X-ray radiation source. The instrument was set at 40 kV and 30 mA and the intensity was measured from 5° to 60° 2θ diffraction angle.

2.7.5. Differential scanning calorimetry analysis

DSC analysis of RSV, BNM and RSNM were carried out on DSC-4000 (Perkin Elmer). Samples were scanned from 30 to 350 °C at a rate of 10 °C /min under N₂ environment.

2.8. Storage stability of nanomicelles

The physical and chemical stabilities of nanomicelles were monitored over a period of 48 h in both refrigerator (4 °C) and room temperature (25 ± 5 °C) conditions. The physical parameters (change in particle size and polydispersity index) were measured by the DLS method. The chemical stability was determined by measuring % RSV content by HPLC analysis.

2.9. pH-responsive stability of nanomicelles in GIT fluids

The change in shape/morphology of the RSNM was monitored by the TEM analysis. Briefly, prepared RSNM formulation was incubated overnight in different pH solution i.e. 0.1 N HCl (pH 1.2) and phosphate buffer (pH 6.8). After that, a drop of the sample was placed on carbon-coated copper grid, stained with 3% phosphotungstic acid, dried under vacuum and used for TEM analysis.

2.10. In-vitro drug release from nanomicelles

The release of RSV from pure RSV suspension, FNM and RSNM was

investigated in different pH conditions i.e. 0.1 N HCl (pH 1.2) and phosphate buffer (pH 6.8) at 37 °C. Briefly, 2 mL of formulation was added to dialysis bag (MWCO = 1000), which was immersed into 50 mL of buffer solutions. At predetermined time intervals, 2 mL of sample was taken and analysed for %RSV released using HPLC analysis. The withdrawn volume of release media was replenished with fresh media.

2.11. Cell culture

The human colorectal cancer cells HCT 116 cells, obtained from American Type Culture Collection (ATCC, United States), were grown in DMEM medium supplemented with 10% fetal bovine serum and 1% penicillin streptomycin antibiotics. Cells were maintained in a CO₂ incubator at 37 °C and 5% CO₂. For the experiment, cells were harvested using 0.05% of the trypsin-EDTA solution.

2.12. Antiproliferative assay

The cytotoxic effect of the pure RSV, BNM and RSNM were analyzed using the MTT assay. The MTT assay was performed against both treated and untreated HCT 116 cells. About 5 × 10³ cells/well were seeded in 96-wells plate and left to adhere for 12 h before the treatment of the different RSV formulations. The cells were incubated with the different concentrations of RSV, BNM and RSNM formulations for 24 and 48 h. It was followed by the addition of 0.5% of MTT reagent per well and incubation at 37 °C in the dark for 4 h. After removal of media, a fix volume (200 µL) of DMSO was added in each well to dissolve formazan crystals formed in cells and to give purple-colored solution having the intensity directly proportional to the number of live cells. The absorbance of the obtained solution was measured at 570 nm using a microplate reader (Synergy H1 Hybrid Reader, Biotek; Winooski, VT, USA). Untreated cells were considered as control and the IC₅₀ values were calculated using the probit software.

2.13. Acridine orange-ethidium bromide assay

For acridine orange-ethidium bromide staining assay, about 4 × 10⁴ HCT 116 were seeded in a 24-wells plate. Cells were allowed to adhere to the plate for 24 h followed by incubation with RSV, BNM and RSNM. After completion of the incubation period, cells were trypsinized and centrifuged at 1800 rpm for 5 min. The supernatant was removed and pellet of centrifuged cells was resuspended in 50 µL of PBS. Further, 10 µL of suspension was mixed with 1 µL of acridine orange (5 µg/mL) and 1 µL of ethidium bromide (5 µg/mL) and mounted on the coverslip. Microscopic images of cells were acquired at 200X magnification using a fluorescence microscope (Olympus, Japan).

2.14. Hoechst 33342 staining

To study the induction of apoptosis and nuclear fragmentation caused by the RSV, BNM and RSNM, cells were seeded in 35 mm cell culture dishes at a seeding density of 1.5 × 10⁵ cells/dish. The cells were incubated with the RSV, BNM and RSNM for 24 and 48 h. The cells were washed with PBS and were fixed with 4% paraformaldehyde. A volume of 100 µL Hoechst solution (1 µg/mL in 1X PBS) was added to the fixed cells and incubated for 15 min at room temperature in dark to selectively stain the nucleus of the cells. The stained cells were washed with PBS to remove excess unbound dye and observed immediately with the help of fluorescence microscope (Olympus, Japan) at 200X magnification to detect apoptotic cells by observing the intensity of the blue-colored nuclei.

2.15. Cellular uptake study

HCT 116 cells were seeded in a 35-mm cell culture dish at a seeding density of 1.5 × 10⁵ cells/dish and incubate for 24 h to adhere. The

cells were treated with pure coumarin (C6) and coumarin loaded nanomicelles (C6NM) for 0.25, 0.5, 1, 2, 4 and 8 h. After incubation, cells were washed three times with cold PBS and observed under a fluorescent microscope (Olympus, Japan) at 200X magnification.

2.16. Pharmacokinetic studies

The oral pharmacokinetics study of RSV or RSNM was performed in Sprague Dawley rats having weight about 180–200 g. Before the experiment, animals were fasted overnight with water availability. Twelve rats were randomly divided into two groups ($n = 6$). The first group was given RSV suspension (50 mg/Kg body weight) orally while another group was administered with the same dose of RSNM formulation. RSV suspension was prepared by dispersing RSV powder in 2% gum acacia. The blood samples (300 μ L) were collected at pre-determined intervals from retro-orbital plexus in tubes containing EDTA. After that, samples were centrifuged at 2000 \times g for 10 min and the supernatant (plasma fraction) was stored at -80 °C until analysis.

2.17. Plasma processing and analysis

For analysis of RSV by HPLC in plasma, 100 μ L of plasma sample was mixed with 5 μ L of internal standard carbamazepine (50 μ g/mL) and mixed well by vortexing for 1 min. Thereafter, 0.5 mL ethyl acetate and 40 μ L of PBS (30 mM, pH 6) were added and vortexed for 1 min. The organic layer and denatured protein were separated by centrifuged at 10,000 rpm for 10 min at 4 °C. The organic layer was collected and evaporated to dry used high vacuum. The residue was re-dissolved in 100 μ L of mobile phase, filtered through 0.22 μ m filter paper and 20 μ L of sample was injected into HPLC system for RSV analysis.

2.18. Statistical analysis

All the experiments were performed in triplicate and the data are represented as mean \pm standard deviation. Statistical significance was analyzed using Student's *t*-test for two groups and one-way ANOVA test for multiple groups. A probability (*p*) value < 0.05 was considered statistically significant.

3. Results and discussion

3.1. Synthesis and characterization of INU-F68-SA bioconjugate

The INU-F68-SA bioconjugate was synthesized according to the three steps synthesis reaction scheme representing in Fig. 1a. First, SA was directly conjugated to F68 through the esterification reaction. Thereafter, the SA-F68 conjugate was successfully converted into succinoyl SA-F68 by ring opening mechanism with the help of succinic anhydride and DMAP under anhydrous conditions and characterized by FTIR and 1 H-NMR. Finally, INU was conjugated to succinoyl SA-F68 in the presence of EDC and HOBt under inert conditions. Each step of above conjugation reaction scheme was confirmed and characterized by FTIR and 1 H-NMR represented in Figs. S1-S2 and 1 b, respectively.

The FTIR spectra of SA, F68 and SA-F68 are presented in Fig. S1, which confirmed the successfully conjugation of SA-F68. The characteristic FTIR signals of SA were observed at 2917 and 2849 cm^{-1} (C–H stretching), 1703 cm^{-1} ($>$ C=O stretching), 1464 cm^{-1} (C–H bending), 1311, 1225 and 1099 cm^{-1} (C–O stretching), 934, 720, 638 and 545 cm^{-1} (C–H bending) (Jain et al., 2020). Pure F68 showed peaks at 3499 cm^{-1} (O–H stretching), 2886 cm^{-1} (C–H stretching), 1956 and 1458 cm^{-1} (C–H bending), 1353, 1284 and 1110 cm^{-1} (C–O stretching), 953, 841, 525 cm^{-1} (C–O bending) (Cai et al., 2016). The synthesized SA-F68 showed the signals at 3554 cm^{-1} (O–H stretching), 2888 cm^{-1} (C–H stretching of SA and F68), 1970 cm^{-1} (C–H bending of F68), 1736 cm^{-1} ($>$ C=O stretching), 1577, 1541 and 1468 cm^{-1} (C–H bending SA and F68), 1350, 1343, 1281, 1242 and 1112 cm^{-1}

(C–O stretching), 963, 842 and 529 cm^{-1} (C–O bending). Thus, the shift in the peak of $>$ C=O moiety of carboxylic group of SA (1703 cm^{-1}) to of $>$ C=O moiety of ester group of SA-F68 (1736 cm^{-1}) confirmed the formation of SA-F68 conjugate.

After the –OH group modification of SA-F68 (formation of succinoyl SA-F68), the obtained FTIR peaks at 1737 and 1650 cm^{-1} which are corresponding to $>$ C=O stretching of free –COOH and –COO– (ester) (Kulhari et al., 2016; Wu et al., 2015), confirmed the successfully transformation of SA-F68 into succinoyl SA-F68 (Fig. S2). This available free –COOH group of succinoyl SA-F68 was used for INU conjugation via EDC and HOBt reaction. The pure INU showed FTIR peaks at 3309 cm^{-1} (O–H stretching), 2935 cm^{-1} (C–H stretching), 1436 cm^{-1} (C–H stretching pyranose ring), 1336, cm^{-1} (β O–H stretching), 1132 cm^{-1} (C–O–C stretching), 1033 cm^{-1} (C–O stretching), 940 – 598 cm^{-1} (α -D-Glcp residue) (Petkova, Sherova, & Denev, 2018). The synthesized INU-F68-SA bioconjugate showed the FTIR peaks at 3358 cm^{-1} (O–H stretching), 2892 cm^{-1} (C–H asymmetric stretching), 2737 cm^{-1} (C–H symmetric stretching), 1737 cm^{-1} ($>$ C=O stretching of ester), 1541 cm^{-1} (C–H bending), 1470 cm^{-1} (C–H pyranose ring), 1357, 1342 cm^{-1} (β O–H stretching), 1281, 1244 cm^{-1} (C–O stretching of SA-F68), 1143, 1113 cm^{-1} (C–O–C stretching of INU), 1056, 1034 cm^{-1} (C–H stretching of INU), 988, 961 cm^{-1} (C–O bending of SA-F68), 941, 842, 646, 602, 530 and 466 cm^{-1} (α -D-Glcp residue).

In 1 H-NMR analysis, pure F68 shows the signals at δ 1.01–1.43 ppm (CH_3 –O of PPO), δ 2.18–3.04 ppm (CH_2 –CH(CH_3)–O of PPO) and δ 3.41–3.79 ppm (CH_2 –O of PEO) (Jangid et al., 2019; Liu et al., 2016). Pure SA shows the signals at δ 0.88 ppm (terminal CH_3), δ 1.26–1.63 ppm ($-\text{CH}_2$), δ 2.34 ppm (α - CH_2), and δ 11.98 ppm ($-\text{COOH}$) (Jain et al., 2020). The synthesized SA-F68 showed the signals at δ 0.78 ppm (terminal CH_3 of SA), δ 1.05–1.45 ppm (CH_3 –O PPO and $-\text{CH}_2$ of SA), δ 2.22 ppm (CH_2 –CH(CH_3)–O in PPO and α - CH_2 of SA) and δ 3.41–3.73 ppm (CH_2 –O PEO) (Jangid et al., 2019). In succinoyl SA-F68, the observed peaks at δ 2.76 and δ 2.84 ppm confirmed the presence of succinoyl group (Kulhari et al., 2016; Wu et al., 2015). The pure INU showed the signals at δ 5.28 – 4.71 ppm (terminal glucose), δ 4.03–4.14 ppm (fructose residue), δ 3.71–3.96 ppm (fructosyl), and δ 3.32–3.58 ppm (β -D fructofuranosyl) (Lopes et al., 2017). The synthesized INU-F68-SA bioconjugate showed the peaks at δ 4.62–5.32 ppm (terminal glucose of INU), δ 4.06–4.14 ppm (fructose residue of INU), δ 3.81–3.98 ppm (fructosyl of INU), δ 3.08–3.78 ppm ($-\text{CH}_2$, $-\text{CH}$ of PPO of F68 and β -D fructofuranosyl of INU), δ 2.34–2.60 ppm ($-\text{CH}_2$ of succinoyl) and δ 1.48 – 0.79 ppm ($-\text{CH}_3$ of SA and PPO of F68) (Fig. 1b). Therefore, the observed peaks suggested the successfully synthesis of INU-F68-SA bioconjugate.

3.2. Determination of CMC of INU-F68-SA

CMC is a vital key parameter for self-assembly behaviour of amphiphilic molecules. The synthesized INU-F68-SA bioconjugate contains both hydrophobic (SA and PPO in F68) and hydrophilic (INU + PEO in F68) and these should show self-assembled behaviour in aqueous media. The CMC of INU-F68-SA bioconjugate was determined by fluorescence method using pyrene hydrophobic probe. Fig. 2a shows the change in the fluorescence intensities ratio (I_{383}/I_{373}) of pyrene with increasing INU-F68-SA concentration 50–500 μ g/mL. The emission fluorescence ratio I_{383}/I_{373} versus concentration bioconjugate (μ g/mL) increased with polymer concentration due to internalization of pyrene into hydrophobic core of nanomicelle. The sharp change in intensity ratio was observed at 100 μ g/mL of bioconjugate concentration which confirms CMC of the INU-F68-SA bioconjugate. The observed CMC of bioconjugate was 3.3 times less than pure F68 (Song et al., 2014). The observed results clearly depict the role of hydrophobic and hydrophilic segments present in the bioconjugate which could able to make the more compact and stable nanomicelles.

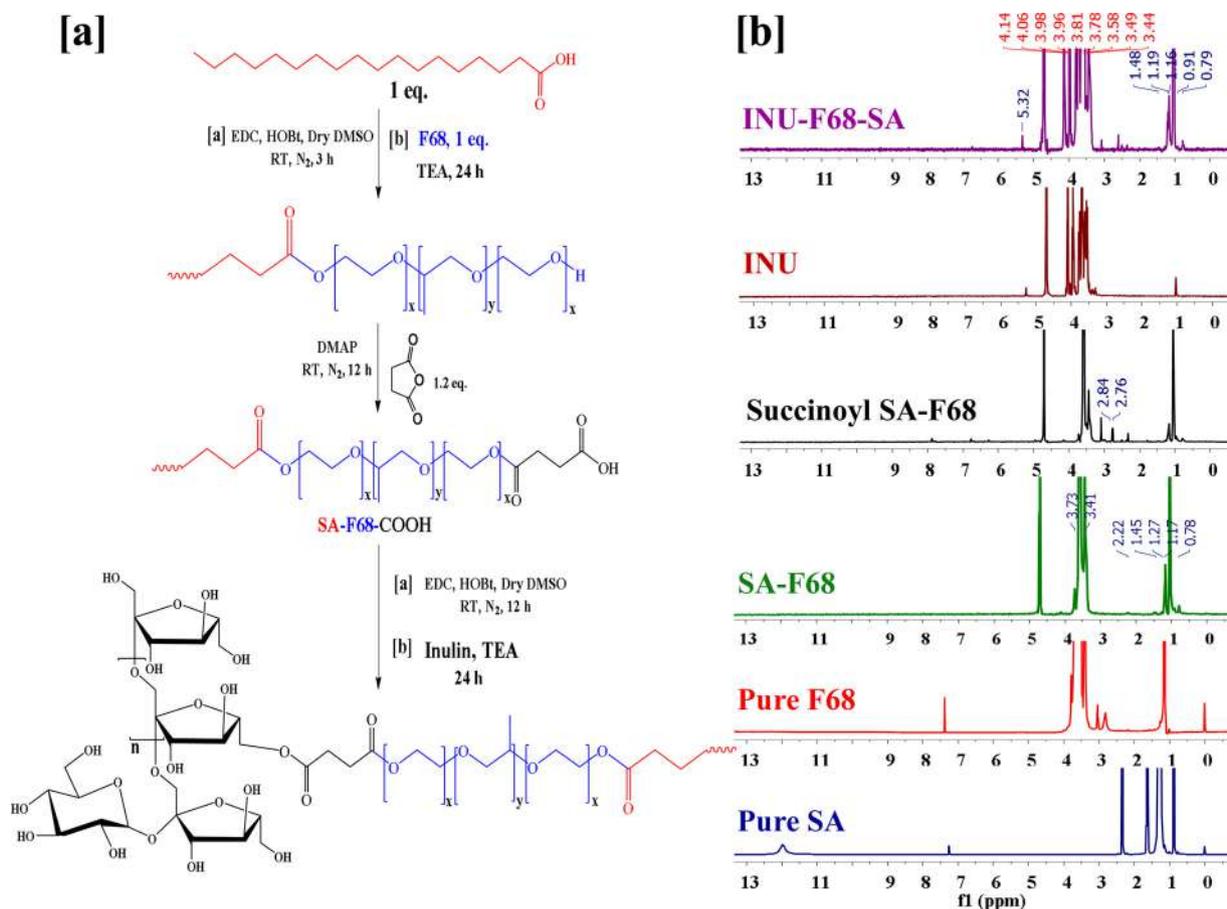


Fig. 1. (a) The scheme represents the reaction procedure of INU-F68-SA conjugation and (b) ¹H-NMR of pure pluronic F68 (F68), pure stearic acid (SA), stearic acid and pluronic F68 conjugate (SA-F68), succinoyl SA-F68 conjugate, pure inulin (INU) and stearic acid-pluronic F68-inulin (INU-F68-SA) bioconjugate.

3.3. Preparation and characterization of nanomicelles

The RSV-loaded INU-F68-SA nanomicelles were prepared *via* solvent evaporation method. For comparative studies, RSV loaded nanomicelles were also prepared using plain F68 and SA-F68 conjugate. The percent encapsulation efficiency (%EE) was found to be $32.9 \pm 1.3\%$ with plain F68, $52.3 \pm 0.8\%$ with SA-F68 conjugate, and $56.03 \pm 1.0\%$ with INU-F68-SA bioconjugate (Fig. S3). The observed %EE was highest for INU-F68-SA bioconjugate followed by SA-F68 conjugate and plain F68. These results suggest that the incorporation of SA *i.e.* hydrophobic segment in the core of the nanomicelles improved loading of hydrophobic RSV. The incorporation of hydrophilic INU does not have significant role in loading of RSV.

DLS results including particle size, polydispersity index (PDI) and zeta potential are reported in Table 1. The particle size and PDI observed 64.9 ± 0.7 nm and 0.475 ± 0.034 for RSV-loaded SA-F68 nanomicelles (FNM), 124.8 ± 6.3 nm and 0.179 ± 0.048 for blank INU-F68-SA nanomicelles (BNM), and 172.3 ± 2.1 nm and 0.237 ± 0.004 for RSV-loaded INU-F68-SA nanomicelles (RSNM). The zeta potential for FNM, BNM and RSNM were observed as -11.1 ± 2.4 , -19.1 ± 3.5 and -18.2 ± 0.15 mV respectively.

The larger size and more negative zeta potential of nanomicelles prepared with INU-F68-SA than that of SA-F68 is due to presence of hydrophilic, high molecular weight and negatively charged INU on the surface of nanomicelles. The increase in size of RSNM as compared to BNM may be due to encapsulation of RSV which led to expand the hydrophobic core of nanomicelles. Results also indicated that INU-F68-SA nanomicelles were more monodisperse than F68-SA nanomicelles.

TEM analysis was carried out to study the shape and size of the prepared RSNM. Fig. 2b–d shows the predictive scheme of RSNM

formation, TEM image and size histogram of RSNM which exhibited spherical shape and monodispersity with ~ 165 nm. The size from TEM observed approximately similar to DLS measurements and depicted the formation of spherical stable nanomicelles.

The presence of RSV in INU-F68-SA bioconjugate was analysed by the FTIR and ¹H-NMR techniques. Fig. 3a shown the FTIR spectra of RSV, BNM and RSNM, where RSV shows the peaks at 3274 cm^{-1} (–OH stretching), 1592 cm^{-1} (C=C stretching of aromatic ring), 1507 and 1451 cm^{-1} (C=C trans double bond stretching), 1380 cm^{-1} (C–O–C stretching), 1153 cm^{-1} (Phenolic C–O stretching), 968 cm^{-1} (trans C=C stretching), 827 cm^{-1} (=C–H vibration bands of phenolic ring), 671 , 614 , 571 and 515 cm^{-1} (=C–H vibration bands of trans double bond) (Porto et al., 2018). The RSNM showed the FTIR peaks at 3440 cm^{-1} (–OH stretching of BNM and RSV), 2888 cm^{-1} (–CH₂ stretching of BNM), 1609 cm^{-1} (C=C stretching of aromatic ring of RSV), 1511 and 1456 cm^{-1} (C=C trans double bond stretching), 1358 and 1302 cm^{-1} (C–H bending and C–O–C stretching of BNM and RSV, respectively), 1249 , 1152 and 1108 cm^{-1} (C–O stretching of BNM and RSV), 948 cm^{-1} (trans C=C stretching), 840 cm^{-1} (=C–H vibration bands of phenolic ring), 684 , 606 and 511 cm^{-1} (=CH– vibration bands of trans double bond). These observed results suggested that RSV was successfully encapsulated in INU-F68-SA bioconjugate *via* hydrophobic interactions or hydrogen bonding. Fig. 3b shows the ¹H-NMR spectra of RSV, BNM and RSNM where the pure RSV shows peaks at δ 9.58 ppm (4' O–H), 9.22 ppm (3,5 O–H), δ 6.80–6.94 ppm (α H of double bond), δ 7.39 ppm (2', 4' C–H), δ 6.76 ppm (3', 6' C–H), δ 6.38 ppm (2,6 C–H) and δ 6.11 ppm (4 C–H) (Cardia et al., 2019). RSNM shows the peaks at δ 9.59 ppm (4' O–H), δ 9.23 ppm (3,5 O–H), δ 6.80–6.94 ppm (α H of double bond), δ 7.38 ppm (2', 4' C–H), δ 6.75 ppm (3', 6' C–H), δ 6.37 ppm (2,6 C–H) and δ 6.10 ppm (4 C–H), δ 5.19–4.65 ppm (terminal

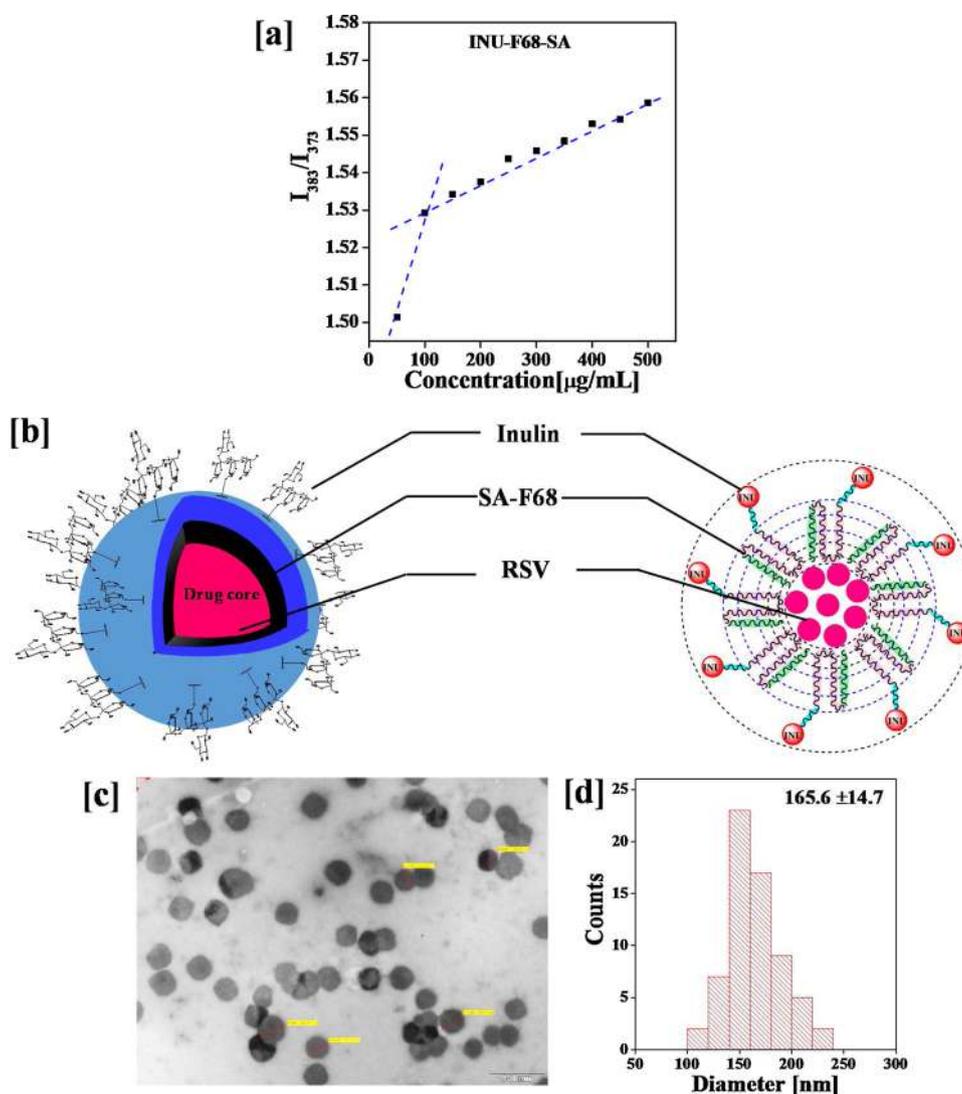


Fig. 2. (a) Critical micelle concentration of synthesized stearic acid-pluronic F68-inulin (INU-F68-SA) bioconjugate, (b) Predictive scheme represents the formation of typical resveratrol loaded INU-F68-SA nanomicelles (RSNM), (c) TEM micrograph of RSNM and (d) average size diameter of RSNM from TEM images by Image J software.

Table 1
Physicochemical parameters of different nanomicelles (Mean \pm SD, n = 3).

Formulation	Particle size (nm)	PDI	ZP (mV)	%EE
FNM	64.9 \pm 0.7	0.475 \pm 0.034	-11.1 \pm 2.4	52.3 \pm 0.8
BNM	124.8 \pm 6.3	0.179 \pm 0.048	-19.1 \pm 3.5	-
RSNM	172.3 \pm 2.1	0.237 \pm 0.004	-18.2 \pm 0.15	56.03 \pm 1.0

FNM: Pluronic SA-F68 nanomicelles; BNM: Blank INU-F68-SA conjugate nanomicelles; RSNM: Resveratrol loaded INU-F68-SA nanomicelles.

glucose of INU), δ 4.06 ppm (fructose residue of INU), δ 3.80 ppm (fructosyl of INU), δ 3.59–3.42 ppm ($-\text{CH}_2$, $-\text{CH}$ of PPO of F68 and β -D fructofuranosyl of INU), δ 2.50–2.09 ppm ($-\text{CH}_2$ of succinoyl) and δ 0.85–1.23 ppm ($-\text{CH}_3$ of SA and PPO of F68). The $^1\text{H-NMR}$ spectra of RSV showed all protons after the encapsulation in synthesized bioconjugate confirming the presence of RSV while there was a slight shift in the peaks of bioconjugate.

The physical state of RSV present in nanomicelles was determined by the powder XRD and DSC analysis. Fig. 3c shows the XRD patterns of RSV, BNM and RSNM where pure RSV showed sharp instance peaks 2θ at 6.6°, 10.1°, 13.2°, 16.4°, 19.3°, 22.3°, 23.6°, 25.2°, 28.4° and 29.1°, and BNM shows peaks at 2θ at 19.1° and 23.3°. However, the RSV peaks

were not appeared in RSNM which clearly suggested that RSV was present in amorphous form in the core of the INU-F68-SA bioconjugate. Fig. 3d shown the DSC scans of pure RSV, BNM and RSNM, where the RSV shows the sharp melting point peak at 265 °C which was not appeared RSNM. These observed results further confirmed that the RSV present in amorphous form or molecularly dispersed in the lipid-polymer moiety of the micelles.

3.4. Storage stability of nanomicelles

Stability of the prepared RSNM formulation was determined by measuring % drug content, particle size and PDI after storage at refrigeration temperatures (FT) and room temperatures (RT) over the time. Fig. S4a-b shown the drug content analysis of RSV and RSNM at RT and FT, respectively. The % RSV content was observed as 12.1 and 22.2% for RSV solution while 98.15 and 98.85% for RSNM at RT and FT conditions, respectively. The observed results suggested that RSV was highly stable in nanomicelle formulation which could be favorable for the further *in-vitro* and *in-vivo* studies. Similarly, we have also measured the particle size and PDI of the RSNM formulation for the period of 48 h after storage at RT and FT conditions. It was observed that initially RSNM showed 172.28 \pm 2.14 nm particle size and 0.237 \pm 0.004 PDI,

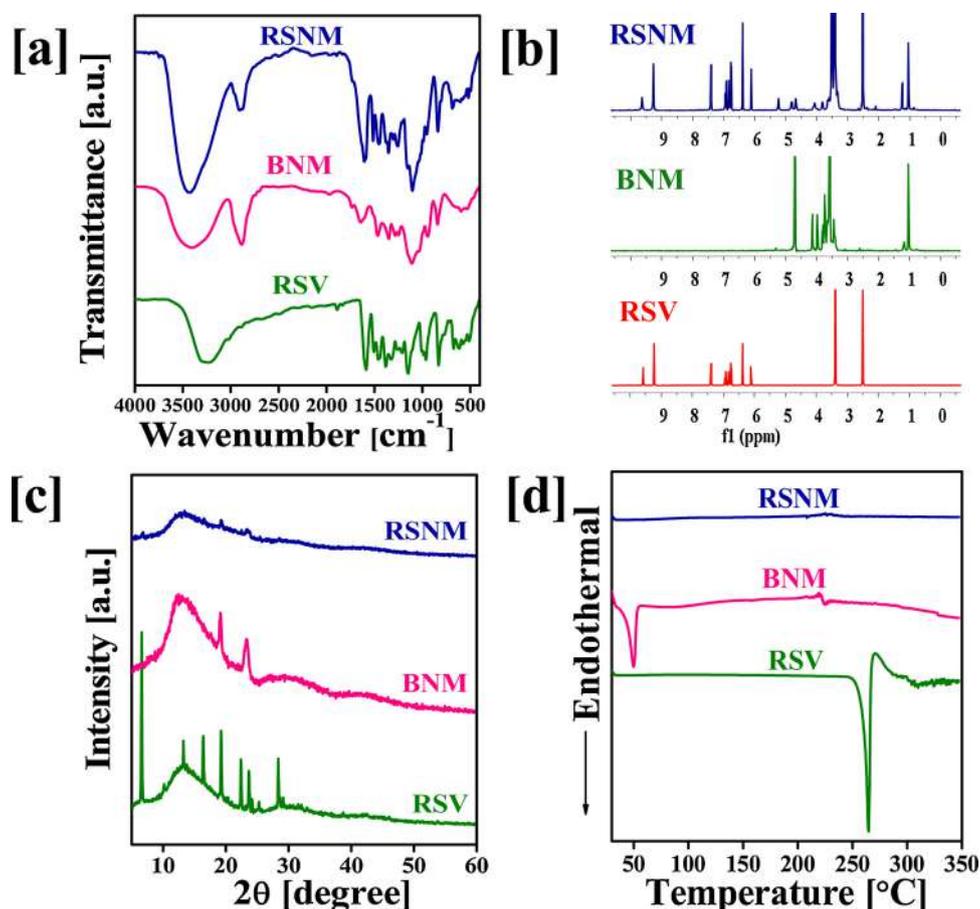


Fig. 3. (a) ¹H-NMR spectra of resveratrol (RSV), blank INU-F68-SA nanomicelles (BNM) and RSV loaded INU-F68-SA nanomicelles (RSNM), (b) FTIR spectra of RSV, BNM and RSNM, (c) Powder XRD patterns of RSV, BNM and RSNM, and (d) DSC spectra of RSV, BNM and RSNM.

which were not significantly changed after storage period of 48 h at RT and FT. The change in particle size are 188.8 ± 1.79 and 180.37 ± 2.77 at RT and FR conditions respectively, and the changes in the PDI values are 0.333 ± 0.017 and 0.241 ± 0.012 at RT and FR conditions respectively (Fig. S4c-d). RSV was highly unstable at both temperature conditions. The % RSV content of pure RSV solution was less than 50% within 12 h. After 48 h, the RSV content was 12% at RT and 22% at FT. Comparatively, RSNM were more stable at FT than RT with respect to size and PDI. Thus, the observed size and PDI results also suggested the stability of the nanomicelles. The prepared RSNM formulation was also visually observed for the physical stability determination. There was no sign of precipitation of formulation during the storage period which further confirmed the stability of the RSNM formulation.

3.5. pH-responsive stability of nanomicelles in GIT fluids

The change in morphology of the prepared RSNM were analyzed by TEM analysis after incubating in 0.1 N HCl (pH 1.2) and phosphate buffer (pH 6.8) for 12 h. As observed in Fig. 4a where the spherical shape of RSNM was not significantly changed but the size was decreased to ~ 82 nm, indicating the formation of more compact structure in acidic condition. This change in morphology of RSNM probably be due to the degradation of INU present at surface of RSNM in pH 6.8. Hence, the presence of INU at micelles surface does not allowed the prompt and controlled release for the RSNM in acidic condition.

3.6. Drug release study from nanomicelles

In-vitro release profile of the pure RSV suspension, RSV-loaded SA-F68 nanomicelles (FNM) and RSV-loaded INU-F68-SA bioconjugate

nanomicelles (RSNM) were evaluated in two different buffer (0.1 N HCl, pH 1.2 and phosphate buffer, pH 6.8) solutions. From the RSV suspension about 95% of RSV was release within 2 h, and about 64% of RSV was released from the FNM in pH 1.2 media, indicating that FNM could not able to hold RSV for long time (Fig. 4b). After 2 h dialysis, in 0.1 N HCl, about 95% and 64% of RSV was released from RSV suspension and FNM indicating that these formulations could not hold RSV for a long time. However, RSNM showed only 35% release of RSV. The initial release of RSV observed in pH 1.2 may be because of the release of RSV present on the nanomicelles surface or near to periphery. In pH 6.8, nanomicelles showed the sustained RSV release behaviour, which clearly indicate that RSV is embedded within the hydrophobic core of INU-F68-SA bioconjugate. The observed results suggested the slow degradation and high stability of proposed nanomicelles. Thus, the pH and media sensitive behaviour of INU bioconjugate could be exploited for a designing of a formulation for modified and sustained release of the drug after the oral administration.

3.7. Antiproliferative activity of resveratrol-loaded nanomicelles

The *in-vitro* cytotoxicity of the pure RSV, BNM and RSNM was evaluated against HCT 116 human colon cancer cells by the MTT assay. As shown in Fig. 5, the BNM did not show cytotoxicity at the high concentration revealing that the BNM are biocompatible and suitable for formulation an effective drug delivery system. The pure RSV and RSNM caused dose and time-dependent cytotoxicity against HCT 116 cancer cells (Fig. 5). The cell viability decreased with the increase in concentration of RSV and RSNM (3.75–90 μ g/mL). The half-maximal inhibitory concentration (IC₅₀) of pure RSV and RSNM were found to be 26.3 ± 2.8 and 17.5 ± 2.4 μ g/mL, respectively. Thus, the IC₅₀ of RSNM

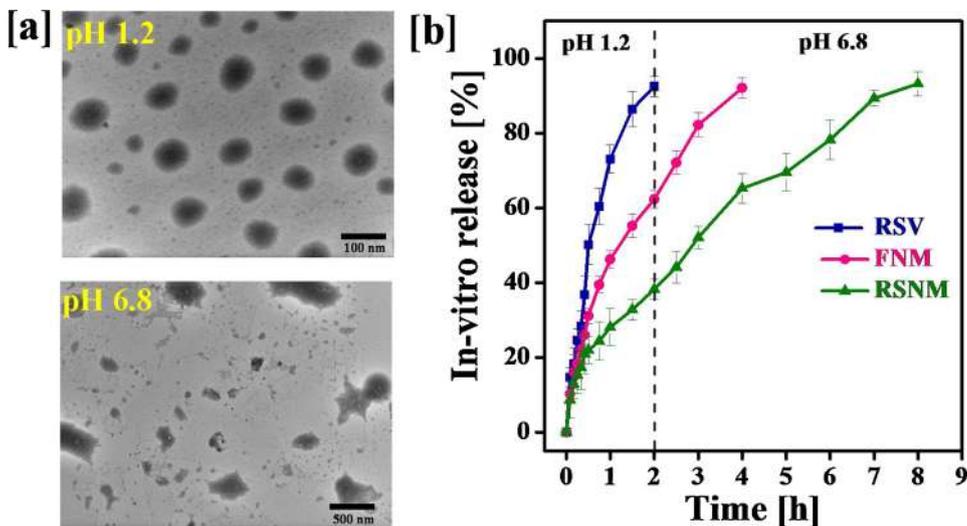


Fig. 4. (a) Morphology of the resveratrol-loaded INU-F68-SA nanomicelles (RSNM) in different media 0.1 N HCl (pH 1.2), phosphate buffer (pH 6.8) and (b) Cumulative release of RSV from RSV suspension, RSV loaded SA-F68 nanomicelles (FNM) and RSV loaded INU-F68-SA nanomicelles (RSNM) after 2 h at pH 1.2 and up to 8 h in pH 6.8 media.

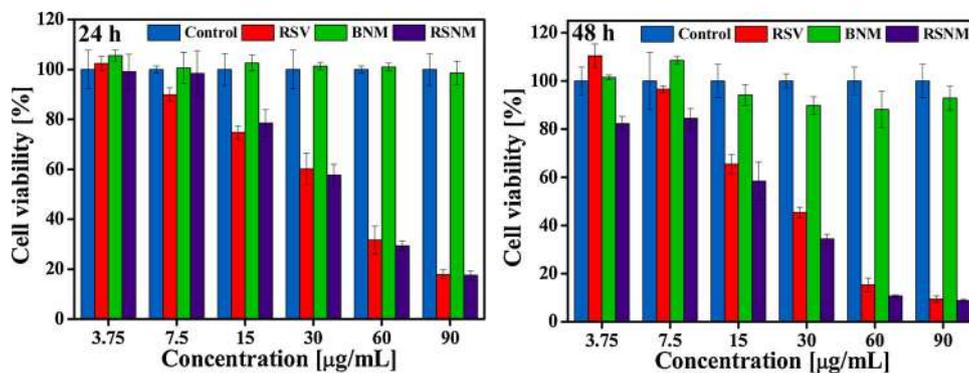


Fig. 5. Anti-proliferative activities of resveratrol (RSV), INU-F68-SA blank nanomicelles (BNM) and resveratrol loaded INU-F68-SA nanomicelles (RSNM); against HCT 116 colorectal cancer cell lines at 24 h and 48 h (n = 4).

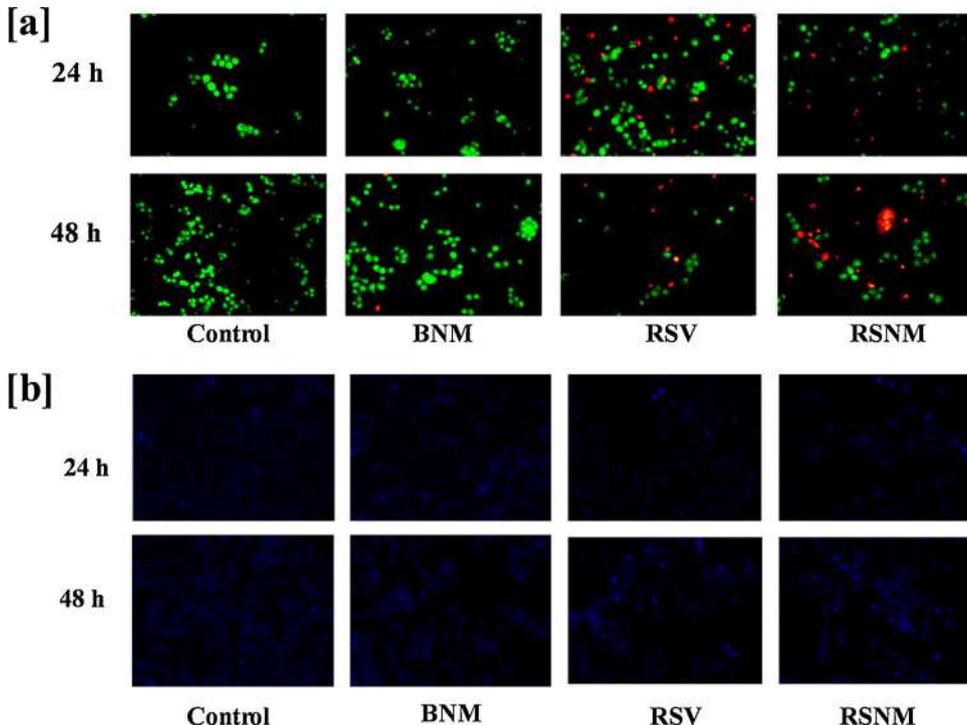


Fig. 6. (a) Acridine orange-ethidium bromide staining of HCT 116 colorectal cancer cell lines after exposure of resveratrol (RSV), blank INU-F68-SA nanomicelles (BNM) and RSV loaded INU-F68-SA bioconjugate nanomicelles (RSNM); (b) Hoechst staining for the detection of apoptotic nuclei against HCT 116 colorectal cancer cell lines after exposure of RSV, BNM and RSNM.

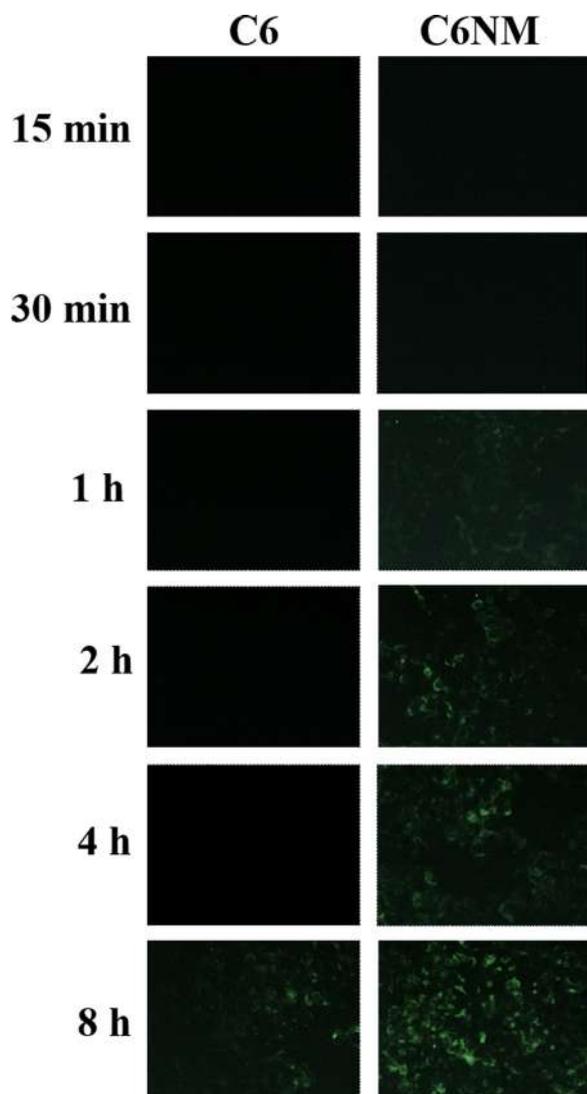


Fig. 7. Cellular uptake study in colorectal cancer HCT 116 cell line. Cells were treated with 100 ng/mL of pristine coumarin 6 (C6) and C6 loaded INU-F68-SA bioconjugate nanomicelles (C6NM) at different time intervals of 15 min, 30 min, 1 h, 2 h, 4 h, and 8 h. Images were taken using fluorescence microscopy at 200X magnification.

was significantly ($p < 0.001$) less than the pure RSV. This was probably due to better uptake of RSNM compared to the free drug.

3.8. Acridine orange/ethidium bromide (AO/EB) staining assay

Double staining combination of AO and EB is used to distinguish normal, early apoptotic, late-apoptotic and necrotic cells. To examine the nuclear morphology changes of HCT 116 cells after treatment with RSV, BNM and RSNM, the AO/EB assay was performed. The AO could penetrate in both normal and early apoptotic cells, which emits green colour after bind with DNA. On other side, EB could enter in late-apoptotic and necrotic cells which gives orange-red fluorescence colour. The AO/EB staining results of control, RSV, BNM and RSNM are shown in Fig. 6a with change in morphology of treated cells. The HCT 116 cells treated with BNM did not show any sign of apoptosis or morphological changes after 24 and 48 h of incubation. But the change in morphology and obtained orange colour in HCT 116 cells was observed after treatment with 30 $\mu\text{g/mL}$ of RSV or RSNM. It indicated the induction of apoptosis in cells. Comparatively, the number of apoptotic or necrotic cells were higher in RSNM than pure RSV which may be due

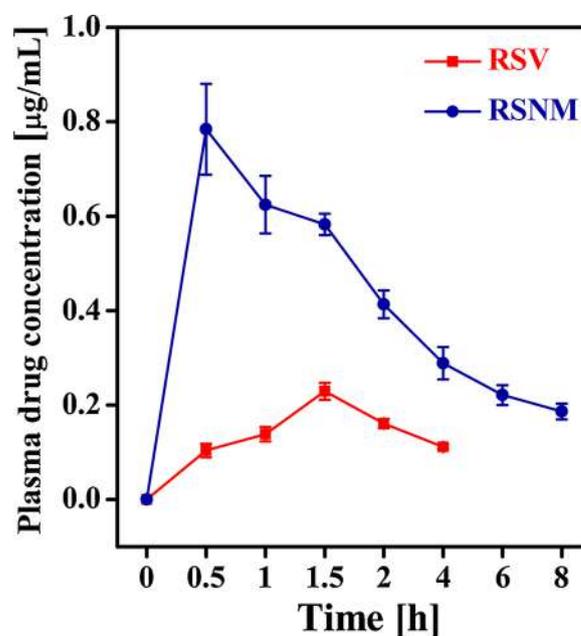


Fig. 8. In-vivo pharmacokinetic performance, represented as plasma drug concentration vs time for resveratrol (RSV) and resveratrol-loaded INU-F68-SA bioconjugate nanomicelles (RSNM) after oral administration in male Sprague Dawley rats. (Data represent as mean \pm SD, $n = 6$).

Table 2

Pharmacokinetic parameters of pure resveratrol (RSV) and RSV-loaded INU-F68-SA bioconjugate nanomicelles (RSNM) (mean \pm SD, $n = 6$).

Parameter	RSV	RSNM	Unit
$t_{1/2}$	2.699 ± 0.163	5.762 ± 0.822	h
T_{max}	1.500	0.500 ± 0.001	h
C_{max}	0.229 ± 0.018	0.784 ± 0.096	$\mu\text{g/mL}$
$AUC_{0-\text{inf,obs}}$	0.983 ± 0.027	4.254 ± 0.208	$\mu\text{g/mL} \times \text{h}$
$MRT_{0-\text{inf,obs}}$	4.627 ± 0.201	7.907 ± 0.841	h
Cl/F_{obs}	50.887 ± 1.401	11.771 ± 0.561	$(\text{mg/kg})/(\mu\text{g/mL})/\text{h}$

$t_{1/2}$ = Plasma half-life; T_{max} = Time to reach peak plasma concentration; C_{max} = maximum plasma concentration; AUC = area under the curve; MRT = Mean residence time; Cl = clearance.

to improved delivery of RSV with RSNM than its suspension formulation.

3.9. Hoechst nuclear staining assay

Nuclear morphology changes of HCT 116 cells was evaluated by staining the cells Hoechst 33342 dye. A change in nuclear morphology is considered as one of the major characteristics of cell apoptosis which can be easily detected by staining the cells with the Hoechst 33342 dye. Untreated control and BNM treated HCT 116 cells showed normal a spherical nuclei and staining of chromatin. In another, HCT 116 cells treated with 30 $\mu\text{g/mL}$ of RSV or RSNM for 24 and 48 h presented bright, chromatin, condensation and nuclear fragmentation which are typical characteristic of apoptosis. With RSNM treatment, more bright nuclei were detected as compared to pure RSV treatment (Fig. 6b). Hence, the observed results confirmed that RSNM significantly induced apoptosis in HCT 116 cells.

3.10. Cellular uptake study

The cellular internalization of prepared nanomicelles in HCT 116 cells line was studied by fluorescent microscopy. For this, fluorescent nanomicelles were prepared used coumarin-6 (C6) as a fluorescent

probe which replaced RSV in the nanomicelles. Further, to determine time-dependent uptake, cells were incubated with pure C6 and C6-loaded nanomicelles (C6NM) for different time period and observed by fluorescent microscopy. As shown in Fig. 7, most of the green fluorescence colour was observed in the cytoplasm of the HCT 116 cells. The green fluorescence colour of C6 was not observed up to 4 h in HCT 116 cells incubated with plain C6.

3.11. Pharmacokinetic study

The influence of prepared nanomicelles on the pharmacokinetic (PK) of RSV was determined. The RSV plasma concentration versus time pattern is shown in Fig. 8, and the PK parameter results are presented in Table 2. After the oral administration of RSV suspension, plasma concentration was detectable up to the first 4 h only. Interestingly, after encapsulation in synthesized bioconjugate, the RSV plasma concentration was detectable up to 8 h which indicated the sustained RSV release in plasma. The relative oral bioavailability of RSV was found to be higher than in the form of nanomicelles after the oral administration of RSNM formulation. The RSNM outperformed RSV in major PK parameters.

The area under the curve (AUC) of the RSNM ($4.254 \pm 0.208 \mu\text{g/mL} \times \text{h}$) was 4.3 times significantly higher ($p < 0.0001$) than the pure RSV ($0.983 \pm 0.027 \mu\text{g/mL} \times \text{h}$). The time taken to reach maximum plasma concentration (T_{max}) for RSV was found to be 1.5 h and which was 3 times significantly higher ($p < 0.0001$) than RSNM 0.5 h. The increased AUC and plasma concentration (C_{max}) values clearly confirmed the higher absorption increase in oral bioavailability of the RSV after encapsulation in synthesized bioconjugate. In addition, both $t_{1/2}$ and MRT values of RSV in plasma after oral administration were found to be significantly higher ($p < 0.0001$) than the pure RSV suspension oral administration. The clearance (Cl) was observed for RSV ($50.887 \pm 1.401 \text{ (mg/kg)/}(\mu\text{g/mL)/h}$) significantly higher than the RSNM ($11.771 \pm 0.561 \text{ (mg/kg)/}(\mu\text{g/mL)/h}$). Hence, these observed results were consistent with the obtained physicochemical data like amorphous nature, solubility, sustain drug release and stability. These results clearly indicating the difference between PK parameters of pure RSV suspension and RSNM formulation from significantly improved solubility, sustain drug release, surface to volume ration (nano size) thereby enhancing oral bioavailability.

4. Conclusions

In conclusion, we successfully synthesized a polysaccharide-surfactant-lipid bioconjugate. Being amphiphilic in nature, the bioconjugate can self-assemble in double-folded micelles that could hold a high amount of hydrophobic RSV and deliver it in pH-dependent manner. The prepared micelles demonstrated nanoscale size, high physicochemical stability up to 48 h in colloidal form and pH-sensitive degradation. In *in-vitro* anticancer studies RSNM showed greater control over the proliferation of human colorectal cancer cells than the pure RSV by inducing higher apoptosis and improving delivery of drug through higher cellular uptake. Finally, the RSNM were orally administered to animals to evaluate the pharmacokinetic parameter which revealed that RSNM improved the oral bioavailability of RSV. Therefore, the designed INU-based nanostructures could be useful in the pH-responsive delivery of hydrophobic molecules in the GIT.

CRedit authorship contribution statement

Ashok Kumar Jangid: Conceptualization, Methodology, Writing - original draft, Software. **Krunal Patel:** Investigation. **Poonam Jain:** Data curation, Software. **Sunita Patel:** Validation. **Nitin Gupta:** Formal analysis. **Deep Pooja:** Writing - review & editing, Visualization. **Hitesh Kulhari:** Conceptualization, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.carbpol.2020.116730>.

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रिश्तों पर लगती 'ठेस' और रेणु की परंपरा

प्रमोद कुमार तिवारी

पिछली सदियों की सबसे बड़ी त्रासदियों में से एक यह है कि हम मन और मस्तिष्क से कई गुना अधिक पेट और शरीर से संचालित होते जा रहे हैं। विज्ञान के अभूतपूर्व किंतु एकांगी विकास ने जहाँ एक ओर मानव श्रम में लगातार कटौती करते हुए भौतिक सुविधाओं की बाढ़ सी ला दी है वहीं दूसरी ओर भाव और संवेदना के स्तर पर हमें लगातार कमजोर करने का भी काम किया है। यह सिर्फ संयोग नहीं है कि जहाँ पहले पढ़ाई का मतलब भाषा, साहित्य और दर्शन हुआ करता था वहीं आज ये तीनों ही लाभ के गणित से संचालित शिक्षा-प्रबंधकों के लिए बोझ जैसे बनते जा रहे हैं। मानविकी से जुड़े ज्यादातर विषय न केवल विद्यार्थियों की कमी से जूझ रहे हैं बल्कि विश्वविद्यालयों के सबसे दयनीय विभागों में उनकी गणना होती है। ऐसे परिवेश में साहित्य की बात करना और साहित्य को अभूतपूर्व ऊंचाई देने वाले साहित्यकारों की बात करना वर्तमान समय के साथ-साथ मनुष्यता की सबसे बड़ी जरूरतों पर बात करना लगता है।

आर्थिक भाव से दिनोंदिन समृद्ध होते जाने वाले समाज में मानवीय भाव की लगातार बढ़ती दरिद्रता को समझने में जो रचनाकार हमारे लिए बहुत मददगार हो सकते हैं उनमें से एक फणीश्वर नाथ रेणु हैं। भाव की प्रबलता को उसकी पूरी सघनता के साथ पाठकों के दिलों में अलग ही तरह से पिरो देनेवाले दुर्लभ कथाकार रेणु की जन्मशती चल रही है। लगभग 70 साल पहले अपने महान उपन्यास 'मैला आंचल' में रेणु ने समाज के सबसे बड़े कीटाणु के

रूप में 'गरीबी और जहालत' को रेखांकित किया था। मुझे ऐसा लगता है कि अगर रेणु आज समाज के कीटाणुओं को पकड़ने निकलते तो समृद्धि और महत्वाकांक्षा के खाद पानी से लहलहा रहे उन दीमकों की चर्चा करते जो भीतर ही भीतर चुपचाप आत्मीय रिश्तों को खाते चले जा रहे हैं। अपनी ज्यादातर रचनाओं में रेणु ने रिश्तों के मरजाद को महत्व दिया है। हर तरह की मुसीबत झेलने के बावजूद उनके पात्र रिश्तों की रक्षा करते हैं। तमाम विपरीत परिस्थितियों में जो चीज उन पात्रों को हारने नहीं देती है उसे रिश्तों की गर्माहट के रूप में रेखांकित किया जा सकता है। वे रिश्ते चाहे तीसरी कसम, रसप्रिया, संवेदिया जैसी कहानियों के हों या मैला आंचल जैसे उपन्यासों के। यह ध्यान देने की बात है कि तमाम प्रकार की खींच-तान और संकटों से जूझने के बावजूद रेणु की कहानियाँ हमें निराश नहीं करतीं। ज्यादातर निम्न वर्ग के पात्रों के जीवन को रेणु उठाते हैं, जहाँ आर्थिक विपन्नता और दूसरे संकटों की कोई कमी नहीं है परंतु रिश्तों की उष्णता और उत्सवधर्मिता के साथ अपनी ही तरह की सांस्कृतिक समृद्धि इन कहानियों में नजर आती है जिसे रेणु की कहानियों की बड़ी ताकत के रूप में देखा जा सकता है।

रेणु की जन्मशती के अवसर पर उनकी कुछ कहानियों के पुनर्सृजन के अनूठे प्रयोग की बात जब कथाकार और आलोचक राकेश बिहारी जी से पता चली तो जाने क्यों मुझे आंतरिक खुशी मिली। राकेश बिहारी भाषा और संवेदना दोनों स्तरों पर जिस तरह नवाचार को बढ़ावा देते हैं, हिंदी साहित्य

को इसकी विशेष जरूरत है। राकेश जी ने जब मुझसे एक लेख लिखने की बात कही तो अच्छा लगा, केवल इसलिए नहीं कि रेणु मेरे प्रिय रचनाकारों में से एक हैं बल्कि इसलिए कि मन में एक जिज्ञासा थी कि हमारे आज के रचनाकार रेणु की कहानियों को कैसे रचते हैं? रिश्तों की बुनियाद पर टिकी रेणु की कहानियों के साथ आज के कथाकारों का व्यवहार कैसा होता है? निश्चित रूप से यह कहना बहुत मुश्किल है कि आज रेणु होते तो इन कहानियों को कैसे लिखते, पर अपने समय और समाज से संपृक्त रूप में जुड़े और अपने समय के आंदोलनों में सक्रिय रूप से हिस्सा लेने वाले रेणु के बारे में कुछ अनुमान तो लगाया ही जा सकता है।

कहानी के पुनर्सृजन के इस जरूरी प्रयोग में मेरे हिस्से में रेणु की जो कहानी आयी है वह है— 'ठेस'। 21वीं सदी के 'ठेस जारी...' के रचनाकार हैं राकेश दूबे। पहले ही साफ कर दूँ कि राकेश दूबे, रेणु नहीं, राकेश हैं और इस कहानी का और राकेश होना भी इसी बात में निहित है कि यह राकेश की कहानी है जो 21वीं सदी में लिखी गयी है। निश्चित रूप से रेणु के कथानक और भाव संवेदना को लेकर लिखी गयी है। 'ठेस जारी...' कहानी को समझने का एक और सूत्र हमारे पास है। रेणु ने 'ठेस' कहानी 1955 के करीब लिखी थी, इसी भाव संवेदना को लेकर रेणु ने एक और कहानी लिखी थी 'भीति चित्र की मयूरी' जिसे उनके अंतिम दौर की कहानी के रूप में देखा जा सकता है, जो नवंबर 1972 में प्रकाशित हुई थी। (उनकी अंतिम

Shiju Sam Varughese

Kerala's Engagements with Modernity: Genealogies and Geographies

Three methodological coordinates were proposed in the editorial introduction of the volume, *Kerala Modernity: Ideas, Spaces and Practices in Transition* (Bose and Varughese 2015: 10) to underscore a transition in our understanding of Kerala's engagements with modernity. First, region is not a subnational entity completely subsumed to the nation. Second, region is not an inert substrate but active, multiple and constantly being de/re-territorialised. Third, regions have deep, heterogeneous and constantly changing connections with global modernity. These coordinates were founded upon the conceptualisation of multiplicity of space-times a region rhizomatically assembles together: in other words, the region is now conceived as a geographical category that makes possible fresh analyses of the regional processes, moving away from an earlier, exclusive focus of scholarly literature on subject, community and language.



Print Media and Contestations over Knowledge

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KEYWORDS: CIVIC EPISTEMOLOGY, DIGITAL PUBLICS, MEDIA CONVERGENCE,
SCIENCE JOURNALISM, SCIENTIFIC PUBLIC SPHERE

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Analysing the historical role of print media and journalism in shaping public engagement with science, technology, and medicine in the Indian context is commonly anchored on the theoretical foundations of technological modernity, a national or subnational imagined community, and a print media-generated public sphere. In this brief discussion, let us examine how these methodological premises create hurdles for our theorisation of print media's relationship with science, technology and medicine. This paper endeavours to go beyond these much-exercised theoretical presuppositions so that the relationship can be understood in more nuanced and complex ways. This is inevitable in the current context of digital convergence of all media forms including print media that altered journalistic practices and science reporting. Such an attempt is performed here by taking cues from the essays in the current section to identify blockages in media theory, and for further extending the fresh and exciting insights offered by the authors.

It seems important to defy the reductionist assumption of technological modernity that informs our engagement with print media as bringing forth social and political transformation through reconfiguring language, community, and knowledge, three domains central to the socio-political life.

Publication Trends in the Informal Sector Innovation Research

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ABSTRACT

Innovation research, representing other than the formal sector, has gained momentum in the last two decades and different terms like grassroots innovations, frugal innovations, *jugaad*, inclusive innovations and informal sector innovations are used. These key terms have specific and contextual meaning and represent different sets of innovations that are different from its traditional or Schumpeterian understanding of innovations. The Scopus and Google Scholar database suggest that since 2005, the number of publications has increased and focus is given on publishing research on informal sector innovations. Further scientometric analysis of Scopus database on parameters of the subject area, document type, country of publication, source journal and institutional affiliation is conducted. The results suggest that in the last one decade the relevance of such innovations have been recognised by academicians and policymakers alike and most of studies or cases are coming from developing economies, especially India. This also suggests that many government and non-government efforts have been made to identify the role of informal sector innovations in developing economies in a major way.

Keywords: Grassroots Innovations, *Jugaad*, Inclusive Innovations, Frugal Innovations, Informal Sector Innovations, Scientometrics.

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INTRODUCTION

In the last two decades, the informal sector innovation research has caught the attention of academicians, policymakers and enthusiasts alike. There are several terms, often used interchangeably, such as grassroots innovations, green innovations, inclusive innovations, frugal, informal sector innovations and *jugaad* to denote innovations other than the formal sector. However, their nature and meaning are contextual and represent a diverse set of innovations. For instance, grassroots innovation term is not very old. However, it has been practised by many cultures for centuries under different names.^[1] Similarly, *jugaad* is used for innovations both in the formal as well as informal sector innovations. The informal sector, considered as one of the largest sectors for employment generation and contributor to the gross domestic product (GDP) in many economies, especially developing countries, had a pessimistic understanding. This sector was looked as unproductive and a hindrance for development in the larger context.^[2] Later on, it was realised that rather than having a negative outlook of the informal sector, one should

develop a better understanding of it from other perspectives.^[1,2] The recent trend in looking at this sector as a knowledge generator and breeding ground for various forms of innovations has attracted scholars to understand the context and relevance of innovations coming out of the informal sector. Such innovations are considered to be by, for and at the grassroots.^[3] There are various interest groups, such as policymakers, scholars and promoters of informal sector innovations, have started looking into the innovations from the informal sector and addressed them using various terms as mentioned above. There are certain country or context-specific terms one can find in the literature, for instance, *Jua Kali* in Kenya, *Système D* in France, *DIY* in the USA, folklore innovations in China and *Gambiarra* in Brazil.^[1] This has not only increased research publications, but also funding has been pumped into it to tap the potential to address various problems such as accessibility, environmental, inclusivity and so forth. For instance, Honey Bee Network (HBN), an informal movement, started in the late 80s in India by Prof. Anil K Gupta and his associates to tap the potential of knowledge in the informal sector.^[4] Later, this movement has been able to successfully institutionalise the grassroots innovations by establishing Society for Research and Initiatives for Sustainable Technologies and Institutions (SRISTI) and National Innovations Foundation (NIF).^[5] They have attracted funds not only from civil society organisations, but the Indian government has also helped them. The activities of these organisations are to scout, document, give awards,

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Examining the Research and Technological Impact of Survismeter

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ABSTRACT

The chemical solutions are backbone of industrial formulation as this helps in manufacturing of various industrial products. Each formulation goes through the structural changes which requires precise instruments to capture the structural characteristics. Understanding these structural changes are also active area of research. Survismeter was designed in an academic setting in India to understand structural characteristics of chemical solutions. The instrument was further modified and was successfully transferred to a firm. It was demonstrated that the instrument not only has relevance for chemical sciences but has functional relevance in different domains especially pharmaceuticals, nanomaterials, functional materials formulations and biophysics. The study examines the research impact of the instrument 'Survismeter'. This paper seeks to analyze the use, development and publications indexed in the database of Scopus to understand the impact of this instrument. Furthermore, there is also focus on the patents granted to this instrument and its commercial transfer.

Keywords: Survismeter, Friccohesity, Borosil, Patent, Instrument.

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INTRODUCTION

Background

Borosil Mansingh Survismeter (where the term Mansingh refers to scientist/inventor/innovator Man Singh), is a green science Trusted Sustainable Analytical Device (TSAD) used for measuring surface tension, interfacial tension, wetting coefficient, particle surface area and size, viscosity and friccohesity simultaneously of aqueous, non-aqueous, aprotic dipolar, polar, protic polar and non-polar solvents and mixtures within wider ranges. Figure 1 and Figure 2 highlights the schematic and physical structure of this instrument

Survismeter is based on the combination of three terms sur + vis + meter that means surface tension, viscosity and meter (measuring parameter). It works on the theory of R4M4 [Reduce Reuse Recycle Redesign–Multipurpose Multidimensional Multifaceted Multitracking] of materials and methods with highly précised and accurate experimental results. The instrument can be used in the quality analysis of various products used in agrochemicals, biochemical, pharmaceuticals, cosmetics, petroleum and oils, polymer and proteins, food and beverages, inks, sol-gels, soaps and detergents, insecticides, pesticides, colloids, emulsions,

lubricating, viscous materials (high or moderately). The focus of this device is on surface tension, viscosity, interfacial tension and wetting coefficient determinations.^[1-12] The study examines the impact of this instrument in academic research.

Objective and Study Method

The study attempts to capture the importance of Survismeter in research and industry by examining research outputs emerging from usage of this instrument. Chronological publication growth pattern (as time series); Authorship pattern; Geographical scattering of publication are examined in this context. Further through personal interviews with the inventor and examining the patents granted to this instrument and its commercial transfer, the study attempts to draw the research and commercial impact of this instrument.

Findings

There were thirty-two research papers that was identified based on the instrument 'Survismeter'. The papers were identified based on the search string 'Survismeter' applied in the keyword field covering the period 2005 to 2019. Figure 3 shows the chronological order of publications as per the Scopus database from the year 2005 to 2019 with respect to term Survismeter. It may be possible that some paper published earlier has not been indexed in the Scopus database.

Most of the documents published are in the form of articles (approximately 97%) and the remaining as conference papers. The progresive decline of the research papers can possibly be understood when one looks more closely at the charactersits

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Title: Role Of Information Communication Technology In Higher Education In Ranchi

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Abstract. This study highlights the role of information and communication technology (ICT) in higher education in the Ranchi district of Jharkhand, India. Other considerations influence how ICT and higher education systems are integrated to improve teaching and learning. The study uses Everett Rogers's 1962 diffusion of invention framework, highlighting student adoption of technologies and organizations influenced by state policies. The data was collected from Ranchi's higher educational institutions. The results indicate that most students are in favour of using ICT in the teaching-learning process. It also suggests the significance of collaboration among the numerous players who have learned new ICT techniques in educational systems. It assumed that government participation would aid in the spread of ICT in education.

Keywords: Higher Education, Information communication technology, Policy, Adoption, Collaborative learning approach.

INTRODUCTION

Education aims to raise a nation's desired standard of living and change in human behaviour. Education should be accepted socially, culturally and reflect in a transfer of knowledge, skills, attitude, and understanding. Traditional education is only to get knowledge but, now it is changed into the invent. Students need to build their understanding of each scientific concept. A teacher's key challenge is to offer teachings and develop a relevant knowledge base to use them further. (Achimugu, Oluwagbemi, & Oluwarnti, 2010). The capacity to learn information and implement it is essential for every country's economic and social well-being. The nations having higher and better knowledge and skills respond more effectively and quickly to globalisation challenges and opportunities.

Education is detecting a significant change regarding access, equity, and quality in India. This transition is being accelerated by the exponential growth of ICT in the world's educational systems. Making full use of ICT diffusion resources in education is a significant obstacle for educational institutions. The Indian higher education system's governing bodies are advanced in science and technology, especially in ICTs that play a dynamic role in all educational process segments. For instance, internet technology has boost ICT and influenced every sector of the Indian education system. With this context, Snehi (2009) argued institutions had used computers in their academic programs for producing a better quality of knowledge output and learning in the higher education sector (Snehi, 2009). Higher education raises awareness, creates intelligence, and gives the recipient a more comprehensive view of the global

Kunal Sinha

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Benefits of Assistive Technology and Policy Implications

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Abstract--According to the World Bank, 15% of the worldwide population, experiences some type of disability, with the incidence of impairment being greater in developing nations. A fifth of the world's population, estimated to be between 110 and 190 million people, has major impairments. In India, the ratio of assistive innovation technologies to innovative technology adaptation is relatively low. Adaptation of ATs has been a term in technology education and research in recent years. This technology connects and involves a variety of technologies, with a focus on assistive innovation technologies. This results in assistance for those who use assistive innovative technology to tackle their problems. People with physically weaker impairment benefit greatly from assistive innovative technologies. It is not only for persons with disabilities but also for the elderly. The benefits of ATs at the national innovation and grassroots level, as well as crucial information about ATs, are explored in this research article.

Keywords---assistive technology, benefits, health, innovation technologies, socioeconomic.

Introduction

Indian manufacturers are happy with Prime Minister Narendra Modi's mantra of "Vocal for Local and Local for Global." This is an appropriate moment to draw attention to Indian innovations on Assistive Technology (AT). As indicated by the World Bank, one billion groups, or 15% of the world's population, experience the side effects of a type of disability, also, equality is high in developing nations. One-fifth of total appraisals worldwide, between 110 million and 190 million, experience massive disabilities. For decades, Persons with Disabilities (PWDs) in

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I, Neerja A Gupta, hereby declare that the particulars are true to my knowl-
edge and belief.

Sd.
(Neerja A Gupta)

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Editorial

Dear Readers,

Indian culture is diverse and comprises various customs, ideas, social attributes, and beliefs. India contains different cultures and communities that differ prominently in their traditions, food habits, languages, and traditions. It is an amalgamation of different traditions and customs and how different communities present and organize themselves in terms of morality and etiquette. Our most valuable and most instructive materials in the history of man are treasured up in India.

In recent decades the sustainable conservation of cultural heritage has become a crucial global challenge. In this context, the trend towards large scale urbanization raises questions as to how new development can take place which respects and maintains the intrinsic values and unique qualities which have been handed down from previous generations, particularly within urban areas.

The Indian culture varies like its vast geography. People speak in different languages, dress differently, follow different religions, eat different food but are of the same temperament. So whether it is a joyous occasion or a moment of grief, people participate whole-heartedly, feeling the happiness or pain. A festival or a celebration is never constrained to a family or a home. The whole community or neighbourhood is involved in bringing liveliness to an occasion. Likewise, an Indian wedding is a celebration of union, not only of the bride and groom, but also of two families, maybe cultures or religion too. Similarly, in times of sorrow, neighbours and friends play an important part in easing out the grief.

As Swamy Vivekananda said "If anyone dreams of the exclusive survival of his own religion and culture, I pity him from the bottom of my heart and point out that upon the banner of

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Life across the Dark Waters: Saga of Indian Indentureship in the Caribbean

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Introduction

Migration is an inseparable and inevitable part of human civilisation and history. From the time immemorial to the present-day era of human history we have witnessed the migration of people, animals, plants etc. from one place to another. However, all the migrations are not the same. Migration differs in terms of its kind, magnitude and form. In spite of many hurdles and taboos Indians have crossed their national boundaries to land in different countries of the world as traders, religious preachers, labourers, professionals etc. by the dint of their determination, hard work and struggling nature Indians have succeeded in their mission and have achieved a great niche for themselves as well as have created their name for India wherever they have gone. Indian migration to the Caribbean was made under the diabolic system of Indentureship, which was in true sense a new system of slavery (Tinker 1993). In spite of their indenture assistance, Indians, by their sheer hard work and struggles have climbed the ladder of success. In some of the countries

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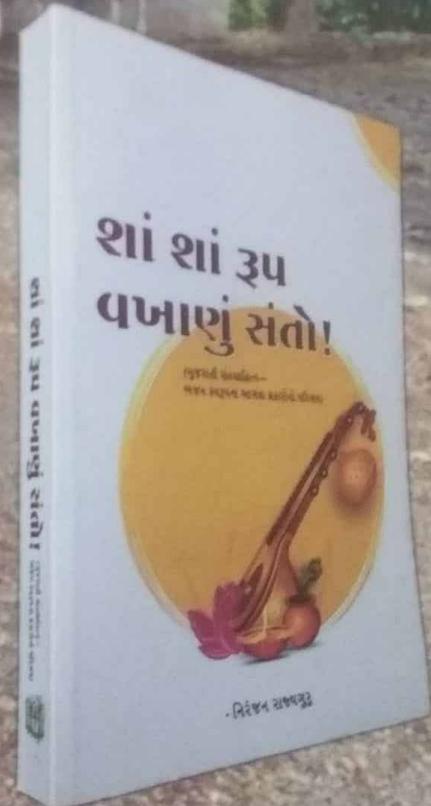
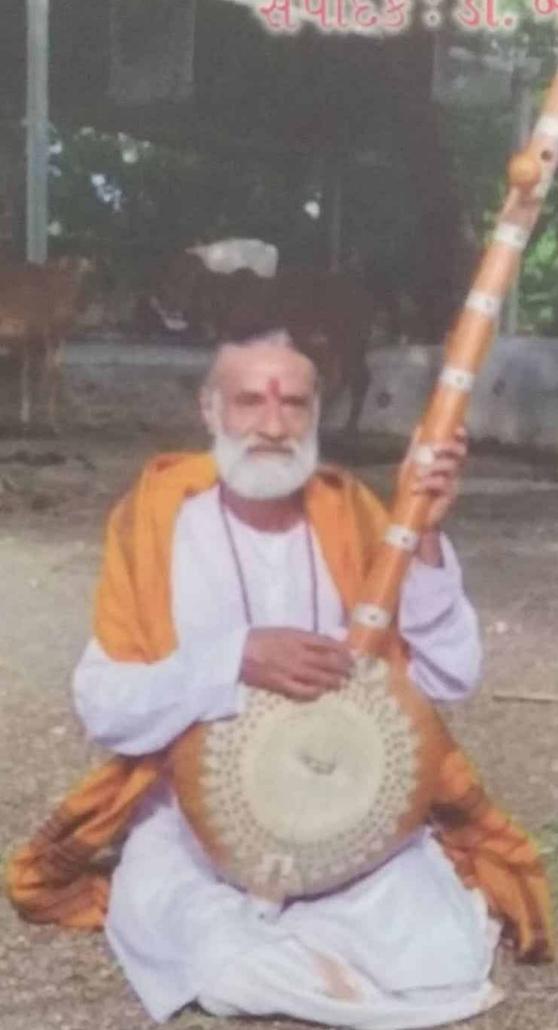
લોકગુર્જરી



સર્ળંગ અંક : એકસઠ

(ત્રૈમાસિક : દસમું વર્ષ, બીજો અંક, સપ્ટેમ્બર-૨૦૨૧)

સંપાદક : ડૉ. બળવંત જાની



શ્રી ઝવેરચંદ મેઘાણી લોકસાહિત્ય કેન્દ્ર

સૌરાષ્ટ્ર યુનિવર્સિટી, રાજકોટ-૫

લોકગુર્જરી

સળંગ અંક : એકસઠ

(ત્રૈમાસિક : દસમું વર્ષ, બીજો અંક, સપ્ટેમ્બર-૨૦૨૧)

: સંપાદક :

ડૉ. બળવંત જાની

શ્રી ઝવેરચંદ મેઘાણી લોકસાહિત્ય કેન્દ્ર

સૌરાષ્ટ્ર યુનિવર્સિટી

રાજકોટ-૫

અનુક્રમણિકા

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૯. ગગાભાએ ન્યાય તોળ્યો	ડૉ. પુલકેશી જાની	૧૦૩
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૧૧. કાવ્યજ્ઞશિક્ષણાભ્યાસની પ્રવૃત્તિ: 'બાળજોડકણાં'	ડૉ. વિનોદ જાડા	૧૨૧
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લોકશિક્ષણ અને મનોરંજનનું માધ્યમ : 'લોકનાટ્ય ભવાઈ'

ડૉ. બળદેવ પ્રજાપતિ

લોકગીત, લોકનાટ્ય, લોકસંગીત આ બધી બાબતો લોકો સાથે જોડાયેલી છે. આ આપણી લૌકિક વિદ્યા તો ખરી જ...! પરંતુ લોકસંસ્કાર, લોકસંસ્કૃતિ અને લોકશિક્ષણની પ્રાચીન પરંપરા પણ છે. કેટલાક પૂર્વ સંદર્ભો આપણને મળે છે કે- 'મધ્યકાળમાં સંસ્કૃત નાટકો રચાયાં અને ભજવાયાં પણ હતાં.' અંગ્રેજ શાસન પૂર્વે ગુજરાતમાં રંગભૂમિ ન હતી. આ અર્વાચીન રંગભૂમિ પશ્ચિમના સંપર્કના કારણે ઉદ્ભવી છે. આ સમય દરમિયાન ગુજરાતી ભાષામાં નાટકો ન હતાં ત્યારે લોકસમુદાયને મનોરંજન પૂરું પાડવાનું કામ આપણા લોકનાટ્ય ભવાઈએ કર્યું હતું. એ સમયે આધુનિક યુગના થિયેટરની કલ્પના ન કરી શકાય. તે સમયે ખુલ્લા આકાશનું થિયેટર એટલે ભવાઈ. ખુલ્લા આકાશની નીચે લોકસમુદાયમાં ભવાઈ ભજવાતી. ગ્રામલોકો ખેતી કે અર્થોપાર્જનની અન્ય પ્રવૃત્તિમાંથી નિરાંત મેળવી મનોરંજન માટે રાત્રે ભવાઈનો આશરો લેતા હતા. ખૂબ જ ટાંચાં સાધનોમાં ગામની વચ્ચે ચમકદમકથી પર ફક્ત નૃત્ય અને સંવાદના સહારે વર્તુળાકારે ગોઠવાયેલા પ્રેક્ષકોની મધ્યે ભવાઈ વેશ ભજવાતો હતો. ભવાઈ પરંપરા સમગ્ર ભારતમાં ભજવાતી એવું પણ જોવા મળે છે. દરેક રાજ્યના લોકનાટ્યો અલગ અલગ નામે ઓળખાય છે, જેમ કે મહારાષ્ટ્રમાં 'તમાશા', 'વાસુદેવ', 'દશાવતાર',

મધ્યપ્રદેશમાં 'માય', કર્ણાટકમાં 'યક્ષગાન' વગેરે નામોથી લોકનાટ્ય પ્રચલિત છે એવી જ રીતે ગુજરાતનું લોકનાટ્ય ભવાઈ છે.

હિન્દુસ્તાનના સંસ્કારવારસાને સમૃદ્ધ કરવામાં, તેની સંસ્કૃતિ ટકાવી રાખવામાં, પરંપરા જાળવવામાં વિવિધ રાજ્યોનાં લોકનૃત્ય અને નાટ્યસ્વરૂપોએ મહત્વનું પ્રદાન કર્યું છે. એમાં ગુજરાતનું મહત્વનું લોકનાટ્ય સ્વરૂપ 'ભવાઈ' છે. આ ભવાઈમાં ઘણી જૂની લોકનાટ્ય પરંપરા જાળવાઈ રહી છે. વૃક્ષોની જેમ માણસને પણ મૂળિયાં હોય છે, સંસ્કૃતિનાં. જેના દ્વારા માનવી પોષણ અને સંસ્કાર મેળવે છે. આવું ગુજરાતનું અને એમાંય ખાસ કરીને ઉત્તર ગુજરાતનું સંસ્કાર મૂળિયું એટલે 'લોકનાટ્ય ભવાઈ' આ કલા ભૂમિગત અને સ્વયંસ્ફુરિત છે. ગ્રામ્યજનોનાં ઊર્મિ અને આનંદ એમાં સમાયેલા છે. મોટે ભાગે જૂની ગુજરાતીમાં ભવાઈનું સાહિત્ય સચવાયું છે. કેટલાક જૈન કથાનકોમાં વાત જાણવા મળે છે કે 'એક વખત અણહિલવાડ પાટણના રાજા સિદ્ધરાજ જયસિંહ નાટક જોવા બેઠા હતા.' આ નાટકમાં ઉત્તર ગુજરાતની લોકનાટ્ય પરંપરા ભવાઈનું મૂળ જોઈ શકાય છે.

ગુજરાતમાં લોકનાટ્ય ભવાઈનું બીજ તેરમી-ચૌદમી સદીમાં નખાયું હોવાનું કહેવાય છે. 'ભવાઈ' શબ્દના અર્થને સ્પષ્ટ કરતાં અનેક વિદ્વાનોએ વિધાનો કર્યા છે. ભગવદ્ગોમંડલમાં ભવાઈનો મૂળ અર્થ આ પ્રમાણે છે કે- "આ શબ્દનું મૂળ ભવવહી એટલે આપણા ભવની વહી. માણસ એ વહી જુએ ને પોતાની જાતને ઓળખતાં શીખે, એવો ભવવહી કે ભવાઈનો મૂળ અર્થ છે"^૧ તો સ્વ. જયશંકર ભોજક (સુંદરી) ૧૯૩૭માં ભરાયેલ રંગભૂમિ પરિષદમાં 'ભવાઈ અને તારગાળા' વિષય પર આપેલ વ્યાખ્યાનમાં "ભવાઈ શબ્દ ભવવહીમાંથી જન્મ્યો તેમ જણાવ્યું હતું".^૨ તો ડૉ. ચીનુભાઈ નાયક કહે છે કે- "ભારતના અન્ય ત્રણ વિભાગોમાં મણિપુરી, કથક, ભરતનાટ્યમ્ ઈત્યાદિ નૃત્યવિશેષની જેવી પ્રણાલી જ હતી તેવી ઉત્તર ભાગની ભવાઈ એ વાસ્તવમાં એક નૃત્ય સહજ પ્રણાલિકા છે. આપણા લોકનાટ્ય ભવાઈની ઉત્પત્તિનું સ્થળ ઉત્તર ગુજરાત છે. ઉત્તર ગુજરાતનું પ્રાચીન નામ આનર્ત છે. તેની વ્યુત્પત્તિ નર્તન કરનાર લોકોનો પ્રદેશ એવી અપાઈ છે. ભવાઈ કરનાર ભવૈયાઓના વસવાટની ભૂમિ આ પ્રદેશ છે એ આ અર્થમાં સાર્થક છે"^૩ અમદાવાદના સ્વ. હરણીશંકર મુન્શીએ ભવાઈ એટલે "ભવ (અંબા) આઈ (માતા) ભવાની માતા સમક્ષ રજૂ થાય તે ભવાઈ એવો અર્થ જ તારવ્યો છે"^૪ તો અનંતરાય રાવળ નોંધે છે કે - "ભવ એટલે સંસાર પરથી લીલાનું અનુકરણ તે ભવાઈ એમ માનવું ઠીક છે"^૫ આમ વિવિધ વિદ્વાનોએ 'ભવાઈ' શબ્દના મૂળ સુધી પહોંચવાનો પ્રયત્ન કર્યો છે. જો સમગ્ર રીતે કહેવું હોય તો કહી શકાય કે

'ભવાઈ' એટલે શિવ-શક્તિની ઉપાસના, ભક્તિભાવ, વીતી ગયેલા ભવનું કથન, સાથે-સાથે ભારતીય સંસ્કૃતિના કલાવારસાને જાળવતો લોકનાટ્ય પ્રકાર છે.

ભવાઈના વેશની શરૂઆત ૧૪મા સૈકામાં થઈ એમ કહી શકાય. આ સંદર્ભે ડૉ. મધુસૂદન પારેખ નોંધે છે કે - "એ વેશોનો રચનાર તે 'હંસાઉલી' પદ્યવાર્તાનો લેખક અસાઈત, એનો સમય અંદાજે ઈ.સ. ૧૩૨૦થી ૧૩૮૦નો માનવમાં આવે છે." આમ જુદા જુદા વિદ્વાનો અસાઈતનો સમયગાળો બતાવે છે. પણ એટલું ચોક્કસ છે કે-'હંસાઉલી' ના કર્તા અસાઈત છે. તેનો સમય ઈ.સ. ૧૩૬૧ છે તેથી તેને આધારે તેનો સમય ઈ.સ. ૧૩૦૦થી ઈ.સ. ૧૪૦૦નો કહી શકાય. આ અસાઈત ઠાકર સિદ્ધપુરના વતની હતા. તેમના પિતા રાજારામ ઠાકર યજુર્વેદી ભારદ્વાજ ગોત્રના ઔદિચ્ય બ્રાહ્મણ સાથે સાથે વિદ્વાન અને કથાકાર હતા. જે ગાયન-વાદન સાથે કથાય કહેતા, જેથી આજુબાજુનાં ગામોમાં એમની ખ્યાતિ સારી હતી. પુત્ર અસાઈત વાકપટુ હોવા સાથે કવિતા પણ સારી લખતો અને ગાઈ જાણતો. આથી આજુ-બાજુનાં ગામોમાં તેની પ્રતિષ્ઠા ઘણી જામી હતી. આમ અસાઈત અને ઊંઝાના હેમાળા પટેલ વચ્ચે ગુરુ યજમાનનો સબંધ હતો. એ સમયગાળા દરમિયાન હિન્દુસ્તાનમાં અલ્લાઉદ્દીન ખલજીના રાજ્યકાળમાં જહાનરોજ નામનો સરદાર દિલ્લી-કનૌજ પર ચઢાઈ કરી, ગુજરાત પર ચડી આવ્યો, એ સમયગાળામાં ઊંઝાના હેમાળા પટેલની દીકરી ગંગાના રૂપ-ગુણની પ્રસંશા સાંભળીને તેણે ઊંઝામાં પડાવ નાખ્યો. સરદારની મરજીને માન આપીને સિપાઈ ગંગાને સરદારના તંબુમાં ઉઠાવી લાવે છે. આ વાતની જાણ અસાઈત ઠાકરને થતાં, તે દીકરીના શિયળની રક્ષા માટે સીધો સરદારની પાસે જઈ વાકપટુતા, ગાયન-વાદન દ્વારા સરદારને ખુશ કરી, તે બદલામાં દીકરી ગંગાની માંગણી કરે છે. પણ આ દીકરી અસાઈતની નથી એવી શંકા સરદારને જાય છે તેથી, સરદાર પરીક્ષા રૂપે અસાઈતને ગંગા સાથે એક ભાણે બેસીને જમવાનું કહે છે. એ સમયમાં બ્રાહ્મણસમાજ જ્ઞાતિ-જાતિના ભેદમાં માનતો, પણ અસાઈત જ્ઞાતિ-જાતિના બંધનોથી પર હતા. એટલું વિચાર્યું કે, મિત્રની પુત્રી બચાવતાં બ્રાહ્મણત્વ ભ્રષ્ટ નહીં થાય. એમ ગંગા સાથે એક ભાણે ભોજન કરી તેના શિયળની રક્ષા કરે છે. આ વાત એ સમયે ઊંઝા-સિદ્ધપુરના બ્રાહ્મણોમાં ફેલાય છે. બ્રાહ્મણસમાજ અસાઈતને જ્ઞાતિ બહાર મૂકી, રોટી-બેટીનો વહેવાર બંધ કરી એકલો પાડી દેવામાં આવે છે. આથી તેણે પોતાનો રોષ અલ્લાઉદ્દીનના સરદાર સામે કે, રૂઢિચુસ્ત બ્રાહ્મણસમાજ સામે ઠાલવવાને બદલે તેમણે પોતાની શક્તિને સર્જનાત્મક તરફ વાળી ને એમાંથી લોકનાટ્ય ભવાઈનો જન્મ થયો. અસાઈત પોતાનું વતન સિદ્ધપુર છોડી, તેના ત્રણ પુત્રો નારણ, માંડણ અને જયરાજ સાથે ઊંઝા આવીને વસ્યા. તેમના શિષ્ય હેમાળા

પટેલે ત્રણેય પુત્રોને ઘર બંધાવી આપ્યા. જમીન-જાગીર આપી. સાથે-સાથે તામ્રપત્ર પર કડવા પાટીદાર જ્ઞાતિએ વંશપરંપરાગત અમુક હકો લખી આપ્યા. આથી ઊંઝામાં સ્થાયી થઈ તેમણે ભવાઈના ૩૬૦ વેશોની રચના કરીને ત્રણે પુત્રોની સહાયથી તે ભજવવાના શરૂ કર્યો. અસાઈત સંગીત અને શાસ્ત્રના જાણકાર હતા એટલે ભવાઈના સ્વરૂપને શક્તિપૂજા રૂપે વિકસાવ્યું. કહેવાય છે કે તેમને ત્રણ દીકરા નારણ, માંડાણ અને જયરાજ નામે હોવાથી 'ત્રણ ઘરવાળા' કહેવાયા. આ ત્રણ ઘર પરથી 'ત્રણ ઘરાળા' ને 'તરગાળા' જ્ઞાતિ ઊપજી. આમ ભવાઈનો વેશ કરનારને 'ભવૈયા' કે 'ભવાયા' તરીકે ઓળખીએ છીએ. આ પ્રકારે ભવાઈનો માર્ગ અપનાવી પોતાની શક્તિને સર્જનાત્મક રૂપ અર્પી, માતાજીની પૂજા-અર્ચના, સમાજસુધારણા તરફ વાળીને ભવાઈના વેશોની રચના કરી. પ્રત્યેક દિવસનો એક વેશ એમ પૂરા એક વર્ષના વેશ રચ્યા અને ભજવ્યા. એમાંના ઘણા વેશો અત્યારે અપ્રાપ્ય છે. એ વેશો દ્વારા સમાજમાં મનોરંજન સાથે લોકશિક્ષણનું કામ ભવાઈ દ્વારા વિવિધ રીતે વર્તમાન સમયમાં પણ ઉપયોગી સાબિત થયું છે.

ભવાઈની શરૂઆત આમ તો માતાજીની આરાધના માટે થઈ, પણ સમય જતાં લોકનાટ્ય ભવાઈ લોકશિક્ષણ અને મનોરંજનનું માધ્યમ બની. લોકો સાંજના સમયે ખેતીકામ, અન્ય કામથી પરવારી નવરા પડે ત્યારે રાત્રીના પહેલા પ્રહરમાં ભવાઈ શરૂ થાય. એ વેશોમાં સમાજસુધારણા, ભક્તિ, જ્ઞાન, મનોરંજનના વિષયો પીરસવામાં આવતા. રજની વ્યાસ આ બાબતે નોંધે છે કે- "ભવાઈનું લક્ષ્ય સામાન્યતઃ લોકશિક્ષણ સાથે મનોરંજનનું. પણ તેનો વિકાસ બે દિશામાં થયો. એક બાજુ માતાના મંદિરના પ્રાંગણમાં ચાચર ચોકમાં રમાતી ભવાઈ તરીકે, માતાની ભક્તિ તરીકે તેનો વિકાસ થયો. બીજી બાજુ પ્રાકૃત મનોરંજનનો ઉદ્દેશ વધુ પડતું પ્રાધાન્ય ભોગવતાં તેમાં પ્રાકૃતતા હદ સુધી વકરી"^૭ મધ્યકાળમાં મોટા ભાગની પ્રજા અભણ હતી. શિક્ષણનું પ્રમાણ નહિવત હતું, એટલે એ સમયે શિક્ષણ માટેનું કોઈ લોકમાધ્યમ બન્યું હોય તો તે 'ભવાઈ' છે. ગમ્મત સાથે જ્ઞાન પીરસી લોકોની અંધશ્રદ્ધા, વહેમો દૂર કરવાનું કામ કર્યું છે. ભગવદ્ગોમંડલમાં નોંધેલ છે કે - "જનસમાજ ઉપર તો ખાસ કાર્ય કર્યું છે. અસંસ્કૃત લોકોનાં મન ઉપર સચોટ અસર પહોંચાડનારું એ સાધન હતું. જોકે ભવાયા અભણ હતા અને તેમની યાચકવૃત્તિએ તેમનામાં કોઈ ઉચ્ચ આદર્શ રહેવા દીધો ન હતો, તોપણ સ્નેહનાં, શૌર્યનાં અને દુરાચારનાં ચિત્રો બહુ જ અસરકારક રીતે તેઓ લોકો આગળ રજૂ કરતા. જ્યાં સુધારણાના કોઈ પણ સંસ્કાર પહોંચી શકે તેવું નહોતું, ત્યાં તેઓ મેલાઘેલા પણ કંઈક આદર્શ રજૂ કરતા."^૮ ભવાઈનું મુખ્ય પ્રયોજન તો આનંદપ્રમોદનું પણ લોકશિક્ષણ દ્વારા સમાજની કેટલીયે સમસ્યાઓ સામે ભવાઈ

મેદાનમાં ઊતરી ને એ કામ ભવાઈ કલાકારોએ પાર પાડ્યું છે. અને એ સમયના સામાન્ય જનનો પણ આર્થિક અને અન્ય રીતે સહકાર મળ્યો છે. આ લોકકલાના કલાકારોની સામાન્ય જન ઉપર ખૂબ જ ઘેરી અસર હતી. એટલે ગામમાં જ્યારે ભવાઈ થવાની હોય ત્યારે ગામલોકો ભેગા થઈ, તેમનું સામૈયું કરતા અને તેમની દરેક પ્રકારની વ્યવસ્થા કરવામાં આવતી આ અંગે ડૉ. કૃષ્ણકાંત કડકિયા નોંધે છે કે- "મધ્યયુગીન પશ્ચિમ ભારતમાં વિશેષ કર ગુજરાત સૌરાષ્ટ્ર મેં ભવાઈ કા નાટ્યપ્રકાર જનશિક્ષણકા પ્રભાવશાલી માધ્યમ રહા હૈ । અસાઈત ને સામાજિક પરિવેશ કા સૂક્ષ્મ અધ્યયન કરકે ભવાઈ કે વેશો કી રચના કી । ઇન વેશો કી અભિવ્યક્તિ સે સમાજ કા ન કેવલ મનોરંજન હુઆ બલ્કિ દીર્ઘકાલ સે સમાજ મેં પ્રચલિત રૂઢિયો, બન્ધનો, મહમો ઓર કુરિવાજોં પર કરારા વ્યંગ ભી કિયા ગયા જો સમાજસુધાર મેં સહાયક સિદ્ધ હુઆ ।"

લોકનાટ્ય ભવાઈએ જનશિક્ષાની સાથે સમાજનું અધ્યયન કરી સમાજની બદીઓ દૂર કરવાનો પ્રયત્ન કર્યો, માત્ર મનોરંજન પૂરું નથી પાડ્યું. આ ઉપરથી એવું લાગે છે કે અસાઈતે સમાજનું ખૂબ જ અધ્યયન કર્યું હશે. એટલે ભવાઈ હરતીફરતી શાળાનું માધ્યમ બની છે. પ્રા.જનક દવેના શબ્દોમાં જોઈએ તો "ભવાઈના વેશો બેશક મનોરંજનનું સાધન હતું અને છે, પરંતુ એ દ્વારા સમાજ શિક્ષણ પણ અપાયું જ હતું. એ દષ્ટિએ ભવાઈ રમનારી મંડળીઓને આપણે હરતાં-ફરતાં વિદ્યાલયો ગણવાં જોઈએ." એ સમયમાં વિદ્યાલયોનું, મીડિયાનું કામ 'ભવાઈ'એ કર્યું છે. અને અસાઈતનો હેતુ પણ સમાજસુધારણાનો હતો એ ભવાઈ વેશો પરથી સ્પષ્ટ રીતે જોઈ શકાય છે. એક સમય એવો હતો કે બધા જ સમાજ ઉપર કહેવાતા ઉચ્ચ વર્ગની પક્કડ હતી. ઊંચ-નીચના ભેદભાવ, કજોડાં લગ્ન વગેરે સમસ્યાઓ હતી. આવા સમયમાં સમાજની નબળાઈઓ સમાજ સમક્ષ મૂકી સમાજસુધારણાનું કાર્ય કર્યું છે. તો ભવાઈ ઘર - ઘર સુધી ટેલિવિઝનના માધ્યમથી પહોંચી છે. એના વેશના વિષયો માટે પણ કોઈ મર્યાદા નથી. વર્તમાન સમયને સ્પર્શતા વિષયો ઉપર કેટલાય વેશો રચાયા અને ભજવાયા, પણ એની યાદી બનાવીએ તો ખૂબ લાંબી થાય. અને નવીન વેશો પણ અનેક વિષયો પર લખાઈ શકે છે. સરકાર પણ પોતાની યોજના માટે કે વિવિધ અભિયાન માટે પણ ભવાઈનો પ્રયોગ કરે છે. જેથી લોકો સુધી સાચો વિષય પહોંચે છે. પણ સાચા અર્થમાં એ જાહેરાતોનું માધ્યમ નથી, સાહિત્યિક માધ્યમ છે. તેના વિષયો વર્તમાન સમયમાં પણ યોગ્ય જ છે. પણ સારા અદાકારોની ખોટ વર્તાઈ રહી છે. બાકી અત્યારે પણ કાન-ગોપી વેશો ઠેર-ઠેર ભજવાય છે. રામપીરનો વેશ પણ ખૂબ

ભજવાઈ રહ્યો છે. અરવલ્લી જિલ્લામાં આવી કેટલીયે મંડળીઓ આ વેશો માટે બનેલી છે. એના કારણે યુવાનો દારૂ, જુગાર કે અન્ય ખરાબ આદતોથી પર થયા અને સમાજમાં શ્રદ્ધાનું વાતાવરણ ઊભું થયું છે. તો સરડોઈના મોતીભાઈ નાયકે ભવાઈને માધ્યમ બનાવી 'કન્યાકેળવણી' કે 'બેટી વધાવો' વગેરેમાં ભવાઈના માધ્યમથી ખૂબ સારાં પરિણામો મેળવ્યાં છે. હજુ કેટલાય કલાકારોની અદાકારી જોઈને આજના ભણેલા-ગણેલા ભાન ભૂલી જાય છે. એટલે આ ભવાઈ સાવ લુપ્ત થઈ એવું નથી, સનેડો ગીત પણ ભવાઈની ઊપજ છે. એ ગીત ઉપર ભણેલા નાચે છે. આ લોકશિક્ષણ અને મનોરંજનનો પ્રભાવ છે. વર્તમાન સમયમાં પણ લોકશિક્ષણ માટે ભવાઈ માધ્યમ બની રહી છે. હજુ પણ પાટીદાર સમાજમાં દીકરાનાં હાલરડાં ગવાય છે. આ સંસ્કારની પ્રક્રિયા છે. હજુ પણ ઊંઝા, અંબાજી, બહુચરાજી, ખેડબ્રહ્મા, સરડોઈ, ધનસુરા, પાટણ, આવાં સ્થાનો પર ભવાઈ ભજવાય છે. ભવાઈ વિષય ઉપર બહુશ્રુત વિદ્વાનોએ સંશોધન કાર્ય કરીને ભવાઈને પોષણ આપ્યું છે. હાલ ઊંઝામાં 'અસાઈત સાહિત્ય સભા' દ્વારા એને બચાવવાના પ્રયત્નો થઈ રહ્યા છે. હાલ ભવાઈને કેન્દ્રમાં રાખીને સંશોધનના વિષયો બનાવવાની જરૂર છે. તો સાચું લોકશિક્ષણ થઈ શકશે. એટલે આટલા લાંબા સમય સુધી સમાજને મનોરંજન અને શિક્ષણ આપ્યું છે, તો એના માટે આપણે ચિંતા અને ચિંતન કરવાની જરૂર છે.

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M.K. GANDHI'S CRITIQUE OF MODERNITY: HIS RELEVANCE DURING THE CRISIS OF COVID-19 PANDEMIC

**Dr. Jagannatham B
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Abstract

The paper is an attempt to understand M.K. Gandhi and his philosophy, particularly his critique of modernity, western civilisation. It further comprehends the spread of COVID pandemic and examines the indigenous and traditional medicines to cure the COVID patients. In addition, the paper is an attempt to focus on the village swaraj, decentralization, Naturopathy and Human Psychology advocated by Gandhi. It touches upon the preventive measures of the governments both Union and states. Lastly, the paper comes up with concluding remarks.

Key words:

Gandhian philosophy, Modernity, decentralization, Corona virus, ingenious preventive measures

Introduction

India witnesses unprecedented problems such as unemployment, poverty, black money, and a corrupt political system. In addition, the country faces varied problems due to the spread of the deadly Coronavirus pandemic which has exposed the current neoliberal model of development, lifestyle, and human greed. This global pandemic has made an effect on day-to-day lifestyle health as well as human psychology. The current pandemic has affected many people in the world in different ways. It has forced to rethink the daily lifestyle, basically the things which were adopted from western culture and modernity. The pandemic situation has further exposed the drawbacks of western culture and it has made people to think about the vitality nature and ethical values and own indigenous lifestyle. Moreover, the degradation of moral values, continuous violence and socio-economic inequalities became a regular phenomenon.

Indian society for centuries carried rich cultural values and a nature-centric atmosphere. Since the majority of the population in India live in rural areas, they were exploited during colonial rule. Despite all the efforts to save and protect villages, many villages in India still lack basic amenities. In view of this, the paper intends to understand the Gandhian philosophy which seeks decentralization of power and promotion of village-level development.

Map as Critical Pedagogy

DHANANJAY RAI

Eklavya's extraordinary nature-society series construes geography as an important signpost of inquiry. The complexity of the environment and well-being of human and other life forms can substantially be churned out, along with understanding it beyond territorial gratification in the form of unravelling the layers of processes and its offshoots like the place, people, questions and alternative. Using geography for people per se necessitates rearranging the engagement with the map as critical pedagogy. This refutes its reductionist use for homogenisation, territorialisation, and empiricisation of beings. Yemuna Sunny, the author of *Maharashtra*, under the nature-society series, metamorphoses the map substantially into critical pedagogy.

Critical Consciousness and Alternative

Construing and presenting a map as critical pedagogy requires a substantive methodological shift—to relook, read and realise about critical consciousness (“what it is” followed by “why is it”) and critical alternative (what ought to be done). Critical pedagogy combines critical consciousness with the critical alternative. In the first aspect (critical consciousness), critical pedagogy is “enacted through the use of generative themes to read the word and the world and the process of problem posing generative themes involves the educational use of issues that are central to students’ lives as a grounding for the curriculum” (Kincheloe 2008: 10). Critical pedagogy requires more than critical thinking in terms of direct engagement cum acquaintance with people. “Distinct from critical thinking (a term that has been hijacked by many anti-critical teachers and textbook publishing companies), critical pedagogy calls for an active engagement with oppressed and exploited groups” (Duncan-Andrade and Morrell 2008 cited in Kirylo et al 2010: 332).

Maharashtra (Nature Society Series) by Yemuna Sunny, Bhopal: Eklavya, 2020; ₹80.

Therefore, it is not “... formulaic, it isn’t stagnant, and it isn’t an is” (Steinberg 2007: ix). Herein, critical ontology (new self) is going to play an indomitable role. “Critical pedagogy’s notion of a new self (a critical ontology) and new modes of exploring the world are grounded on the human ability to use new social contexts and experiences to reformulate both subjectivity and knowledge” (Kincheloe 2008: 147).

In the second aspect (critical alternative), critical pedagogy proposes “... a social and educational vision of justice and equality ... [by way of] ... alleviating human suffering” (Kincheloe 2008: 10). It becomes “a critical agent” (Giroux 2007: 1) to challenge socio-economic structures in which schools do operate (Kirylo et al 2010: 332) for social transformation (McLaren 2007: 310). It promotes social justice and democracy by way of using education as “counter-socialisation” (Stanley 2007: 371). The foremost task of critical pedagogy “... is to clarify the legitimacy of the ethical-political dream of overcoming unjust reality” (Freire 2016: 19). Critical pedagogy becomes assiduously crucial for combining critical consciousness with critical alternative.

In this book series, the map meant for children brings forth substantial critical consciousness. The map is neither a cryptic code of administrative parlance nor a homogenising tool. The state of Maharashtra is more than its capital and second capital. Twenty-one languages (Marathi, Malwani, Konkani, Hindi, Gujarati, English, Jhadiboli, Vahadi, Ahirani, Gondi, Mavchi, Nimari, Dhanki, Rathi, Warli, Kolami, Maria, Naiki, Korku, Kurmi and Koli), presence of multiple religions (Hinduism, Islam, Buddhism, Jainism, Christianity, Sikhism, Adivasi religion and not specified religions), multi-culinary food habits

(fish, rice, wheat, jowar, bajra, and ragi) and availability of a heterogeneous range of vegetables (lentils, poultry, meat, coconut and groundnut oil) make reality as multilayered exploration and acquaintance. Geographical heterogeneity is explained with three crucial components—providing a critical alternative to any homogenised world view—nature-society relations, people of Maharashtra, and the environment.

Synthetic Convergence

The section on nature-society relations explains the crop and region. It not only succinctly highlights the intricate relationship between geography and history but also the symmetry/asymmetry between cultivations and regions. Seafront, sustenance, trade, wars, and forts are intertwined. To generate curiosity, the question being asked is apt: “Can you find out from other sources why forts were built, and what were their uses?”

The question of water availability comes uniquely. In Maharashtra, 64% of people do cultivation and related activities. The pattern of crop and regions is as follows: cotton and orange: Konkan; banana: Khandesh; rice and coconut: Marathwada; grapes: Vidarbha; ragi: Submountain; jowar and bajra: Sahyadri. The description of sugar cane is telling and forces us to rethink our priorities. Maharashtra has only 18% cultivable land and sugar cane production consumes 71% of the irrigated water. Solapur district of Maharashtra contributes significantly to sugar production. These descriptions are crucial for the reader to further explore the twin enigmatic developments in Solapur, which is the leading producer of sugar on the one hand and worst sufferer of drought on the other. This is sufficient to draw attention to the consumption of water for a particular crop and democratisation of water in a district which is drought-prone. It also highlights the trajectory of BT cotton. Though BT cotton remains immune from several insects, it is not completely free from all insects. This requires the use of additional pesticides along with the higher cost of BT cotton seeds. This information becomes significant in the context of farmer suicides.

People are beyond enumeration. They are not a fact but life.

Not Merely Enumeration

The book underlines that the diversity of religions, languages, and food habits are crucial aspects of Maharashtra. Life suffers from inequality in access to resources. The presence of 47 tribal communities and non-tribal societies do provide heterogeneity. Non-tribal society by way of caste does deprive people of development and landownership. This gives rise to traditions that seek socio-economic justice. This gives rise to traditions that seek socio-economic justice. This aspect is crucial for imagining the presence of a critical alternative.

Warkari tradition and Dalit movement are examples mentioned. The pre-14th century Warkari tradition by Dnyaneshwar, Eknath, Tukaram, and others emphasises “equality, compassion, love, non-violence, humility, and peaceful living.” These developments were crucial for anti-caste and anti-gender discrimination movements. Pandharpur is not merely a point on the map but encompasses a history of struggle. Maharashtra is also known for the Dalit movement and pioneering contribution of Jotirao Phule, Savitribai Phule, and B R Ambedkar towards an egalitarian world. The map of Maharashtra encompasses such a rich tradition of transformation. The author asks aptly in this backdrop: “In your life experiences, do you see caste, poverty, and gender-based inequalities decreasing? Do you think we still have much to do for making a just society?”

Raising curiosity in the minds of young readers without pre-empting a response, forces them to not only know but also constantly interrogate our socio-economic engagement and overt and discreet discriminations—ever-present, justified, and normalised. Therefore, the complacency towards discrimination is broken through goading inquiry and inquisitiveness.

Moreover, the author draws attention towards patterns of development in farming and industrial productions relating to problems for the unemployment and livelihood requirement of the people. It is important to understand the correlation

among expensive seeds production by multinational companies along with fertilisers and pesticides production by companies, expensive farming, and loans for agricultural purposes. Due to the interplay of these myriad factors, the cotton farmers faced the highest number of suicides.

Moreover, the disappearance of textiles due to the shifting of land use for other commercial activities and resistance of textile owners towards higher wages caused unemployment among people, and affected their children’s school education.

Diversity and Democratisation

This book also informs us about the environment as both a repository of the diversity of all life forms and as a site for the democratisation of resources (in this case water availability). It highlights the diversity of medicinal plants, trees, plants, animals, birds and other living beings in the forest. The Sahyadri region of Maharashtra has UNESCO-recognised world heritage sites (Koyna, Radhanagari, Bhimashankar wildlife sanctuary, Kassi plateau, and Chandoli National Park). These sites are pointed on maps as a symbolic representation of the protection of the diversity of all forms of life.¹

The author invites the attention of students towards excessive car availability, pollution, waterlogging, flooding of houses and interruption of railways during monsoon in Mumbai. Water consumption patterns offer an insight into the process of democratisation of resources. Industrial consumption of water is voluminous. Thermal plants are placed on the map while suggesting that they consume a large amount of water. Moreover, industrial waste is released into rivers which contaminates the water. Excessive use of water in sugar cane cultivation drops the groundwater level. Together, these insights are represented on the map, enabling the readers to conclude for themselves, the factors that attribute to acute water scarcity in an area.

The author uses Warli painting brilliantly to highlight and provide meaning from the intricate relation between tribes and art. Peculiar shapes, inhabitants, nature, festivities, animals, farming and

trees are the best representatives of the richer profundity of environment and ecology.

A Quintessential Read

The pithy book is a required methodological innovation. It lays out the backdrop for further exploration. It does not do away with the explanation part but asks students to develop it further on their own. The map is exploratory in nature while facilitating an understanding of nature, biodiversity, humans, and allied activities. The book understands the consequences for human beings vis-à-vis the presence of diversity of all forms of life. The map becomes a signpost of critical pedagogy concerning critical consciousness and a critical alternative. This book bridges the gap between essentialist and people-centric understanding of geography/map.

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NOTE

- 1 Some of the concerns around “Sahyadri-Radhanagari” can be seen in Government of India report on *Status of Tigers, Copredators and Prey in India* (Jhala et al 2020: 82).

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Micro-morphological and anatomical response of groundnut (*Arachis hypogaea* L.) cultivars to ground-level ozone

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ABSTRACT

Tropospheric ozone is a phytotoxic gaseous pollutant with global warming potential that disrupts the plants growth and development directly or through climate change. Ozone enters into a plant's body through stomatal pores and develops oxidative stress, which results in injury to foliage and modifies leaf micro-morphology and anatomy. A field study was conducted to assess the morphological, micro-morphological, and anatomical response of groundnut cultivars (*Arachis hypogaea* L.) to enhance the level of ozone. This study observed ozone-like visible injury symptoms on all groundnut cultivars. Visible injury was maximum in cultivar Dh-86 and minimum in cultivar TPG-41. Micro-morphological characteristics, such as increase in stomata, epidermal cells number, and its index, were also increased under enhanced ozone-exposed plants. The highest stomatal index was found in cultivar TPG-41 and lowest were noted in cultivar GG-20. Cultivars TAG-24 > TG-37A > and Dh-86 show moderate modification in the morphological and micro-morphological characteristics of plants. Elevated ozone also affected the stomatal movement and leaf internal tissue. Most of the stomata of all the groundnut cultivars were observed as closed during the enhanced ozone exposure, suggesting a protective mechanism from ozone stress. The study concluded that the micro-morphological and anatomical characteristics are important aspects to determine the effect of ozone on plants and to influence plants sensitivity to ozone. On the basis of these characteristics, cultivar TPG-41 was found to be less sensitivity, while cultivar Dh-86 was found to be highly sensitive to ozone pollution.

1. INTRODUCTION

Rapid industrialization, growing cities, and increasing vehicular load have caused serious environmental pollution and have affected plant life [1–3]. Ozone is a secondary air pollutant in the tropospheric atmosphere, formed by the reaction of sunlight and originator gases, including SO_x, NO_x, and volatile organic carbon, generated through anthropogenic activities [4,5]. The rapid increase in ground-level ozone concentration has come to be a global concern due to its direct phytotoxic effect or indirect effect through global warming [6–8]. Researches from the past four to five decades acknowledge the detrimental effects of ozone on plants. A higher ozone concentration could constrain photosynthesis [9], reduce yields and biomass [10], and also

change the allocation of photosynthesis in plant organs. Ozone is also reported to cause foliar injury [11,12] and changes to stomatal conductance [13].

Ozone primarily enters the plant through stomatal pores [14] and generates oxidative stress [7,15]. To counteract this stress, the plant develops a series of defense response. However, most of the studies describing plants defense response to ozone are based on antioxidative efficiency and/or stomatal conductance. However, previously it was shown that balancing the density of stomata and cell division in developing leaves is directly connected with the plant's response to environmental stress [16–18]. The study of Navea et al. [19] reveals a specific defense mechanism in case of drought stress in which plants turn down the number of stomata and refer to it as 'stomata abortion'. Chaudhary and Rathore [3], Paakkönen et al. [20], and Aasamaa et al. [21] found decreased stomatal pores or aperture size due to the abiotic stress. Studies on plant response to air pollution, including ozone, have observed an increased stomatal density of plants [22,23]. Ferdinand et al.

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[23] observed an ozone-sensitive clone *Prunus serotina*, which has larger stomatal density and a lesser ratio of palisade per spongy parenchyma. The authors also asserts that resistance can be described by separate gas permeability, which is based on extending the structure of the leaf, as gas resistance of palisade tissue is larger than that of spongy tissue.

Alteration to stomata and abundance as a response to stress is also expressed as a major pollutant absorption controlling mechanism [24]. Stengleina et al. [25] stated that the density of stomata is basically affected by both the beginning of stomatal development and the build-up of epidermal cells (EC). The build-up of EC turns into a function of various environmental and developmental variables; the altitude of the experimental site can even influence stomatal index (SI) and its density. The redox state of plant cells gets unbalanced due to ozone, which leads to modifications in metabolic processes and gene expression, which affects cell growth and development. Cell division on the whole is sensible to the redox state of plant cells whereby oxidative stress hinders the cell cycle and hence proliferation [26]. Ozone sensitivity was dissimilar among plant species. However, a lot of unanswered questions come forward regarding the possible mainspring of ozone sensitivity or resistance. Furthermore, the effects of ozone on internal tissues of plants are also missing.

Therefore, the present experiment was conducted to assess the effects of ozone on plant development, micromorphology, and anatomy of groundnut (*Arachis hypogaea* L.) cultivars and their possible role in plant defense. This study hypothesized that the stomatal distribution and movement influence plant sensitivity to ozone.

2. MATERIALS AND METHODS

2.1. Site Description and Ozone Treatment

Five cultivars of groundnut (*Arachis hypogaea* L), viz. TG-37A, TPG-41, TAG-24, GG-20, and Dh-86, were selected for the present experiment. Crop and cultivars were selected on the basis of popularity among the formers in the region. The experiment was carried out in open top chambers (OTCs), sized 4 × 4 × 3.5

m, established at the research field of the Central University of Gujarat (23.2156°N, 72.6369°E) during February to May 2017. OTCs were made up of multi-layered clear polycarbonate sheet (3 mm thick) to provide maximum available sunlight. Plants were grown in plots sized 1 m² using a regular agronomical practice for groundnuts. The soil of the field was measured with the help of a pH meter and was found to be slightly alkaline (pH 7.4). The soil texture was determined based on sand, silt, and clay percentages and was found to be sandy loam of medium fertility. Each plot was mixed with 250 g vermicompost during field preparation and was applied with the recommended dose of NPK in the ratio of 40:40:20 kg/ha. The plots were regularly irrigated ensuring sufficient water supply and weeds were managed manually. A randomized block design was opted with two treatments, i.e., enhanced ozone (provided -75.91 ± 11.42 ppb of mean ozone concentration for 4 hours) and ambient ozone (ozone concentration varied between 13.6 and 40 ppb) (Fig. 1). Ambient ozone concentration and temperature were checked with the help of an ozone analyzer [Genesis (LEDM)] and temperature sensor (open top chamber with temperature sensor (HK Tempsensor), data logger (Ambetric, TC800D), ozone generator, Genesis Technologies, India) throughout the study period. Ozone fumigations were conducted from 11.00 am to 03.00 pm every day from seed germination till harvesting.

2.2. Plant Morphology and Ozone Visible Injury

The morphological characteristics of plants are illustrated by the image and number of roots, leaves and branches that were counts at 20, 40, and 60 days after sowing (DAS). Ozone visible injury was also identified at 20, 40, and 60 DAS of plants. Various parameters were measured for visible injury, such as the number of injured plants per plot, the number of injured leaves per plant and the number of chlorosis and necrosis spots per plant.

2.3. Micro-Morphological and Anatomical Characteristics

2.3.1. Microscopic observation

Micro-morphological characteristics of the leaf were measured using a digital microscope (Milton Instruments, Mumbai,

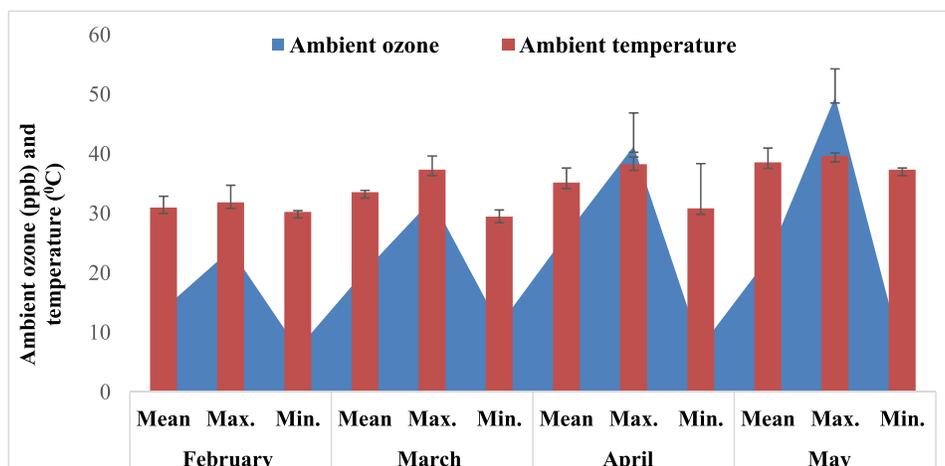


Figure 1: Monthly average ambient ozone (ppb) and temperature (°C) during the study periods of groundnut cultivars. (Mean ± standard deviation of three replicates presented by thin vertical bars.)

Maharashtra) and scanning electron microscopy (SEM) (Model: EVO 18, Make: Carl ZEISS). The sample for micro-morphological studies was collected and shifted to the laboratory in desiccators to avoid the effect of humidity. For stomatal study through a compound microscope, leaf abaxial surfaces were peeled off using dissecting needles and forceps and were finally washed with clean water. After that, each specimen was stained with safranin (1% aqueous) for 3–10 minutes and excess stains were washed using deionized water and then the stained cuticle was mounted on glycerine jelly and detected under a microscope. The number of stomata and EC and its index was calculated as per the equation of Salisbury [27], i.e., $(SI = S \times 100/S + E)$ using a compound microscope.

where SI = stomatal index, S = No. of stomata/unit leaf area, and E = number of EC/unit leaf area.

2.3.2. SEM analysis

For SEM [Model: EVO 18, Make: Carl ZEISS] analysis, the specimen of the collected leaf sample was cut in 2–4 mm pieces and fixed (Primary fixation) to 2.5% Glutaraldehyde/Karnovsky's fixative for 6 hours at 4°C. After that, the samples were washed in 0.1 M phosphate buffer, for three changes each of 15 minutes at 4°C. After primary fixation, the same sample was ready for post-fixation.

For post-fixation, 1% osmium tetroxide was used for 2 hours at 4°C.

With regard to washing and dehydration, the sample was washed in 0.1 M phosphate buffer for three changes each of 15 minutes at 4°C to remove the unreacted fixative. After that, the specimen was dehydrated using increasing concentrations of acetone to remove water by the following procedure:

For dehydration, increasing concentrations of acetone, such as 30%, 50%, 70%, and 90%, were used for 30 minutes of each step, and finally 100% (dry acetone) was used.

After that, the specimen was dried by air and critical point drying (critical point, i.e. 31.5°C at 1,100 p.s.i.) and kept in desiccators.

With regard to specimen mounting and coating, the specimen was mounted on aluminum stubs with a carbon tape. The sample was coated using a sputter coater to make the sample conductive.

2.4. Leaf Anatomy

The anatomy of the selected groundnut leaf sample was analyzed by a compound microscope. A fresh leaf of groundnut sample was taken for the observation of anatomical characteristics. Fine transverse sections of the leaf were taken and stained with safranin and mounted on glycerine. The specimens were covered with a cover slip after mounting to be observed in a compound microscope.

2.5. Statistical Analysis

Data were analyzed with three replicates (mean \pm standard deviation). MS Excel 2010 was used for standard deviation. Significant correlation and variance of treatment, cultivars, and parameters were calculated with the help of regression. All data, such as morphology, visible injury, and micromorphology, were correlated with injured plants per plot of selected cultivars. Data were also analyzed through three-way analysis of variance (ANOVA) test using Statistical Package for the Social Sciences (SPSS) (SPSS Inc., version 17.0) for assessing the significance of quantitative changes in different parameters of groundnuts' response to ozone treatments at different sampling intervals.

3. RESULTS

3.1. Visible Injury

Foliar injuries on the plants are the first visible symptoms of the ozone pollution. Intervascular chlorosis and necrotic stippling were observed on the adaxial surface of the leaves of enhanced ozone-treated groundnut plants (Table 1 and Fig. 2). Chlorotic spots

Table 1: Ozone-like injury such as total number of plants injured, number of leaves injured, chlorosis per plant, and necrosis of groundnut cultivars.

Cultivars	DAS	Total no. of plant plots ⁻¹	Total No. of plant injured plots ⁻¹	No. of leaf injured plant ⁻¹	Chlorosis plant ⁻¹	NECRO plant ⁻¹
TG-37A	20 DAS	50	2.33 \pm 0.45	7.66 \pm 0.67	5.66 \pm 0.87	2.33 \pm 0.33
	40 DAS	20	13.33 \pm 0.73	13.66 \pm 0.86	11.66 \pm 0.66	2.66 \pm 0.67
	60 DAS	15	7.66 \pm 1.67	18.33 \pm 1.33	15.33 \pm 0.78	3.00 \pm 1.33
TPG-41	20 DAS	50	2.33 \pm 1.33	5.33 \pm 0.93	3.66 \pm 0.12	2.00 \pm 1.20
	40 DAS	20	12.33 \pm 1.33	13.33 \pm 0.83	11.33 \pm 1.50	3.00 \pm 1.50
	60 DAS	15	6.66 \pm 0.67	15.66 \pm 1.67	12.33 \pm 2.12	3.33 \pm 0.93
TAG-24	20 DAS	50	1.33 \pm 1.33	7.33 \pm 0.63	4.00 \pm 1.1	2.00 \pm 1.10
	40 DAS	20	8.33 \pm 0.83	11.00 \pm 1.80	8.33 \pm 1.33	2.66 \pm 0.67
	60 DAS	15	8.00 \pm 0.1	14.33 \pm 0.33	10.00 \pm 1.50	4.00 \pm 1.10
GG-20	20 DAS	50	3.66 \pm 1.7	13.00 \pm 1.56	8.33 \pm 1.03	3.33 \pm 0.73
	40 DAS	20	15.33 \pm 0.63	18.33 \pm 1.33	16.00 \pm 1.02	3.66 \pm 0.55
	60 DAS	15	9.66 \pm 0.57	21.00 \pm 1.52	17.33 \pm 1.23	4.33 \pm 0.80
Dh-86	20 DAS	50	2.66 \pm 1.67	7.33 \pm 1.30	8.66 \pm 1.57	3.66 \pm 0.66
	40 DAS	20	16.66 \pm 1.87	19.00 \pm 1.11	16.33 \pm 1.33	4.33 \pm 0.50
	60 DAS	15	12.66 \pm 1.97	22.00 \pm 1.28	19.00 \pm 1.38	5.33 \pm 0.28



Figure 2: Ozone-like visible injury on selected groundnut cultivars. (A) ambient ozone, (B) chlorosis and necrotic spots, and (C) visible injury on TG-37A, TPG-41, TAG-24, GG-20, and Dh-86.

were found to be higher than the necrotic spots in all the selected cultivars. Injury was identified as ozone-like because it was either absent or very less in ambient ozone (data not mentioned). Ozone visible injury was higher in older leaves than in younger leaves. The number of injured leaves was increased with the duration of treatment and was found to be maximum at 60 DAS in all the cultivars tested. The maximum number of injured plants was noted in cultivar Dh-86 (12.66 m^{-2}) and the minimum injury was noted in cultivar TAG-24 (6.66 m^{-2}). The number of injured leaves was also higher in cultivar Dh-86 (22 plant^{-1}) at 60 DAS and the minimum number of injured leaf was found in cultivar TPG-41 (5.33 plant^{-1}) at 20 DAS. The trends of injury among the selected cultivars were GG-20 > Dh-86 > TG-37A > TPG-41 > TAG-24 under the acute level of ozone (Fig. 2) at all the sampling durations.

3.2. Plant Morphology

An acute level of ozone changed the morphology of tested groundnut cultivars. At the initial growth stage, the number of

leaves of all the cultivars remained similar for enhanced ozone and control plants; however, it was highly affected by an enhanced level of ozone at latter growth stages. Maximum variation in the total number of leaves per plant was noticed in cultivar Dh-86 (-40%) at 40 DAS, while minimum reduction was found in cultivar TPG-41 (-6.52%) at 60 DAS due to the enhanced level of ozone (Table 2).

Similar to the number of leaves, shoots and root branching of selected groundnut cultivars were also affected by enhanced ozone exposure (Table 2). The effect of ozone on branching of groundnut cultivars was higher during the early growth stage. Branching of the shoot was highly affected in cultivar TAG-24 (-66.66%) at 40 DAS and cultivar TPG-41 showed minimum reduction in the number of shoot branches (-16.75%) at 60 DAS. A higher reduction in root branching was found in cultivar TG-37A (-36.60%) at 60 DAS, while minimum root branching reduction was found in cultivars GG-20 (-2.30%) at 40 DAS.

Table 2: Number of roots, number of leaves, and number of branches of groundnut cultivars under enhanced ozone and ambient ozone.

Cultivars	Treatments	No. of root			
		20 DAS	40 DAS	60 DAS	
TG-37A	Enhanced ozone	4.00 ± 0.06	13.66 ± 0.67	15.00 ± 0.53	
	Ambient ozone	5.00 ± 0.05	16.00 ± 0.25	23.66 ± 0.66	
	No. of leaf				
	Enhanced ozone	4.00	8.00 ± 0.3	16 ± 0.57	
	Ambient ozone	4.00	10.00 ± 0.5	19.66 ± 0.67	
	No. of branch				
	Enhanced ozone	0.00	3.00 ± 0.36	3.33 ± 0.33	
	Ambient ozone	0.00	3.00 ± 0.54	4.66 ± 0.66	
	No. of root				
	Enhanced ozone	4.33 ± 0.33	10.33 ± 0.33	13.33 ± 0.33	
	Ambient ozone	5.00 ± 0.33	13.66 ± 0.67	16.33 ± 0.33	
	No. of leaf				
TPG-41	Enhanced ozone	4.00	7.00 ± 0.50	14.33 ± 0.33	
	Ambient ozone	4.00	11.00 ± 0.50	15.33 ± 0.33	
	No. of branch				
	Enhanced ozone	0.00	2.66 ± 0.67	3.33 ± 0.33	
	Ambient ozone	0.00	3.66 ± 0.67	4.00 ± 0.68	
	No. of root				
	Enhanced ozone	4.66 ± 0.66	10.33 ± 0.33	12.00 ± 0.65	
	Ambient ozone	5.66 ± 0.70	14.66 ± 0.66	16.00 ± 0.33	
	No. of leaf				
	TAG-24	Enhanced ozone	4.00	6.00 ± 0.30	10.00 ± 0.65
		Ambient ozone	4.00	8.00 ± 0.01	14.33 ± 0.33
		No. of branch			
Enhanced ozone		0.00	1.00 ± 0.28	1.66 ± 0.67	
Ambient ozone		0.00	3.00 ± 0.24	4.60 ± 0.67	
No. of root					
Enhanced ozone		5.33 ± 0.33	14.00 ± 0.35	15.00 ± 0.08	
Ambient ozone		6.33 ± 0.33	14.33 ± 0.33	15.66 ± 0.66	
No. of leaf					
GG-20		Enhanced ozone	4.00	7.00 ± 0.2	18.00 ± 0.25
		Ambient ozone	4.00	9.00 ± 0.25	22.00 ± 0.22
		No. of branch			
	Enhanced ozone	0.00	2.00 ± 0.25	2.33 ± 0.33	
	Ambient ozone	0.00	3.00 ± 0.34	3.66 ± 0.67	
	No. of root				
	Enhanced ozone	4.66 ± 0.7	12.00 ± 0.36	13.00 ± 0.54	
	Ambient ozone	6.00 ± 0.75	12.66 ± 0.67	14.00 ± 0.58	
	No. of leaf				
	Dh-86	Enhanced ozone	4.00	6.00 ± 0.6	13.00 ± 0.35
		Ambient ozone	4.00	10.00 ± 0.8	15.00 ± 0.22
		No. of branch			
Enhanced ozone		0.00	2.66 ± 0.67	3.00 ± 0.35	
Ambient ozone		0.00	3.66 ± 0.67	5.00 ± 0.57	
No. of root					

3.3. Micro-Morphological Characteristics

The result of the study found that the enhanced level of ozone modified the micro-morphological characteristics of groundnut

cultivars. Enhanced level of ozone increased the number of stomata and EC in all selected groundnut cultivars (Fig. 3). Among the tested crop cultivars, maximum increase in stomata was recorded in cultivar TPG-41 (62.5%) at 20 DAS, while a minimum increase in cultivar GG-20 (10%) at 40 DAS was noted. The trend of increase in stomata due to enhanced ozone was TPG-41 > TAG-24 > Dh-86 > TG-37A > GG-20. While the highest increase in EC was noted in cultivar TPG-41 (33.33%) at 60 DAS, the lowest number of EC count was noted in cultivar TG-37A (5.45%) at 20 DAS. The trends of increase were TPG-41 > GG-20 > TAG-24 > Dh-86 > TG-37A. Higher SI was also recorded among the plants grown under an enhanced level of ozone (Fig. 3). Maximum SI was found in cultivar TG-37A (27.06) at 60 DAS and minimum in cultivar Dh-86 (14.63) at 20 DAS. The trends of increase in SI under enhanced ozone exposure is TG-37A > TPG-41 > TAG-24 > Dh-86 > GG-20.

Enhanced ozone also interferes with the stomatal opening of plant leaves. The present study shows that exposure of groundnut plants to enhanced ozone deduced stomata openings and maximum stomatal pores were closed in enhanced ozone-exposed plants than ambient ozone (Figs. 4 and 5).

3.4. Anatomical Modification

Elevated ozone also influences the internal structure of the plant leaf (Fig. 6). An enhanced ozone-treated plant showed thin epidermis than ambient ozone-treated plants. Xylem and phloem of the foliage were also affected under enhanced ozone exposure. Vessel elements of xylem reduced in size, despite the increase in the number. In contrast to xylem, the phloem tissue was expanded but became significantly disorganized and collenchyma was reduced (Fig. 6). An enhanced level of ozone also affected the palisade cells and the damage can be seen in Figure 6B. Mesophyll cells were also reduced in size than ambient ozone plants. Xylem and phloem of elevated ozone-treated plants become compact with minimum distancing and size (Fig. 6C and D).

3.5. Regression and Three-Way ANOVA Test

Data were analyzed by regression and ANOVA (three factors) test for significant variation between treatments, age, and cultivars of selected parameters. Pearson's correlation analysis with R^2 values nearest to one shows a strong relationship with the number of injured plants per plot (Fig. 7). The number of stomata and EC shows a strong relationship when compared to other parameters, while the number of injured leaves per plant was highly correlated with chlorosis of leaves per plant in cultivar TG-37A. The highest R^2 (37%) value was found in the number of injured leaves per plant. In cultivar TPG-41, the highest R^2 (75%) value was noted in necrotic spots per plant. Chlorosis (CHLO) and necrotic showed a strong relationship with each other, while the number of injured leaves slightly correlated with these parameters. EC, chlorosis, and the number of stomata highly correlated in cultivar TAG-24 with R^2 (95%) values are same in all three parameters, while the number of injured leaves is slightly correlated (Fig. 7). For cultivar GG-20, the plant necrotic spots showed higher R^2 values (99%) and chlorosis of the plant was slightly correlated with R^2 value (73%). The higher R^2 value of cultivar Dh-86 was found in SI (92%) and

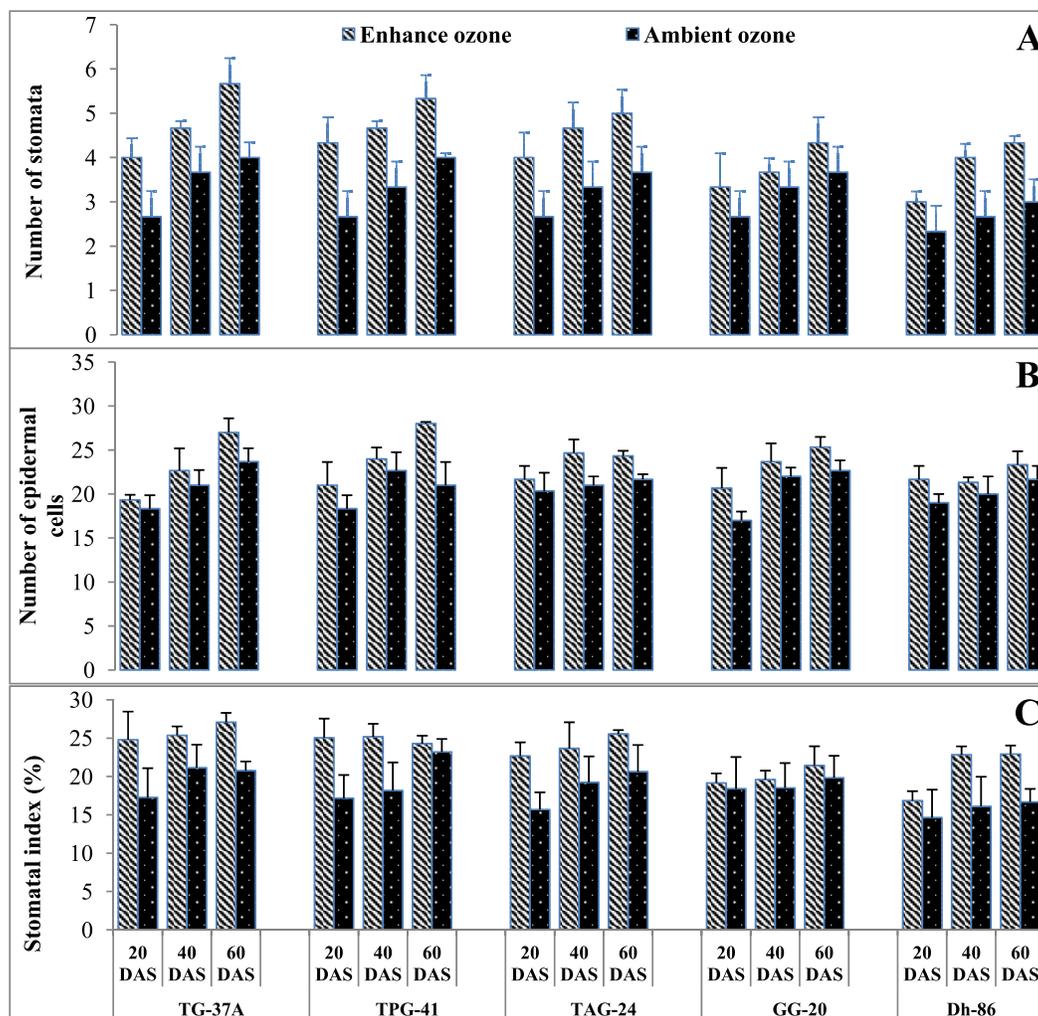


Figure 3: Microscopic observation. (A) Number of stomata, (B) number of EC, and (C) SI (%) of groundnut cultivars under enhance and ambient ozone-treated plants. (Mean \pm standard deviation of three replicates presented by thin vertical bars.)

the lowest R^2 value was for the number of EC (10%). The number of injured leaves, stomata, and chlorosis was slightly correlated with SI. Overall, sensitive cultivars showed a strong relationship with the number of injured plants per plot. In sensitive cultivars, the number of injured plants per plot was higher; therefore, the number of injured leaves per plant and chlorosis necrosis was also higher (Fig. 7).

Three-way ANOVA test (three-factor ANOVA) confirmed the significant levels of treatment, DAS, and cultivars (Table 3). Cultivars wise the number of stomata significantly varied at $p < 0.01$ level and the level of significance of SI was $p < 0.05$, while the number of EC showed no significant levels. Selected parameters, such as the number of stomata, EC, and SI of groundnut, were highly significant with treatments ($p < 0.001$). The level of significance of the number of stomata and EC showed the same values ($p < 0.01$), while the significant level of SI was $p < 0.05$. Selected parameters' relationship with Cult.*Treat. Cult.*DAS, Treat.* DAS, and Cult.*Treat.*DAS showed no significant level, except the number of EC; the significant level of the number of EC was $p < 0.05$.

4. DISCUSSION

The present study shows that the enhanced level of ozone caused a negative effect on groundnut cultivars. The micro-morphological and anatomical modifications observed in selected cultivars show higher variability in the injuries of plants. On the basis of morphological, micro-morphological, and anatomical modification, ozone-sensitive cultivars were highly affected than ozone-tolerant cultivars. Besides having global warming potential, ozone is one of the major gaseous pollutants that directly affected plant growth and productivity [7,9,10,28]. However, its effect depends on the genotype and prevailing environmental condition [29,30].

Foliage is the primary plant organ that is exposed to ozone and shows visible symptoms. Foliar injury is generally the first visible sign of injury to plants from ozone exposure and indicates lessened physiological processes in the leaves [31]. However, these symptoms vary with the genotypes and are restricted to sensitive species and the plant site exposed to the ozone [32]. All the groundnut cultivars tested in the present experiment showed injury under enhanced ozone exposure. However, the total number

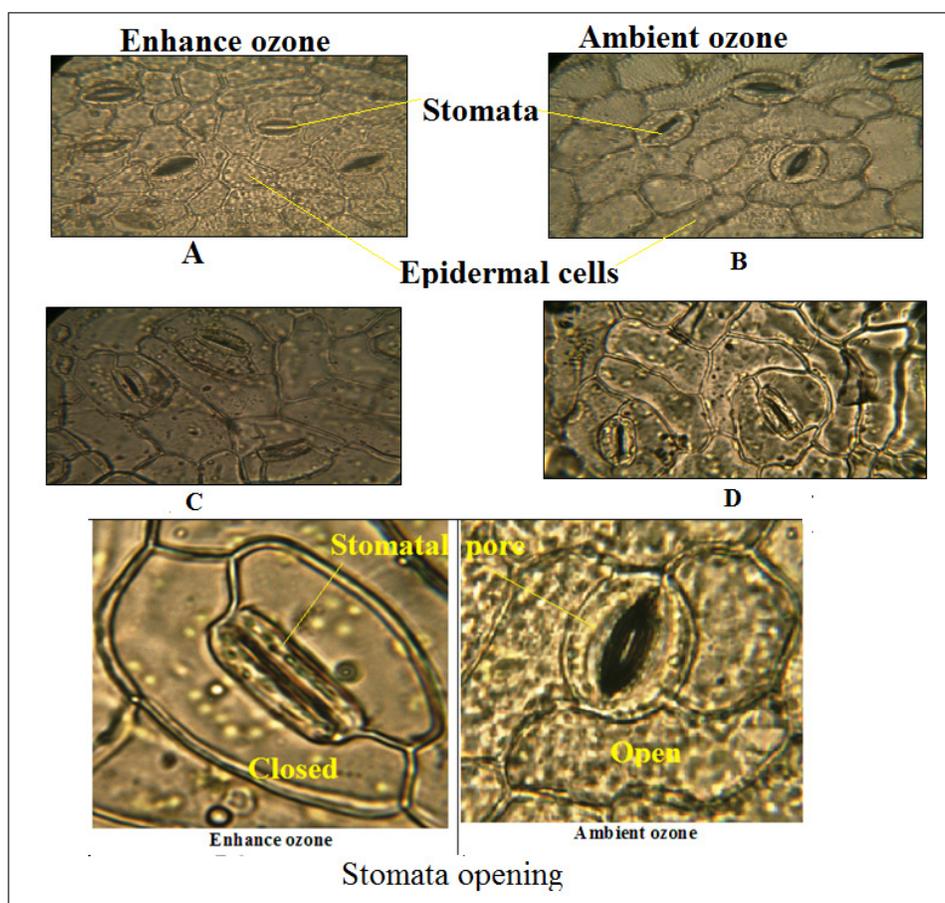


Figure 4: Microscopic observation of the number of stomata, number of EC, and stomatal opening of groundnut cultivar TPG-41. (A) Enhanced ozone, (B) ambient ozone and cultivar Dh-86, (C) enhanced ozone, and (D) ambient ozone.

of plant affected and the number of leaves affected in each plant was varied. Leung et al. [28] explained that the sensitive species had a higher injury than resistant species. A higher number of chlorosis and necrotic spots were found in cultivar Dh-86, suggesting its higher sensitivity to ozone, while cultivar TPG-41 showed reduced sensitivity to ozone with the least number of chlorosis and necrotic spots. Similar to this study, Basahi et al. [33] and Islam et al. [34] also reported visible ozone injury under ambient ozone to olive and mung bean plants, respectively. Hayes et al. [35] reported increased ozone-induced leaf injury in *Phaseolus vulgaris* with increased ozone exposure. In the present experiment, we also found that the ozone-induced injury was higher in mature leaves. This may be due to the longer duration to ozone exposure to older leaves than younger ones.

The reduction in the number of leaves per plant is an indicator of an unhealthy and stressed condition. The reduction in leaves per plant reduced total carbon assimilation and NPP of the ecosystem. The reduction in leaves was seen in all the groundnut cultivars, despite their sensitivity to ozone. However, a higher sensitive cultivar Dh-86 had a higher reduction and tolerant cultivar TPG-41 had the least reduction in leaves. Consistent production of new leaves can be a reason for lesser leaf reduction in tolerant cultivars. Leung et al. [28] reported the production of new leaves in tolerant cultivar of *Phaseolus vulgaris* which reduces the percentage of ozone-

affected leaves. Furthermore, the least affected branching of tolerant species also maintained the number of leaves. Similar to our study, Cotrozzi et al. [36] also reported 25%–60% phylloptosis in *Quercus* cultivars under ozone exposure. The minimum reduction in the number of branches per plant in cultivar TPG-41 is also an indication of tolerance of this cultivar than other cultivars. Tsukahara et al. [37] reported ozone-induced reduction in rachis branches in two cultivars of rice and explained this as the effect of ozone on genes near RM3430 markers. However, a surprising reduction in branching of root under enhanced ozone cannot be explained as ozone effect as the effect of ozone on underground plant part is not direct [38].

Stomata development during cell differentiation is confirmed to be regulated by genes that are simultaneously regulating physiological parameters, such as stomatal conductance. The present study found an increase in stomata as well as SI under enhanced ozone exposure in all the groundnut cultivars, suggesting a reduced size of EC under ozone pollution. Islam et al. [34] reported that the ozone pollution interferes with stomatal functioning causing increases in conductance, sluggish stomatal response to environmental factors, or stomatal closure, depending on the species and ozone exposure. Wang et al. [39] and Taiz and Zeiger [26] suggested that between sensitive leaves, young mature ones that developed after the plant had been exposed to significant

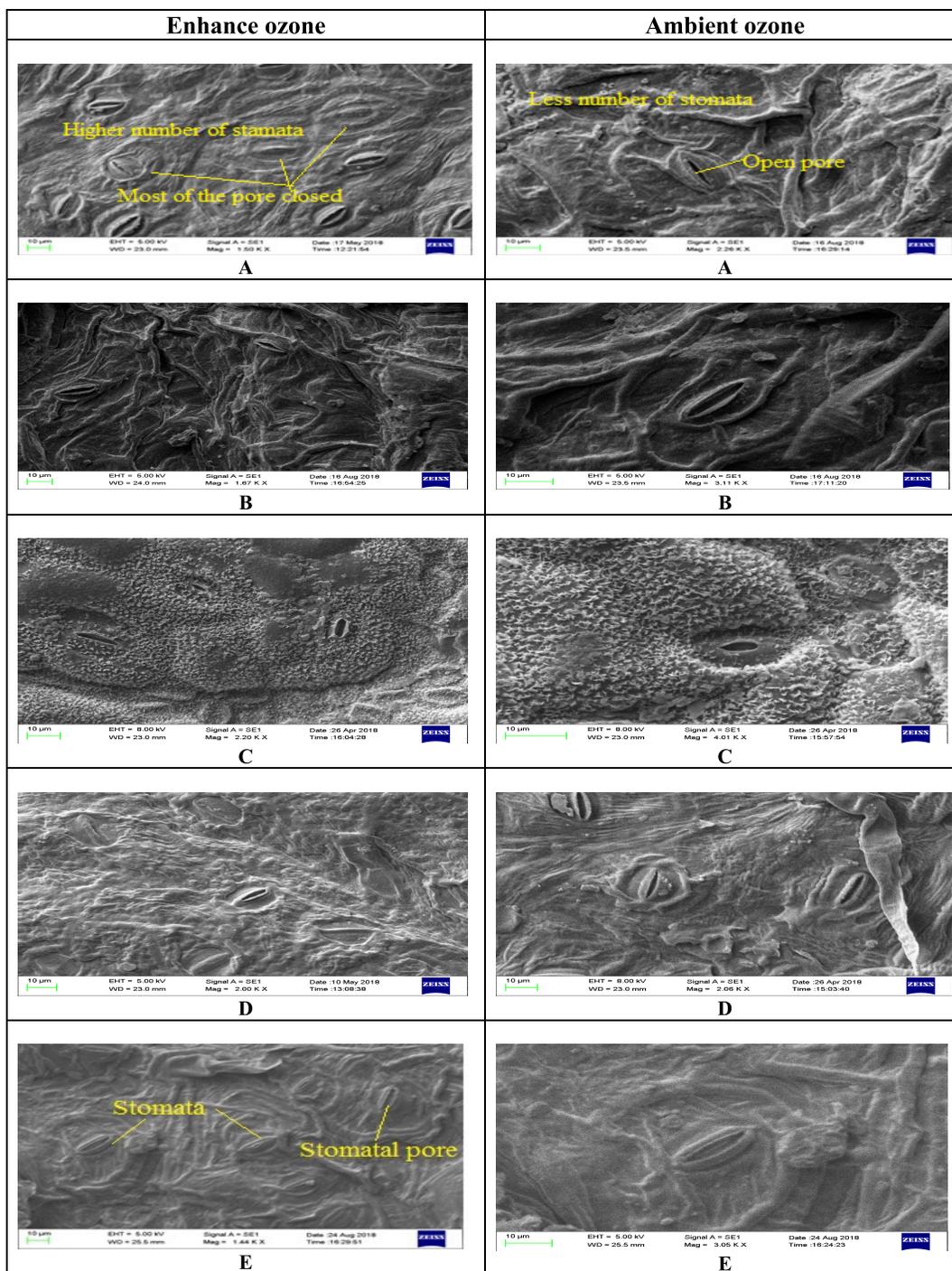


Figure 5: Micro-morphological modification identified by SEM of selected groundnut cultivars (A) TG-37A, (B) TPG-41, (C) TAG-24, (D) GG-20, and (E) Dh-86 under enhanced and ambient ozone.

cumulative doses of ozone stopped cell division of EC earlier than the old ones did. From another aspect, leaves which developed later maintained generous cell growth for a longer duration. This phenomenon may be due to ozone-convicted oxidative stress, which changes the redox state of cells and hence proliferation. The expansive growth of cell decreased the stomata number per mm² of epidermis in sensitive strain and increased EC size. Guard cells development changed similarly after the plants had been exposed

to the climate of the summer season and high cumulative ozone doses; the recently developed leaves of sensitive plants had higher-sized guard cells and thus found the larger stomatal apparatus. Cultivar TPG-41 showed maximum increase in EC suggesting its adaptability to enhanced ozone by reducing epidermal cell size and stomatal pores to reduce conductance. The idea of SI normalizes the epidermal cell expansion effect on the density of stomata [25]. SI (Fig. 2) of sensitive strain was mainly lower, but

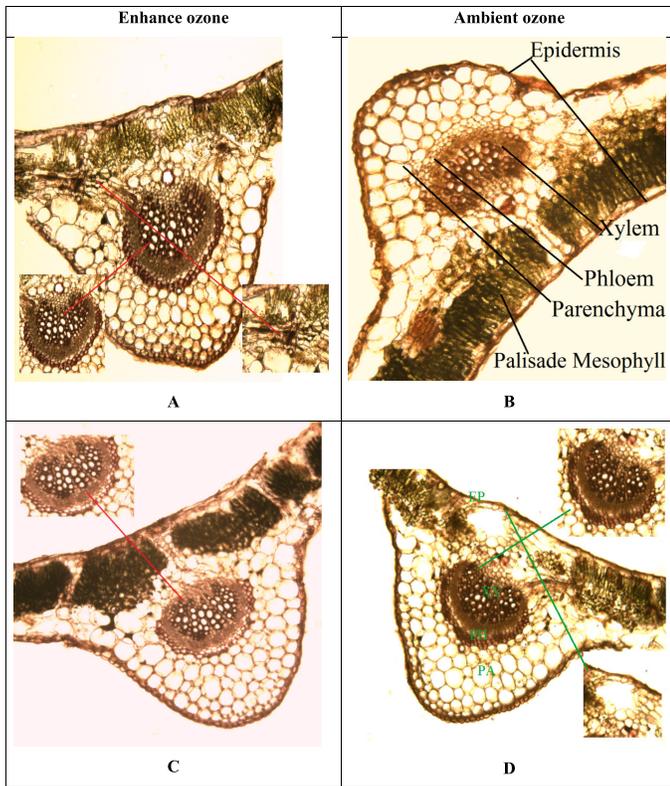


Figure 6: Cross-section of groundnut ozone-tolerant cultivar TPG-41. (A) Enhanced ozone, (B) ambient ozone and ozone-sensitive cultivar Dh-86, (C) enhanced ozone, and (D) ambient ozone.

this difference was more significant in resistant genotypes, which developed an increase in stomata numbers, while sensitive plants had a lesser number of stomata either as an attempt to reimburse the adverse effects of ozone or as a consequence of ozone which would have caused the change in cell division and developments. If the purpose of this change had been to lower the accession of the contaminant, it should have also resulted in lower gas condition and/or transpiration rate. The reason of the different stomatal developments could have possibly been ozone-inhibited cell division and/or increased expansive growth of cells, which comparatively decreased the stomata numbers per mm² epidermal area. Chaudhary and Rathore [2] and Qadir et al. [40] reported higher stomata indexes due to oxidative stresses.

Moreover, the present experiment also found that the enhanced ozone leads to closure of stomata of all the experimental groundnut cultivars. This would reach a reduction in evapotranspiration and water use efficiency, including reduced capability to uptake soil water and an increase in sensible heat flux as seen in soybean by VanLoocke et al. [41] and Bou Jaoudé et al. [42]. Although stomatal closure seems to be the principal response in crops, previous studies have proposed that under chronic ozone exposure, ozone-induced eminent production of stress ethylene can lead to a reducing of the abscisic acid signal [43, 44], which would normally lead to decreases in gas to conserve water in dry soils [45]. This could result in the crop losing control of stomatal closure, impairing water loss, and enhancing ozone uptake that would otherwise be limited by soil water stress, thus generating

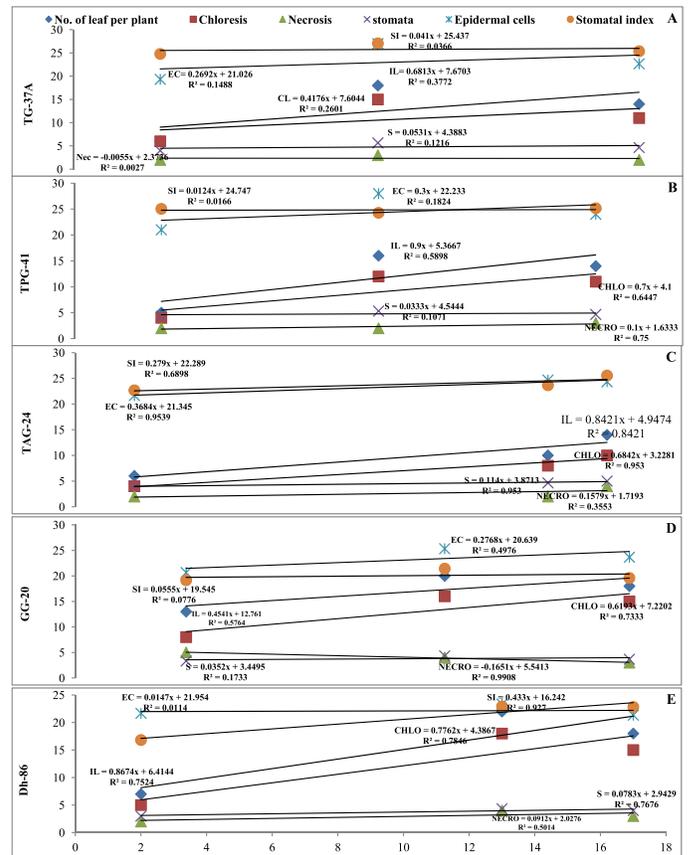


Figure 7: Pearson's correlation analysis between the total number of injured plants at 60 DAS of plant in response to number of leave injured per plant (IL), CHLO, Necrosis (NECRO), number of stomata (S), number of EC, and SI of groundnuts cultivars (A) TG-37A, (B) TPG-41, (C) TAG-24, (D) GG-20, and (E) Dh-86. Coefficient (R^2) value close to 1 expressed the strong positive correlation in between the subsets.

Table 3: *F* ratio and level of significance of number of stomata, number of EC, and SI of groundnut cultivars obtained by ANOVA test.

Parameters	Cult.	Treat.	DAS	Cult. × Treat.	Cult. × DAS	Treat. × DAS	Cult. × Treat. × DAS
Number of stomata	3.53**	40.35***	13.00***	0.67 ^{NS}	0.12 ^{NS}	0.14 ^{NS}	0.14 ^{NS}
Number of EC	1.18 ^{NS}	37.57***	33.40***	0.57 ^{NS}	1.97*	1.24 ^{NS}	0.94 ^{NS}
SI	2.50*	19.49***	2.92*	0.70 ^{NS}	0.13 ^{NS}	0.08 ^{NS}	0.35 ^{NS}

Significant levels, * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; NS = not significant.

a feedback loop that enhances ozone damage. The present study also found that the elevated ozone caused stomatal and EC modifications and caused succulents guard cells under exposure of enhanced ozone.

Ozone-induced anatomical changes in groundnut cultivars were also observed in the present study. Mitu et al. [46] reported changes in spongy parenchyma, epidermis, and vascular bundles of leaves and stems of mango, mahogany, and koroï due to continuous exposure of pollutants. Reduced vessel size in leaves of groundnut suggested reduced water transport and can be correlated with stomatal closure. It would be vital to emphasize that a more

detailed anatomical assessment is needed with variable species and cultivars to justify the effect of ozone on internal tissues of plants.

5. CONCLUSION

Ozone is a toxic gaseous pollutant that causes a negative effect on the micromorphology and anatomy of groundnut plants. The study observed ozone-like visible injury symptoms on all the groundnut cultivars using OTC. The results of the study showed that the visible injury was maximum in cultivar Dh-86 and minimum was in cultivar TPG-41. Micro-morphological characteristics, such as the number of stomata, EC, and SI, were increased due to elevated levels of ozone. The higher SI was found in cultivar TPG-41, while cultivars TAG-24 > TG-37A > and Dh-86 showed moderate values and the lowest SI was noted in cultivar GG-20. The enhanced level of ozone injured the leaves, micromorphology, and anatomy of all the groundnut cultivars. The results also confirmed that the stomatal closure and anatomical characteristics such as xylem, phloem, collenchyma, and mesophyll cells disorganization are important characteristics for the identification of ozone-resistant variety. On the basis of stomatal movement and distribution, anatomical changes in cultivar TPG-41 were shown as the most ozone-resistant groundnut variety among the tested cultivars. However, more studies with variable plant species and different cultivars are needed for a substantial conclusion.

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SUPPLYMENTARY FIGURES

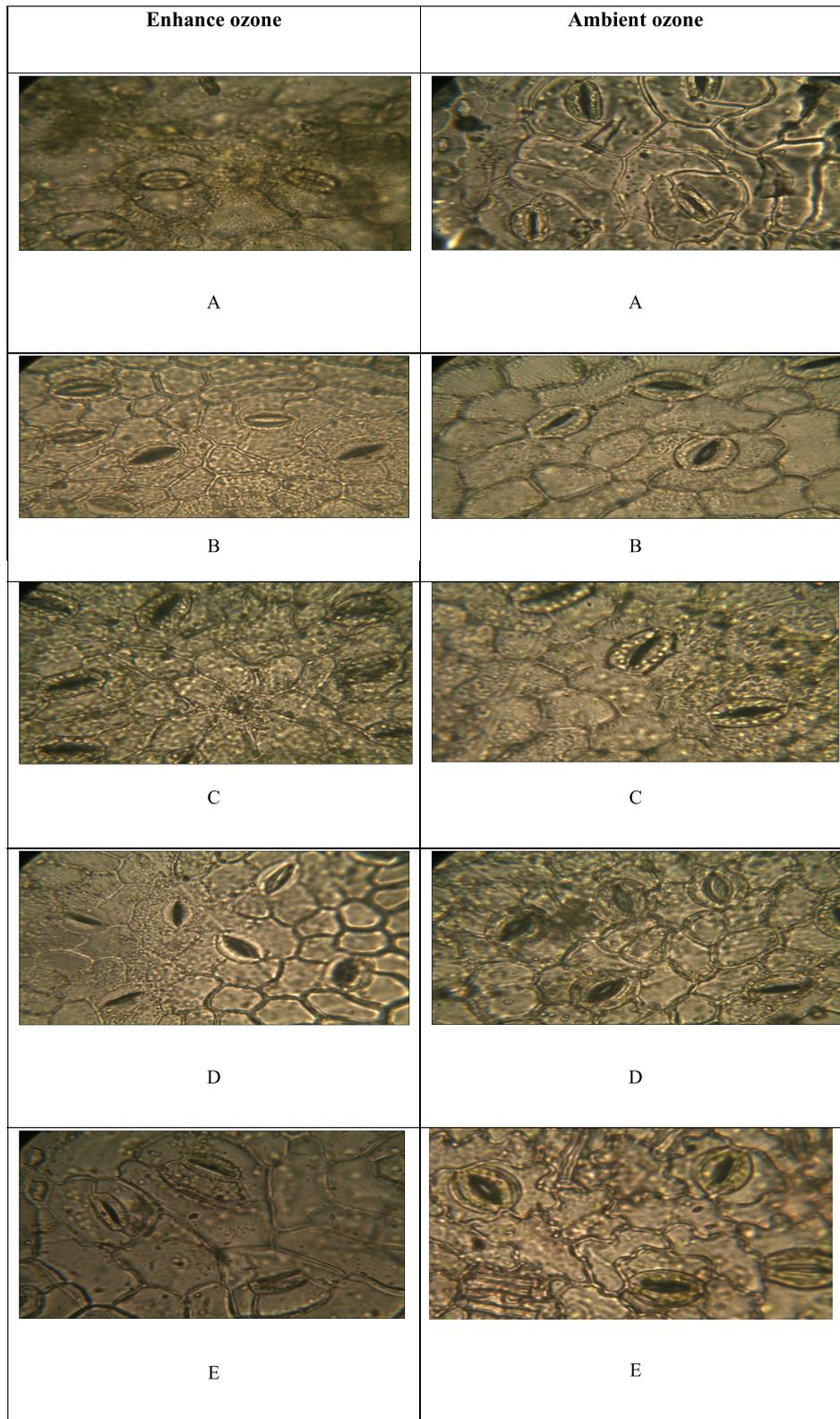


Figure S1: Modification of micro-morphological characteristics such as number of stomata, number of EC, and stomatal opening of groundnut cultivars (A) TG-37A, (B) TPG-41, (C) TAG-24, (D) GG-20 and (E) Dh-86 under enhanced ozone and ambient ozone.

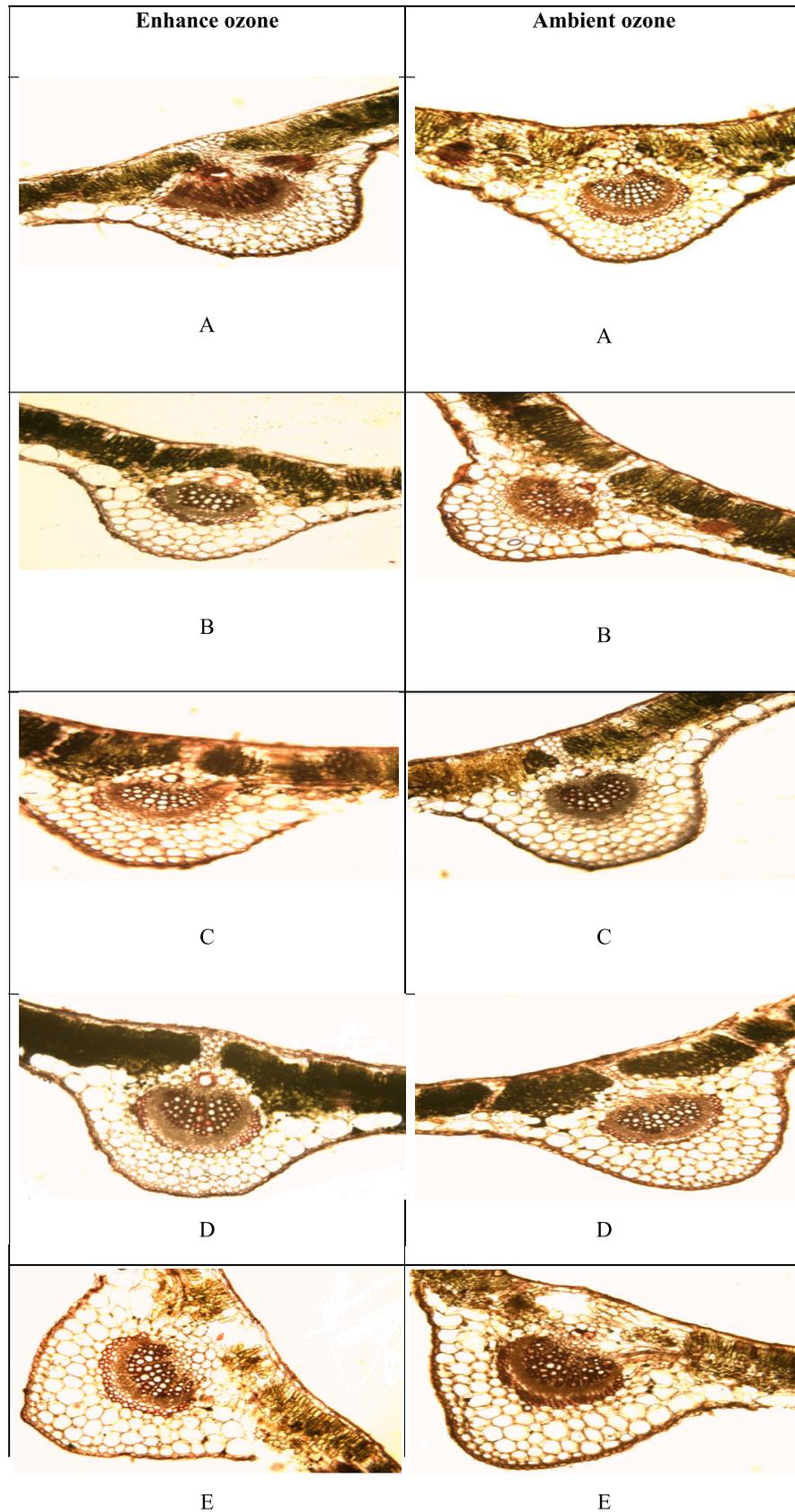
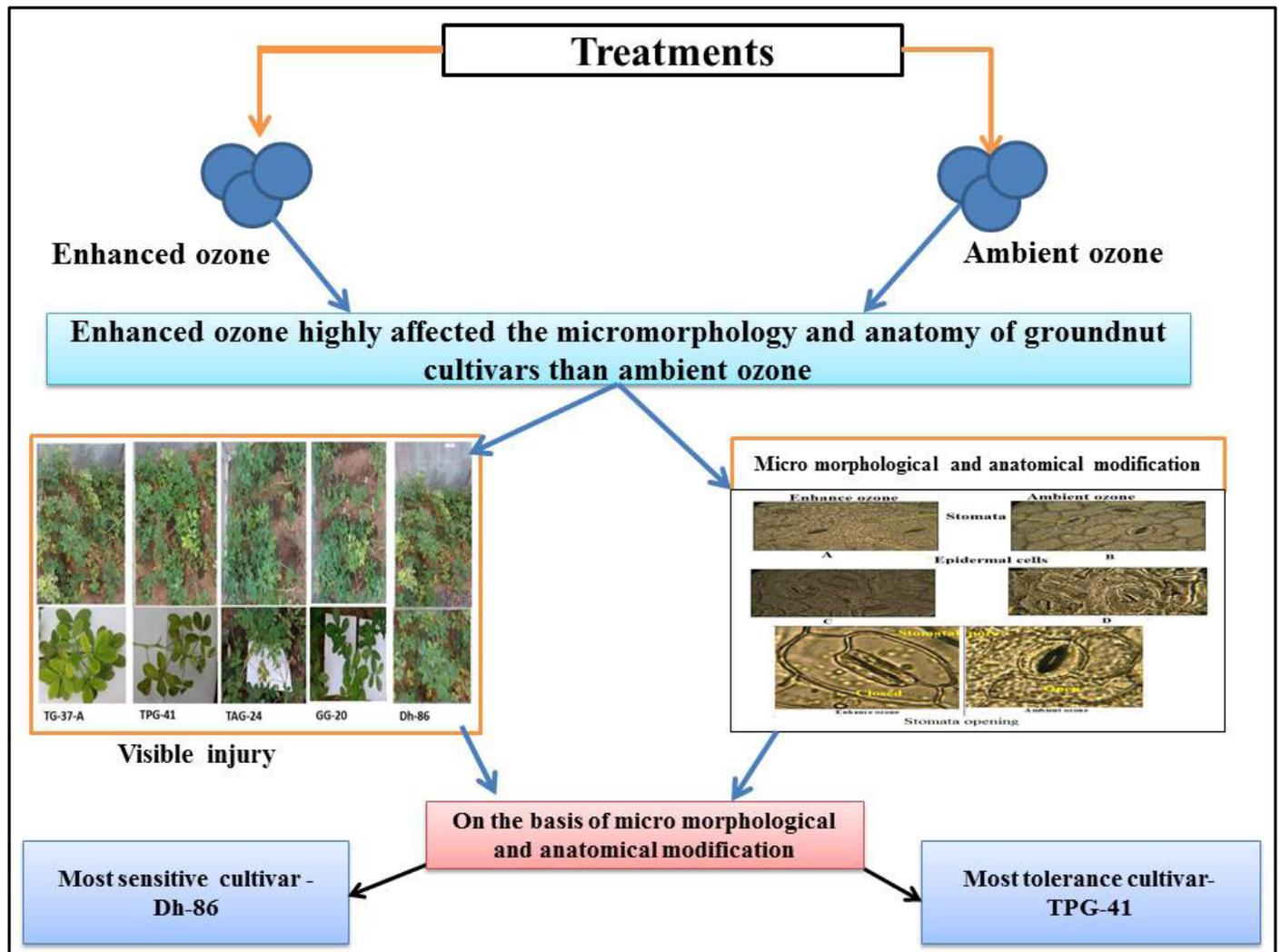


Figure S2: Cross-section of anatomical characteristic changes such as epidermis, xylem, phloem, parenchyma and palisade mesophyll cells of groundnut cultivars (A) TG-37A, (B) TPG-41, (C) TAG-24, (D) GG-20 and (E) Dh-86 under enhanced ozone and ambient ozone stress.

GRAPHICAL ABSTRACT



Modulating the Delivery of 5-Fluorouracil to Human Colon Cancer Cells Using Multifunctional Arginine-Coated Manganese Oxide Nanocuboids with MRI Properties

Poonam Jain, Krunal Patel, Ashok Kumar Jangid, Anupam Guleria, Sunita Patel, Deep Pooja,* and Hitesh Kulhari*

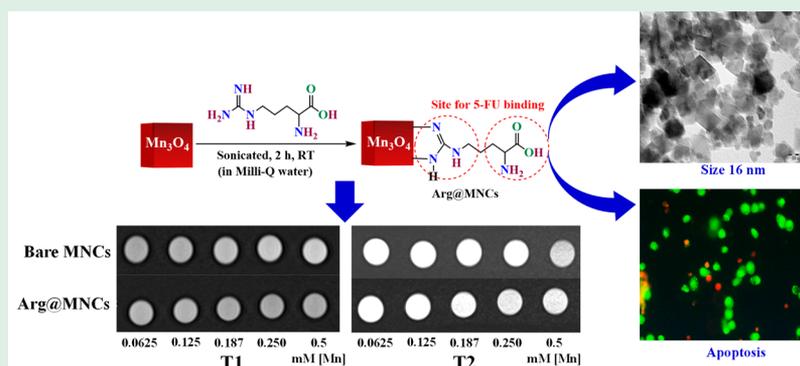
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ABSTRACT: 5-Fluorouracil (5-FU) is one of the most prescribed drugs and the major component of chemotherapy for the treatment of colorectal cancer. In this study, we have designed arginine-functionalized manganese oxide nanocuboids (Arg@MNCs) for the effective delivery of 5-FU to colon cancer cells. Arginine was used as multifunctional agent to provide stability to MNCs, achieve high drug loading, control the release of loaded drug, and improve delivery to cancer cells. The synthesized Arg@MNCs were characterized by DLS, TEM, XRD, FTIR, XPS, TGA, and VSM analysis. The structural and morphological analysis by TEM showed cuboid-shaped MNCs with average particle size ~ 15 nm. Biodegradation studies indicated that the Arg@MNCs were degraded at endolysosomal pH in 24 h while remaining stable at physiological pH. Hemolytic toxicity studies revealed the safety and nontoxic nature of the prepared MNCs. 5-FU-loaded Arg@MNCs showed significant control over the release of 5-FU, decrease in the hemolytic toxicity of loaded 5-FU but higher in vitro anticancer activity against HCT 116 and SW480 human colon cancer cells. Importantly, both the bare MNCs and Arg@MNCs showed excellent T1 and T2MR relaxivity under 3.0 T MRI scanner. Thus, the nanostructures developed in this study, i.e., 5-FU-Arg@MNCs could overcome the issues of both MNCs (stability) and 5-FU (low drug loading and nonspecificity) and may be used as a multifunctional theranostic nanocarrier for colon cancer treatment.

KEYWORDS: manganese oxide, L-arginine, 5-fluorouracil, colon cancer, magnetic resonance imaging

1. INTRODUCTION

Colorectal cancer (CRC) is the third most death-defying cancer worldwide. According to a GLOBOCAN 2018 report, an International Agency for Research on Cancer (IARC) of the World Health Organization (WHO), CRC was responsible for 10.2% of the incidence and 9.2% of the mortality of total cancers.¹ Colon cancer is a type of neoplasia and generally starts from the transformation of the inner linings of the large intestine or colon or rectum.² The advanced application of screening by colonoscopy helps in prevention of cancer by identification and removal of precancerous lesions. Chemotherapy is the most promising therapy for colon cancer treatment, but poor delivery modality and chemoresistance hamper its clinical use.³

5-Fluorouracil (5-FU) is a clinically approved anticancer drug for the treatment of solid tumors including colorectal, gastric, skin, and breast cancers.^{4,5} It is used either alone or in combination with other chemotherapeutic agents. 5-FU is widely used as a first-line chemotherapeutic agent for the treatment of metastatic colon cancer along with oxaliplatin and

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leucovorin.⁶ Structurally, 5-FU is a pyrimidine analogue that blocks the action of thymidylate synthase and inhibits DNA synthesis, leading to cytotoxicity and cell death. However, its high metabolic rate and short half-life (10–20 min) require continuous administration of high doses to maintain the serum concentration, which results in severe toxicities such as hematological, myelosuppression, neural, and gastrointestinal adverse effects.^{7,8} In addition, conventional formulations of 5-FU show uncontrolled biodistribution. Uncontrolled biodistribution means the inconsistent or nonspecific circulation of drug molecules after administration into a biological system. It leads to the distribution of drug molecules to and interaction with the nontargeted tissues and cells, which results in a low level of drug at the target site. Thus, uncontrolled biodistribution of 5-FU is responsible for its low therapeutic efficacy and high mortality rate.⁹ Therefore, to overcome these drawbacks, we need an effective carrier system for an appropriate cancer therapy using 5-FU. Further, the carrier system should be biocompatible and biodegradable and able to provide a high drug payload, controlled drug release, and targeted drug delivery.

Manganese (Mn)-based nanocarriers such as MnO, MnO₂, and Mn₃O₄ nanoparticles have been gaining attention in the past few years because of their theranostic applications.^{10–12} MnO, MnO₂, and Mn₃O₄ nanoparticles are commonly investigated Mn-based nanocarriers for their applications in drug delivery and diagnosis.^{13–19} However, like other inorganic nanoparticles, Mn-based nanoparticles also agglomerate or precipitate out rapidly in the aqueous solutions and physiological buffers. Therefore, a stabilizing agent is needed to increase their colloidal stability in biological fluids. In this study, we selected L-arginine (Arg) as a multifunctional stabilizing agent to improve the stability of Mn₃O₄ nanocuboids (MNCs). Arg is a nonessential amino acid and is required for the synthesis of various proteins in the body. Chemically, it contains a strongly basic guanidinium group, making it cationic in nature. Various types of nanoparticles have been functionalized and stabilized using Arg as the capping and stabilizing agent.^{20,21} Recently, Arg-functionalized and ciprofloxacin-loaded mesoporous silica nanoparticles have been used for the treatment of salmonella through cationic amino acid transporters (CAT).²² As CAT are also overexpressed on cancer and hence, arginine functionalization to MNCs may improve the delivery of the drug to the cancer cells.²³ Apart from providing stability and targeting, being a cationic molecule, it may help in loading of drugs like 5-FU, which is otherwise difficult to load on inorganic nanoparticles.

The use of MNCs as drug carrier also helps in diagnosis by simultaneously monitoring the disease progress. MNCs have also been found as good contrast agents (CAs) in the magnetic resonance imaging (MRI) technique. MRI is a noninvasive and soft-tissue imaging modality that provides excellent anatomical details at high resolution.^{24,25} A range of nanoparticles and chelates has been investigated for their application as CAs in MRI. The widely explored agents are paramagnetic gadolinium^{26–28} (Gd) and manganese (Mn)-based chelates^{16,29–32} for positive or T₁-weighted imaging, and superparamagnetic iron oxide nanoparticles^{33–37} (SPIONs) for negative or T₂-weighted imaging. However, Gd-based CAs show nephrogenic systemic fibrosis in the patients, whereas intrinsic dark MR signals of SPION hamper the understanding of deep tissue level.³⁸ Recently, Mn-based nanostructures have emerged as alternative CAs because of their biocompatibility and bright contrast MRI. Therefore, in this study, we designed a theranostics nanocarrier

system using Arg-functionalized Mn₃O₄ nanocuboids to provide stability to these nanoparticles in biological fluids, achieving loading of 5-FU on nanoparticles, improving the delivery of loaded drug to cancer cells, and achieving MRI diagnosis.

2. MATERIALS AND METHODS

2.1. Materials. Manganese chloride tetrahydrate (MnCl₂·4H₂O, M_w 125.84) was purchased from TCI Chemicals Pvt. Ltd. (Chennai, India). Sodium hydroxide was purchased from Merck (Mumbai, India). L-Arginine was purchased from Sigma-Aldrich (Saint Louis, MO, USA). Cell culture media RPMI-1640, penicillin-streptomycin-amphotericin B cocktail, 0.25% Trypsin-EDTA, and fetal bovine serum (FBS) were purchased from Thermo-Fisher Scientific (Waltham, MA, USA). 3-(4,5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was purchased from Himedia laboratories (Mumbai, India).

2.2. Synthesis and Surface Modification of MNCs. Mn₃O₄ nanocuboids (MNCs) were synthesized by the precipitation method as reported previously.^{39,40} Surface modification of the prepared MNCs with Arg was performed by the ultrasonication method with 20 kHz ultrasonic frequencies at 25 °C. First, 4% w/v aqueous solution of Arg was added into MNC (5 mg/mL) dispersion and sonicated for 2 h. The dispersion was washed thrice with Milli-Q water to remove non-functionalized or free Arg. The obtained surface-modified MNCs (Arg@MNCs) were lyophilized and stored in a storage vial.

2.3. Loading of 5-FU on Arg@MNCs Nanocuboids. An aqueous dispersion of Arg@MNCs (2.5 mg/mL) was mixed with different amounts of 5-FU solutions (0.5–2.5 mg/mL) and mechanically stirred for 2 h at room temperature at 100 rpm. After that, the mixture was centrifuged at 14 000 rpm for 20 min. The absorbance of the supernatant was measured at 266 nm to determine the remaining 5-FU concentration as unloaded drug content. The drug loading efficiency was calculated as follows:

$$\text{loading efficiency (\%)} = \frac{\text{weight of initial drug added} - \text{weight of drug in supernatant}}{\text{total weight of drug and nanoparticles}} \times 100$$

2.4. General Characterization. MNCs and Arg@MNCs were characterized by TEM, powder XRD, PSA, FTIR, XPS, and TGA analysis. Transmission electron microscopy (TECHNAI-20-G2) was used for determination of the morphology and particle size of prepared samples. Powder XRD measurement was carried out using Bruker D8 advance X-ray diffractometer equipped with Cu K alpha ($\lambda = 0.154$ nm). Particle size, polydispersity index (DLS), and zeta potential (ZP) of MNCs and Arg@MNCs were examined by dynamic light scattering technique using a Zetasizer Nano ZS90 series (Malvern Instruments). Particles were dispersed in Milli-Q water at a concentration of 0.1 mg/mL and sonicated for 20 min for complete dispersion of MNCs and Arg@MNCs. For the FTIR study, the samples were mixed with KBr, palletized, and scanned in the wavenumber region of 4000 cm⁻¹ to 400 cm⁻¹ (PerkinElmer Spectrum 65 series). The elemental composition and oxidation state of Arg@MNCs were measured by photoelectron spectrometer (SPECS, Surface Nano Analysis GmbH) with monochromatic Al K-alpha (1486.61 eV) X-rays as an excitation source at 13 kV, 100 W. Thermal gravimetric analysis (TGA) was performed on the TGA instrument (TG/DTA7300 EXSRAR) set for temperature from 30 to 800 °C under a nitrogen flow and a heating rate of 10 °C/min. Magnetic measurement of Arg@MNCs was carried out using vibrating sample magnetometer (VSM) instrument (3472–70 electromagnet).

2.5. In Vitro Drug Release Study. In vitro drug release of 5-FU from Arg@MNCs was studied through the centrifugation method. 5-FU-loaded Arg@MNCs containing about 1 mg of 5-FU were dispersed in 10 mL of release medium, i.e., phosphate buffer saline pH 7.4 or sodium acetate buffer pH 5.0, placed at 37 °C in a shaker incubator. At the predetermined time interval, the samples were centrifuged and the 5-FU content was measured at 266 nm by UV–vis spectrophotometer (UV Vis 1800 Shimadzu).

2.6. Stability of 5-FU in Arg@MNCs. To determine the stability, we redispersed freshly prepared 5-FU-loaded Arg@MNCs in two

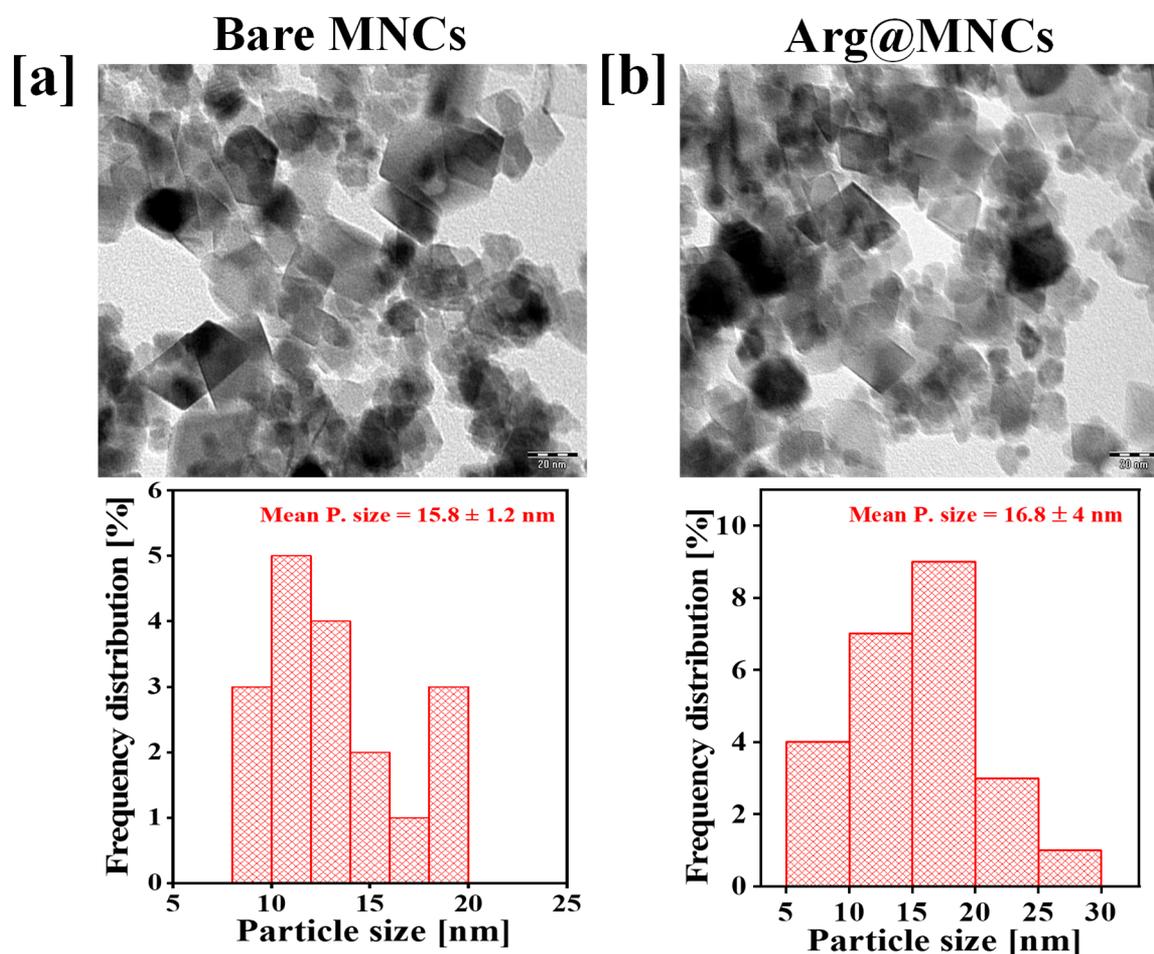


Figure 1. (a) TEM images of bare MNCs and Arg@MNCs. (b) Particle size distribution histogram of bare MNCs and Arg@MNCs.

physiological mediums, PBS (pH 7.4) and normal saline (0.9% w/v NaCl), and stored at room temperature (25 ± 5 °C) and refrigeration temperature (4 °C). At predetermined time intervals, samples were centrifuged and the supernatant was analyzed by UV–vis spectrophotometry at 266 nm to determine the 5-FU loss during storage.

2.7. Hemocompatibility Studies. A hemocompatibility study was performed as described previously.⁴¹ Human blood was collected into EDTA solution (5% w/v) containing a falcon tube under aseptic conditions. Whole blood was centrifuged (4000 rpm, 10 min). The settled down red blood cells (RBCs) were collected and washed thrice with normal saline (0.9% w/v) solution. The washed RBCs were resuspended in the normal saline to make 2% RBC suspension. Bare MNCs, blank Arg@MNCs, pure 5-FU, and 5-FU-Arg@MNCs at different concentrations (0, 5, 10, 25, 50, 100, 150, and 250 $\mu\text{g}/\text{mL}$) were added into 2% RBCs suspensions and incubated at 37 °C with mild shaking up to 60 min. The samples were centrifuged (4000 rpm, 10 min) and the optical density (OD) was measured at 540 nm using a microplate reader (Synergy H1 Hybrid Reader, Biotek). Normal saline and Milli-Q water as a negative (0% hemolysis) and positive (100% hemolysis) control, respectively was used. The hemolysis percentage (HPs) of all samples were calculated according to the given equation:

$$\text{hemolysis (\%)} = \frac{\text{OD(T)} - \text{OD(NC)}}{\text{OD(PC)} - \text{OD(NC)}} \times 100$$

where T is test sample, NC is negative control, and PC is positive control.

2.8. Biodegradation Studies. The biodegradation study of bare MNCs and Arg@MNCs was determined by quantifying the % Mn content in physiological media, i.e., phosphate buffer saline (PBS, pH 7.4), sodium acetate buffer (SAB, pH 5.0), and 50% fetal bovine serum

(FBS) at 37 °C using atomic absorption spectroscopy (AAS) (Thermo scientific, AAS: iCE 3000 SERIES) as reported previously.⁴⁰

2.9. Cell Culture Study. **2.9.1. In Vitro Cytotoxicity Studies.** In vitro cytotoxicity of bare MNCs, free 5-FU, 5-FU-loaded MNCs, and 5-FU-loaded Arg@MNCs were evaluated against SW480 and HCT 116 human colon cancer cells. Cells were cultured in RPMI medium supplemented with 10% fetal bovine serum. For MTT assay, 1×10^4 cells were seeded in a 96-well plate and incubated overnight. Cells were treated with the different concentrations (0.125–4 $\mu\text{g}/\text{mL}$) of samples and further incubated for 24 or 48 h. At the end of the incubation period, the media was removed and 200 μL of fresh media containing 0.5% MTT reagent was added in wells and plates were incubated at 37 °C in the dark for 4 h. Finally, the media was removed. A volume of 200 μL of DMSO was added to each well and the absorbance was measured at 570 nm using a microplate reader (Synergy H1 Hybrid Reader, Biotek). Untreated cells were considered as control.

2.9.2. Cellular Uptake. For cellular uptake studies, 4×10^4 cells (SW480 and HCT 116) were seeded per well of a 24-wells plate and were allowed to attach. Cells were treated with 10 $\mu\text{g}/\text{mL}$ of bare MNCs and Arg@MNCs for different time periods. The cells were further processed as reported previously and the cellular uptake of bare MNCs and Arg@MNCs was quantified by the determination of Mn concentration.⁴⁰

2.9.3. Apoptosis Studies. Acridine orange and ethidium bromide staining was performed to assess the cellular changes induced by the 5-FU treatment. About 4×10^4 cells (SW480 and HCT 116) were seeded in a 24-wells plate. After 24 h of seeding, cells were treated with 1 $\mu\text{g}/\text{mL}$ and 2 $\mu\text{g}/\text{mL}$ of free 5-FU, bare MNCs, 5-FU-loaded MNCs, and 5-FU-loaded Arg@MNCs for 24 h. The cells were further processed as reported previously,⁴² and microscopic images of cells were acquired.

2.10. MRI Measurement. The T_1 and T_2 relaxation times were measured using a 3.0 T MRI scanner (Siemens Skyra) equipped with head coil. The T_1 - and T_2 -weighted phantom images were acquired on the aqueous dispersions of synthesized MNCs and Arg@MNCs with varying concentrations using an inversion recovery and multi spin echo pulse sequences, respectively. The parameters used for inversion recovery pulse sequence were as follows: matrix size = 256×320 , slice thickness = 2 mm, spacing gap = 0, field of view (FOV) = 15 cm, phase (FOV) = 1, repetition time (TR) = 5000 ms, time of echo (TE) = 12 ms and inversion time (TI) = 100 to 4000 ms. T_2 weighted images were obtained at echo times with matrix size = 410×512 , the repetition time (TR) = 6000 ms, and the spin echo time (TE) from 40 to 230 ms. Other parameters were similar to that of inversion recovery sequence. The signal intensity of the appropriate region of interest (ROI) were obtained at each TI and TE for the T_1 - and T_2 -weighted images, respectively, using the Siemens software and the corresponding T_1 and T_2 relaxation times were obtained by fitting these MRI signal amplitude with the nonlinear least-squares regression eqs 1 and 2 as given below and described previously in detail.⁴³

$$M(TI) = M_0(1 - 2\exp(-TI/T_1)) \quad (1)$$

$$M(TE) = M_0\exp(-TE/T_2) \quad (2)$$

The r_1 and r_2 relaxivities were calculated from the slope of the plots of inverse relaxation times ($1/T_1$ and $1/T_2$) versus Mn concentration, respectively.

2.11. Data Analysis. The experiments were performed in triplicate ($n = 3$) and the values are expressed as mean \pm SD. The p value < 0.05 was considered as statistically significant during data analysis.

3. RESULTS AND DISCUSSION

3.1. Synthesis and Characterizations of Bare MNCs and Arg@MNCs.

First, bare MNCs were synthesized by our

Table 1. Particle Size, Polydispersity Index (PDI), and Zeta Potential (ZP) of MNCs and Arg@MNCs

NP system	TEM size (nm)	DLS size (nm)	PDI	ZP (mV)
MNCs	15.8 ± 1.2	152.5 ± 8	0.591 ± 0.07	-9 ± 0.7
Arg@MNCs	16.8 ± 4	158.8 ± 7	0.191 ± 0.03	-7.4 ± 0.5

optimized chemical precipitation method⁴⁰ and then modified with Arg to form stable MNCs. The surface functionalization of MNCs with Arg was optimized by varying Arg concentration (2–8% w/v), and its effect on the particle size and stability in the water as well as in cell culture media were observed. A 4% w/v concentration of Arg was found to be an optimum concentration for providing stable Arg@MNCs. Formation of nanostructures was confirmed by different techniques such as TEM, DLS, XRD, FTIR, XPS, and TGA techniques. Figure 1 shows the TEM images of MNCs and Arg@MNCs. The size of particles were uniform with a size distribution of 15.8 ± 1.2 nm and 16.8 ± 4 nm for MNCs and Arg@MNCs, respectively, and having cuboid shape. The DLS measurements of prepared MNCs and Arg@MNCs further confirmed the formation of nanosized particles.^{40,41,44} The particle size, PDI and ZP of MNCs and Arg@MNCs are presented in Table 1. These results suggested that the surface functionalization of MNCs with Arg does not significantly increase the size of MNCs.

The phase purity and crystal structure of synthesized MNCs and Arg@MNCs were identified by the XRD pattern as demonstrated in Figure 2a. The diffraction pattern of bare MNCs showed peaks at 2θ values 18.1, 28.9, 31, 32.4, 36.2, 38.2, 44.5, 50.7, 53.9, 58.5, 60.1, and 64.8° belonging to the crystal planes (101), (112), (200), (103), (211), (004), (220), (105), (312), (321), (224), and (400), respectively. The observed peaks were correlated with the powder diffraction file no. 24–0734 (Joint Committee on Powder Diffraction Standards).^{39,40} Pure L-Arg showed peaks at 11, 14.9, 16.9, 18.4, 19.1, 19.5, 23.1, and 27.4° .⁴⁵ After the surface modification of MNCs by Arg, the Arg@MNCs showed the peaks at 2θ values of 18, 28.9, 30.8, 36.1, 38.0, 44.4, 50.8, 53.9, 58.5, 59.9, and 64.6° . The observed results suggested that no other impurities have found in the phase purity measurements. Hence, MNCs and Arg@MNCs indicated the presence of no other oxidation state of Mn.^{32,39,46}

The FTIR spectrum of bare MNCs shows two broad peaks at 617 and 513 cm^{-1} (Figure 2b) which correspond to the Mn–O stretching modes of tetrahedral and octahedral sites of MNCs. A peak at 429 cm^{-1} is assigned to the bending vibration of octahedral sites. Therefore, the FTIR spectrum further

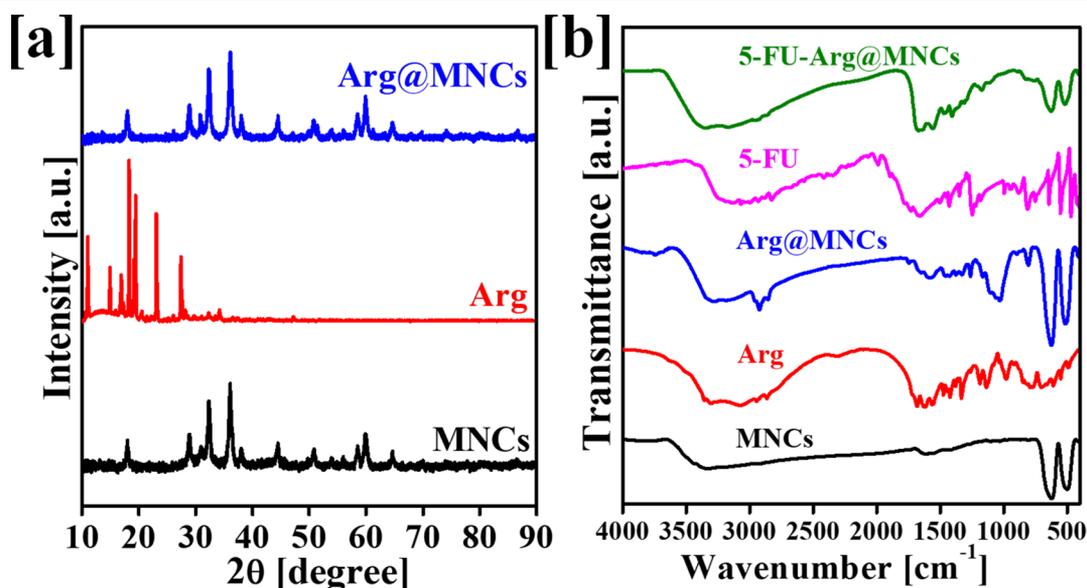


Figure 2. Characterizations of synthesized bare MNCs and Arg@MNCs: (a) powder-XRD spectra of bare MNCs, Arg, and Arg@MNCs. (b) FTIR spectra of bare MNCs, Arg, Arg@MNCs, 5-FU, and 5-FU-Arg@MNCs.

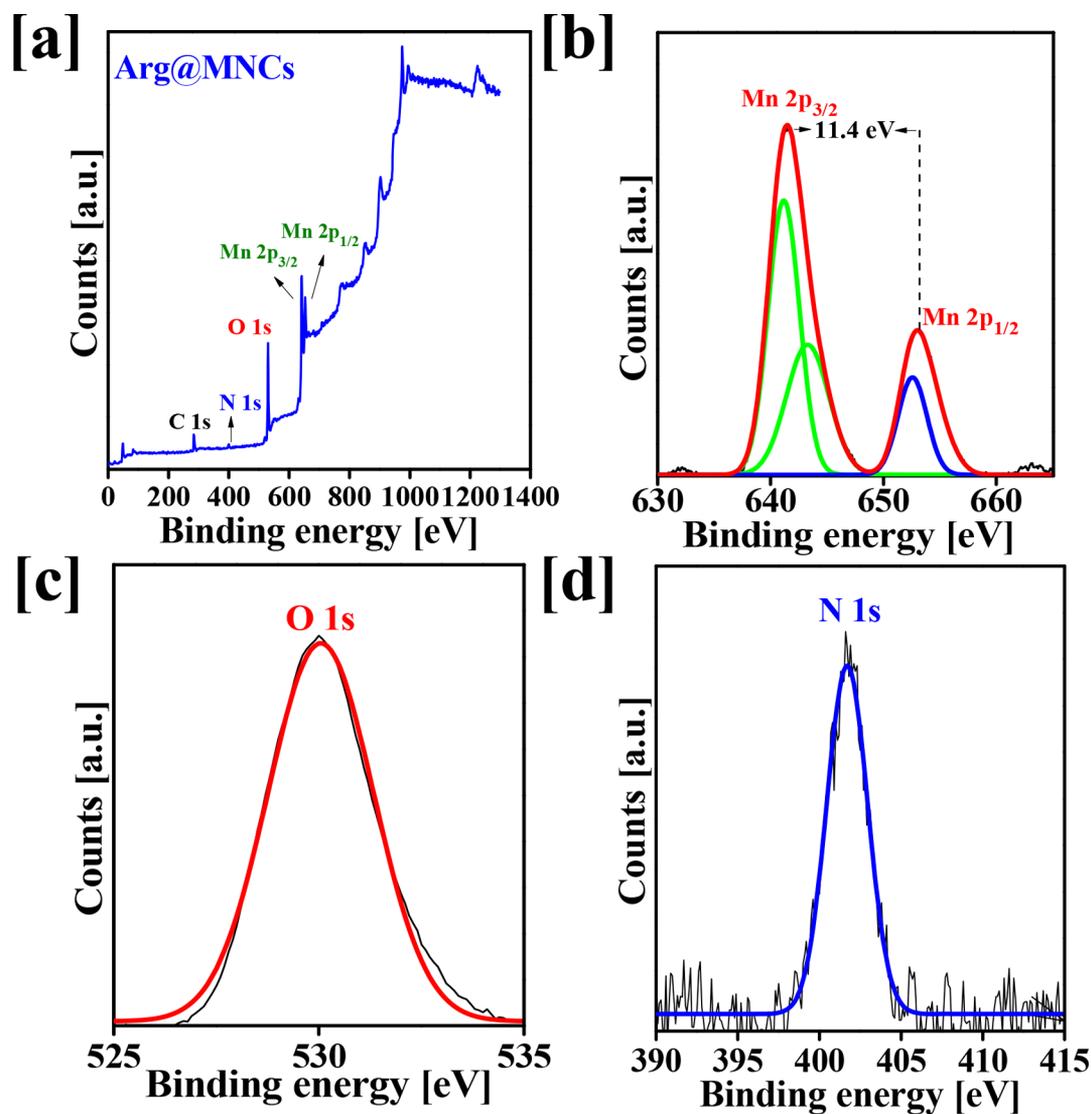


Figure 3. XPS spectra of Arg@MNCs: (a) survey scan, (b) deconvoluted spectrum of Mn 2p_{3/2} and Mn 2p_{1/2} levels, (c) XPS spectrum of oxygen (O 1s), and (d) XPS spectrum of nitrogen (N 1s).

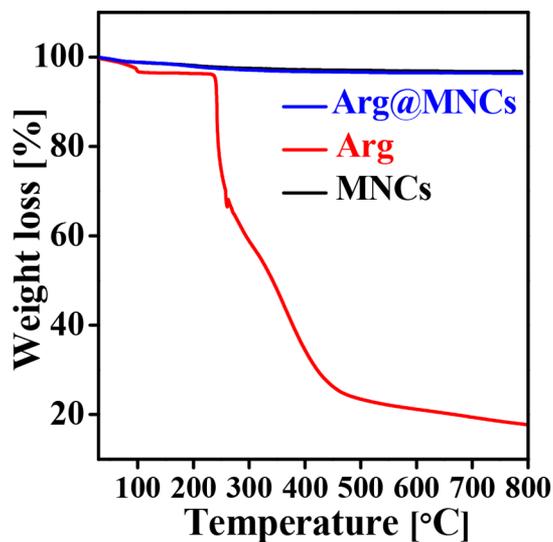


Figure 4. TGA thermogram of MNCs, pure L-arginine (Arg), and Arg@MNCs.

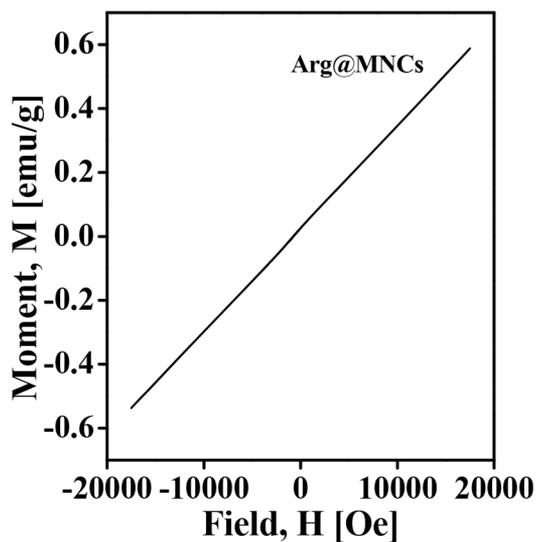


Figure 5. $M-H$ curve of Arg@MNCs at room temperature.

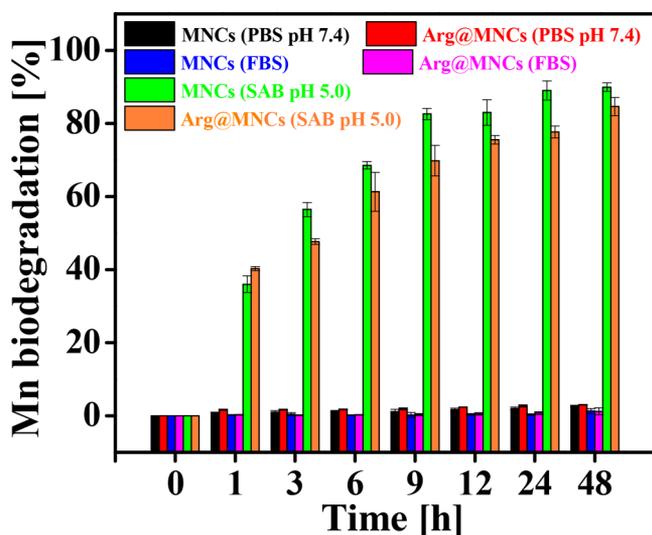


Figure 6. Biodegradation profile of MNCs and Arg@MNCs in sodium acetate buffer (SAB, pH 5.0), phosphate buffer saline (PBS, pH 7.4), and 50% fetal bovine serum (FBS).

confirmed the formation of MNCs.⁴⁶ FTIR spectrum of pure Arg shows the characteristic vibration peaks at 3296 cm^{-1} ($-\text{NH}$ stretching), 3082 and 2855 cm^{-1} ($-\text{CH}$ stretching), 1682 and 1631 cm^{-1} ($\text{C}=\text{N}$ stretching), 1550 cm^{-1} ($\text{C}=\text{N}$ bending), 1423 and 1326 cm^{-1} (CH_2 bending), and 1186, 1131, and 986 cm^{-1} ($-\text{CO}$ stretching).⁴⁷ Arg@MNCs shows the IR peaks at 3279 cm^{-1} ($-\text{NH}$ stretching), 2922 and 2861 cm^{-1} ($-\text{CH}$ stretching), 1744 and 1648 cm^{-1} ($\text{C}=\text{N}$ stretching), 1578 cm^{-1} ($\text{C}=\text{N}$ bending), 1452 cm^{-1} (CH_2 bending), 1260, 1032, and 806 cm^{-1} ($-\text{COH}$ stretching) with the two major characteristic peaks of bare MNCs at 625 and 511 cm^{-1} . These observed peaks confirm the successful coating of Arg on the surface of the MNCs. The FTIR spectrum of pure 5-FU shows the peaks at 3236 cm^{-1} ($-\text{NH}$ stretching), 2981 and 2833 cm^{-1} ($-\text{CH}$ stretching), 1734 and 1665 cm^{-1} ($-\text{C}=\text{N}$ stretching), 1427 and 1348 cm^{-1} (CH_2 bending), 1252 and 988 cm^{-1} ($-\text{COH}$ stretching). 5-FU-Arg@MNCs shows the IR peaks at

3177 cm^{-1} ($-\text{NH}$ stretching), 2947 and 2848 cm^{-1} ($-\text{CH}$ stretching), 1655 cm^{-1} ($\text{C}=\text{N}$ stretching), 1560 cm^{-1} ($\text{C}=\text{N}$ bending), 1399 cm^{-1} (CH_2 bending), 1161, 1090, and 812 cm^{-1} ($-\text{COH}$ stretching), 623 and 504 cm^{-1} ($\text{Mn}-\text{O}$ stretching). Therefore, the presence of IR peaks of pure 5-FU proved the drug was loaded successfully into the 5-FU-loaded Arg@MNCs.

Figure 3a shows the XPS analysis of Arg@MNCs. XPS analysis was performed in order to estimate the oxidation state and elemental composition. The XPS survey scan of Arg@MNCs does not show any signs of impurities. As shown in Figure 3b, c, obtained binding energy values of Mn 2p_{3/2}, Mn 2p_{1/2}, and an O 1s peak were 641.1, 652.6, and 530.04 eV, respectively. The binding energy values of Mn 2p_{3/2} and Mn 2p_{1/2} showed differences between the spin-orbit splitting level of 11.4 eV, which also confirms the formation of Mn₃O₄ NCs.^{39,48} The binding energy value of O 1s at 529.7 and 531.9 eV confirmed the presence of Mn²⁺, and Mn³⁺ ion in lattice oxygen of manganese oxide shown in Figure 3d shows the survey spectra of Arg@MNCs, where a peak of N 1s observed at 401.6 eV, indicating that the Arg is successfully modified on the surface of MNCs.⁴⁹

3.2. Thermal Gravimetric Analysis (TGA). Figure 4 depicts the TGA analysis of bare MNCs and Arg@MNCs from 30 to 800 °C, in which the amount of Arg on the surface of MNCs was quantified. The bare MNCs showed a weight loss of 2.8% up to 800 °C, whereas Arg@MNCs showed a percent weight loss of 3.6% up to 800 °C, which clearly indicate the coating of Arg on the MNCs via physical adsorption and strong electrostatic interaction.⁵⁰ Pure Arg showed the percent weight loss of about 82.2%, indicating the thermal degradation of Arg. Hence, it is thermally stable up to 230 °C. This indicated that Arg@MNCs is a thermally stable nanocarrier system and Arg was successfully modified on the surface of MNCs through physical interaction.

3.3. Vibrating Sample Magnetometer (VSM). The magnetization curve of Arg@MNCs at room temperature was shown in Figure 5. The $M-H$ curve between magnetization (M) and applied magnetic field (H) was found to be linear with the applied magnetic field magnetic properties.^{51,52} The $M-H$ curve

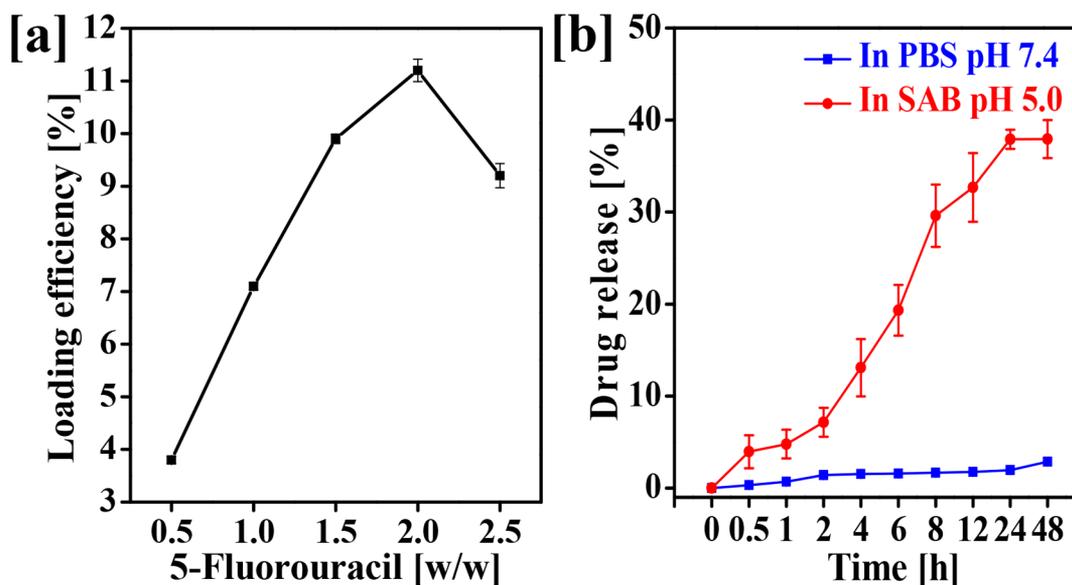


Figure 7. (a) Optimization of 5-FU loading on the Arg@MNCs and (b) In vitro 5-FU release profile from Arg@MNCs at 37 °C.

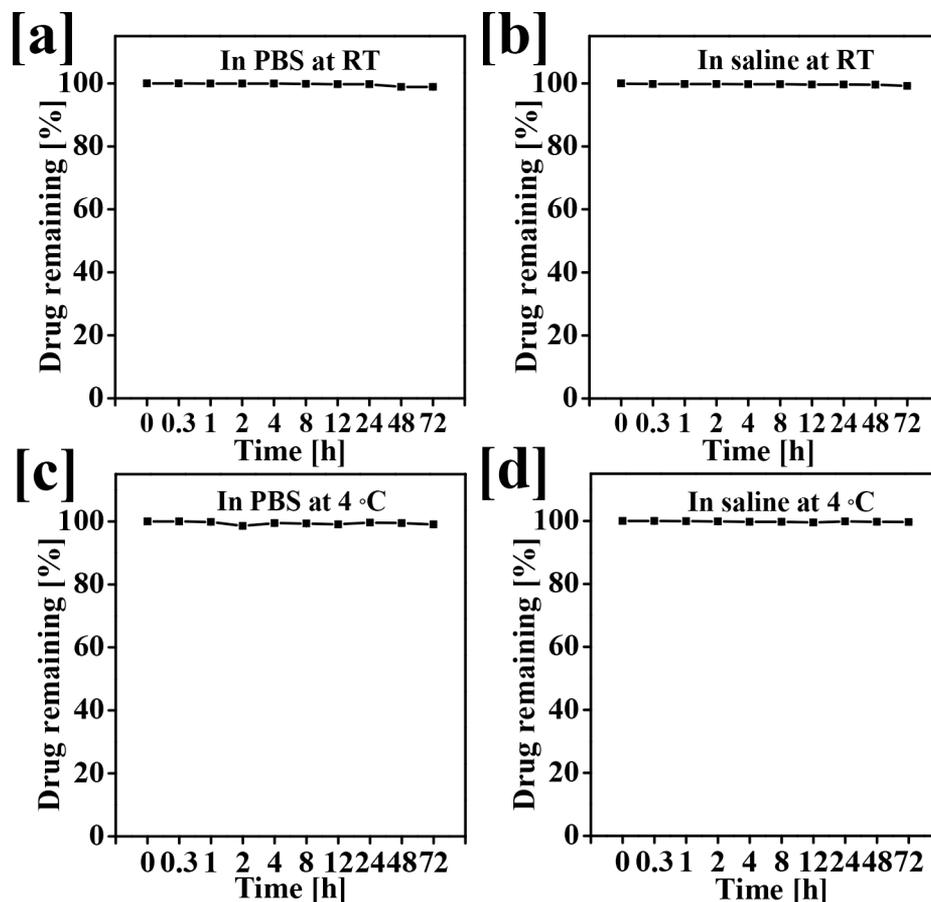


Figure 8. (a–d) Stability of 5-FU-loaded Arg@MNCs in phosphate buffer saline and normal saline at room and refrigeration temperature.

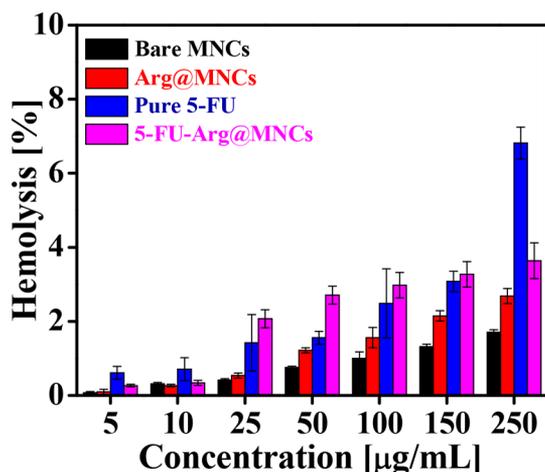


Figure 9. Hemocompatibility studies of bare manganese oxide nanocuboids (MNCs), arginine-modified MNCs (Arg@MNCs), pure 5-fluorouracil (5-FU), and 5-fluorouracil-loaded arginine-modified manganese oxide nanocuboids (5-FU-Arg@MNCs) at concentrations of 5, 10, 25, 50, 100, 150, and 250 $\mu\text{g/mL}$.

also did not show any hysteresis, indicating that Arg@MNCs exhibited paramagnetic properties at room temperature.

3.4. Biodegradation of MNCs. After incubation of MNCs and Arg@MNCs in PBS pH 7.4, SAB pH 5.0, and 50% FBS, the observed Mn content at different time intervals are depicted in Figure 6. As compared to PBS and 50% FBS, the bare MNCs and Arg@MNCs exhibited faster degradation in SAB. About $89 \pm$

2.6% and $77.7 \pm 1.6\%$ of MNCs and Arg@MNCs were degraded in 24 h in SAB, respectively. On the other hand, about $2 \pm 1.1\%$ and $2.7 \pm 0.9\%$ in PBS or $0.33 \pm 0.2\%$ and $0.75 \pm 0.4\%$ degradation in FBS were observed for MNCs and Arg@MNCs, respectively. This higher degradation of MNCs and Arg@MNCs in SAB reflects the diffusion of Mn^{2+} ions under the acidic condition. On the other side, the slow degradation of MNCs and Arg@MNCs in PBS and FBS showed the stability of MNCs at blood pH.^{53,54} It suggested that these nanostructures may remain stable during the circulation in the blood but immediately start to degrade after reaching to the endosomes or lysosomes of the cells. Hence, the Arg@MNCs could improve the blood circulation time of the developed drug-loaded nanocarrier.

3.5. 5-FU Loading Efficiency and In Vitro Release Study of Arg@MNCs. Figure 7a shows the drug loading efficiency of Arg@MNCs, where the loading of 5-FU was optimized by increasing the drug concentration while maintaining constant Arg@MNC concentration. The %LE was increased with the increase in 5-FU concentration up to 5:2 w/w of Arg@MNCs/drug. The highest loading of 5-FU was found to be about $11.2 \pm 0.2\%$. An in vitro release of 5-FU from Arg@MNCs in PBS and SAB is shown in Figure 7b. In PBS, the 5-FU was released about 1.6% in the first 6 h, whereas about 20% of 5-FU was released in SAB for the same period. This pH-dependent difference in the release of 5-FU from Arg@MNCs was continued in subsequent determinations. After 24 h, about 38% of 5-FU was released in SAB in comparison to only 3% release in PBS. The stronger hydrogen bonding between 5-FU and Arg@MNCs in PBS may be responsible for the slow drug

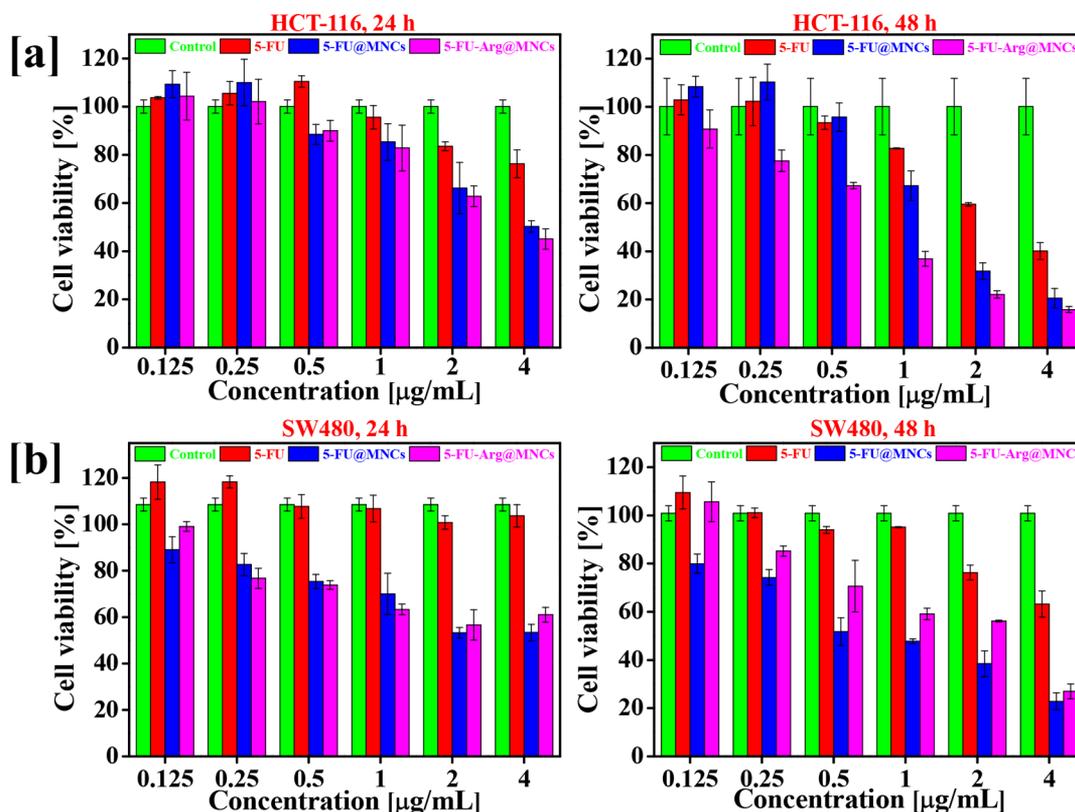


Figure 10. Effect of 5-FU, 5-FU@MNCs, and 5-FU-Arg@MNCs on cell viability in (a) HCT 116 and (b) SW480 cell lines. Cells were treated with different concentrations of 5-FU (0.125–4 µg/mL), 5-FU-loaded Mn₃O₄ nanocuboids (5-FU@MNCs) (0.125–4 µg/mL), and 5-FU-loaded arginine-functionalized Mn₃O₄ nanocuboids (5-FU-Arg@MNCs) (0.125–4 µg/mL) for 24 and 48 h.

Table 2. Half-Maximal Inhibitory Concentration Values of 5-Fluorouracil (5-FU), 5-FU-Loaded Mn₃O₄ Nanocuboids (5-FU@MNCs), and 5-FU-Loaded Arginine-Functionalized Mn₃O₄ Nanocuboids (5-FU-Arg@MNCs) against Colon Cancer Cell Lines HCT 116 and SW480 after 24 and 48 h Incubation

samples	HCT 116		SW480	
	24 h	48 h	24 h	48 h
5-FU		2.84 ± 0.14	5.93 ± 0.15	
5-FU@MNCs	3.74 ± 1.23	1.63 ± 0.12	4.73 ± 0.98	0.82 ± 0.13
5-FU-Arg@MNCs	3.17 ± 0.23	0.077 ± 0.06	4.38 ± 0.90	1.16 ± 0.09

release. The higher release of 5-FU in SAB suggested the weak ionic interaction between 5-FU and Arg@MNCs, and it may be beneficial for the intracellular delivering of the drug.

3.6. Stability of 5-FU-Loaded Arg@MNCs. The physicochemical or colloidal stability of a drug-loaded nanoformulation is an important parameter for its clinical and practical application. Figure 8a–d shows the stability of 5-FU on the Arg@MNCs. The 5-FU content was measured up to 72 h. About 99% of 5-FU content was found in the formulation in both PBS and normal saline at room and refrigeration temperature. The observed results suggested that Arg@MNCs is a highly stable system and serves as an excellent carrier for the 5-FU in both conditions.⁵⁵ This stability of 5-FU in Arg@MNCs could be attributed to the strong electrostatic interaction between drug and Arg@MNCs, therefore showing resistivity toward drug loss.

3.7. Hemocompatibility Study. A hemocompatibility study was performed to evaluate the toxic effect of prepared nanoparticles to RBCs.⁵⁶ Hemolytic toxicities of bare MNCs, Arg@MNCs, pure 5-FU, and 5-FU-Arg@MNCs after incubation with RBCs are shown in Figure 9. Up to 5% hemolysis is considered a safe or nontoxic level.^{57,58} The bare MNCs ($1.7 \pm 0.1\%$) and Arg@MNCs ($2.7 \pm 0.2\%$) did not show any hemolysis. Pure 5-FU exhibited slight hemolysis, i.e., $7 \pm 0.4\%$, at higher concentration, whereas hemolysis caused by 5-FU-loaded Arg@MNCs was negligible ($3.6 \pm 0.5\%$ hemolysis) at all tested concentrations. These results indicated that bare MNCs, Arg@MNCs, and 5-FU-loaded Arg@MNCs had excellent hemocompatibility and could be used for the pharmaceutical applications. Importantly, it can also be noted that the toxicity of 5-FU was decreased after loading into the Arg@MNCs. It can be explained by the interaction of 5-FU with Arg@MNCs and then slow release of loaded 5-FU. Thus, the loaded 5-FU is not available for the interaction with RBCs.

3.8. Induction of Cytotoxicity. In vitro anticancer activity of free 5-FU and 5-FU-loaded MNCs and 5-FU-Arg@MNCs was investigated against SW480 and HCT 116 human colon cancer cells. The cell viability results for cells treated with free 5-FU, 5-FU@MNCs, and 5-FU-Arg@MNCs at a concentration range from 0.125 to 4 µg/mL for 24 and 48 h are presented in Figure 10. Cell viability of both the cancer cells was reduced significantly after treatment with drug-loaded MNCs than for free 5-FU. The observed IC₅₀ values of 5-FU@MNCs and 5-FU-Arg@MNCs against SW480 cells were 4.73 and 4.38 µg/mL, respectively (Table 2). Free 5-FU did not show cytotoxicity up to 24 h. After 48 h of treatment, the IC₅₀ value for free 5-FU, 5-FU@MNCs, and 5-FU-Arg@MNCs were 5.93, 0.82, and 1.16

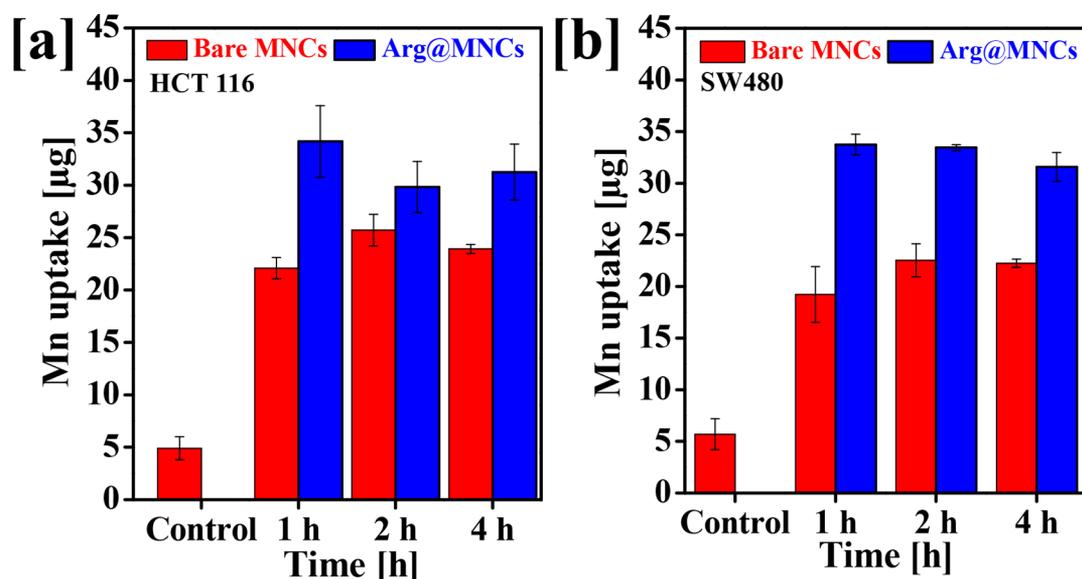


Figure 11. Cellular uptake study of bare MNCs and Arg@MNCs in (a) HCT 116 cell line and (b) SW480 cell line. Cells were incubated for 1, 2, and 4 h and untreated cells were left as control.

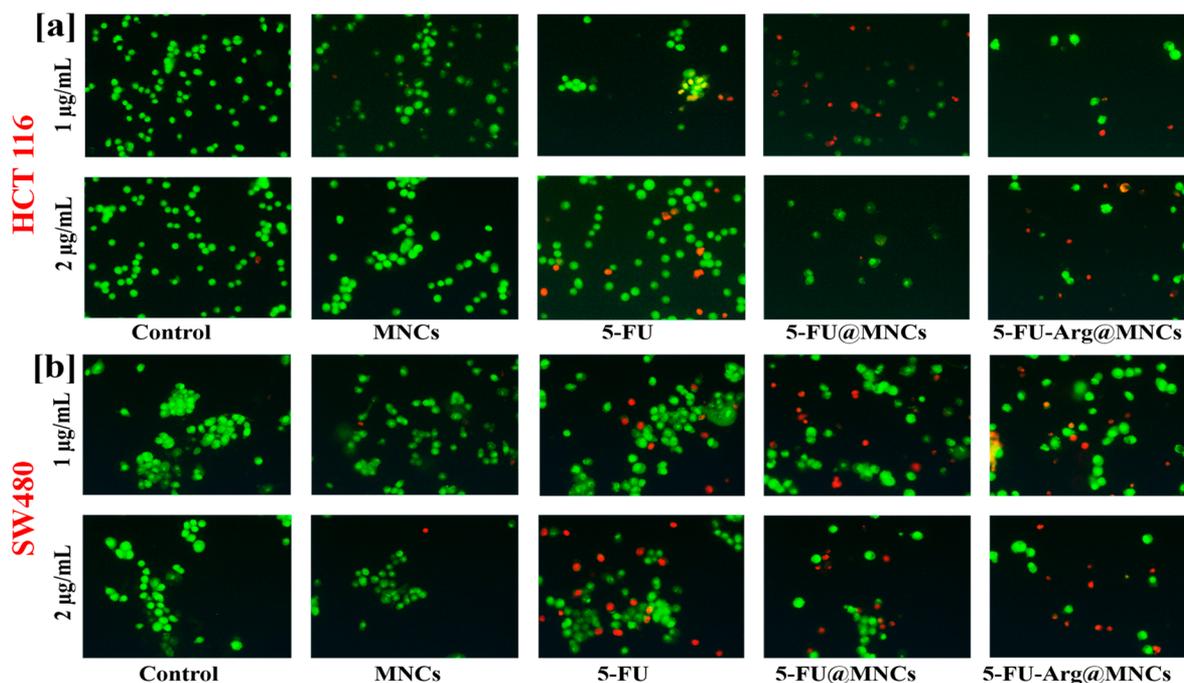


Figure 12. Acridine orange-ethidium bromide assay: (a) against HCT 116 cells and (b) against SW480 cells. Cells were treated with different concentrations of 5-FU (1 and 2 $\mu\text{g/mL}$), blank Mn_3O_4 nanocuboids (MNCs) (1 and 2 $\mu\text{g/mL}$), 5-FU-loaded Mn_3O_4 nanocuboids (5-FU@MNCs) (1 and 2 $\mu\text{g/mL}$), and 5-FU-loaded arginine-coated Mn_3O_4 nanocuboids (5-FU-Arg@MNCs) (1 and 2 $\mu\text{g/mL}$) for 24 h.

$\mu\text{g/mL}$, respectively. The insignificant difference between the IC_{50} value of the 5-FU@MNC and 5-FU-Arg@MNC systems suggested that both systems had the same cytotoxicity against SW480 cells.^{59,60} Comparatively, after 24 h, the IC_{50} values for pure 5-FU, 5-FU@MNCs, and 5-FU-Arg@MNCs against HCT 116 cells were 8.27, 3.74, and 3.17 $\mu\text{g/mL}$, respectively. After 48 h of treatment, the IC_{50} values for free 5-FU, 5-FU@MNCs, and 5-FU-Arg@MNCs were 2.81, 1.63, and 0.77 $\mu\text{g/mL}$, respectively.^{59,61} These results suggest that 5-FU-Arg@MNCs were more effective than free 5-FU (3.6 times) or 5-FU@MNCs (2.1 times). It further indicates that Arg-functionalized MNCs

with a combination of 5-FU was more pronounced to HCT 116 cells than SW480 cells.

3.9. Cellular Uptake Study. To further investigate the role of Arg@MNCs as an in vitro drug delivery vehicle, we incubated cells with bare MNCs or Arg@MNCs ($10 \mu\text{g mL}^{-1}$) and uptake of these nanocarriers by HCT 116 and SW480 human colon cancer cells were investigated. The cells were digested after 1, 2, and 4 h of incubation and processed for AAS analysis to quantify Mn concentration.^{62,63} The Mn ions amount was found to be approximately $22.1 \pm 1.0 \mu\text{g}$ and $24 \pm 0.4 \mu\text{g}$ in HCT 116 cells (Figure 11a) and $19.2 \pm 2.7 \mu\text{g}$ and $22.24 \pm 0.4 \mu\text{g}$ in SW480 cells (Figure 11b) after 1 and 4 h of incubation, respectively,

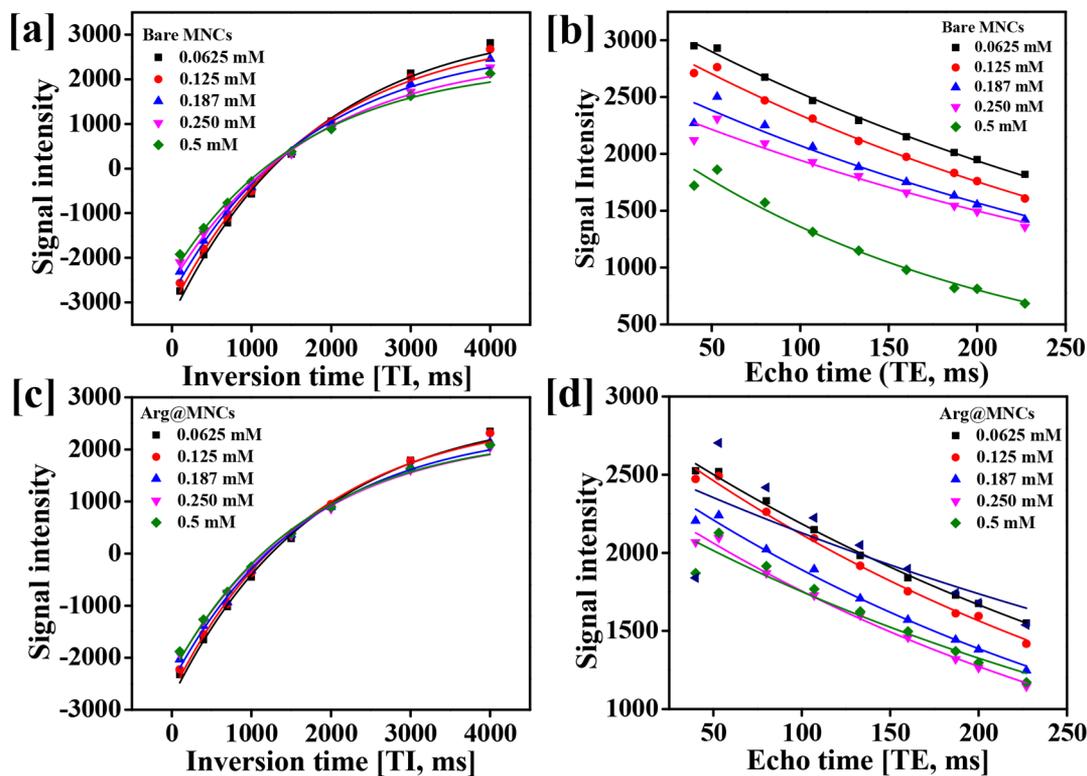


Figure 13. Plots of (a, c) longitudinal [T_1] and (b, d) transverse [T_2] relaxation signal intensities as a function of inversion time and echo time for aqueous dispersion of bare MNCs and Arg@MNCs.

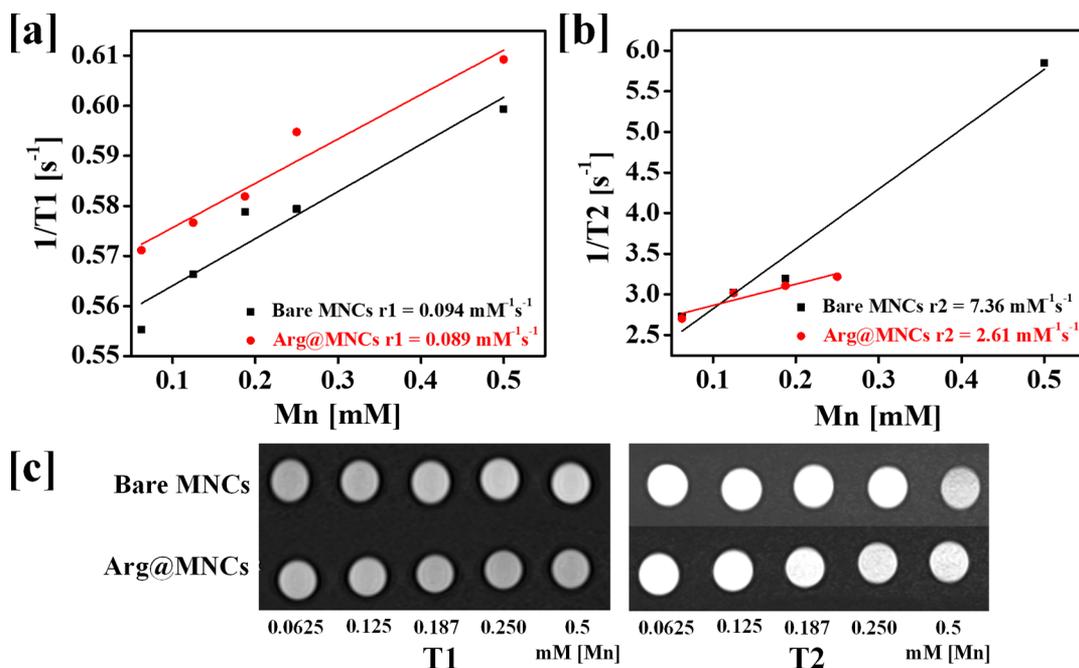


Figure 14. Plots of (a) T_1 and (b) T_2 versus Mn concentration for bare and Arg@MNCs. (c) T_1 -weighted MR images (left) and T_2 -weighted MR images (right) of the MNC and Arg@MNC aqueous solutions at various Mn concentrations.

with the bare MNCs. The observed Mn ions amount was higher in the cells incubated with Arg@MNCs. About $34.18 \pm 1.0 \mu\text{g}$ and $31.2 \pm 3.0 \mu\text{g}$ of Mn ions was found in HCT 116 cells after 1 and 4 h of incubation. Similarly, about $33.7 \pm 0.3 \mu\text{g}$ and $31.6 \pm 1.4 \mu\text{g}$ of Mn ion was found in SW480 after 1 and 4 h of incubation. Thus, after 4 h of incubation with Arg@MNCs, approximately 1.2- and 1.4-times higher Mn ions were observed

in HCT 116 cells and SW480 cells, respectively, than those incubated with bare MNCs. The increased uptake of bare MNCs ($ZP = -9.02 \pm 0.74$) and Arg@MNCs ($ZP = -7.37 \pm 0.53$) was due to the interaction with the negatively surface charged cell membrane. Our results suggested that Arg coating on the surface of Mn_3O_4 NCs may be also responsible for internalization inside

the cell membrane and simultaneous delivery of 5-FU nearby cancerous cells.

3.10. Induction of Cellular Apoptosis by 5-FU, MNCs, 5-FU@MNCs, and 5-FU-Arg@MNCs. Figure 12 shows fluorescent images of SW480 and HCT 116 cells after treating with 1 and 2 $\mu\text{g}/\text{mL}$ of 5-FU, MNCs, 5-FU@MNCs, or 5-FU-Arg@MNCs. For the determination of cell-death mechanism, the cells were incubated with DNA intercalating dyes viz. AO/EB. AO binds to double standard DNA and emits green fluorescence, but EB emits orange-yellow fluorescence only after entering into the membrane compromised cells.^{63,64} Control or untreated cells showed normal morphology with intense green fluorescence. Cells treated with free or pure 5-FU had almost negligible apoptosis to the SW480 cells and a marginal effect on the HCT 116 cells. Blank nanocuboids were also studied to examine the apoptosis induced by NCs alone.

No apoptotic sign was observed in two colon cancer cell lines after treatment with bare NCs. However, a combination with Arg@MNCs, 5-FU increased the apoptotic cells in HCT 116 cancer cells than SW480 cells. It indicated that the apoptosis induced by the drug loaded NCs was due to the improved delivery of 5-FU into the cells by MNCs. As evident from Figure 12a, b, HCT 116 and SW480 cells treated with 1 $\mu\text{g}/\text{mL}$ of 5-FU@MNCs or 5-FU-Arg@MNCs showed both green and orange fluorescent nuclei due to presence of early and late apoptosis cells. However, the cell treated with higher concentration (2 $\mu\text{g}/\text{mL}$) showed a greater number of late apoptosis cells and observed that a greater number of apoptosis characteristic such as apoptotic body formation, nuclear fragmentation and also few necrotic cells observed as cell blebbing. On the other hand, the untreated cells exhibited spherical and intact nuclei.

3.11. In Vitro MRI imaging. Figure 13a–d show the longitudinal and transverse MRI signal intensities as a function of inversion time and echo time, respectively, for aqueous dispersions of bare MNCs and Arg@MNCs at various Mn concentrations. The longitudinal MR signal intensities of aqueous dispersion were increased and transverse MR signal intensities were decreased with increase in Mn concentration. These signal intensities were fitted to the nonlinear least-squares regression eqs 1 and 2 to determine the respective longitudinal (T_1) and transverse (T_2) times, respectively. To investigate the contrast enhancement of bare MNCs and Arg@MNCs, longitudinal relaxivity r_1 and transverse relaxivity r_2 of bare MNCs and Arg@MNCs were calculated from the linear fitting of $1/T_1$ or $1/T_2$ plot against Mn (mM) concentrations (Figure 14a, b). The values of r_1 were estimated to be 0.094 and 0.089 $\text{mM}^{-1} \text{s}^{-1}$ for bare and coated MNCs or values of r_2 were estimated to be 7.35 and 2.16 $\text{mM}^{-1} \text{s}^{-1}$, respectively,^{65–67} for bare and Arg@MNCs. As shown in Figure 14c, bare and coated MNCs showed excellent T_1 and T_2 contrast enhancement. T_1 -weighted images exhibit bright contrast increment with an increase in the concentration of bare NPs, whereas a decay of signal intensity was observed in T_2 -weighted images.

4. CONCLUSIONS

In the present studies, we have successfully developed a theranostics nanocarrier system by functionalizing Mn_3O_4 nanocuboids with arginine amino acid. The Arg-modified MNCs were evaluated for loading of a hydrophilic anticancer drug 5-FU and for delivering loaded drug to human colon cancer cells. The pH-dependent degradation of MNCs could be useful in the selective delivery of the drugs to cancer cells. In

hemocompatibility studies, Arg@MNCs were not only nontoxic but also decreased the toxicity of 5-FU. The in vitro anticancer studies against human colon cancer cells indicated that the delivery of 5-FU by Arg@MNCs was superior as compared to the pure 5-FU solution. The unique feature of this system was the presence of Arg, which acts as a multifunctional agent. The Arg not only stabilized the MNCs in physiological solutions, but also controlled the release of drug and improved its delivery to the human cancer cells. Finally, Arg@MNCs also showed high relaxivity during MRI imaging and could therefore be an effective tool in diagnosis.

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Notes

The authors declare no competing financial interest.

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Molecular insights of metastasis and cancer progression derived using 3D cancer spheroid co-culture *in vitro* platform

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ABSTRACT

The multistep metastasis process is carried out by the combinatorial effect of the stromal cells and the cancerous cells and plays vital role in the cancer progression. The scaffold/physical cues aided 3D cancer spheroid imitates the spatiotemporal organization and physiological properties of the tumor. Understanding the role of the key players in different stages of metastasis, the molecular cross-talk between the stromal cells and the cancer cells contributing in the advancement of the metastasis through 3D cancer spheroid co-culture *in vitro* platform is the center of discussion in the present review. This state-of-art *in vitro* platform utilized to study the cancer cell host defence and the role of exosomes in the cross talk leading to cancer progression has been critically examined here. 3D cancer spheroid co-culture technique is the promising next-generation *in vitro* approach for exploring potent treatments and personalized medicines to combat cancer metastasis leading to cancer progression.

1. Introduction

Cancer is considered one of the most fatal diseases. The deadly characteristic attributed to the cancer is its potential for metastasis. The choice of a secondary metastatic site doesn't happen randomly. The malignant cell could self-facilitate meaning to a metaphor of favorable soil for the seed in the "seed and soil" hypothesis proposed by Stephen Paget (Langley and Fidler, 2011). Generally, the initialization of the metastasis process could be the result of genetic (Lara et al., 2020) or epigenetic (Galle et al., 2020) trigger in most cases. Furthermore, compatible niche assisted by the tumor microenvironment (TME) (Hill et al., 2020), signaling pathway mutations (Chang et al., 2020a; Porcheri and Mitsiadis, 2020), suppression of the inhibitory mechanism (Waku et al., 2020), evading immune system regulation (Wu et al., 2020) all these comprehensively assist the cancer cell metastasis and growth of the tumor at the secondary site. The mechanism of cancer metastasis comprises mainly 6 steps: 1) Invasion and Migration, 2) Angiogenesis and Intravasation, 3) Survival from the host defence mechanism during circulation, 4) Extravasation, and 5) Colonization and 6) Advancement of the secondary tumor (Hapach et al., 2019). These steps are depicted below in Fig. 1. In the whole process, many molecular mechanisms and signaling cascades are involved which will be further elucidated in detail wherever relevant for better and in-depth understanding.

In vitro approaches like co-culture or co-cultivation involves the use

of two or more cell types that are grown together or culture together for multiple purposes. The co-culture techniques are reported to study drug resistance (Xin et al., 2019; Wei et al., 2019) autocrine and paracrine signaling, cell-cell interaction, cell migration, the effect of the micro-environment (Wu et al., 2020; Pausch et al., 2020; Lugo-Cintrón et al., 2020). Cancer cell also behaves differentially when grew in co-culture condition. For example when human pancreatic stellate cells (hPSC) co-cultured with a pancreatic cancer cell (Panc-1), increased expression of KI-67, fibronectin, Smooth Muscle Actin (a-SMA) was observed. This suggested proliferation and activation of stellate cells as a result of cross-talk between hPSC and Panc-1 in the co-culture system. Lack of E-cadherin (an epithelial marker) in Panc1 cell spheroid indicated a loss of epithelial character and induction of Epithelial to Mesenchymal Transition (EMT) in co-culture (Norberg et al., 2020). These studies demonstrated the potential of co-culture and its relevance in recreating the TME.

Co-culture can be classified as, direct and indirect co-culture (Kook et al., 2017). Direct co-culture, involves the growth of one type of cell on a monolayer formed by another cell (as shown in Fig. 2a-II), 3D spheroid formation by mixing two different cell types (as shown in Fig. 2c) (Norberg et al., 2020), and one cell type forming monolayer while other cell type forms spheroid on it (Kook et al., 2017).

Indirect co-culture can be subdivided into the use of conditioned media or transwell system. In co-culture involving conditioned media,

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two different cell types are grown separately and the conditioned media of one cell type is used to study its effect on the other type of cell population (As shown in Fig. 2b-II). It allows studying the effect of soluble secreted factors including growth inducers and cytokines. However, the lack of direct cellular contact influences the contact-based cell fate process. Transwell-based co-culture (As shown in Fig. 2I & III) includes a chamber with a defined pore size membrane where one cell type is grown at the bottom chamber and the other on the upper chamber on a semi-permeable membrane (Truong et al., 2016). Indeed the existing co-culture techniques can be improvised by incorporating the concept of 3D culture either with a scaffold or without a scaffold.

For the sake of advancement in cancer biology research, the limitations of two-dimensional (2D) cell culture led to the implementation of three dimensional (3D) cell culture system. In 3D cell culture, cells are induced to be present in different morphology depending upon the substrate or the environment it is present in. As observed through many types of research, the 3D cell culture facilitates cancer cells to be in similar cellular topography, gene expression, metabolism, and signaling alike to the cancer cell in the physiological condition (Nanki et al., 2020; Jacob et al., 2020). It is found that in many research articles there are different nomenclature used for 3D spheroids and have been used sometimes interchangeably. Hence, it is advisable to define and use the terms according to certain criteria for accurate representation. Hereby we postulate certain nomenclature with their probable definition hoping to define their usage in an accurate manner in Table 1. This kind of particular nomenclature pattern if followed it will be easy and clear to the reader and researcher about the origin, composition, and what that 3D cell aggregate represented in the experiment.

The 3D spheroid culture has been trending and accepted as an *in vitro* model nearest to the *in vivo* micro tumors in context to the spatial organization, physiological characteristics, the micro-environmental factors, and the molecular blueprint of *in vivo* micro tumors. Thus, the 3D spheroid recapitulates the nodal structure of solid tumors by mimicking various such aspects. 3D spheroids can mimic the nutrient gradient present in the *in vivo* tumors in which monolayer cultures cannot recapitulate which was demonstrated in an amino acid uptake study conducted on spheroids compared to monolayer culture (Pawlik et al., 2000). Studies have also demonstrated the heterogeneous zonal cell

population in the tumor (Pawlik et al., 2000). Also, the spheroids replicate pH gradient, with a lower pH core and periphery showing little high pH and the hypoxic condition alike to the *in vivo* micro tumors (Al-Husari et al., 2014; Langan et al., 2016; Mukomoto et al., 2021). The drug resistance is also co-related to the pH gradient in the tumor (Swietach et al., 2008; Evans et al., 2011; Hulikova et al., 2011; Shirmanova et al., 2015; Zagaynova et al., 2017). Heterogeneous population, hypoxic condition, and pH gradient all together mimic the avascularized tumor. This interaction is shown in Fig. 3, where various stromal cells interact with tumor cells in the TME.

The interaction of the tumor-associated stromal cells (TASCs) and the cancer cell is all that creates this deadly play named 'cancer progression'. Thus, it is very important to study and elucidate various aspects minutely involved in this mechanism to find different diagnostic and therapeutic strategies to fight cancer. Co-culturing these TASCs with the cancer cell in the spheroid form of the specific tissue in turn attempting to mimic TME and tissue stiffness *in vitro* like the *in vivo* condition could help to understand the exact scenario which imposes and facilitates the cancer progression. One such study also demonstrated the same, as shown in Fig. 4, where spheroid-based triple co-culture of PANC-1 (Pancreatic cancer cell): MRC-5 (fibroblast): Human umbilical vein endothelial cells (HUVEC) were directly co-cultured together as a spheroid. In this multicellular spheroid, HUVEC is present in the center of the spheroid, while the fibroblast is in the core. Pancreatic cancer cells were homogeneously distributed in the spheroid (Lazzari et al., 2018). This spheroid co-culture mimic the complex heterogeneity of tumor in the tumor microenvironment.

The molecular insights derived using 3D cancer spheroid co-cultured with the stromal cells and/or immune cells *in vitro* contributing to cancer metastasis have been meticulously discussed ahead in this review. Cancer progression endowed in each crucial step of metastasis elucidated through 3D cancer spheroid co-culture system has been further reviewed in-depth.

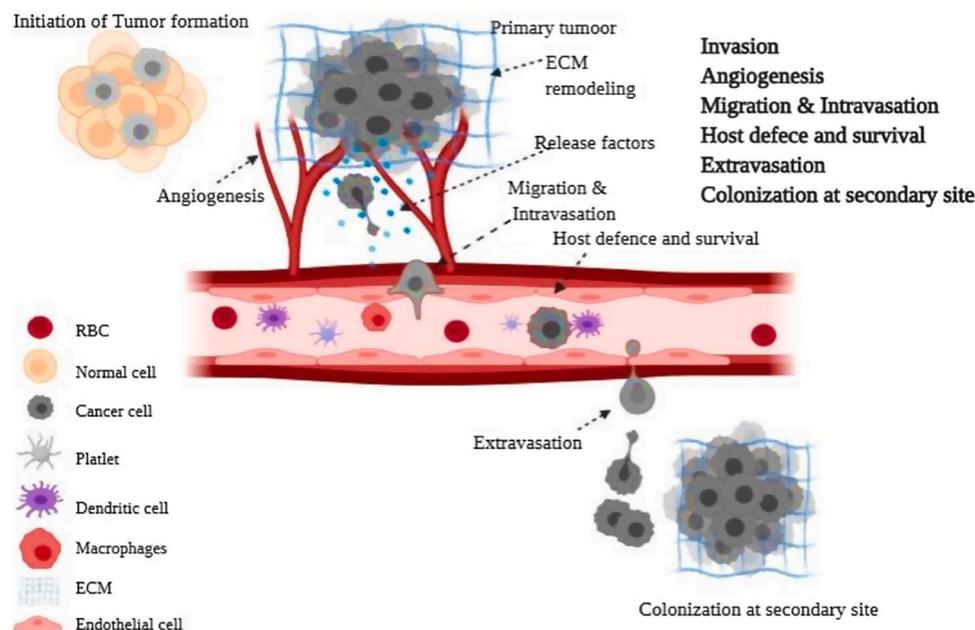


Fig. 1. The Schematic describes the stages involved in the metastasis process; Invasion and migration, Angiogenesis, Intravasation, Host defence and survival, Extravasation, Colonization at secondary site. In the process of metastasis there are many stromal and tumor associated cells which facilitates the process of metastasis.

2. Co-culture of cancer spheroids to study vascularization/angiogenesis

2.1. Vascularization and its role in cancer progression

Tumor proliferation and growth led to increment in the size of the tumor. Increase in the tumor size, limit the nutrient and oxygen supply to the tumor cells which in turn hinders its further growth and proliferation. This leads to the activation of signals that promote neo-vascularization in the tumor. This avascular to vascular phase transition in tumor is often known as “angiogenic switch”. The formation of new blood vessels provide an adequate supply of nutrients as well as oxygen, which are the basic requirement for tumor growth. Various signals from the tumor microenvironment that promote angiogenesis are Vascular endothelial growth factor (VEGF), Fibroblast growth factor (FGF), Hypoxia inducible factor-alpha (HIF-1 α), Platelet-derived growth factor-beta (PDGF- β), Tumor necrosis factor-alpha (TNF- α), and angiopoietin. (Joo et al., 2019; Semenza, 2003; Goel and Mercurio, 2013).

Angiogenesis can be induced by a balance between pro-angiogenic and anti-angiogenic factors. Pro-angiogenic factors are the one which induces an angiogenic switch to form neovascularization (Kazerounian and Lawler, 2018). Cancer cells adapt different strategies to form new blood vessels. Such strategies are angiogenesis, vascular co-option, vasculogenesis, and vasculogenic mimicry (Zuazo-Gaztelu and Casanovas, 2018). Out of these strategies, most studies have explored angiogenesis through an angiogenic switch. Whenever proangiogenic molecules are higher or anti-angiogenic molecules are lower, an angiogenic switch is triggered leading to neovascularization in the tumor. In this switch the cancer cells secrete VEGF (Goel and Mercurio, 2013; Zheng et al., 2006) and FGF in the tumor microenvironment. On the other hand, the endothelial cells of normal blood vessels express the receptor for VEGF. Also, Perivascular cells that surround the endothelial cells (EC) get detached and thus, exposes the basement membrane. Altogether, the above mentioned mechanisms disrupt the normal vessel integrity or form leaky vasculature. This combinatorically further promotes angiogenesis (Zheng et al., 2006; Weis and Cheresh, 2011).

In a study reported by Thomas et al., the interaction between a cancer cell and fibroblast and its influence over angiogenesis was demonstrated. The condition media from Normal Human Dermal

Table 1

Postulated nomenclature for *in vitro* spheroids and their probable definitions.

No.	Nomenclature	Postulated definition	References
1.	Spheroids	A 3D cellular aggregate formed of any type of cells showing compactness that has altogether a spherical morphology. It is better to say it as “ <u>cancer spheroid</u> ” to define that the spheroid consists of cancerous cells.	(Han et al., 2020a; Theard et al., 2020; Rodríguez-Dorantes et al., 2021)
2.	Multicellular tumor spheroids (MCTS)	They are similar to spheroid, but it additionally defines that there are multiple types of cells in the spheroid representing the different cells present in <i>in vivo</i> TME.	(Lazzari et al., 2018; Lee et al., 2018)
3.	Organoids	The term can be specifically coded to 3D spheroid formed from the cells from the patient-derived tissue section. More precisely if it is a tissue section from a tumor of a particular organ e.g., liver or lung adenocarcinoma it can be stated as “Liver cancer organoid or lung adenocarcinoma organoid”.	(Schnalzer et al., 2019; Rosenbluth et al., 2020; Dijkstra et al., 2020)
4.	Tumoroids	3D cancer cell aggregate having shape other than spherical e.g., grape shaped or irregular shaped aggregates, representing tumor of specific tissue/organ.	(Wang et al., 2020a; Karakasheva et al., 2018; Lee et al., 2019)

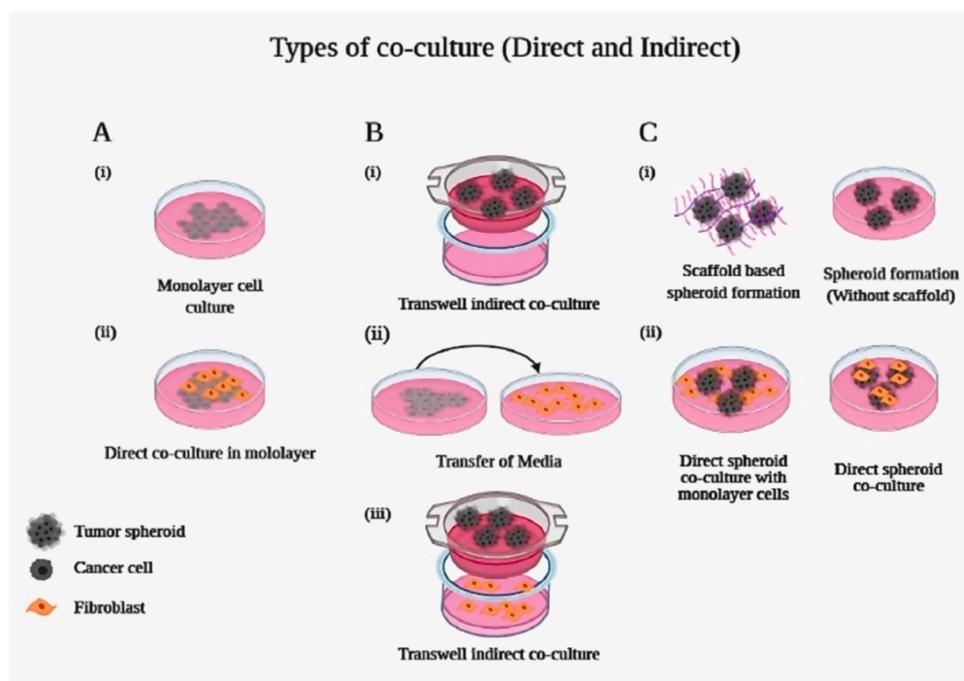


Fig. 2. Schematic diagram describing the different co-culture methods. (a) (i) Monolayer cell culture (ii) Direct co-culture of more than one cell type. (b) (i) Spheroid culture on upper chamber of transwell (ii) Conditioned media derived from one cell type used treat on another cell type (iii) Spheroid culture on the upper chamber of the transwell co-cultured with another type of cells cultured on the lower chamber of the transwell-indirect co-culture. (c) (i) Scaffold based spheroid formation and spheroid formation without scaffold (ii) Spheroids directly co-cultured with another type of cells and spheroid co-culture of cancer cells along with stromal cells.

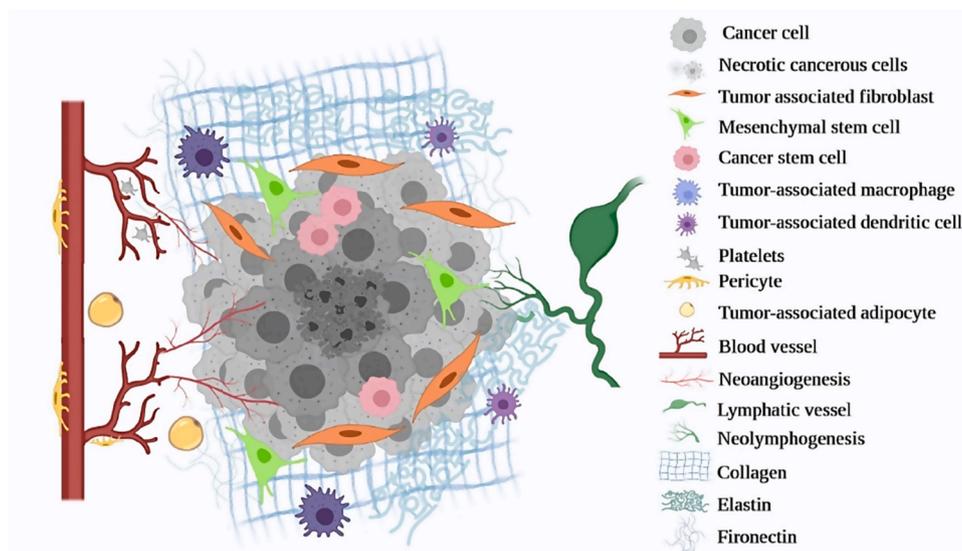


Fig. 3. The schematic diagram shows the stromal cells and other tumor associated cells interacting with the cancer cells in the tumor microenvironment.

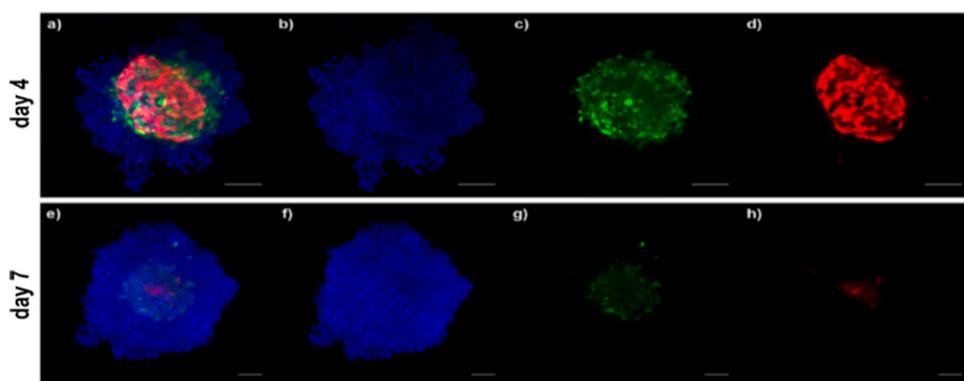


Fig. 4. SPIM 3D topography of MCTS, the triple co-culture (PANC-1; MRC-5; HUVEC) at day 4 and day 7. (a, e) Overlay of blue (Hoechst 33342, nuclei), green (GFP-expressing MRC-5 fibroblasts) and red (RFP-expressing HUVECs) fluorescence; (b, f) single blue channel (Excitation/emission 405/440 nm) showing all cell nuclei; (c, g) single green channel (Excitation/emission 488/525 nm) showing GFP-expressing MRC-5 fibroblasts; (d, h) single red channel (Excitation/emission 561/605 nm) with RFP-expressing HUVEC cells (Lazzari et al., 2018) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Fibroblast (NHDF), T3M4, and co-culture (T3M4, NHDF) were used to culture HUVEC cells. HUVEC grown in co-culture conditioned media demonstrated a two-fold angiogenic effect, longer branches/tube formation as compared to controls where starving media and VEGF were provided externally. This study suggests that cancer cells and fibroblast did not increase angiogenesis, instead cancer cell-activated fibroblast influence angiogenesis. From secretome analysis of co-culture media, pure expression of chemokine (C-C motif) ligand 2 (CCL2) and C-X-C Motif Chemokine Ligand 8 (CXCL8) was observed. Further, the CCL2 and CXCL8 expressions were found to be contributed by the fibroblasts (Pausch et al., 2020). WNT2 (Wingless type MMTV integration site family member expressed by CAF enhances angiogenesis by affecting endothelial cells. Co-culture with WNT2 overexpressing fibroblast BJ1 and CAF individually with HUVEC formed larger vessels, increased vessel area, and branch point than control (Daniela et al., 2020).

Macrophages also enhance the angiogenic potential of tumor cells. A recent study co-cultured adipocyte with the macrophages to demonstrate a mechanism called immunomodulation (Yadav et al., 2020). The treatment of leptin, insulin, IL-6, and TNF- α increases VEGF levels in the J774.A1 and THP-1 (human monocytic cell) macrophages. Co-culture with adipocytes enhances IL-8, Serpin E1/PAI1, Matrix metalloproteinase 9 (MMP9), Pentraxin3 (PTX3), pro-metastasis, and pro-angiogenic protein expression. Increased tube formation was observed in HUVEC cells, when co-cultured condition media was supplied, as compared to the macrophages condition media (Yadav et al., 2020). A study was done where the role of synthesized peptide (JP3) on

angiogenesis was demonstrated. The JP3 peptide inhibits the angiogenesis in the tumor. An anti-angiogenic peptic JP3 treated Gastric cancer (GC) cells condition medium inhibits tube formation *in vitro*. JP3 ubiquitinylate K610 amino acid in SP1 protein, followed by its degradation. Co-immunoprecipitation reveals the interaction between TRIM25 and SP1. JP3 phosphorylation decreases TRIM25 ubiquitination, thus increases its stability. JP3 treatment induces TRIM25 phosphorylation which further degrades SP1 by ubiquitinylation at K610 position, thus inhibiting MMP2 transcription and further action. JP3 indirectly inhibits angiogenesis. JP3 treatment decreases tumor progression *via* inhibiting angiogenesis (Chen et al., 2020a). Cancer associated fibroblast and macrophages promote tumor angiogenesis and tumor progression. Inhibiting angiogenesis by inhibitors or drugs can be a better therapeutic option for cancer treatment. Angiogenesis is the first step towards promoting invasion and migration of tumor as well as providing nutrient and oxygen to the cancer cell driving towards cancer progression.

2.2. Co-culture of cancer spheroids to study vascularization

Angiogenesis in the tumor can be best viewed by a co-culture-based study. Angiogenesis involves or is initiated *via* the complex crosstalk of cancer cells with neighbouring cells like endothelial, fibroblast, and macrophages. In a study, the angiogenic potential was demonstrated by two types of co-culture spheroids: Mixed spheroid containing endothelial cells (ECs) with mesenchymal stromal cells (MSCs) and shell

spheroid containing spatially arranged ECs in the periphery of MSCs. Compared to homotypic MSC spheroid, the mixed co-culture spheroid exhibited more number of branches (51.3 ± 35.3 vs. 72.9 ± 18.9), total segment length ($2501.6 \pm 2337.7 \mu\text{m}$ vs. $5533.5 \pm 1679.5 \mu\text{m}$), branch length ($2265.3 \pm 1397.6 \mu\text{m}$ vs. $3523.1 \pm 836.7 \mu\text{m}$), number of segments (68.1 ± 60.6 vs. 124.4 ± 34.0). Mixed co-culture spheroid has higher segment length (2237.8 ± 242.0 and $829.3 \pm 139.8 \mu\text{m}$), a number of segments (50.5 ± 11.0 and 19.3 ± 5.2) than shell co-culture spheroid. siRNA silencing of NOTCH3 lowers the sprouting potential of MSC in the mixed spheroid than control (spheroid were not treated with siRNA) (Vorwald et al., 2020). Thus, cell-cell communication was demonstrated by higher expression of NOTCH3 in mixed spheroid compared to shell spheroid.

In a study, the role of Ionizing radiation in the functioning of the endothelial cell was determined. Where Ionizing radiation (IR) exposure increases endothelial cell dysfunction and endothelial cell apoptosis. Caveolin (CAV-1) presence increased EC survival while survival pathway AKT was decreased in CAV1 deficient EC. Radiation increased cell death by activating the p38 MAPK pathway. IR irradiation was given to CAV-1 deficient EC, because of which sphingomyelinase (ASMase) level was increased. Ceramide was also accumulated upon IR treatment in CAV-1 negative EC. Radiation therapy to spheroid co-culture of prostate carcinoma cell (LNCaP) and CAV-1 deficient EC decreased growth and induced cell death. CAV-positive EC cells were resistant to ionizing radiation, CAV deficient cells were sensitive to it, and induced cell death by ASMase/ceramide lipid rafts in the cancer cell. CAV-positive EC, as well as cancer cells, were resistant to radiation therapy (Ketteler et al., 2020). Angiogenesis is the key component for tumor progression that also drives tumor metastasis. Knowledge regarding the angiogenesis mechanism or signaling cascade can be better known by spheroid co-culture *in vitro*. The use of specific inhibitors, drugs to target angiogenic mechanisms will be better elucidated by a co-culture-based study.

3. Co-culture of cancer spheroids to study invasion

3.1. Introduction to invasion and role in cancer progression

Invasion is the first step towards metastasis. Tumors at the primary site proliferate then the metastatic cell from the tumor invade the surrounding stroma by dissolution/breaking the basement membrane (Valastyan and Weinberg, 2011). Majorly the process of invasion can be described in three steps. The first step, where no membrane protrusion is observed and no invadopodia formation is observed. This step is marked as a non-invasive step where cells reside within the cellular mass. In second, which is also known as the invadopodium stage, the actin-rich invadopodia is formed. The third step, known as invasive can be characterized as the relocation of the leader cell nucleus and cells are observed to be invading the surrounding stroma by moving out from the cellular mass followed by ECM adhesion, its degradation by MMPs and proteases, leading to invasion (Hwang et al., 2019; Alfonso et al., 2017).

Cancer cell detaches from the primary tumor and invade as individual cell or undergo collective cell migration by initially indulging in phenotypic change - EMT or partial EMT and then through performing amoeboid movement or collective amoeboid migration. Factors such as Ras homolog family member A (RhoA) are important for single-cell amoeboid invasion. Also Rac, proteases, and integrin play key roles in mesenchymal single-cell migration. (Valastyan and Weinberg, 2011; Krakhmal et al., 2015). In the initial stage, the cells within the tumor are round in morphology, then after they change into an elongated morphology, and start to invade the stroma. Epidermal growth factor (EGF) is reported for increasing the invasion of MCF-7 cells (Truong et al., 2016). Cancer-associated fibroblasts present in the tumor stroma act as chemo-attractants which promotes the invasion of SUM-159 cells (Truong et al., 2016). Further two types of invasions, bottleneck and multiclonal were observed based on the clonal evolution in colorectal

cancer. Bottleneck represents the new tumor cells that arise within a tumor. These have higher metastatic potential and are invasive. Whereas, in multiclonal tumor cells have the potential for invasion irrespective of whether they arise earlier or late in the tumor (Ryser et al., 2020).

3.2. Co-culture of cancer spheroids to study invasion

As compared to monoculture spheroids, the invasive property of cells in co-culture spheroids is reported to be increased by 85 % (Hwang et al., 2019). The co-culture condition media delivers factors like IL-6, IL-8, CXCL1, Tissue inhibitor of metalloproteinases 1 (TIMP-1), Tissue inhibitor of metalloproteinases 2 (TIMP -2), VEGF-A, macrophage colony-stimulating factor (M-CSF), Granulocyte-macrophage colony-stimulating factor (GM-CSF) which play key roles in the invasion of the tumor cells. Indirect co-culture of pancreatic cancer cells (Panc-1) with pancreatic stellate cells increased α -SMA (1.88 fold) and Transforming growth factor-beta 1 (TGF- β 1) (1.47 fold) expression with spindle shape morphology of stellate cells suggesting activation of a pancreatic stellate cell into CAF. Collagen1 deposition increased with changes in fiber orientation at the outer boundary of a spheroid. Also, an increase in fibronectin expression altogether suggests invadopodia formation (Hwang et al., 2019). In another study, varied invasive potential of CAF subtypes was reported. Out of four CAF subtypes (CAF-S1, S2, S3, S4) identified in breast cancer lymph node metastasis, only two CAF-S1 and CAF-S4 invade more in the lymph node. CAF-S4 presence increases the invasion of MDA-MB-231 cells than CAF-S1 when investigated in the 3D culture system *via* promoting the NOTCH pathway (Pelon et al., 2020). IL-32 secreted from CAF binds to the arginine-glycine-aspartic acid (RGD) domain of integrin β 3 on breast cancer cells and activates p38 MAPK signaling leading to invasion and EMT. Normal fibroblast does not show this kind of invasion-promoting characteristics (Wen et al., 2019). Similar paracrine crosstalk between CAF and gastric cancer cells was observed. CAF secreted IL-33 binds to the ST2L receptor on gastric cancer (GC) cells and activates the ERK1/2 that in turn induces SP1 binding to ZEB2 promoter activating ZEB2. On the other hand, as a part of crosstalk GC releases TNF- α that binds to TNFR2 and activates NK-KB/IRF-1 signaling that leads to IL-33 secretion from CAF that further promotes invasion, migration, and EMT (Zhou et al., 2020).

Macrophages present in the tumor microenvironment also enhances the invasion of cancer cells. Co-culture of alternatively activated macrophages (AAM) and high-grade serous ovarian cancer (HGSO) cell line (OVCAR3, OVCA433, OV90) was performed to study the spheroid spreading/invasion, detachment from a primary tumor. AAM decreases the OVCAR3 spheroid spreading whereas increases the spreading of OV90. Ligands like FMS-like tyrosine kinase 3 ligand (FLT3L), Heparin-binding EGF-like growth factor (HB-EGF), IL-8, Leptin were secreted from AAM and its receptor found on the HGSO cell line were upregulated in presence of AAM. The influence of the AAM secreted ligands in turn activated JAK/STAT3 pathway further aided the release of MMP9 that increases cancer cell spreading and influences invasion (Fogg et al., 2019). CCL5 and CCL2 are the two important cytokines secreted by Tumor-Associated macrophages (TAM), which regulate cancer cell growth in the tumor microenvironment. THP-1 derived Tumor-associated macrophages secrete CCL5 ligand which binds to a CCR5 receptor present on prostate cancer cell activate β -catenin/STAT3 pathway, leads to increased invasion, migration, and EMT (Huang et al., 2020a). Further, as an example of stromal crosstalk in the tumor, the lung carcinoma cells aided M2 polarization (TAM-like) of the co-cultured macrophages. Additionally, the TAM induced A549 and NCI-H1975 cells invaded up to longer distances by facilitating upregulation of α B-Crystallin (CRYAB) under the influence of TAM conditioned medium (Guo et al., 2019). The stromal cell such as endothelial cells was co-cultured with Glioblastoma cells in a collagen 1 gel. The presence of endothelial cells enhances the invasive potential of glioblastoma cells in spheroid co-culture (McCoy et al., 2019). This is shown in Fig. 5. Thus,

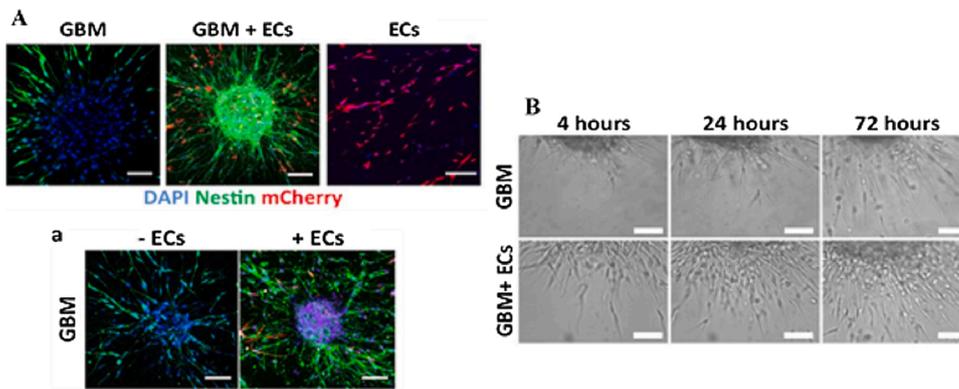


Fig. 5. Representative image of co-culture of cancer spheroids to study invasion. The Glioblastoma cell spheroid co-cultured with endothelial cells showed more invasiveness into the collagen matrix (a) Confocal micrographs of immuno stained invasive Glioblastoma cells (green) in the presence of Endothelial cells (mCherry). (b) Time lapse light microscopic image over 72 h, showing enhanced invasiveness of the Glioblastoma cell spheroid in the presence of endothelial cells (McCoy et al., 2019) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

the cancer-stromal cell crosstalk seems to play a vital role in promoting cancer cell invasiveness leading to enhanced metastasis. Co-culture systems provide relevant *in vitro* conditions to study such important phenomena.

4. Co-culture of cancer spheroids to study migration

4.1. Introduction to migration and role in cancer progression

Metastasis refers to the migration of tumor cells from the primary site to a secondary site or destined secondary organ. It involves steps as invasion, EMT, intravasation into circulation, survival within the blood circulation and arrest at the capillary site, extravasation into a secondary organ where they either proliferate or remain dormant for a prolonged period, then leading micro-metastasis to macro-metastasis by colonizing at secondary site. Migration can occur early when the primary tumor is unable to detect or in the later stages. Tumor cells adopt the new microenvironment to become highly proliferative. TGF- β , EGF, and CSF-1, L- and P- selectin, PDGF, $\alpha v \beta 3$ and $\alpha v \beta 5$ integrin, Small GTPase, Rho, Rac and cdc42, P120, and E-cadherin are some of the factors that help in metastasis of various cancers. Breast cancer cells metastasize to bone, liver, lung, and lymph nodes (Valastyan and Weinberg, 2011; Mego et al., 2010; Chambers et al., 2002; Lambert et al., 2017; Venhuizen et al., 2020; Chen et al., 2018).

Macrophage and fibroblast cells enhance the migration of cancer cells. TAM was observed to be recruited by cancer-derived succinate. Succinate promotes migration of MCF-7, PC3, LLC, A549, and HT-29 cancer cells in a dose-dependent manner. Macrophages increase LLC cancer cell migration via IL-6 secretion. Cancer cell releases succinate that binds to SUCNR1 receptor on the cancer cell and promotes HIL-1A signaling in an autocrine manner. Succinate increases the migration of cancer cells via direct and indirect ways through macrophages (Wu et al., 2020). TAM enhances the invasive ability of HSPCs tumor cells by secreting the CXCL1 ligand. XIAOPI formula decreases the CD206, CD163, and Arg1 marker expression, thus inhibit tumor invasion (Zheng et al., 2020). CAF-S1 induces breast cancer migration via CXCL12/TGF β pathway (Pelon et al., 2020). SOX2 higher expression confers the proliferative advantage and promoted invasion and migration of breast cancer cells. SOX2 expression increases adhesion to brain microvascular endothelial cells and transmigration. Breast cancer cells with higher SOX2 expression induce expression of FSCN1 and HBEGF genes via AKT and β -catenin signalling and in turn promotes brain metastasis (Xiao et al., 2020).

4.2. Co-culture of cancer spheroids to study migration

Tumor cell migration can be influenced by different ECM components. Lugo-Cintrón et al., used different ECM mimicking matrix (collagen, fibronectin with collagen) with fibroblast incorporated in it to demonstrate the migration of tumor cells. They developed a microfluidic

model mimicking the complex tumor microenvironment of breast cancer. Cross-talk between fibroblast and cancer cells was determined via co-culture technique. The liquid bridge was formed between two cell populations that separate them but allow soluble factor exchange. Breast cancer cells activate the human mammary fibroblast (HMF). This cross-talk influences migration of cancer cells on different matrices like collagen and fibronectin rich-collagen. The average number of migrating cancer cells (146 ± 70) was less in the collagen matrix and high (319 ± 62) in the fibronectin-rich matrix in co-culture with HMF cells. When co-cultured with CAF, number of migrating cancer cells found in collagen were 224 ± 76 and 380 ± 61 cells in fibronectin rich matrix. Suggest that the fibronectin-rich matrix with CAF or HMF fibroblast increases the migration potential of cancer cells. The average migrating distance of MDA-MB-231 when co-cultured with HMF was reported $139.9 \pm 20.4 \mu\text{m}$ in a collagen matrix and $189.6 \pm 16.3 \mu\text{m}$ in fibronectin rich matrix. Migration can be viewed as the degradation of ECM protein by MMP secretion, which enhances cell migration. An increase of MMP-2 (4.3-fold), MMP-9 (2.3-fold), and MMP-3 (2-fold) was observed in the fibronectin rich matrix with HMF. Whereas the increase in MMP-3 (14 fold) and MMP-9 (3 fold) was observed in the fibronectin-rich matrix when co-cultured with CAF. Thus, suggesting tumor microenvironment with different fibroblasts influences breast cancer migration (Lugo-Cintrón et al., 2020). Further on direct co-culture of Pancreatic tumor cells (PANC-1 and BXP-3) with the Pancreatic stellate cells (PSC) showed increased Vimentin, TGF- β , CTGF, TIMP-1 markers expression. PANC-1 spheroid had high expression of MT1 (membrane type-1)-MMP, MMP-2, MMP-13, and cleaved collagen with invadopodia formation - a characteristic of individual cell migration. Whereas BxPC-3 showed both collective and individual cell migration (showed the mesenchymal and amoeboid mode of individual cell migration) as shown in Fig. 6. The number of pancreatic cancer cells increased and migrated up to $200 \mu\text{m}$ when co-cultured with PSC cells which were not observed in pancreatic cell monoculture conditions showing the influence of the stromal cells on the pancreatic cancer cell migration (Kim et al., 2020a).

Another interesting study to understand the role of interstitial flow in cancer cell migration was determined using a microfluidic device. In presence of interstitial flow, average speed was $0.30 \pm 0.01 \mu\text{m}/\text{min}$, and in control $0.23 \pm 0.01 \mu\text{m}/\text{min}$ was observed. Mean square displacement (MSD) and migration speed enhanced in presence of flow for MDA-MB-231 cells. Migration speed was increased in presence of flow ($0.33 \pm 0.01 \mu\text{m}/\text{min}$ in comparison to control ($0.20 \pm 0.02 \mu\text{m}/\text{min}$). Thus, the interstitial flow was observed to be influencing the cell-cell adhesion within the tumor promoting the cancer cell migration (Huang et al., 2020b). Spheroid co-culture with tumor stroma and ECM mimicking scaffold can view invasion and migration similar to *in vivo* condition. As well as steps of cancer metastasis like an invasion, intravasation, survival in the circulation, and extravasation into secondary organs can be determined via spheroid co-culture.

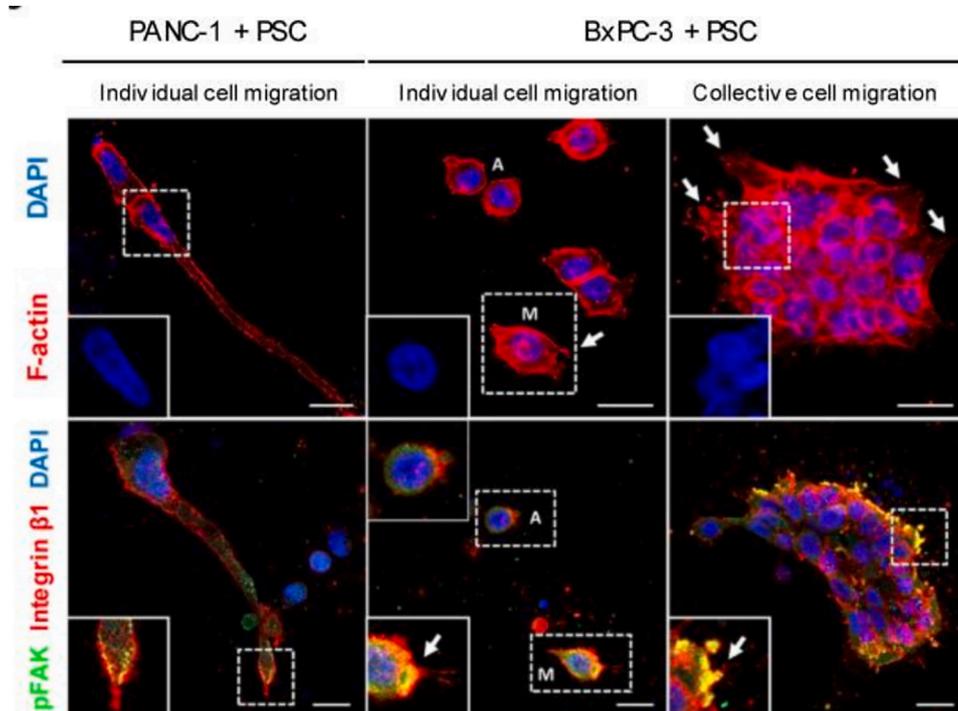


Fig. 6. pFAK expression at the leading edge of protrusion and morphology of podium between BxPC-3 and PANC-1 cells in co-culture condition. M: mesenchymal mode and A: amoeboid mode White arrow: spike-like filopodia. Scale bar: 20 μ m (Kim et al., 2020a).

5. Co-culture of cancer spheroids to study EMT

5.1. Role of EMT in cancer progression

Cancer is a multifaceted disease. The cellular population involved in cancer is always smarter than the healthy cell as they find and activate different cellular types of machinery. Out of them Epithelial to Mesenchymal Transition of cancer cells is pronouncedly considered to be a key player in cancer progression. Altering molecular signalling, genomic, or epigenetic (Galle et al., 2020) regulation related to EMT can facilitate its survival, drug resistance plus aids its progression too. Hypoxic condition in tumor elevates epithelial cell adhesion molecule (EPCAM) N-glycosylation in turn increasing the expression of EMT markers – Vimentin and N-cadherin (Zhang et al., 2020). Also, modulation of E-cadherin is important for induction of EMT and regulating various stages of metastasis (Na et al., 2020; Kim et al., 2020b). MicroRNAs play important role in modulating EMT, where miRNA delivery via exosomes also plays an important role (Lin et al., 2020). miR-708-5p lower expression aids EMT via modulating ZEB-1 expression (Feng et al., 2020). The lower microRNA lethal-7 expressing cells were found to be more potent in stemness and spheroid formation and less EMT score (Chirshv et al., 2020). According to a high-grade serous ovarian cancer (HGSOC) patient-derived xenograft (PDX) study and based on the growth pattern observed from its orthotopic PDX, the epithelial phenotype had characteristics of tumorigenicity and self-renewal. Whereas the mesenchymal phenotypic cells had more invasive and migratory potential. Similar roles played via epithelial and mesenchymal state switching of cancer cell was observed using RFP + to GFP + transiting cell depending upon the activation of Cre expression symbolic to pre and post EMT tumor cells respectively. The once E to M transited cell reverting to epithelial state again gives rise to secondary tumors. This signifies the role of cell plasticity in metastasis and cancer progression (Lourenco et al., 2020).

Further, a study stated the association of HOX transcript antisense intergenic RNA (HOTAIR) (long non-coding RNA) to the hybrid Epithelial-Mesenchymal phenotype leading to partial EMT. The c-MET

expression is suppressed via caveolin-1 modulation by the over-expression of HOTAIR giving the cell ability to survive in an anchorage-independent environment and increasing its collective migration ability, overall supporting the cancer metastasis (Topel et al., 2020).

For evaluating the EMT, methods of calculating EMT scores are being implemented where various approaches have emerged. EMT score = Average of RNA sequence z score of mesenchymal marker genes - Average of RNA sequence z score of epithelial marker genes (Chae et al., 2018), EMT score = Mean of Mesenchymal marker expression value - Mean of epithelial marker expression value (value normalized by the expression value of housekeeping gene) (Chirshv et al., 2020) and another approach; EMT score = Ratio of (sum of expression value of mesenchymal gene) to that of (sum of expression value of epithelial gene) (Lourenco et al., 2020). The more the EMT score, the more is the EMT potential.

Cancer cells accomplish the event of EMT aided by the co-ordination of signalling pathways like NOTCH1-HEY1 (Xie et al., 2020), WNT/ β -catenin (Kim et al., 2019a; Bai et al., 2020), and TGF- β signalling (Camerlingo et al., 2019). As an apt plan of action, elucidation of phytochemicals or synthetic compounds facilitating reversal or suppression of EMT is one of the best strategies to inhibit cancer progression. Triptolide (Diterpenoid derived from *Tripterygium wilfordii* Chinese herb) acts on the EMT mechanism by lowering the Twist, SNAIL, and SLUG expression (Acikgoz et al., 2020). Many metabolic inhibitors targeting EMT are studied such as Rolipram, Simvastatin, Suramin, Eto-dolac, etc. Further elucidation of the combinatorial effect of such EMT targeting drugs and for gaining more in-depth information regarding EMT, co-culture spheroid system could be an appropriate and efficient *in vitro* model.

5.2. Recent studies - co-culture of cancer spheroids to study EMT

EMT is considered to be the main mechanism involved in cancer metastasis leading to cancer progression. The 3D culture system is more relevant to the *in vivo* condition where more cells are found to be in EMT state than in the 2D culture system. Emphasis should be given to

developing better *in vitro* models which can mimic cell heterogeneity as present in the tumor microenvironment and then the EMT could be studied in more relevant culture condition (Hum et al., 2020). The controversy on the effect of MSCs in induction or suppression of EMT still prevails. Many studies have shown that MSCs have an anti-metastatic effect and suppressive effect on EMT markers (He et al., 2018; Meleshina et al., 2015; Casson et al., 2018; Brown et al., 2019). Whereas, in a study MCF-7, a non-metastatic breast cancer cell line was co-cultured with bone marrow-derived MSCs forming spheroid, loss of epithelial characteristic of MCF-7 was observed. E-cadherin was down-regulated and SNAIL was upregulated, but no significant change was observed in TWIST and ZEB1 expression. Co-culturing MCF-7 with MSCs provided increased metastatic characteristics to MCF-7 (Pal et al., 2020).

Not all EMT markers can define the EMT in a particular cell type at a particular instance. Thus, a range of EMT marker expression studies can help to get a clearer and wider picture of EMT. Many studies have proven that TWIST and SNAIL work independently too and induce EMT (Pal et al., 2020; Yang et al., 2004; Carver et al., 2001). Cancer co-culture with stellate cells had enhanced N-cadherin expression and low E-cadherin expression compared to the mono-cultured cancer spheroid signifying the EMT-inducing effect of stromal cells (Chen et al., 2019). The effect of stellate cells may be different in context to EMT depending upon the cancer cell it is co-cultured with. This aspect should be kept in mind for developing a model for testing drug effect targeting anti-stromal strategy (Nam et al., 2019). It would be difficult to analyse the phenomenon of EMT in the spheroid co-culture system especially to visualize the phenotypic changes, which is more difficult in the case of partial EMT. Thus, EMT related marker expression analysis at transcript and protein level of the cells type sorted through FACS may be appropriate to study the effect of one cell type in the spheroid co-culture system in context to EMT. Also, the circulating tumor cells (CTCs) population was found from the blood specimens of 18 patients out of 20 patients. Out of which Epithelial-CTCs in 15 patients and Mesenchymal-CTCs were observed in all 18 patients. Whereas, the CTCs having hybrid expressions are not able to be identified due to technical difficulties. These CTCs undergone EMT and are responsible for the secondary tumor formation which should be targeted to combat the cancer metastasis (Liao et al., 2019a). Apart from EMT, endothelial to mesenchymal transition (EMT) has been also observed for imparting compactness and drug resistance against Cisplatin and Gefitinib in the lung cancer cells and HUVEC cells co-culture spheroid system compared to that observed in Lung cancer cells and fibroblasts co-culture system. The aggressiveness observed in the former system was credited by the GSK-3 β activation, depicting the cellular crosstalk in TME directing cancer progression (Kim et al., 2019b).

All together EMT plays a major role in cancer progression and to target, this mechanism appropriate *in vitro* models are desired which can mimic the effects on EMT mechanism due to the cross-talk between cancer and stromal cells so that appropriate screening of existing drugs and discoveries of new drugs targeting EMT can be successfully carried out. The use of anti-EMT compounds synergistically with other chemotherapeutic compounds could very effectively reduce the possibility of cancer progression and will be effective in treating cancer.

6. Co-culture of cancer spheroids to study evasion of host defence

6.1. Interaction of immune cells with cancer cells and role in cancer progression

Macrophages, NK cells, and cytotoxic T cells are important immune cells that are involved in host immune surveillance. The immune system within a normal person functions to suppress tumor growth, the proliferation of tumor cells *via* NK cells, CD8⁺ cells, and macrophages. These are known as tumor suppressor cells. Tumor evades this surveillance mechanism adopted by the host and alter the function of immune cells

(Wu et al., 2020). Fig. 7 shows the schematic representation where various immune cells interact with the cancer cell and the tumor associated immune cells instead helps the cancer cells in surpassing the immune surveillance. Tumor-associated Macrophages are the most abundant immune cells found in the tumor microenvironment (Yin et al., 2020). Monocytes are differentiated into either M1 phenotype (CD 80⁺) or M2 phenotypes (CD 206⁺, CD163⁺) known as TAM. M1 marker TNF, CD80, CD86, IL-12p40 expressed were lower whereas M2 phenotype related mRNA (VEGFA, VEGFC, IL-10,) and MMP1 were expressed higher (Yamaguchi et al., 2016).

In a co-culture study, THP-1 macrophages were co-cultured with human breast adipocytes. The mRNA expression of macrophages demonstrated an increase in M1/M2 markers including IL-6, IL-10, Cyclooxygenase-2 (COX2) as compared to control. (Yadav et al., 2020). Cancer cells interact with immune cells in an either autocrine or paracrine manner and suppress immune responses, making the tumor microenvironment more favourable for tumor growth (Wei et al., 2019; Fogg et al., 2019; Zhao et al., 2019).

Tumor secreted factors, retinoic acid, succinate, and CCL2 stimulates macrophage polarization towards tumor-associated macrophages and recruits it towards the tumor microenvironment (Wu et al., 2020; Wei et al., 2019). Cytotoxic T cell function is altered/blocked through Programmed death-ligand 1 (PD-1 L) and Programmed cell death protein 1 (PD-1), immune checkpoint by tumor cells (Zhao et al., 2019). CSF1 released from cancer cells promotes tumor-associated macrophage proliferation. Macrophages promote invasion, tumor growth, and proliferation by the release of EGF molecules which binds to the Epidermal growth factor receptor (EGFR) on the cancer cells (Yin et al., 2020). Alternatively, activated macrophages promote ovarian cancer invasion by JAK2/STAT3/MMP9 pathway (Fogg et al., 2019). Macrophages derived CCL5/CCL2 activate CCR5/ β -catenin/STAT3 pathway promotes invasion, migration, EMT, and stemness of prostate cancer (Huang et al., 2020a). Interaction between adipocytes and macrophages enhances pro-angiogenic potential (Yadav et al., 2020). Cold Plasma treated macrophages increased cancer death and decreased invasion, EMT, and stemness (Kaushik et al., 2019).

In a study A549, LLC, MCF7, PC3, HT-29, and HCT116 cell lines' conditioned media was supplied to macrophages. The increase in Arginase 1 (ARG1), Fizz1, Mgl1 mRNA, and protein level was observed indicating TAM activation/polarization. Higher CD163, IL-10, IL-1 β , IFN, TNF marker expression indicates both M1 and M2 phenotype. The Cancer cell secreted succinate driven macrophage polarization. The presence of succinate induces CD11c, VCAM1, markers on the TAM surfaces (Wu et al., 2020; Wei et al., 2019). A549 and NCI-H1975 cells co-culture with macrophages induce its polarization into M2 type. M2 macrophages positively express CD206 and F4/80 markers (Guo et al., 2019).

Tumor-derived retinoic acid enhances monocytes differentiation into TAM than into Dendritic cell (DC), which further promotes tumor growth. Presence of Retinoic acid block DC differentiation *via* targeting Irf4 (a transcription factor). Retinoic acid indirectly inhibiting T-cell mediated immune suppression *via* targeting dendritic cell differentiation. TAM, DC, and monocytes were isolated from tumor tissue and were co-cultured with splenic T cells. T cell proliferation was suppressed by TAM and monocytes but was enhanced by the DC. GM-CSF and IL-4 treatment to tumor monocytes, differentiated it into DC, not in a TAM (Devalaraja et al., 2020). HC116 cell and TAM when co-cultured, increased IL-6 mRNA expression compared to that in TAM monoculture. TAM co-cultured with CRC showed increment in p-JAK2, p-STAT3 in the CRC cells. Further, TAM has been also studied to be enhancing FoxQ1 transcription factors in HCT116 cells in the co-culture system. Macrophages secreted IL-6 inducing EMT in the CRC cells through FoxQ1/STAT3 pathway (Wei et al., 2019). SNHG14 (Small nucleolar RNA host gene 14) a long noncoding RNA was upregulated in DLBCL (diffuse large B cell lymphoma) cells, found to affect DLBCL proliferation, EMT, and migration. miR-5590-3p and SNHG14 were

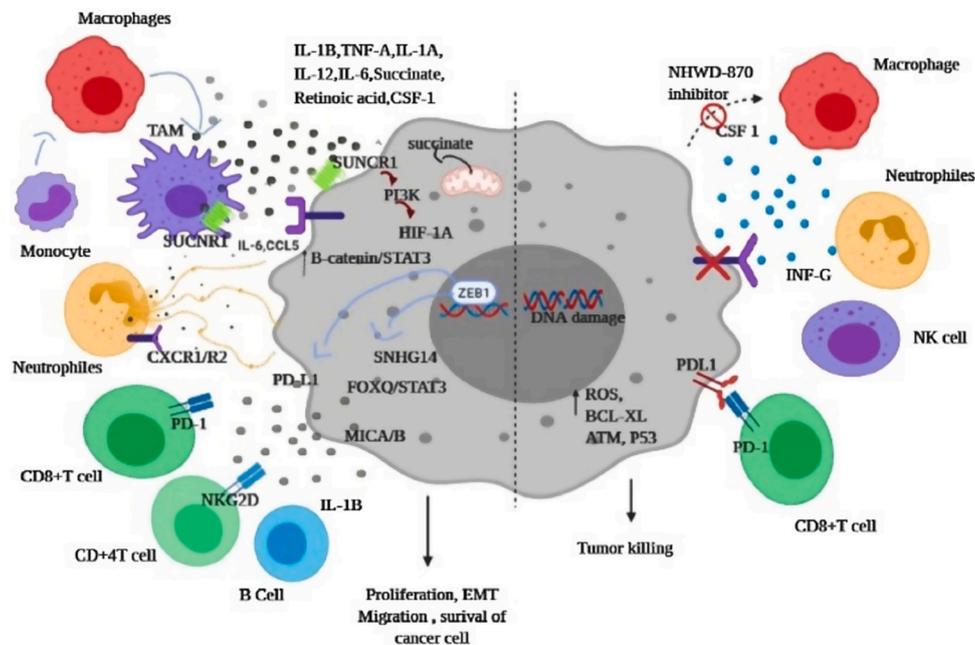


Fig. 7. Representative image showing interaction of immune cells and cancer cells in the tumor microenvironment (Pawlik et al., 2000).

inversely related to one another. SNHG14 acts as an immune suppressor to decrease cytotoxic T (CD8⁺) cell activity. DLBCL and CD8⁺ cell interaction was governed by SNHG14/miR-5590-3p, and it induced apoptosis in CD8⁺ by targeting the PD-1/PD-L1 immune checkpoint. SNHG14 functions as an immune suppressor and activator of cancer survival by activating PD-L1 ligand from DLBCL cell through miR-5590-3p/ZEB1. This study suggests the role of SNHG14 in immune evasion by targeting PD-L1/PD-1 immune checkpoint. Immune therapy may be improved by targeting SNHG14 (Zhao et al., 2019).

NHWD-870, a potent BRD (Bromodomain) inhibitor decreases CSF1 release from a cancer cell and thus inhibits TAM proliferation. NHWD-870 treated A2780 cell 3D co-cultured with TAM, decreased TAM proliferation, colony formation (8 fold), and dose-dependent growth. CSF1 is highly expressed in ID18 and B16 cancer cells and its receptor expression observed was high on TAM. Co-culture activates PI3K/AKT signalling in TAM. Inhibition of tumor associated macrophage proliferation inhibits cancer growth and proliferation. NHWD-870 acts as a potent inhibitor of CSF1, a molecule required for macrophage growth, via targeting BRD4, an upstream molecule for CSF1 release (Yin et al., 2020).

6.2. Co-culture of cancer spheroids to study evasion of host defence

Immune cells are the key player in host defence. Immune cell infiltration into the tumor spheroid was determined by flow cytometry. The higher proportion for NK cells than the T cells was observed to be infiltrating into per cancer spheroid. NK cells and CD8⁺ cells infiltrated into spheroid displaying express of CD25 (activation marker) and CD107a (degranulation) marker (Courau et al., 2019). MICA/B (MHC class I polypeptide-related sequence A/B) ligand expressed on a colorectal cancer cell, while its receptor NKG2D on CD4 T cells. Antibody against NKG2D enhances tumor survival. Cancer cell releases MICA/B ligand bound to its NKG2D receptor on immune cells and decreases immune cell activity, thus enhances tumor survival. Inhibiting MICA/B expression of cancer cells reveals the antitumor function of NK cells. NKG2A and HLA-E interaction help cancer cells to evade immune response (Courau et al., 2019).

Neutrophil extrudes its DNA outside the cell known as NETosis - a kind of death extruded DNA bound to neutrophil protein and form neutrophil extracellular traps (NETs). Neutrophils affect the function of

NK cells and T cells. CXCR1 and CXCR2 receptors are present on the neutrophils, these receptor induction leads to NETosis in neutrophils. Melanoma and colon cancer cell supernatant induces NETosis in healthy neutrophils. A human colorectal adenocarcinoma (3D HT29) spheroid cells were co-cultured with healthy neutrophils. SYTOX green nuclear staining reveals NET extrusion of neutrophils. In presence of NET inducer, tumor spheroid and neutrophils were preliminarily cultured then co-cultured with CD8⁺T or NK cells. The presence of NK and CD8⁺T cells did not affect the function of cancer cells, instead the cancer cells survived in the presence of effector immune cells. Tumor spheroid induces NETosis in neutrophil forming NET. These NET prevent CD8⁺T cell-mediated killing of cancer cells in the co-culture system. Tumor cells survived (80 %) when co-cultured with NK cells. NET formed by neutrophils protect tumor cells from killing from NK and CD8⁺T cells by creating protective cover surrounding the tumor. NET also prevents CD8⁺T and NK cell penetration towards tumor cells (Tejreira et al., 2020).

Loss of interferon-gamma (IFN- γ) signalling makes tumor cells resistant to killing by CD8⁺ cells but sensitive to killing by NK cells. In a study, transduced B610 tumor cell was co-cultured with NK cell. The NK cells kill tumor cells via a perforin-dependent manner in co-culture system. Deletion of antigen presentation and IFN- γ signalling genes sensitizes tumor cells for killing via NK cells. Deletion of genes involved in IFN- γ signalling [Ifn γ r2, Janus kinase 1 (Jak1), and Jak2] and antigen presentation [Beta 2 Microglobulin (B2m), Tap1 and Antigen peptide transporter 2 (Tap2)] fails to represent MHC-1 thus sensitize the B16-F10 tumor cell killing by NK cells. The release of Interferon-gamma from NK cells upregulated and expressed molecules on the tumor cells, that protect them from killing (Freeman et al., 2019). Inhibition of MICA/B induces tumor destruction by increasing NK cell activity. Infiltration of NK and T cells within HT29 spheroid leads to its destruction and apoptosis, correlated by activation of caspase-3 and 7. The addition of IL-15 within co-culture destroys spheroid structure within 48 h. Synergistic effect of anti-MICA/B and anti-NkG2A antibody enhances immune cells mediated killing of cancer cells in co-cultures (Courau et al., 2019).

7. Co-culture of cancer spheroids to study metastasis to the bone as a secondary site

7.1. Metastasis to secondary site and role in cancer progression

Osteoblast and osteoclast are bone forming and bone resorbing cells. The osteoblast secretes multiple biomolecules including TGF- β , Insulin-like growth factor (IGF), Bone morphogenetic proteins (BMP), osteocalcin, and collagen into the bone matrix. Biomolecules like Receptor activator of nuclear factor kappa-B ligand (RANKL) and Osteoprotegerin (OPG) from osteoblast, differentiate osteoclast cells. Whereas Osteoclast secretes factors like BMP6, exosomes, Sphk1, Wnt10b affect/alter osteoblast function (Zhu et al., 2018). Bone is the preferred site of metastasis by breast cancer and prostate cancer. The bone microenvironment acts as a chemoattractant and provides a favourable condition for a cancer cell to grow and proliferate (Allocca et al., 2019). The bone metastasis begins with the arrival of tumor cells at the bone. The process followed by homing and forming a pre-metastatic niche, and the formation of micro-metastasis by overcoming dormancy later forming a metastatic tumor (Liang et al., 2020). IL-6, Lysyl Oxidase (LOX), RANKL, Parathyroid hormone-related protein (PTHrP), MMPs, CXCL12/CXCR4, α v β 3, α 4 β 1, Intercellular adhesion molecule 1 (ICAM-1), VCAM-1, fibronectin, vitronectin, IL-11, IL-1, TGF- β are reported factors for regulating various steps of bone metastasis. The vicious cycle is a well-known mechanism for bone metastasis (Chen et al., 2018; Liang et al., 2020).

Tumor cell arrival at the bone disturbs normal bone homeostasis. Tumor cells activate osteoclast either directly or indirectly via osteoblast cells. It involves complex cross-talk between osteoblast, osteoclast, and tumor cells (Chen et al., 2018; Liang et al., 2020). Bone metastasis is of three types - osteolytic, osteosclerotic, and mixed types. A common one is osteolytic which involves the degradation of bone. Osteosclerotic involves osteoblast cells that form new bone (Macedo et al., 2017). There are multiple challenges associated to study cancer metastasis toward the second organ including different microenvironments, bone composition, poor early detection of metastasis, and a limited number of the human sample (Hao et al., 2018). Breast cancer cells form a niche in the long bone and specifically in the trabecular region of bone which is not dependent on the hormonal status of breast cancer cells. Both MDA-MB-231 and MCF-7 both colonize in the same way in the bone microenvironment (Allocca et al., 2019). Breast cancer cells homing to the bone and their colonization in the bone microenvironment was also demonstrated by the *in vivo* mouse model (Allocca et al., 2019). Indeed the use of co-culture studies will enhance the understanding of cross-talk between cells and the progression of the disease.

There is a large scope to explore the key signalling cascades and their therapeutic targets, which can be better elucidated via 3D spheroid co-culture. The signalling cascade plays an important role in bone metastasis, cancer cell colonization, and cellular dormancy. Androgen signalling and OSM/miRNA-181b-5p axis is an important regulator of prostate cancer to bone metastasis (Han et al., 2020b; Bock et al., 2019). *Ex vivo* bone co-culture demonstrated that CXCL5/CXCR2 pathway is important for breast cancer cell colonization in the bone microenvironment. Bone colonization represents a rate-limiting step for the therapeutic target (Romero-Moreno et al., 2019). Spindle-shaped N-cadherin⁺/CD45⁻ osteoblasts cells (SNO) induce cellular dormancy in breast cancer cells. Breast cancer cells remain dormant in the endosteal niche of the bone. These cells mimic hematopoietic stem-like properties. NOTCH2 play role in the dormancy but its signal disruption leads to colonization. NOTCH2 is crucial for breast cancer cell dormancy in the bone (Capulli et al., 2019).

One of the G-protein coupled receptor- calcium-sensing receptor (CaSR) promote lung cancer bone migration. Patients with lung cancer bone metastasis express a higher level of calcium-sensing receptor expression. Co-culture of CaSR overexpressing cell A549 and macrophages enhance the osteolytic area. RANK/OPG ratio was high when

indicates osteolytic lesion induced by osteoclast cells. PTHrP, IL-6, IL-8, and IL-11 expression were higher in the CaSR group, indicate that PTHrP induces increment in the osteolytic bone areas by activating and promoting differentiation to osteoclast. Lung adenocarcinoma cells with higher CaSR expression enhance their invasion/migration via MMP2, MMP9, and NF- κ B pathways and indirectly promote osteolytic bone lesions via PTHrP (Liu et al., 2020).

Combination treatment of mTOR inhibitor and bone targeted drugs in the co-culture condition is an effective treatment for bone metastasis. mTOR inhibitor in the co-culture condition decreases the number of osteoclast from 41.7 ± 4.6 to 26.8 ± 4.6 . Caki-2 cancer cells' survival was decreased (20.3 %) in presence of inhibitor in the co-culture compared to control (34.9 %). Drug Everolimus (Eve) treatment in the co-culture decreases the number of osteoclasts, cathepsin K (CTSK), osteoclast-associated immunoglobulin-like receptor (OSCAR), nuclear factor of activated T cells 1 (NFATC1), carbonic anhydrase II (CAII) marker expression as compared to untreated co-culture. The presence of Eve increases survival in the co-culture condition than monoculture caki-cells. Osteoclastogenesis was increased in the co-culture condition when treated with Eve, Deno, Zol (zoledronic acid), and Eve + Deno. (Spadazzi et al., 2019). These studies provided compelling evidences that co-culture would be more appropriate platform for understanding the crosstalk between cancer and bone cells. The application of co-culture can be extended for studying the vicious cycle and drug targeting for bone metastasis. Drug targeting the vicious cycle of bone metastasis better studied via spheroid co-culture.

7.2. Co-culture of cancer spheroids to study metastasis to bone

Metabolic pathways such as oxidative phosphorylation are crucial for bone metastasis. A study was performed in which spheroids were co-cultured with the stromal cells. MCF-7 and T47D cells were co-cultured with the HS5 and HS27 (bone marrow cells) in the 2D as well as 3D cultured cells. 3D co-culture promotes the dormancy of the tumor cells in the bone marrow by affecting cell proliferation, and not the cell viability. Breast cancer cells in the spheroid co-culture system exhibited 1.2–1.3 fold increases in oxidative phosphorylation (OXPHOS) in comparison to monocultured spheroid system. Co-cultured cancer cells with stromal cells also increase Akt expression than ERK. 3D spheroid co-culture demonstrates that breast cancer cells use OXPHOS metabolism and AKT signalling to remain in the dormant state. Oxidative phosphorylation promotes the dormancy of cancer cells in the bone microenvironment (Buschhaus et al., 2020).

When cancer cells metastasize to the bone as a secondary site, cancer cells are observed to be dormant. Such dormancy stating study using bone cells was observed to decrease tumor spheroid area and the tumor size shrunk. Osteocyte like MLO-A5/MLO-Y4 cells spheroid decreases the tumor spheroid area and compact it when they are cultured together. Direct contact proved to be more efficient in reducing tumor spheroid area. Osteocytes cells induces compactness to the tumor spheroid in comparison to osteoblasts. TMD and BMD (clones of MDA-MB-231) spheroid area were reduced in the presence of bone cells. Bone ECM molecules such as biglycan, collagen, and osteonectin decreases the tumor size. Osteocytes cells decrease SLUG, SNAIL, N-Cadherin, EMT related genes expression in the TMD tumor spheroid. Osteocytes upregulated the phosphorylated AKT. Osteocytes also decreased tumor cell migration (Chen et al., 2017). Thus, bone cells promote breast cancer cell colonization, dormancy, compaction and further promoting cancer metastasis to bone.

8. Co-culture of cancer spheroids to study the role of Cancer stem cells (CSCs) in cancer progression

8.1. CSCs and their role in cancer progression

A special sub-group of cells out of the total tumor cell population that

has the characteristic potential of tumorigenicity, differentiation, and self-renewal is known as cancer stem cells (CSCs). These subsets of cells bestow the tumor cell's tendency of reoccurrence, multidrug resistance, tumor initiation further helping in tumor metastasis and progression (Han et al., 2019). This subset of a cancer cell population is also known as tumor initiating cells (TICs). To conquer the relapse event happening after the various strategies implemented to treat cancer, more studies are done on the CSCs to understand its mechanism of aiding cancer progression in more detail. There are various stem cell markers expressed in different tumors, one such novel stem cell marker is Anterior gradient 2 (AGR2). It was elucidated that it was highly co-expressed along with other cancer stem cell surface markers in colon cancer cell spheroid culture. AGR2 regulated the colony-forming capability of cells and was modulated by the canonical Wnt/ β -catenin pathway (Lamichane et al., 2019).

CD44⁺ stem-like undifferentiated cells within the tumor population are associated with increased proliferation and invasiveness contributing to more tumorigenic, metastatic, and aggressive tumors (Vega Moreno et al., 2019). CSCs enhance AKT activation and increase in migratory potential of the cancer cells (Khan et al., 2019). Further, an RNA polymerase II-associated factor 1 complex (PAF1C) component – PAF1 is also considered a marker of CSCs. DDX3 [a DEAD-box protein sub-family of the RNA helicase superfamily which has a distinct motif (Asp-Glu-Ala-Asp/His referred to as DEAD/H)] interacts with PAF-1 and PHF5A (PHD finger protein 5A) mediating NANOG expression and thus, downstream stemness contributing marker genes in pancreatic cancer (Karmakar et al., 2020).

8.2. Co-culture of cancer spheroids to study CSCs

By decoding the various mechanistic linkages at the tumor-stroma interface, many targets for drug discovery can be interpreted. Research has been already a step ahead in this path, where spheroid co-culture facilitates the journey of findings. For evaluating therapy based on cytokine-induced killer (CIK) cells, the CIKs were co-cultured with epithelial ovarian cancer (EOC) cells (Bu et al., 2019). There was distinct evasion of EOC spheroid cell from the cellular lytic effect of CIK, where spheroid cells highly expressed Nanog (2.1 fold) and aldehyde dehydrogenase 1 family member A1 (ALDH1A1) (1.5 fold) [stem cell markers] compared to adherent cultured cells. This resistance was assisted by the downregulation of intercellular adhesion molecule-1 (ICAM-1) via hypoxia-inducible factor-1 α (HIF1A) (Bu et al., 2019).

In a study, the immunosuppressive tactic driven by CSCs was explored (Raghavan et al., 2019). The CSCs avail WNT ligands and activates M2 polarized macrophages (CD206⁺) which in turn augments ALDH⁺ cells within the CSC compartment of the co-cultured spheroid through IL-6 signalling. This grants immunosuppressive mode to the spheroid, chemo-resistance, and greater invasiveness supplementing tumor progression (Raghavan et al., 2019). Also, via STAT3/IL-8 signalling macrophage induced stemness in the ovarian cancer stem-like cells when co-cultured with the macrophage (Ning et al., 2019). Hyaluronic acid aided by tumor-associated macrophage (TAM) increases PI3K-4EBP1-SOX2 signalling via binding to CD44. As a result, CSC fraction also increased. On the other hand, Ezrin/PI3K signalling was promoted by VCAM-1 CD44 binding observed in the TAM and CSCs (Gomez et al., 2020). Further, in another co-culture study, it was demonstrated the importance of CAFs for spheroid forming potential of CD44⁺ OSCC cells expressing stable OCT4 by its Lactate production. This contributes to the enhancement of oxidative phosphorylation activity of CSCs as protein expression of TOM20 (a marker for functional mitochondrion) and PGC-1 (mitochondrial metabolism regulator) in CD44⁺ CSCs was observed highly facilitating the tumor progression. Such signalling pathways and regulators can be targeted as an advanced strategy to target cancer stemness and tumor-initiating property lying with the tumor attributed by the CSCs and lead to the discovery of a potent strategy to stop tumor progression. The cross-talk between the

endothelial cells and the cancer stem cells in the tumor microenvironment has been studied to be enhancing stemness and malignancy in the 3D co-culture based system (Qiao et al., 2021).

3D culture HUVEC cells were co-cultured with CSCs encapsulated in alginate based hydrogel microcapsule. The paracrine signalling between HUVEC and CSCs triggered more stemness and enhanced expression of MMP9 suggesting more malignant potency in co-cultured CSCs than the 3D CSCs alone. Also, the expression of MDR1 involved in imparting multiple drug resistance to the CSCs was observed to be highly expressing in CSCs-HUVEC 3D co-culture than 3D CSCs alone as shown in the Fig. 8 (Qiao et al., 2021).

The cancer cells develop an extracellular microenvironment which can induce stemness in normal cells and can further trigger chemo-resistance (Park et al., 2019). Thus, cancer cell-stromal cell interaction plays a key role in the increment of chemo-resistance. Cancer-associated MSCs (CA-MSCs) via their PDGF secretion increased cancer stemness, EMT potential and contributed to the chemo-resistance capability of co-cultured CSCs. IC₅₀ of Carboplatin in CSCs alone was 25.7 μ M whereas in presence of CA-MSCs was 67 μ M. On inhibiting the PDGF signalling in CA-MSCs by using siRNA for PDGF subunit B, IC₅₀ then was 46.2 μ M. Further with a combination of Sunitinib (multitargeted tyrosine kinase inhibitor), IC₅₀ was 39.4 μ M (Raghavan et al., 2020). Many spheroid co-culture studies have been reported where the stromal cells are co-cultured with the cancer cells and different concentrations of the drug targeting particular aspect are evaluated and the chemo-resistance and tumor growth potential are observed (Ning et al., 2019; Shao et al., 2021; Fang et al., 2019).

9. Co-culture of cancer spheroids to study the role of exosomes in metastasis

9.1. Introduction to exosomes

Extracellular vesicles are generally categorized into two subgroups (1) Microvesicles (~100 to 1000 nm) (2) Exosomes (~30 to 150 nm) depending upon their size and biogenesis (Patel et al., 2019; Paulaitis et al., 2018; Rajagopal and Harikumar, 2018). Exosomes are formed from endosome membrane inward invagination. Exosomes have a characteristic “donut” like shape and express classical exosome markers – TSG101 and ALIX (Bellmunt et al., 2019). Also, CD63, CD9, and CD81 are used as exosome markers. Exosomes act as suppliers of cargo containing important biological entities like DNA, mRNA, and miRNA contributing to the cell-cell communication (Kruger et al., 2014; Fernando et al., 2017).

Here in this part of the review, we have discussed studies, findings, and contribution of exosomes ranging in of ~30 to 150 nm size in context to cancer progression. As an example of differential roles of exosomes in cancer, the DNA present in exosomes derived from malignant pleural effusions can be used for the detection of EGFR mutation in lung adenocarcinoma patients (Qu et al., 2019). Also, it was noted that the exosomes derived from Icotinib resistant EGFR^{19 del} NSCLC cells – HCC827 triggered invasion and migration of the Icotinib sensitive cells. This was observed as the exosomes contained MET mRNA (a proto-oncogene, Hepatocyte Growth Factor Receptor also known as c-MET) and contributed to acquiring drug resistance and thus, cancer progression (Yu et al., 2019). Also, the hypoxic environment developed in tumor-induced miR-410-3p rich exosome secretion and activates PI3K/AKT signalling, thus contributing to tumor progression (Hu et al., 2020). The fact that the exosome of the tumor can recognize and home to the cells of their genesis can be converted into a therapeutic strategy for cancer treatment (Qiao et al., 2020). But while engineering a plan of action using exosomes, one should take into consideration the fact that exosomes from the tumor cell carry many cancer-promoting biological molecules.

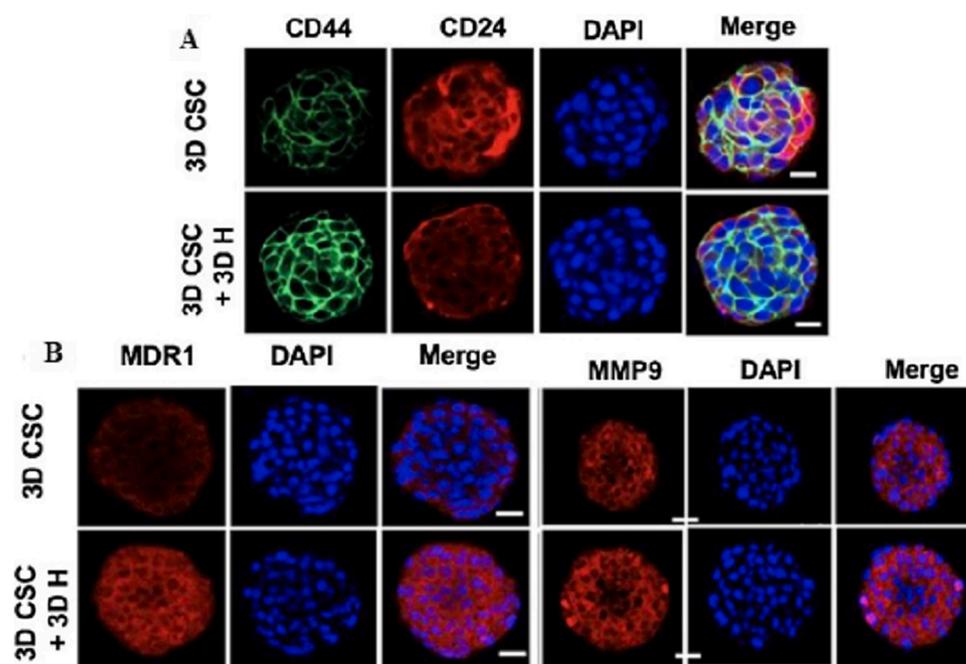


Fig. 8. Representative images illustrating significance of spheroid culture in enhancing cancer stem cell properties. The immunofluorescence image showing expression of (a) enhanced CD44 and lower CD24 marker expression in 3D CSCs co-cultured with HUVEC cell than in 3D CSC alone. (b) enhanced MDR1 and MMP9 expression in 3D CSCs co-cultured with HUVEC cell than in 3D CSC alone (Qiao et al., 2021).

9.2. Co-culture of cancer spheroids to study exosomes in metastasis

In a co-culture study, it was indicated that the metastatic breast cancer cells secreted exosomes trigger host organ fibroblast to CAFs. These CAFs in turn develop a pre-metastatic niche which facilitates further tumor cell recruitment indicating the metastasis (Zhun et al., 2020). Another such study stating the symbiotic effect of exosomes released by the macrophages and cancer cells in the tumor microenvironment was done with the help of a co-culture system. Macrophage exosomes mediated STAT3 signalling promoted cancer cell migration. Whereas macrophage co-cultured with cancer cell-derived exosomes induced IL-10 directing towards immunosuppression (Bellmunt et al., 2019). Also, M2 polarization of the macrophage was triggered which is not facilitated by the exosomes secreted by the normal epithelial cells (Pritchard et al., 2020).

Moreover, exomes derived from tumor cells facilitate macrophages to induce programmed death-1 (PD-1) expression (Morrissey and Yan, 2020). Further, in hypoxic condition cancer cells release exosomes carrying miR-301a-3p which induces the co-cultured macrophage polarization to M2 phenotype by stimulating PTEN/PI3K γ pathway (Wang et al., 2020b). Altogether through such a mechanism immune machinery is suppressed and tumor progression is encouraged. Additional proof of contribution of exosomes in the process of cancer progression was observed where miR-23a containing exosomes promoted angiogenesis by inhibiting PTEN and activation AKT signalling. It enhanced VEGF and reduced Thrombospondin-1 (TSP-1) protein expression in HUVEC co-cultured with gastric cancer-derived exosomes (Du et al., 2020). Further, miRNA-221-3p harbouring cervical cancer cell exosome promotes angiogenesis by reducing MAPK10 in co-cultured microvascular endothelial cells (Zhang et al., 2019).

Signifying the relevance of 3D culture, the RNA profiling of 3D culture-derived exosomes was ~ 96 % similar to the small RNA profiling of circulating exosomes derived from cervical cancer patient plasma done by using next-generation sequencing (NGS) compared to 2D exosome small RNA profile (Thippabhotla et al., 2019). The patient-derived gastric organoids, the gene expression of miR-25, miR-658, miR-210, miR-649, miR-192, and miR-302c in presence of esophageal

adenocarcinoma (EAC) exosomes was 4.48, 2.94, 4.01, 3.82, 2.17 and 3.21 fold high compared to that of EAC condition media devoid of exosomes. Out of them miR-25, miR-210, and miR-192 are well-known reported oncogenic biomarkers. The Ki-67 marker expression was also high in gastric organoids co-cultured with EAC exosomes indicating high proliferative activity (Ke et al., 2017).

In another study, the colon cancer cell spheroid having the highest resistance to 5-fluorouracil co-cultured on lymphatic endothelial cells created the most “circular chemo-repellent induced defect” (CCID) than the non-resistant colon cancer spheroid co-cultured with lymphatic endothelial cells (LEC). This depicted the more invasive nature of the resistant colon cancer cell spheroid. It was observed that the exosomes derived from the non-resistant colon cancer spheroid carried more miR-200c than observed in the highest resistant colon cancer spheroid. Here, the different role of exosome has been highlighted as the exosomes carrying less miR-200c expression is directly facilitating the migration potential of LEC pointing towards the enhanced potential of intravasation mechanism. Also, miR-200c regulated the EMT markers ZEB1 and SLUG (Senfter et al., 2015). The, miR-200c cargo containing exosome released from colorectal cancer spheroid had the same CCID effect on blood endothelial cells and regulated EMT markers ZEB2, SNAI, and TWIST, modulating the cancer progression (Holzner et al., 2016). For the in-depth exploration of the inter-tumor microenvironment cell interaction, a heterotypic cell spheroid was cultured including PC3 (human prostate cancer cell line producing GFP tagged CD63⁺ exosomes) was co-cultured with PBMCs (human peripheral blood mononuclear cells) as shown in Fig. 9. 0.3–5.8 % CD8⁺ T cells interacted with GFP tagged exosomes of PC3 cells whereas 15.7–24.1 % interacted with GFP tagged PC3 exosomes out of the total CD3⁺T cell population through macropinocytosis exosome internalization. Further, exosomes interacted with B cells via majorly binding to B cell surface and less through B cell internalization (Sadovska et al., 2018).

Alike the observation stated above for exosome behaviour in hypoxic 2D culture cells, in the hypoxic environment the exosomes derived from primary tumor-derived cell spheroid were taken up more by the same native cells when exposed to the hypoxic primary tumor derived cell spheroid compared to its normoxic counterpart. The uptake of PKH67

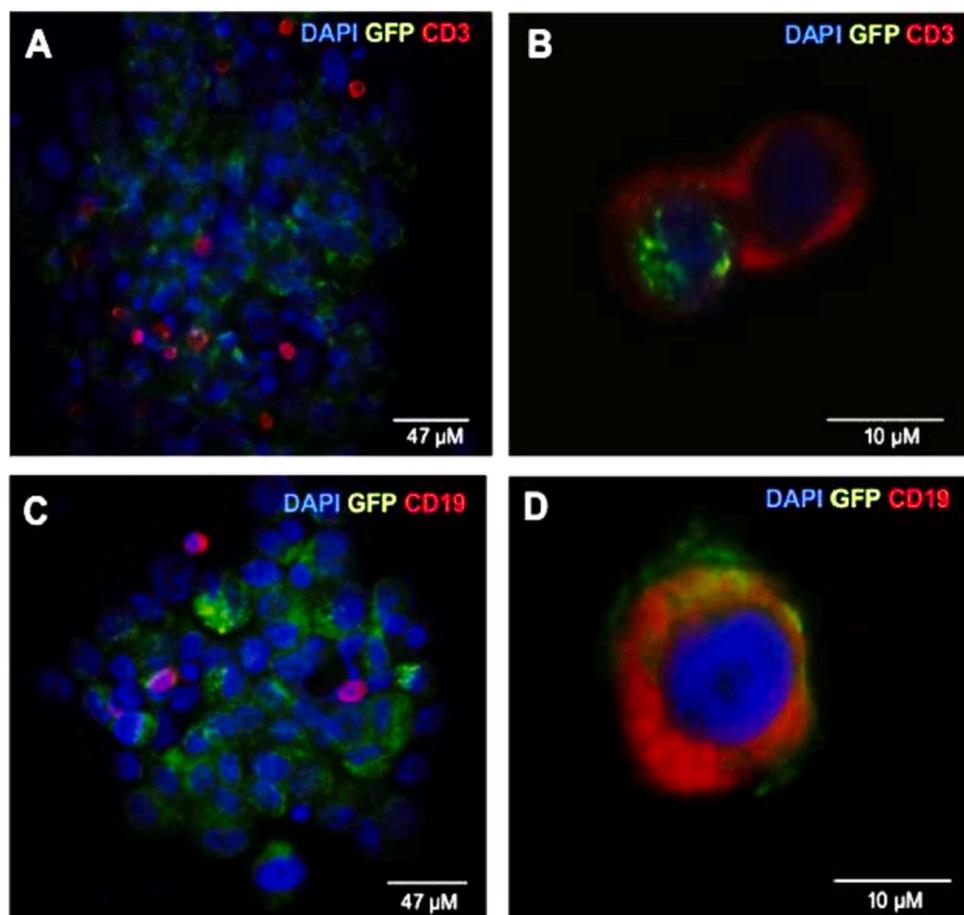


Fig. 9. The PC3-CD63-GFP and PBMC heterotypic 3D co-culture model. (a) Anti-human CD3 labelled 3D spheroid immunofluorescence image. (b) CD63-GFP-positive EVs uptake and localization in CD3⁺ T cells. (c) Anti-human CD19 labelled 3D spheroid immunofluorescence image. (d) CD63-GFP-positive EVs binding to CD19⁺ B cells. Nuclei counterstained with DAPI (blue) (Sadovska et al., 2018) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

labelled hypoxia SW480 derived EVs by the naïve Hypoxic SW480 was observed. EVs were found located in the cytoplasm of the cells. Further, the spheroid forming potential, migration, and invasive capacity were observed more in exosome treated primary tumor derived cells compared to the metastasis derived cells in hypoxic conditions. It seems that a hypoxic environment may alter exosome surface molecules such that it could facilitate the exosome intake as shown in or interaction in the cancer cells (Endzeliņš et al., 2018). Taken together exosome related studies done using spheroid co-culture can help in the clear picturization of different means implemented by the exosomes for cell-cell interaction and different roles of exosomes in cancer progression.

10. Co-culture of patient-derived cells spheroid to study metastasis

10.1. Significance of using patient-derived cells to study cancer

Clinically different patients are encountered suffering from different types of cancer and each is diagnosed at a different stage of cancer. Each patient has a different gene expression profile (Liao et al., 2019b) and should have different epigenetic causative factors too (Lara et al., 2020; Darwiche, 2020). The key reasons for the occurrence of cancer in each patient would be different out of the group of patients diagnosed with the same type of cancer and so would be their treatment response. For instance, it was observed in a study that the organoids established *in vitro* from the lung cancer patients with gene alteration - BRCA2 mutant, EGFR mutant and EGFR mutant/MET amplified responded to Olaparib, Erlotinib, and Crizotinib drug respectively (Kim et al., 2019c). Thus, when the causative reason for the occurrence is unique then the treatment given needs to be personalized. Therefore, treating patients with personalized cancer therapy planned based on the 3D cancer spheroid

culture done using patient-derived cells of a particular cancer type would be more appropriate to elucidate the chemotherapeutic drug (de Witte et al., 2020; Narasimhan et al., 2020), photoactive drug (Yakavets et al., 2019) and natural compound screening (Li et al., 2020), to study the drug dosage, regimen, resistance (Nanki et al., 2020) and selection of the drugs for synergistic strategy (Antonia et al., 2020).

Drug testing on patient-derived cancer cell spheroid or patient-derived organoid can be done in a shorter time period and comparatively with less expense than the *in vitro* model of patient-derived xenografts as the pleural effusion and malignant ascites can avail the target cells (Chen et al., 2020b). It also mimics the tumor heterogeneity, pattern, and level of the various marker expression profile, histological and genomic features of the patients (Nanki et al., 2020; Jacob et al., 2020; Antonia et al., 2020). Thus, studies conducted on the patient-derived cancer cell spheroid are considered to be more realistic. Other than the above-stated advantages there are certain challenges for patient-derived cancer cell spheroid culture. As the starting material of patient-derived cancer cells is the tissue surgical section gained through biopsies, thus the quality is unpredictable. Also, depending upon the type of tissue the yield of viable target cells is obtained which can be used for further experimental purposes. For example, in the case of breast tissue, it comprises more fat cells and fewer epithelial cells whereas, in the colon or prostate tissue higher yield of viable cancerous epithelial cells can be obtained. This could be the reason of more published articles based on patient derived cell studies are related to colon or prostate cancer (Goldhammer et al., 2019; Hofmann et al., 2020). Along with the benefit of more *in vivo* relevance than the 3D culture using cell line, to explore the influence of stromal cells and understand the role of tumor microenvironment, the patient derived cancer cell spheroid/organoid co-culture with the stromal cells or immune cells are now proven to be more applicable and appropriate *in vitro* models.

10.2. Recent studies involving co-culture of patient-derived cells spheroid to study metastasis

With the advancing research in the field of cancer, there is a growing quest for more relevant *in vitro* models. To study breast cancer metastasis where invasion plays an important role, a group of researchers co-cultured MSCs along with estrogen receptor (ER)/ progesterone receptor (PR) positive cells (BR15) and another set with ER/PR negative cells (BR8) derived from Breast cancer patients. The invasion index (perimeter/circumference) observed in ER/PR positive BR15 cells (non-invasive) embedded in basement membrane extract (BME) were enhanced than observed in the mono-cultured spheroid. The localization of GFP tagged MSCs was found to be clustered at the center of the co-cultured MSC-GFP and BR8/BR15 spheroid as shown in Fig. 10a. Under MSC driven signalling, the invasion observed was signified through the finger-like projection in the BR15 and MSC-GFP co-culture spheroid embedded in BME as shown in Fig. 10b (Pal et al., 2020).

In context to the arrangement of the stromal cells, in the spheroid co-culture bearing patient-derived breast cancer cells and human dermal fibroblast cells, no distinct arrangement of epithelial cells and fibroblasts was observed (Hofmann et al., 2020). This observation was in contrast to the positioning noticed in the spheroid co-culture generated using cancer cell line and fibroblastic cell line where fibroblast are arranged primarily in the core and epithelial cells in the periphery (Herter et al., 2017).

Cancer-associated fibroblasts play a distinct role in cancer progression, by influencing tumor metastasis by regulating tissue stiffness because of increased expression of lysyl oxidase-like 2 (LOXL2) and discoidin domain-containing receptor 2 (DDR2) (Nguyen et al., 2019) directing to ECM remodelling. It releases cytokines such as IL-6 and regulates pathways like the JAK/STAT signalling pathway attenuating p53 activity and contributing to the inhibition of prostate cancer cell death (Cheteh et al., 2020). Further, overexpresses TGF- β , MMP7 and releases fibroblast growth factor 2 and 9 (FGF2 & FGF9) enhancing tumor cell growth. Additionally, it transforms Tumor-associated macrophage (TAM) to tumor-supportive M2 phenotype (Hegab et al., 2019) and aids angiogenesis by overexpressing WNT2 contributing to pro-angiogenic signalling (Daniela et al., 2020), and also influences the chemotherapy response. Thus, due to such an important contribution of CAF, it is important to study its interaction in a co-culture system. Lung squamous carcinoma (LUSC) patient-derived cancer cells and CAF cells when co-cultured the presence of CAFs amplified the size and number of spheroids formed. Also, facilitated acini formation when localized close to CAFs making it invasive. The co-cultured cancer cells migrated towards CAFs appearing like a “tear-drop” structure which was not observed in the cancer cell monoculture (Chen et al., 2020c).

Spheroid co-culture studies have demonstrated the contribution of CAFs in modulating oxidative metabolism in the pancreatic ductal adenocarcinoma cells (PDAC) contributing to chemotherapy resistance. The co-culture spheroid formed using primary pancreatic cell line and patient-derived CAFs, the cyclooxygenase-2 (COX2) was highly

expressed compared to mono-cultured cancer cells. COX2 induction is the result of nuclear factor- activation by ROS. On treating the co-cultured spheroid with Metformin (mitochondrial complex-1 inhibitor), the optical redox ratio remained high even after metformin treatment compared to primary pancreatic ductal adenocarcinoma cells (PDAC) monoculture. There is also an indication that in the presence of CAFs, PDAC may opt alternative metabolic pathway for obtaining energy, thus emphasizing on the synergistic or combine treatment strategy could be more beneficial for such cancer (Broekgaarden et al., 2019). Further, a study conducted with the patient derived PDAC showed a reciprocal interaction with the Pancreatic stellate cells (PSC). The PSCs are stimulated by the PDAC cells to secrete Hepatocyte growth factor (HGF) which in-turn enhance the drug resistance and proliferation of the PDAC cells. The hetero-spheroid formed of the PSCs and the PDAC cells sorted for SSEA4 (a human ductal stem cell marker - stage specific embryonic antigen-4) showed augmented gemcitabine resistance and promoted the phospho c-MET expression leading to tumor promoting effect compared to the PDAC cells homo-spheroid (Firuzi et al., 2019). Thus, the PSCs were observed to be acting key role in providing drug resistance (Hessmann et al., 2018) through growth factor secretion (Vonlaufen et al., 2008). Studies have provided evidences that the *in vitro* spheroid co-culture system are an appropriate chemotherapeutic drug screening platform as the combination therapy (gemcitabine + c-MET inhibitor) tested in the PDAC-PSC hetero-spheroid (Firuzi et al., 2019) showed *in vivo* relevant drug efficacy as showed in a study where c-MET inhibitor (Ci), HGF inhibitor (Hi), and gemcitabine different combination therapy was tested on the human PSCs - human pancreatic cancer cells orthotopic implanted Pancreatic cancer mouse model (Xu et al., 2020). Another study done using colorectal cancer patient derived cells and patient derived tumor-infiltrating lymphocytes (TILs) has advised use of autologous cells for co-culture studies. Anti-MICA/B antibodies along with IL-15 and anti-NKG2A in combination could aid in the destruction of the cancer spheroid. Such co-culture technique can more precisely elicit the immunotherapy based research for treating cancer (Courau et al., 2019).

The successful spheroid co-culture ratio of different type of cells opted for co-culture plays an important role and also the optimization of the culture media for the co-culture is very critical (Courau et al., 2019; Hofmann et al., 2020). With co-culture spheroid system involving direct interaction between cancer cells and the stromal and other influencing cells of the same species, allows relevant investigation in the area of cancer progression triggered through paracrine or juxtacrine signalling. Due to the various advantages of using patient-derived cells along with the co-culture technique, there has been enormous recent studies reported listed below in Table 2.

11. Biomaterials and Biofabrication facilitating spheroid co-culture

As discussed earlier in this review, the *in vitro* studies related to various steps of tumor progression, the spheroid co-culture has its many

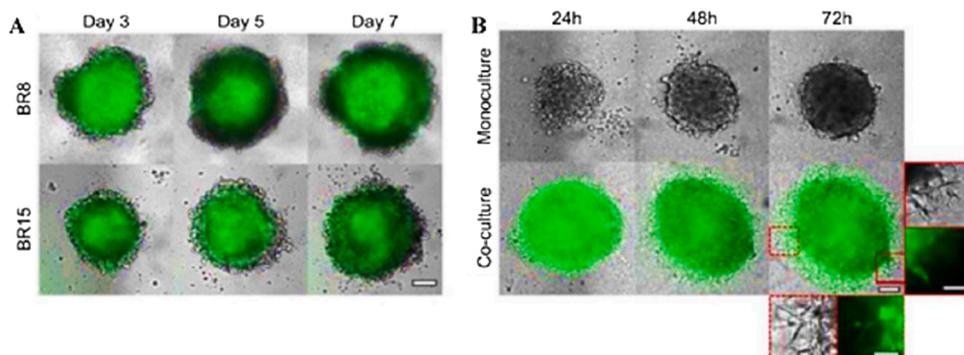


Fig. 10. (a) Spheroid co-culture of GFP tagged MSCs with ER/PR negative (BR8) or ER/PR positive (BR15) cells, where MSCs were found localized at the center of the spheroid. (b) Finger like projections observed in BR15 and MSC-GFP co-culture spheroid embedded in BME. Invasive finger like projection not observed in the BR15 monocultured spheroid edge assessed using fluorescent microscopy and bright- field microscopy at 10x magnification. Scale bar 100 μ m, 50 μ m inset (Pal et al., 2020).

Table 2
List of co-culture studies using patient-derived cells spheroid.

Type of cancer	Cell types involved in spheroid co-culture	Findings	Reference
Breast cancer	ER/PR positive and ER/PR negative breast cancer cells co-cultured with bone marrow-derived MSCs	ER/PR positive (non-invasive) has enhanced invasion under MSC signalling	(Pal et al., 2020)
Breast cancer	Breast cancer patient-derived cells and human dermal fibroblast	Drug screening of Paclitaxel, metformin, 5-Fluorouracil, Epirubicin on co-cultured spheroid generated personalized for multiple patients	(Hofmann et al., 2020)
Lung squamous carcinoma (LUSC)	LUSC patient with derived cancer cells and CAFs	CAFs induce acini formation and gives invasive property to the cancer cell	(Chen et al., 2020c)
Pancreatic ductal adenocarcinoma (PDAC)	Primary PDAC cells and patient-derived CAFs	Interaction of CAFs with PDAC elevates metabolic plasticity of PDAC cells	(Broekgaarden et al., 2019)
Colorectal cancer	Colorectal cancer patient-derived cells and patient-derived tumor-infiltrating lymphocytes (TILs)	HLA-E is upregulated in cancer cells upon infiltration of TILs in spheroid, later also having NKG2A increased aiding to escape from immune recognition mechanism	(Courau et al., 2019)
Head and Neck squamous cell carcinoma (HNSCC)	Macrophage differentiated from monocytes isolated from patient peripheral blood mononuclear cells and cell line established from HNSCC patient xenograft	Macrophage mediated HA availability, on binding to CD 44 triggered PI3K-4EBP1-SOX2 signalling lead to an increment in stemness and CD44-VCAM-1 binding stimulated Ezrin/PI3K signalling leading to increment in invasiveness	(Gomez et al., 2020)
Oral squamous cell carcinoma	CSCs sorted from the organoid formed from patient-derived specimens and Patient-derived CAFs	CAFs secrete lactate which helps in the spheroid formation potential of CSCs which has in turn increased mitochondrial metabolism	(Zhao et al., 2020)
High-grade serous ovarian carcinoma (HGSOc)	Triple co-culture of Patient-derived Fallopian tube epithelial cells (FTEC), and Fallopian tube MSCs with HUVEC	With triple co-culture, spheroid formed was more contracted and concluded that inhibiting Wnt signalling can hinder the spheroid formation	(Chang et al., 2020b)
Ovarian cancer	Patient-derived CSCs and cancer-associated MSCs (CA-MSCs)	CA-MSCs enhance stemness and chemo-resistance in CSCs via PDGF	(Raghavan et al., 2020)

Table 2 (continued)

Type of cancer	Cell types involved in spheroid co-culture	Findings	Reference
		signalling and upregulated Hedgehog ligands	

advantages. The spheroids formation *in vitro* depends on the interaction between the cells and the physicochemical properties of the surface on which cells are cultured. The most commonly used method to induce cell spheroid formation is the hanging drop method, ultra-low attachment (ULA) surface, and other cell repellent surfaces. Hanging drop is the method in which gravitational force triggers cell spheroid formation. Whereas in ULA surface for example Corning® Costar® ULA is a neutrally charged hydrophilic coating of a hydrogel covalently bound to the cell culture vessel. Such properties of the coating trigger cells to be in a suspended state and thus forcing cells to make a spheroid.

Adding on, the round bottom ULA accelerates the spheroid formation by providing the surface aided physical cues. For example, the design claimed in the patent WO2017/142410A1 involves the insert plates consisting of tube structures with concave arcuate. The no-attachment or low attachment base surface leading to the uniform spheroid formation. A comparative study between round bottom ULA, cell repellent surface, and hanging drop method was carried out focusing on various aspects of spheroid formation. Despite various pros and cons of the aforementioned methods; round bottom ULA was observed to be more efficient (Bresciani et al., 2019). The ULA surface plates are also widely used to develop spheroid co-culture systems too (Courau et al., 2019). In a study conducted by Shun Li et al., the authors have very judiciously explored the biomechanical transduction stimulated *via* the geometrical confinement engineered through microprinting (Li et al., 2021). The Yes-associated protein (YAP) [a crucial transcriptional factor in mechanotransduction (Brusatin et al., 2018)] was expressed higher peculiarly in the external part of the 3D spheroids compared to monolayer cells. This phenomenon is speculated due to the elevated mechanical force at the 3D spheroid edge. The regulated cell-cell adhesion through geometrical confinement *via* mechanical transduction governs the cell proliferation and its spatial architecture. Also, the stellate like invasive phenotype of MDA-MB-231 cells observed in this study and co-relating with the previously reported studies (Kenny et al., 2007) suggested that it might be governed by the bio-signalling provided by the ECM protein rather than the biomechanical cues. The aggressive malignant cells have lower cell-cell adhesion and cell stiffness compared to the non-malignant cells (Li et al., 2008; Nikkhah et al., 2011). The edge of the spheroid becomes rougher when the cell stiffness is high and the spheroid is more compact because of high cell-cell adhesion which results into the morphological polymorphism of the spheroid which is in turn influenced by the biochemical and biomechanical cues (Li et al., 2021).

The spheroid co-culture establishment is way more challenging than the spheroid culture. Direct spheroid co-culture involves more than one cell type, thus the most critical aspect is cell culture media optimization, maintenance of spheroid, and monitoring the different cell types. These challenges are a bit surpassed in the indirect spheroid co-culture systems for instance using transwell chambers.

Natural materials such as Dextran and chitosan when modified to oxidized dextran and thiolated chitosan, the surface chemistry and surface topography provided by the hydrogel fabricated using it, tumoroid formation (Kedaria and Vasita, 2017). Further, the collagen (Daniela et al., 2020; Fogg et al., 2019), collagen with fibronectin (Lugo-Cintrón et al., 2020) are widely used for 3D spheroid based co-culture. Also, the Liquid Overlay technique was used, where the gelatin-fibronectin coating of fibroblast facilitated HUVEC adhesion and successful formation of cancer cell, fibroblast, and HUVEC spheroid

triple co-culture (Lazzari et al., 2018). Further gelatin-methacrylate and hyaluronan-methacrylate based in-air and photo-crosslinked microgels facilitated 3D spheroid based co-culture of osteoblast and prostate cancer cells (Antunes et al., 2019).

Techniques like Microfluidics and Bioprinting have emerged as an aid for developing spheroid based co-culture systems. Microfluidics allow incorporation of fluid or media flow. Many studies involving spheroid co-culture model developed with incorporation of Collagen I (Jeong et al., 2016; Saini et al., 2020), Cultrex® (Basement membrane extract) (Liu et al., 2010), Fibrin (Ko et al., 2019) matrix been reported illustrating the advantages of exploring various tumor progression mechanisms. Bioprinting can create complex tumor physiology facilitating orderly cell distribution (Vanderburgh et al., 2017). The triple (Lazzari et al., 2018; Lamichane et al., 2019; Correa de Sampaio et al., 2012; Liu et al., 2016), tetra (Tang et al., 2020) and penta (Neufeld et al., 2021) co-culture models have been developed for better understanding of various biological phenomenon using Microfluidics and Bioprinting. Hence, these techniques help in developing cancer spheroid co-culture models more closely mimicking the physiological tumor and its micro-environment. Teresa et al., have well extensively discussed the various strategies been implemented in urge of remodelling the TME complexity and the underlying physiological mechanism *in vitro* in terms of spheroid co-culture. With the practical challenges such as cell retrieval, reproducibility and comparability; selection of scaffold, cell source and other physicochemical parameters govern applicability of the 3D tumor models (Franchi-Mendes et al., 2021).

Overall, the biomaterials play a vital role in inducing spheroid formation either by its surface chemistry or physical cues or the biomaterial consisting micro pattern forcing cells to form aggregates. A desired characteristic of the material to stimulate the cell spheroid formation are; it should be non-cytotoxic, stable, biologically unreactive, and non-degradable. Due to the different opportunities and obstacles of various methods and used biomaterials for deriving spheroids, there constant pursuit for achieving more appropriate *in vitro* 3D tumor model. Focus is on the reproducibility of spheroid formation considering its size, morphology, and other biological features, efficient spheroid monitoring, retrieval of the spheroids. Also, user-friendly handling, trouble-free fabrication, efficiency to scale up, practically potent for high throughput drug screening is desired. Thus, new combinations of the biomaterials, different shaped or patterned moulded biomaterials are in demand and continuously investigated to match up the above discussed criteria.

12. Conclusion

In the last few decades, the advancement in the *in vitro* cancer spheroid culture has lifted to a state of the art of cancer spheroid co-culture. The spheroid co-culture technique is becoming a preferred choice, especially by the scientific community. With the immense advantages of a 3D spheroid co-culturing with the stromal cells, the cross talk effect can be precisely dug in. Also, more *in vivo* relevant drug response screening can be done speedily, precisely where reproducibility is of key importance to bring the upcoming therapeutics from bench to bedside. *In vivo* models have been mainly used to test the therapeutic response but certainly have drawback translationally due to interspecies differences. The translational based cancer studies for finding molecular mechanisms and therapeutic targets, the cancer spheroid co-culture study has immense potential. The spheroid co-culture technique is proving to be potent *in vitro* tool for elucidating a more realistic therapeutic strategy by using patient derived target cancer cells. Spheroid co-culture is a promising and dynamic technique using which facts and secretes existing unexplored can be found out. Advantages of spheroid co-culture like homo and hetero cell-cell cross talk, cell ECM interaction, cell morphology, and other molecular mechanism are found to be in line with the one observed in the physiological condition. This makes it a more desirable *in vitro* technique than the monolayer cell

culture. Mimicking secondary tumor site environment, mechanism and molecules involved in invasion, migration, angiogenesis, EMT, host defence, exosomes driving cancer metastasis can be studied in the environment nearby/similar to *in vivo* condition.

Consent for publication

All authors read and approved the final manuscript.

Authors' contributions

Rajesh Vasita motivated, supervised, and critically corrected the review manuscript; Unnati Modi conceptualized the review topic, literature review, intellectually drafting of the findings based on literature, schematic diagrams preparation and revisions of the manuscript were equally contributed by Unnati Modi and Pooja Makwana. Further all the others approved the version to be published.

Declaration of Competing Interest

The authors report no declarations of interest.

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Monitoring land use changes and its future prospects using cellular automata simulation and artificial neural network for Ahmedabad city, India

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Abstract The urban sprawl is one of the potential threat for sustainable development. The present study has been carried out on Ahmedabad Municipal Corporation (AMC) area including Ahmedabad city to monitor the urban sprawl from 1976 to 2017. Normalized Difference Vegetation Index, Normalized Difference Built-Up Index, and Built-Up Index are applied to monitor the urban land use change by using multi-temporal and multi-date remote sensing satellite data from Landsat. The future scenario of urban land use prediction of AMC has been modelled by the use of cellular automata (CA) and artificial neural network (ANN) with GIS techniques. The results of land use monitoring indicate that the built-up area has increased by 156.93 km² whereas, agricultural land, open spaces, urban vegetation, and water bodies have

decreased during the last 4-decades from 1976 to 2017. Moreover, the results of CA simulation reveals that the built-up will continue to increase in the future at the cost of other classes. The decadal built-up growth was 2.58 km² during the earlier period and it was the highest (5.98 km²) during the decade of 2007–2017. The validation of results related to CA–ANN has been measured by the Kappa coefficient in which the obtained values of Kappa local, Kappa histogram and Kappa overall were 0.92, 0.75, and 0.69 respectively and the measured percentage of correctness was 78.63%. The predicted urban land use growth reveals that the built-up would cover the maximum part of the AMC area by 2027 provided that the present land use trend, demographic growth, and commercial development does not show any major change. Therefore, the study pertaining to LULC change and its future prediction will be highly helpful for urban planners and administrators to prepare a sustainable city planning for the Ahmedabad city and its surroundings.

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Keywords Multi-temporal Landsat data · Built-up indices · LULC · Cellular Automata (CA) simulation · Artificial Neural Network (ANN) · Sustainable development

**THE NEW EDUCATION POLICY- 2020: POSSIBILITIES, TRENDS AND CHALLENGES
UNDERSTANDING FROM THE PERSPECTIVE OF M.K. GANDHI**

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Abstract

The education system in India has witnessed many changes, trends and faced many challenges. There were many educational Commissions, Committees, Reports, and policy interventions that came into existence to uphold education and the educational system in India. There were many Educational Commissions like Dr. S. Radhakrishnan Commission, Mudaliyar Commission, and Kothari Commission which gave the recommendations-based Gandhi's educational ideology and philosophy and gave the recommendations. Subsequently, National Knowledge Commission and the Right to Education Act further contributed to it. Based on the recommendations, the governments at the Centre and States had formulated the policies to meet the educational aspirations of the people and their overall development. Presently the New Education Policy 2020 has been formulated. In view of this backdrop, the paper is an attempt to conceptualize the notion of education, assess the importance of education, the New Education Policy- 2020, its significance, challenges from the perspective of M.K. Gandhi. In the light of this, it is also an attempt to revisit the Gandhian principles in general, the educational philosophy of Gandhi, and his impact on the current education system, challenges, and prosperities.

Keywords: *M.K. Gandhi, Education, New Education Policy-2020, Inclusion and Integration*

Introduction

Education is the tool for the socio-economic development of all the sections, and it is an instrument to attain social justice and equality. It is to note that one can acquire knowledge through education and influence future development. Education also reduces population growth, empower women, promote democracy and human rights, and safeguard children from exploitation and strive for social change. (Singh, & Nagpal, 2010:119). The Indian education system is designed to benefit India's children and ensure that no child is denied an opportunity to access education. The Indian education system has made significant progress in reducing gender and social class disparities at all levels of schooling.

However, significant disparities still exist within socioeconomically marginalized communities that have historically been neglected in education. (NEP, 2020:24) There are many theorists like John Dewey was with the view that school should be representative of a social environment as the student learns best when it is surrounded by natural social settings. He further believes that all students are unique learners. (Williams, 2017:91). He further noted that children should be at the center of education learnings and advocate for equity and freedom and adhere to democratic virtues and ideals. (Williams, 2017: 920). Jyotiba Phule had recognized that education is as "Tritya Ratna" education opened the door for the liberation of women and the deprived sections of the society. He conceptualized universalization of education and free education for all through which he wanted to transform Indian society. (Joshi, 1991: 1325). In view of this, It is a prerequisite to understanding Gandhian Educational philosophy.

Gandhi's philosophy of Education

M. K. Gandhi had advocated different levels of education: literary education, English Education, and new education which is rooted in the culture and life of the people. This according to Gandhi developed

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परीक्षा सुधारों के संदर्भ में राष्ट्रीय शिक्षा नीति 1968 से 2020 तक की शिक्षा नीतियों की समीक्षा

डॉ. शमीम आरा हुसैन

सार

भारत में शिक्षा ऊर्ध्वगामी गतिशीलता प्राप्त करने का एक महत्वपूर्ण साधन है। वर्तमान दुनिया में शिक्षा का उद्देश्य रचनात्मक और तर्कसंगत विचारक विकसित करना है, जो देश के विकास में महत्वपूर्ण योगदान दे सकते हैं, लेकिन हम इसे स्वीकार करते हैं या नहीं? ये अत्यंत महत्वपूर्ण प्रश्न है। भारत में किसी व्यक्ति का भाग्य आज भी उनके परीक्षा के अंकों से तय होता है। यह समाज में उनकी स्वीकार्यता को भी निर्धारित करता है। एक टॉपर को आदर्श के रूप में देखा जाता है, जबकि असफलताओं को भिन्न दृष्टिकोण से देखा जाता है। हालांकि विकसित देशों में स्थिति काफी भिन्न है, जहां असफलता को बेहतर परिणाम के लिए सुधार का अवसर माना जाता है, लेकिन आज़ादी के 75 साल बाद भी भारतीय शिक्षा प्रणाली अंक-आधारित मूल्यांकन की प्रणाली में फंसी हुई है जिसने रचनात्मक और तार्किक सोच के बजाय स्मृति और रटंत पद्धति पर ज़ोर दिया है। अतः लगभग सभी शिक्षा नीतियों जैसे राष्ट्रीय शिक्षा नीति 1968 से 2020 तकने विद्यालय स्तर से लेकर महाविद्यालय और विश्वविद्यालय स्तर तक की परीक्षा प्रणाली में सुधार के लिये ठोस कदम उठाने पर बल दिया है।

मुख्य शब्दावली : राष्ट्रीय शिक्षा नीति, परीक्षा प्रणाली

परिचय:

विश्वविद्यालय शिक्षा आयोग की सिफारिश (1948-49) से लेकर राष्ट्रीय शिक्षा नीति 2020 तक, परीक्षा प्रणाली के पीछे का विचार आदर्श रूप से रैंक आधारित वितरण होने से लेकर निरंतर मूल्यांकन के आधार पर सीखने के परिणाम पर आधारित है। हालांकि कुछ विद्यालयों या विश्वविद्यालयों के विभिन्न स्तरों पर आज भी यह प्रचलन में है। परीक्षा प्रणाली में अनुसरण किया जाने वाला वर्तमान पैटर्न उच्चतर क्रम कौशल जैसे रचनात्मक कौशल, चिंतन, तर्क, सृजनात्मकता और विश्लेषण का परीक्षण करने के बजाय ज्यादातर रटंत प्रवृत्ति पर निर्भर करता है। यह परीक्षा, संबंधित छात्रों को अनावश्यक बोझ की ओर ले जाता है। यह सुधार की गुंजाइश प्रदान नहीं करता है, बल्कि एक पहचान का हिस्सा बन जाता है। यह किसी को सर्वश्रेष्ठ, औसत या असफल के रूप में नामांकित करता है। अतः लगभग सभी शिक्षा नीतियों ने विद्यालय स्तर से लेकर महाविद्यालय और विश्वविद्यालय स्तर तक की परीक्षा प्रणाली में सुधार के लिये ठोस कदम उठाने पर बल दिया है।

राधाकृष्णन आयोग, 1948 में स्वतंत्रता के बाद पहला विश्वविद्यालय शिक्षा आयोग था, जिसने परीक्षा के संबंध में कहा, “अगर हमें विश्वविद्यालय शिक्षा में एक भी सुधार का सुझाव देना है, तो यह परीक्षाओं में सुधार होना चाहिए।” राधाकृष्णन आयोग ने शिक्षा प्रणाली में रैंकिंग प्रणाली को परिभाषित किया, जहां 70% और अधिक अंकों को प्रथम श्रेणी में और 55% और 69% दूसरी श्रेणी के तहत और कम से कम 40% को तीसरी रैंक के

लिए माना जाना चाहिए। राष्ट्रीय शिक्षा नीति 2016 प्रारूप में पर्सेंटाईल सिस्टम के लिए सुझाव दिया गया है, जो पूरे क्षेत्र, स्कूलों और यहां तक कि वर्षों में एक तुलनीय स्कोर प्रदान करेगा। यह ड्राप-आउट की दर में वृद्धि की ओर जाता है, जिसके कारण 18 से 23 की आयु वर्ग के बीच केवल 18.8 प्रतिशत जनसंख्या उच्च शिक्षा में नामांकित है।

शिक्षा पर राष्ट्रीय नीति, 1968

शिक्षा पर राष्ट्रीय नीति 1968 को भारत सरकार द्वारा इंदिरा गाँधी के नेतृत्व में प्रचारित किया गया था। परीक्षा सुधारों के बारे में, राष्ट्रीय शिक्षा नीति 1968 ने परीक्षा को एक सत्त् प्रक्रिया बनाने पर बल दिया। इसलिए सत्त् व्यापक मूल्यांकन (सीसीई) का विचार शिक्षा पर पहली नीति से उत्पन्न होता है। हालांकि, कार्यान्वयन के स्तर पर इसके सही दृश्य को देखना मुश्किल होगा, क्योंकि अधिकांश शिक्षक इसे कठिन कार्य मानते हैं, जिसके लिए उनके अधिकतम समय की आवश्यकता होती है। अधिकांश छात्रों के माता-पिता भी इस तरह के मूल्यांकन से उलझन में हैं, क्योंकि उन्हें निरंतर मूल्यांकन के बजाय सारांश मूल्यांकन के आधार पर रिपोर्ट कार्ड देखने के लिए जाना जाता है। इसे अपने वास्तविक अर्थों में सत्त् और व्यापक मूल्यांकन योजना को लागू करने के लिए भारतीय शिक्षा प्रणाली के सभी हितधारकों की ओर से एक समझ और इच्छा की आवश्यकता है।

शिक्षा पर राष्ट्रीय नीति 1986 तथा प्रोग्राम ऑफ एक्शन (1992)

शिक्षा पर राष्ट्रीय नीति 1986 ने 1968 की तुलना में परीक्षा सुधार पर व्यापक सिफारिशें दीं। यह भारत सरकार द्वारा प्रधानमंत्री राजीव गाँधी के नेतृत्व में प्रस्तावित किया गया था। इसने सुझाव दिया कि परीक्षा का

उद्देश्य शिक्षा में गुणात्मक सुधार लाना होगा। यह विद्यार्थी के विकास को मापने का एक मान्य और विश्वसनीय तरीका होना चाहिए और शिक्षण-शिक्षण विधियों में सुधार कि लिए एक मजबूत संकेतक होना चाहिए।

1986 की नीति ने बेहतर शिक्षण-शिक्षण विधियों के मापदंडों को परिभाषित किया। यह सीखने की रटंत विधि को समाप्त करना है। सत्त और व्यापक शिक्षा का अभ्यास किया जाना चाहिए, और इसमें शिक्षा के विद्वानों और गैर-विद्वानों दोनों को शामिल किया जाना चाहिए। शिक्षकों, छात्रों और अभिभावकों द्वारा मूल्यांकन प्रक्रिया का प्रभावी उपयोग होना चाहिए। परीक्षा के संचालन में सुधार होना चाहिए। उपर्युक्त सिफारिशों के अनुसार अनुदेशात्मक सामग्री और कार्यप्रणाली में आवश्यक परिवर्तन किए जाने की आवश्यकता है। अंकों के बजाय ग्रेड के उपयोग को प्रोत्साहित किया जाना चाहिए। माध्यमिक स्तर में नियमित और चरण-वार सेमेस्टर प्रणाली होना चाहिए।

वर्ष 1990 में, आचार्य राममूर्ति ने 1986 की नीति की सिफारिशों की समीक्षा की और स्कूल के साथ-साथ विश्वविद्यालय स्तर पर एक परीक्षा आयोजित करने के लिए निम्नलिखित उपाय सुझाए।

प्राथमिक स्तर पर—प्राथमिक स्तर पर किसी भी निरोध नीति की परिकल्पना नहीं की गई थी, जिसका मुख्य विचार यह था कि एक बिंदु स्थापित किया जाए कि मूल्यांकन नैदानिक होना चाहिए ताकि किसी को उसके प्रदर्शन में सुधार करने में मदद मिल सके। राज्य की प्रत्येक संबंधित एजेंसी को प्रारंभिक चरण में सतत व्यापक योजना (सीसीई) की एक योजना तैयार करने की सलाह दी गई थी, ताकि मूल्यांकन सीखने की प्रक्रिया का अभिन्न अंग बन जाए।

माध्यमिक स्तर पर—माध्यमिक स्तर पर, प्रत्येक राज्य बोर्ड को ज्ञान, समझ, संचार, कौशल, समझ, आवेदन, विश्लेषण, संश्लेषण और निर्णयों के आधार पर अपेक्षित स्तर की प्राप्ति को ध्यान में रखते हुए सतत व्यापक मूल्यांकन तैयार करने की सलाह दी गई। इस संदर्भ में सहायता का कार्य राष्ट्रीय शैक्षिक अनुसंधान और प्रशिक्षण परिषद को प्रदान किया गया था।

उच्च शिक्षा स्तर पर— पेशेवर के साथ-साथ तकनीकी परीक्षण के लिए अखिल भारतीय स्तर पर चयन परीक्षा प्रस्ताव रखा। संक्षेप में, ग्रेडिंग प्रणाली के लिए अंक आधारित मूल्यांकन से दूर जाने की सिफारिश की गई। यूजीसी और राज्य सरकार द्वारा राष्ट्रीय मूल्यांकन संगठन की सेवाओं का उपयोग करके उचित डिज़ाइन और प्रशासन के साथ उच्च संस्थान में प्रवेश के लिए प्रवेश परीक्षा को बढ़ावा दिया गया था। इसने जोर दिया कि परीक्षा प्रासंगिक और विकासवादी होनी चाहिए। 1986 की नीति में, बेहतर समन्वय के लिए एक परीक्षा सुधार आयोग की स्थापना करने की सिफारिश की गई थी।

राष्ट्रीय शिक्षा नीति (2016) प्रारूप :

राष्ट्रीय शिक्षा नीति (2016) प्रारूप में जिस बिंदु पर ध्यान केंद्रित करने की आवश्यकता है, वह पृष्ठभूमि हैं, जो मूल्यांकन सुधार की आवश्यकता को निर्दिष्ट करती हैं। 1986 की नीति की तरह, यह स्कूल के प्रदर्शन की असामान्य स्थिति पर भी प्रकाश डालता है। मूल्यांकन, इस समय भी सामग्री ज्ञान को पुनः पेश करने की क्षमता में सीमित है। आज भी प्रौद्योगिकी की दुनिया में चिंता का विषय शिक्षा प्रणाली में गुणवत्ता सुनिश्चित करना है, जो केवल रचनात्मकता और नवाचार के माध्यम से आएगी। यह स्कॉलैस्टिक और को-स्कॉलैस्टिक डोमेन दोनों के आधार पर सीखने के परिणाम का आंकलन करने की बात करता है।

यह तीन विषयों में दो स्तरों पर प्रदर्शित होने का विकल्प देती है जो हैं—गणित, विज्ञान और अंग्रेजी। भाग ए उच्च स्तर का और भाग बी निम्न स्तर को दर्शाता है। जो छात्र उन तीन विषयों के अलावा कार्यक्रमों या पाठ्यक्रम को आगे बढ़ाने की इच्छा रखते हैं और जो व्यवसायिक धारा का चयन करना चाहते हैं, वे उपर्युक्त विषय में भाग बी का विकल्प चुन सकते हैं। राष्ट्रीय शिक्षा नीति (2016) प्रारूपकक्षा 10वीं और 12वीं के अंत में एक केंद्रीय परीक्षा आयोजित करने, प्रत्येक बोर्ड में प्रतिशत के संदर्भ में अंको की गणना करने का सुझाव देता है।

परीक्षा सुधार के बारे में प्रारूप की समग्र संरचना परीक्षा सुधार का उल्लेख एक संक्षिप्त पैराग्राफ में किया गया है, जो मुख्य रूप से विश्वसनीयता में सुधार और परीक्षा की वैधता पर प्रकाश डालता है। परीक्षा सुधार को दो प्रमुख बिंदुओं में लिखा गया है जो परीक्षा के उद्देश्य को छात्र के प्रदर्शन का आंकलन करने का वैध और विश्वसनीय तरीका बताता है और शिक्षण—शिक्षण पद्धति को बेहतर बनाने के लिए आठ मापदंडों को परिभाषित करता है। यहाँ, नीति में शैक्षणिक के साथ—साथ गैर—शैक्षणिक गतिविधियों के निरंतर और व्यापक मूल्यांकन का उल्लेख है। सिफारिश पाठ्यक्रम सुधार के साथ दी गई है और एक अलग खंड 10वीं बोर्ड परीक्षा के लिये समर्पित है। परीक्षा सुधार का प्रमुख उद्देश्य पाठ्यपुस्तक सामग्री को पुनः प्रस्तुत करने के बजाय व्यापक जागरूकता, समझ और उच्च क्रम कौशल का परीक्षण करना है। 10वीं कक्षा की बोर्ड परीक्षा के पुर्नगठन पर अधिक जोर दिया गया है, इसके लिए एक अलग सेक्शन रखा गया है, जो बोर्ड परीक्षा में सुधार और सिफारिश का सुझाव देता है। नीति के अनुसार परीक्षा में गड़बड़ी जैसे प्रश्न पत्र के लीक होने, नकल को रोकने पर ध्यान दिया जाना चाहिए। परीक्षा प्रणाली की विश्वसनीयता पर भी सवाल उठाया जाता है। नीति की मुख्य सिफारिश प्रश्न पत्र सेटिंग में मानक,

मूल्यांकन मानदंडों में परदर्शिता, मूल्यांकन की प्रतिशतता प्रणाली कुछ प्रमुख कदम हैं, जो परीक्षा सुधार के हिस्से के रूप में किए जा सकते हैं।

यदि केवल परीक्षा सुधार पर विचार किया जाता है, तो इस नीति के बाद, प्राथमिक शिक्षा में न्यूनतम स्तर का सीखने अस्तित्व में आया और विचार यह था कि जाति, पंथ, स्थान या लिंग के अतिरिक्त सभी छात्रों को एक तुलनीय शिक्षा तक पहुंच दी जानी चाहिए मानक साथ ही विभिन्न विश्वविद्यालयों में अलग-अलग आवेदन करने के बोझ को कम करने के लिए देश में व्यवसायिक और तकनीकी कार्यक्रमों में प्रवेश के लिए अखिल भारतीय आधार पर एक आम प्रवेश परीक्षा शुरू की गई थी। यद्यपि यह नीति, प्रारूप के रूप में है, इसकी कुछ सिफारिशों को पहले ही लागू कर दिया गया है। परीक्षा सुधार से संबंधित कई प्रणालियाँ पहले से ही लागू हैं।

राष्ट्रीय शिक्षा नीति 2020 :

राष्ट्रीय शिक्षा नीति 2016 प्रारूपसभी सुझावों को देखने-परखने तथा सिफारिशों के आधार पर मानव संसाधन विकास मंत्रालय द्वारा सुप्रसिद्ध वैज्ञानिक डॉ.के.कस्तूरीरंगन की अध्यक्षता में राष्ट्रीय शिक्षा नीति 2020 हेतु समिति का गठन किया गया, समिति द्वारा राष्ट्रीय शिक्षा नीति 2020 को हाल ही में शिक्षा मंत्रालय द्वारा देश को समर्पित किया गया है।

राष्ट्रीय शिक्षा नीति 2020 का प्रथम भाग विद्यालयी शिक्षा तथा द्वितीय भाग उच्च शिक्षा से संबंधित है। प्रारूप में वर्तमान परीक्षा प्रणाली को अन्य शिक्षा नीतियों के समान ही विद्यार्थियों के विकास के लिये अहितकारी कहा गया है। प्रारूप के अनुसार वर्तमान परीक्षा या आकलन पद्धतियाँ विद्यार्थियों को केवल रटंत के लिये ही प्रेरित करता है, इसे

बदलने की आवश्यकता है। परीक्षा के द्वारा विद्यार्थियों में सीखने की प्रवृत्ति का विकास होना चाहिये साथ ही विद्यार्थियों के तर्क, विश्लेषण, रचनात्मकता, अवधारणाओं की समझ तथा स्पष्टता की जाँच भी परीक्षा द्वारा होना चाहिये।

राष्ट्रीय शिक्षा नीति 2020, के अनुसार वर्तमान परीक्षा प्रणाली के स्थान पर फोर्मेटिव और विकासात्मक आकलन की आवश्यकता है। राष्ट्रीय शिक्षा नीति 2020 सम्पूर्ण देश में समान परीक्षा प्रारूप करने की आवश्यकता बताती है, साथ ही वर्तमान बोर्ड परीक्षाओं को ही कोचिंग संस्थानों का जनक बताते हुए विद्यार्थियों का मूल्यवान समय नष्ट होने और अनावश्यक दबाव निर्मित होने पर भी चिंता व्यक्त करती है।

राष्ट्रीय शिक्षा नीति 2020 में परीक्षा संबंधित निम्न अनुशंसाएँ दी गई हैं—

- बोर्ड एवं अन्य प्रवेश परीक्षाओं को पुनर्गठित कर विद्यार्थियों के समग्र विकास हेतु फोर्मेटिव आकलन पर परीक्षाओं का केन्द्रण होना चाहिये।
- बोर्ड परीक्षाओं का प्रारूप सरल होना चाहिये तथा इन परीक्षाओं के माध्यम से विद्यार्थियों की मूल क्षमताओं की जाँच होना चाहीये।
- समस्त विश्वविद्यालयों हेतु नेशनल टेस्टिंग एजेंसी(एन.टी.ए.) द्वारा एक साथ प्रवेश परीक्षाओं का आयोजन किया जाएगा तथा अन्य प्रवेश परीक्षाओं का आयोजन वर्ष में कई बार किया जाएगा।
- विद्यालयों में शिक्षकों द्वारा परीक्षाएँ, क्विज़ तथा पोर्टफोलियों का विकास किया जाना चाहिये।
- परीक्षाओं को कम्प्यूटरीकृत किया जाना चाहिये तथा विद्यार्थियों

को परीक्षा में बैठने के अवसरों की संख्या में वृद्धि की जानी चाहिये।

- कक्षा 3,5 एवं 8 में सेंसस परीक्षाओं का आयोजन किया जाना चाहिये जिनके द्वारा विद्यार्थियों के विभिन्न कौशलों तथा मूल अवधारणाओं की जाँच होगी जो सम्पूर्ण समाज के लिये लाभदायक होगा।
- विद्यालयों में बोर्ड परीक्षाओं के स्थान पर वर्ष में प्रत्येक सेमेस्टर में औसतन तीन बोर्ड परीक्षाओं द्वारा विद्यार्थियों पर पड़ने वाले अतिरिक्त भार को कम किया जा सकता है।
- उच्च शिक्षा में परीक्षा का मुख्य उद्देश्य विद्यार्थियों के विकास के लिये होना चाहिये न कि उनके बारे में राय बनाने के लिये।
- यू.जी.सी. के च्वाइस बेस्ड क्रेडिट सिस्टम (सी.बी.सी.एस.) की समीक्षा कर उसमें आवश्यक संशोधन के साथ नवाचार तथा लचीलापन लाया जाएगा।
- विद्यार्थियों द्वारा स्वयं का आकलन करने हेतु सहपाठी मूल्यांकन, पोर्टफोलियो, परियाजना कार्य, प्रस्तुतीकरण इत्यादी गतिविधियाँ करवाई जा सकती हैं।

परीक्षा के संदर्भ में राष्ट्रीय शिक्षा नीति 1968,1986 / 1992, तथा राष्ट्रीय शिक्षा नीति 2016 और 2020 की समीक्षा

राष्ट्रीय शिक्षा नीति 1968,1986 / 1992 : परीक्षा सुधार पर तीनों नीतियों की सिफारिशों के प्रमुख क्रैक्स को तथ्यों और जानकारी को याद करने की एक यांत्रिक प्रक्रिया के बजाय परीक्षा को एक समग्र प्रक्रिया के रूप में माना जाता है। पहली नीति में परीक्षा सुधार अन्य दो नीतियों की तुलना में काफी संक्षेप में लिखा गया था। इसने प्रदर्शन को प्रमाणित करने

के बजाय परीक्षा को एक सतत प्रक्रिया बनाने का एक प्रमुख बिंदु पर जोर दिया।

1986 की राष्ट्रीय नीति में, परीक्षा की प्रक्रिया के बारे में कई बिंदु बनाए गए थे जहाँ परीक्षा और शिक्षण—शिक्षण प्रक्रिया के बीच एक मजबूत संबंध बनाया गया था और साथ ही साथ एक अच्छे शिक्षण—शिक्षण के मापदंडों को परिभाषित किया गया था। 2016 की नीति के ड्राफ्ट दस्तावेज में परीक्षा के संचालन से संबंधित कुछ और बिंदु जोड़े गए थे। दोनों 1986/1992 की शिक्षा नीति और 2016 के नीतिगत प्रारूप से स्कोलिस्टिक और सह—स्कोलिस्टिक डोमेन पर जोर दिया गया।

उसी प्रकार राष्ट्रीय शिक्षा नीति 2016 और 2020 के अनुसार बोर्ड परीक्षाओं का प्रारूप सरल होना चाहिये तथा इन परीक्षाओं के माध्यम से विद्यार्थियों की मूल क्षमताओं की जाँच होना चाहिये साथ ही राष्ट्रीय शिक्षा नीति 2020 सम्पूर्ण देश में समान परीक्षा प्रारूप करने की आवश्यकता पर बल देता है।

विभिन्न शिक्षा नीतियों की तुलनात्मक समीक्षा के आधार पर परीक्षा प्रणाली पर सिफारिशें

हमारे देश में परीक्षा प्रणाली को हर स्तर पर सुधार की आवश्यकता है।

1. परीक्षा को केवल डिग्री, पास या असफल अंक प्राप्त करने से संबंधित नहीं होना चाहिए, बल्कि उचित कौशल, समझ और निर्णय लेने की क्षमता के परीक्षण से संबंधित होना चाहिए। लेकिन, वास्तव में यह अभी परीक्षण स्मृति पर आधारित हैं। इसलिए विचार स्तर पर बदलाव लाने की जरूरत है। विदाउट लर्निंग विदाउट बर्डन पर राष्ट्रीय सलाहकार समिति ने दसवीं और बारहवीं कक्षा की बोर्ड परीक्षाओं से संबंधित बयान दिया, “दसवीं और बारहवीं कक्षा के अंत में ली गई

बोर्ड परीक्षाएँ कठोर, नौकरशाही और अनिवार्य रूप से अशिक्षित (के रूप में) रहीं हैं, बच्चे कभी नहीं देखते हैं कि वह एक निश्चित तरीके से चिह्नित क्यों है ? और मुख्य रूप से खौफ का एक स्रोत हैं, क्योंकि वे तत्काल याद करने के लिए तैयार तरीके से सूचना की मात्रा की मांग करते हैं ।

2. एक समाधान ओपन बुक एग्जामिनेशन हो सकता है, जहां अप्रत्यक्ष प्रश्न पूछे जाते हैं और छात्र उत्तर लिखने के दौरान अपने तर्कों को प्रतिबिंबित और आधार बनाने के लिए केवल सैद्धांतिक ज्ञान का उपयोग का सकते हैं । यह छात्र की क्षमता का सही आंकलन करेगा क्योंकि ध्यान सामग्री को याद करने पर नहीं होगा, बल्कि एक सैद्धांतिक तर्क द्वारा समर्थित इसके आवेदन भाग पर भी होगा । परीक्षा सुधार पर नेशनल करिकुलम फ्रेमवर्क के फोकस पेपर में, खुली किताब की परीक्षा के महत्व पर उदाहरणों के माध्यम से चर्चा की गई है ।
3. विद्यार्थियों का आकलन न केवल उनके शैक्षणिक ज्ञान पर बल्कि उनके सह-शैक्षिक कौशल और गतिविधियों पर आधारित होना चाहिए, जैसे कि पारस्परिक कौशल, समूह की गतिशीलता, टीमवर्क, नेतृत्व आदि । इसलिए, ऐसे कौशल का परीक्षण एक अभिन्न अंग होना चाहिए । शैक्षणिक ग्रेड के साथ मूल्यांकन । हालांकि, सत्त् व्यापक मूल्यांकन के आने के पीछे का उद्देश्य छात्रों के समग्र कौशल का मूल्यांकन करना है लेकिन तथा यह विद्यालयों में सीखने की गुणवत्ता बढ़ाने में मदद नहीं कर रहा है ।

निष्कर्ष

हमारे देश में, परीक्षाओं को एक अंतिम उद्देश्य के रूप में माना जाता है,

जैसे कि शिक्षा का एकमात्र उद्देश्य परीक्षा उत्तीर्ण करना है। 1968 से 2019 तक की सभी भारतीय शिक्षा नीतियों ने स्मृति-आधारित परीक्षा पैटर्न से चिंतनशीलता की ओर बढ़ने की सिफारिश की है। यह एक सतत् प्रक्रिया होनी चाहिए जो न केवल छात्रों के शैक्षणिक ज्ञान का मूल्यांकन करती हैं बल्कि उनके समग्र विकास का आकलन करती है। हालांकि, विभिन्न कारणों से वास्तविक क्षेत्र में इसे लागू करना मुश्किल है। एक इसके लिए आवश्यक पर्यावरण को स्वीकार करने के लिए एक प्रणालीगत विफलता हो सकती है इसलिए, राष्ट्रीय शिक्षा नीति 2020 में भी पाठ्यक्रम सुधार के साथ परीक्षा सुधार शामिल है, विशेषकर बोर्ड परीक्षाओं में सुधार करने पर अत्यधिक बल दिया गया है।

धनकर ने अपने लेख “व्हाई वी फेल अवर चिल्ड्रन” में बताया कि भारत में शिक्षकों के लिए मौजूद कठोर और निरंकुश प्रणाली में सतत व्यापक मूल्यांकन को अंजाम देना संभव नहीं है। एक सतत व्यापक और मूल्यांकन के लिए एक बाल केंद्रित दृष्टिकोण की आवश्यकता होती है जिसे कठोर प्रणाली में अभ्यास नहीं किया जा सकता है। “यह प्रत्येक व्यक्तिगत बच्चे को ध्यान में रखते हुए, शिक्षक द्वारा स्वतंत्रता, लचीलापन और प्रासंगिक निर्णय लेने की मांग करता है।” (धनकर, 2017)

सतत् मूल्यांकन की एक प्रणाली बनाने के लिए, इसे ग्रेडिंग के पैटर्न के रूप में देखने के बजाय इसे समग्र रूप से समझना सबसे महत्वपूर्ण है। एक बाल-केंद्रित वातावरण इस सतत् मूल्यांकन के लिए दूसरी और सबसे महत्वपूर्ण आवश्यकता है, जो कक्षा में केवल गतिविधियों को करने के बारे में नहीं हैं, बल्कि यह मुख्य रूप से बच्चे के संदर्भ में विषय सामग्री या सिद्धांत

का वर्गीकरण कर रहा है। इसके अलावा, शिक्षक को स्वायत्तता देने की आवश्यकता है, ताकि वह पाठ्यक्रम के लेन-देन के मामले में निर्णय ले सकें और उनके सम्मान के लिए उसका मूल्यांकन कर सकें और विद्यार्थियों को राष्ट्र निर्माण में सहायक बना सकें।

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Politics of Elemental and Eliminative Representation of Women in the Indian Cinema

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Abstract: *Modernization in India came with its own set of issues which raised more questions than answers. It has been observed since ages that no matter how much progressive and modern we claim to be; we are still very much a part of the society where a variety of atrocities and violence against women like killing and aborting the girl child, honour killing, acid attacks, rape, etc. have almost become part of the everyday life. This brutally enigmatic reality of life has always managed to find means of representation and misrepresentation across different mediums. The Indian Hindi Cinema or Bollywood has produced a number of very significant movies where the atrocities and violence against women are depicted. Movies like – Mother India, Mirch Masala, Mrityudanda, Shakti: The Power, Provoked, etc. are some of the very important and impressively directed movies which in some way or the other reveal the darker side of society where women are subjected to torture and subjugation. Women in Bollywood are no longer “decorative objects” but through their realistic and powerful characterization aim to provide a “worldwide awareness of female experience...”. Movies like Bazaar, Provoked, Water and Fire have raised the issues of proper (ideal?) representation of women. This paper attempts to trace the instances of rebellion by such oppressed and abused women. This rebellion was not just at the level of raising their voice against injustice of society but it also penetrated the deep layers of systemic sectarian violence. It then becomes mandatory to look into the acts of violence and counter violence inside and outside the institution of marriage too as the institution of marriage holds the center in discourse of the Indian society. It attempts to look into the acts of articulation, dialogue, sexual aggressiveness and physical torture through a “culture – specific” and “situation specific” mode of analysis. It tries to find the intersections between the “real” and “reel” (mis) representations of women who are trying to make a conscious effort to address the anxieties and angsts of sexuality, gender, caste, class and ethnicity. The paper also tries to read the very phenomenon of modernization as a cultural discourse in the mainstream commercial Bollywood movies as well as in the critically acclaimed art movies.*

Keywords: Atrocities, Bollywood, class, power, subjugation, torture, rebellion, relationship (s), objectification

India and modernization! A lot has been said and claimed to put India into the category of modern nations, but these claims have always been questioned by certain socio-political situations and issues. The issue of violence and other atrocities against women in India stands demanding attention and action since medieval ages. The issues pertaining to violence against women and their lamentable condition in the society were in need of some kind of representation where they could reach to the masses and could generate some sense of awareness. These concerns are not new to the society or to the cinema. These are the stories of the struggles of women who tried to confront a suppressive social structure and their individual intertwined desires.

The Indian Hindi Cinema or Bollywood is a global phenomenon with over 1.2 billion viewers. It has fashioned and articulated the fluctuating structure of modern India. It has also earned itself a bad reputation for objectifying women. In majority of the mainstream cinema women have been portrayed as decorative things. There's another side of the cinema which has depicted them as martyrs or victimizers of other women. There are a few movie makers who have produced a number of very significant movies where the actual atrocities and violence against women is depicted. They tried to make a more “gender sensitized cinema” where the “exploited” and “exploitable roles” are clearly illustrated. These movies in a way made their own political statement where a woman is raising her voice against the injustice in the society. Movies like – *Mother India, Mirch Masala, Mrityudanda, Shakti: The Power, Provoked, Pinjar, Lajja, Water, Earth, Fire, Parched*, etc.

are some of the very important and impressively directed movies which, some way or the other, reveal the darker side of the society where women are subjected to torture and subjugation. Now women in Bollywood are no longer the “decorative objects” but through their realistic and powerful characterization, aim to provide a “world - wide awareness of female experience...” Movies like – *Bazaar, Provoked, Water and Fire* has raised the issues of the proper (ideal?) representation of women. These movies very successfully questioned the larger authoritarian male world order where women are subjugated, tortured, raped and murdered. They visualized “the many layers of a brutal world for women”. These movies in a way also questioned the stereotypical objectification of a woman's body in the Bollywood which was mostly meant for the male gaze. More than that it questioned the commodification of the female body meant for the male consumption in and outside the marital relationship. It tries to look into the issues of different cinematic experiences and instances where the women have been denied their actual identity and have been either intentionally eliminated and/or kept in the margins. The above mentioned movies make a deliberate attempt to present a very problematic and partial picture of women in the society to create a need for awareness on the issues of the social situation of the women. At this juncture it becomes crucial to understand that the movies related to the issues of women are majorly controlled by the prevailing sociopolitical discourses on women and eventually become victims of the very much open or liberal epistemology of conventional feminism which has always looked upon the issues of women as a kind of war of the genders. So there's a need to recognize and scrutinize the representation of

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women in artistic practice which should be able to contend the fundamentally social appeal of all practices.

There have been some efforts in the direction of the social upliftment of women since 1800. It became more organized around 1900 – 1920 with the formation of the organizations like **The Women's Indian Association (WIA, 1917)**, **The National Council of Indian Women (NCWI, 1925)**, and **The All India Women's Conference (AIWC, 1927)**. Some very remarkable women like Kamala Devi Chattopadhyay, Muthulakshmi Reddy, Sarojini Naidu, Margaret Cousins, etc., were the chiefs of such organizations. In their leadership and with the help of some prominent nationalist leaders many legislative acts were passed and proposed like the Sharda Act (1929) restraining the child marriage, Hindu women's rights to property act (1937), the Muslim personal law application act (1937), etc. With the help of such organizations Women's Movement in India was catching pace and tried to bring women in the center from the margins through a number of exemplifications in a variety of mediums like that of art and academia. Sangeeta Datta says in her *Globalisation and Representations of Women in Indian Cinema* that the academic debate of the female representation should move on from the abstract analyses to a more dynamic commitment like the "modalities of image, forms of speech and address to viewers" (Datta, 71). In light of the Women's Movement in India and its repercussions on cinema she further illustrates-

The nationalist rhetoric of the pre - independence years produced films valorizing the mother figure. Mehboob Khan's *Aurat*, a modest film made in the early forties was remade in color as *Mother India* in 1956. The making of a new nation, the projection of Indian culture to the world market, the first International Film Festival in Delhi – perhaps all these factors led to the tremendous reception of the film both at home and abroad. It was the immediate post - independence moment that led to the phenomenal iconisation and identification of the mother and nation in popular consciousness. Nationalist discourse constitutes the female body as a privileged signifier and various struggles are waged over the meaning and ownership of that body. What does it mean for women to be explicitly evoked in theories of nation only when their specificity can serve a particular cause? As the women's movement gained strength in India and highlighted women's oppression and a struggle for an egalitarian society– a series of women film makers brought women from the margins to the center of their texts. An alternative viewpoint and a female gaze brought focus on female subjectivity. A number of films were made by Aparna Sen, Sai Paranjape, Vijaya Mehta, Aruna Raje and Kalpana Lajmi, which were sensitive portrayals of women protagonists, in search of social and sexual identity, women firmly located in specific socio - historical contexts. (Datta, 72 - 74)

Movies like *Bazaar*, *Mandi*, *Nikaah*, *Water* and *Provoked* have socio - economic backdrops which stand at the intersections of the history, real life and reel life. In these movies we do find an articulation of ideas and opinions; wherein we see that somewhere the modern feminism is in correlation with the traditional values. When we see characters like Najma, Rukminibai, Nilofer, kalyani and

Kiranjeet challenging the larger social structure and their personal tangled desires and needs, we notice a cognizance of the female experience. These and many other such movies visualize a multi layered vicious world for women. These are the stories of the exploited and exploitable roles in both – real and reel life; which tries to contest the unfair and uneven situations that instigate violence against women.

The story of *Nikaah* has a Muslim locale. It criticized the use and abuse of woman by her two successive husbands. When I say 'use' and 'abuse' I mean the representation of woman as a 'thing' meant for the consumption of the male. Her condition after her first divorce is still the very condition of the majority of women in India who are looked down as a social stigma and have a hard time settling in the routine life and even finding a decent job without being the subject of scorn and ridicule. There is another point of view which this movie presents before us – the place of divorced/single woman in the society. Through the repeated Nikah (marriage) of Niloufer it is signified that there is no place for a single woman in the Indian society. She needs to have a male protection; preferably a husband's, in order to gain respectable social recognition. The way Niloufer is treated in this movie is very disturbing. She actually becomes a victim of male – her two respective husband's whimsies and insecurities and is tossed around like a damaged and soiled toy! This persecution of the woman within the so called sacred bond of marriage; demonstrating a kind of unseen and obscure violence, is very much intimidating, shocking and precarious.

The notion of woman as an object of consumption and commodification is expressed in a very disturbingly striking manner in the movies like - *Bazaar*, *Mandi* and *Water*. The issues of girl - child marriage, wife shopping and buying, forced prostitution, molestation, widow sexual exploitation, etc. have been bought up in a certain manner which asserts that a female body can be transactional! It can be hustled up and traded for family honour, financial benefit, marriage, sex, flimsy egos and respect. They all portray a sort of perverted politics of majoritarian upper class domination. In *Bazaar* and *Nikaah*; religion plays an important role as the bartering, the trade happens in the guise of religiosity and tradition. In *Bazaar*, throughout the movie we notice that women and money are talked about together. The protagonist of the movie – Najma, belongs to a withered high class family of Hyderabad Nawabs. Her mother tries to force her into prostitution so that they can live a 'respectable' and reasonably lavish life matching their social position. When Najma refuses to do so her mother tries to comfort and then confront her by saying that she just needs to do it in the darkness of night so that no one knows about it and asks her to do so in the name of family's honour, while her brothers are free to roam around without doing anything; because their doing any kind of job will ruin their 'Nawabi thath'. There is a beautiful scene in the very beginning of the movie where Najma is watching herself in the mirror inquisitively and we as audience watch her watching in the mirror. It does provides a kind of visual pleasure to the audience in terms of the scene's cinematography but in a way it also gives a sense of premonition that throughout the movie Najma keeps on questioning her existence, her identity and her actions. The movie questions the despair of

the poor and exposes the decadent Indian society where the poor teenage brides are sold and bought by the old and rich. Najma painfully says that – “aapke bazaar mey agar koi sabse sasti chiz hay – to who hay – aurat” (the cheapest object in your market is – the woman). With such serious and tragic storyline, we notice a very casual atmosphere maintained by most of the male actors throughout the movie. There are very serious matters of love, life and livelihood and it's the poor and helpless people from the society who are affected majorly by these issues. The rich and powerful simply play ignorant and indifferent to the poor people's pleadings. The other female characters in the movie are also silent because they know that teenager Shabnam's marriage to wealthy middle aged Shakir Ali Khan will somewhere clear the way to their own marriage (s) and better future. The tradition of “Meher” in Muslim marriage which makes the bride a property of the groom is confronted in this movie. It makes marriage a legal system of buying and selling humans. It is very unsettling to notice that so far no hardcore social or political hardcore efforts have been made to abolish this practice.

“Mard kharidta hai tabhi toh aurat bikti hai!” (Just because a man buys, a woman is sold.) Movie *Mandi* depicts an even murkier side of the society where the aged old “kalakar” – the artists are forced to reduce themselves into prostitution as their art is no longer appreciated or recognized by the society. In the movie *Rukminibai* – a brothel madam, keeps on lamenting about their downfall. There is a very interesting character of the photographer in the movie who keeps on clicking pictures of the prostitutes from all the angles and if possible in ‘all’ kind of positions and for that he is ready to do almost anything – just like modern paparazzi! In a way this character shows that reality of the society which is full of people who delights in hidden sensual pleasures like looking at the sexy and sensational pictures of women and also wants to earn by selling these pictures to the ‘demanding’ customers. There is also a policeman in the brothel who actually lives in the brothel and has also fathered a child with one of the prostitutes. Instead of taking the responsibility of the child and his mother and taking them home, he rather stays back in the brothel in order to fulfill his sexual desires from almost all the prostitutes! He is actually free from all the responsibilities of being a father or a responsible husband or partner! His 24*7 presence there also in a way depicts a society which has crossed all the limits of decency, responsibility and which has no concern for the needy and the poor. It is society of lecherous and treacherous upper class rich people where the poor people and prostitutes like *Rukminibai* are forced to safeguard their ‘illegitimate secrets’. There is a triple displacement of prostitutes in the movie. They made to relocate again and again because the so called respected and sophisticated people refused to live around them and demanded more and more space for the modernization of their surroundings. The prostitutes were made to live in a dilapidated building near a shrine and they turned it into a thriving business and religious center, where all the “wishes” of people are answered. These displacements also changed the power dynamics inside and outside the brothel. Many well – guarded secrets are revealed and Zeenat, the most valued prostitute runs away with her own step brother in desperation. *Rukminibai* is

kicked out of her own ‘kingdom’ but manages to find a beautiful place with a Shivlinga at the riverbank and poor deaf and dumb Phoolmani running towards her from the assumed Nari Niketan Grih. This scene is very symbolic as *Rukminibai* finds again a place to start her new life with signs of life - water (river), religion (Shivlinga) and woman and man (Phoolmani and Tungrus) and for Phoolmani it's a run from one prison to the other. Throughout the movie the discussion moves around some ‘Nari Niketan Grih’, but it is never actually shown! This could also be interpreted as the larger reality of society where the places or spaces like these which work for the betterment of needy and suffering women do not exist. Their existence is just on paper. Even if they exist, the condition of women in such places is very horrifying. There is a very interesting representation of female characters in this movie which depicts most of them as “in the service of the society”! It includes the prostitutes and the social worker. Another interesting point is that – the people of all caste and creed are welcomed in the brothel. It comes out as a more secular place than the actual educated and so called progressive society. This movie has also very successfully depicted women of almost all the types – innocent women, shrewd women, lusty women, longing women, always pregnant women, smart women, foolish women, constantly worried and working women, pretentious, mysterious and confused women etc.

Bazaar and *Mandi* also attempts to probe into the issues of the identity formation and its deconstruction. Even the titles of both the movies are very apt! *Bazaar* – meaning a proper market with established and trained shopkeepers like Hajjun Bi who serve to the chosen elites or the highest bidder; unfolds the inhuman and pretentious side of the high class society whereas *Mandi* – which means a road side, street market with small vendors shows its availability and access to all. It also shows the hypocritical nature and the vileness of the high class people who are full of contempt for the poor and live dual lives of respectability and debauchery.

These movies, no doubt elementarily talk about the injustices and atrocities on women; have a very eliminative approach of doing so. They depict women in the margins, mostly sidelined. The representation of women in Bollywood is crucial in the mode and manner of their depiction which majorly revolves around them as ‘something’ which could be traded, bartered or replaced.

In contrast to these movies; *Utsav* by Girish Karnad presented a very interesting viewpoint on the lives and condition of women. Though the story is set in the medieval India; it is way modern in terms of its concept. This movie talks about a brothel and a prostitute named Vasantsena in it. This movie offers a very different perspective on the lives of prostitutes as they had a distinct position in the society and were respected. They were under the state's protection. It is not just a story of prostitutes experimenting with sex and helping Vatsayana with his magnum opus – *Kama Sutra*; but a narrative of the power politics - the Marxian concept of entertainment, consumption and sex related to the politics. Yet when it comes to the representation of women, it falls into the stereotype and depicts them as objects of and for entertainment and luxury – meant for the male consumption. As Charudutta's wife Aditi clarifies to Vasantsena that in a

way she is happy that her husband is having an affair with the most sought after prostitute in the state, but at the same time she makes it very clear to her that ultimately she is his wife and therefore holds a better position in the society. The idea of female representation here becomes a bit problematic as on one side the prostitutes are shown as well respected beings in the society but at the same time their representation and existence is mainly for the male gaze and consumption. They are both – elemental to the society but are also in the easily eliminative zone of the society.

There's a need to not only venture into the avenues of the politics of (mis) representation of women in the Indian cinema; but also to bring into fore the trials and traumas of the Indian woman who struggles to find, claim and reclaim her identity as a living – breathing person! The notion of inflicting violence upon women becomes very important here as this violence is multi layered and multi - dimensional. This violence is not just the physical violence; it is also a sexual, emotional and psychological trauma for the woman who is victimized for the centuries which is the result of a number of superstitions and malpractices prevalent in the society at the levels of religion, caste, class and gender. In Deepa Mehta's *Water* there's a very disturbing portrayal of the pre - independence Hindu widows. Where they are denied all the pleasures of life and made to forsake their dignity with no right or hope for their emancipation. Widows like Kalyani and Shakuntala either live a life with no dignity and forced servitude or they just die or commit suicide. These were their only options. Theirs were the silent and silenced narratives. The representation of such narratives can be studied as an attempt to understand and investigate the lives of the pre - independence Hindu widows and the post - independence Hindu widows. With this it needs to be determined whether the society has actually changed?

In Indian society, a woman's strength and virtue is directly proportional to how much suffering they can endure and still do what is expected of them. This paper also attempts to trace the instances of rebellion by such oppressed and abused women. This rebellion was not just at the level of raising their voice against the injustice of the society, but it also penetrated the deep layers of systematic sectarian violence. Movies like *Provoked* are made to address the issues relating to the violence upon women. In spite of suffering severe domestic violence Kiranjit is made to suffer in the prison for long. A battered and provoked victim Kiranjit eventually manages to settle down in her life. In the movie *Provoked* we see a very problematic representation of Indian women. She suffers the violence and reacts violently by killing her husband, but in prison, offers formal prayers with the help of a proper Hindu pandit (priest). One of the reviews about the movie states that - "The construction of the self - conscious Indian images and narratives, a sense of 'Indianness', not only for the Indian audience but for the European market with a touch of glamour." Other reviewer opined – "Phenomenal iconisation and identification of the mother, the wife and nation in popular consciousness." The violence begins at the domestic level and then penetrates deep and silenced in the name of duty, religion and society. Religion demands obedience and silence. This violence is deeply rooted and somewhere also knowingly –

unknowingly inculcated in the future generations. It then becomes mandatory to look into the acts of violence and counter violence in and outside the institution of marriage too as the institution of marriage holds the center in the discourse of the Indian society. The present, now, is the time to look into the acts of articulation, dialogue, sexual aggressiveness and physical torture on women through a "culture specific" and "situation specific" mode of analysis. It tries to find the intersections between the "real" and "reel" (mis) representations of women who are trying to make a conscious effort to address the anxieties and angsts of sexuality, gender, caste, class, and ethnicity because even today they are not able to find strength to tell anyone about their pain – because they don't know if anyone would listen!

From *Mother India* in 1956 to *Parched* in 2016, in the last 60 years of Indian Hindi cinema how little the technique of representation of women has actually changed! These movies raise very important questions not only of the proper representation (s), but also of the situations and circumstances in which such representations are carried out. They are majorly pertaining to some or the other type of violence. Is there any way escaping this violence or we simply need enduring it? Precious Arinze says in *The Women Who Carried Violence In Their Bodies* –

The idea of a female voice that is seen and heard is an inconceivable atrocity to some. The need to censor and police the female body is built on fear. But fear of what? A culture that rids the male body of responsibility and accountability while tattooing blame on the female body is dangerous and benefits no one.

Women are culturally engineered to be custodians of patriarchy as much as men are. The existence of these wonderful, rebellious, independent, audacious women characters opened my eyes to painful realities. I drew inspiration from their experiences, some of which had been mine. Like them, I did what I could to move against the swirl of transgression. (Arinze)

These myriad representations of women in Bollywood in a way attempts to make its own political statement (s). From show pieces to victims and martyrs, they are now emerging as survivors with possibilities of empowerment, emancipation and freedom.

"I am reclaiming my time. I am learning what freedom is. Coming into myself with the audacity of sunshine after a storm. I am verbalizing my defiance."

- Precious Arinze

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Director: MehboobKhan

Released in: 1940

Mother India

Director: Mehboob Khan

Released in: 1956/57

Nikah

Director: B. R. Chopra

Released in: 1982

Bazaar

Director: Sagar Sarhadi

Released in: 1982

Mandi

Director: Shyam Benegal

Released in: 1983

Utsav

Director: Girish Karnad

Released in: 1984

Water

Director: Dipa Mehta

Released in: 2005

Provoked

Director: Jag Mundra

Released in: 2006/2007

Parched

Director: Leena Yadav

Released in: 2015/2016

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PUBLIC EXPENDITURE ON ELEMENTARY EDUCATION IN THE NORTHEAST REGION OF INDIA

Dr. Sonam Choephel

Dr. Sarala Desari

Abstract

The northeast region has achieved remarkable growth in average literacy rate that is more than what the national average has achieved since the independent. However, the literacy rate cannot be justified for better elementary education. The extremely high dropout rate put the northeast region in danger of losing the track of achieving universal elementary education. Being the peripheral region of the country, the sole development of the education system relies largely on government funding. The pattern of government funding in the region is miserable. The large gap between plan and non-plan expenditure on elementary education as well as the negative growth of plan expenditure hinders elementary education development. The share of elementary education to the ratio of total expenditure on education keeps on decreasing. The impressive percentage share of expenditure on elementary education to the ratio of GSDP is misleading since GSDP of the northeast region is comparatively very small that portrays high percentage shares on education.

Key Words: Public Expenditure, Elementary Education, Gross Enrolment Ratio, Drop-out Rate

1.1 Introduction

The idea to provide free and compulsory elementary education was not new in India. When India first adopted its constitution which came into force in 1950 it has not neglected education. Article 45 made a resolution to provide free and compulsory education to all up to age 14 within the next 10 years but it was under the directive principle. Before 1976, education was under the state list where the delivery of education at the district level was the sole responsibility of the state's government. The partnership of Centre and State in education came in 1976 when education becomes the concurrent list where both the government has figured out as to how that finance will materialize.

The financing of education has always remained a problem. The Kothari commission 1964, recommended India should spend at least spend 6 percent of gross national income (GNI) on the education of which 50 percent of the expenditure should be earmarked on primary education. National

Public Policy and Human Rights: Towards Inclusive Society

—Jagannatham Begari

Abstract—The paper attempts to elucidate the importance of public policy and human rights, as both are important spheres in a nation striving for the welfare and wellbeing of its people. There is a significant impact of human rights on policy making and its implementation. Human rights i.e., the right to life, right to liberty, right to association and be part of decision making implicitly or explicitly. A large majority of countries in the world are signatories of international Agreements, Conventions and covenants. Therefore, it is the fundamental duty of all States to respect and follow the provisions of such agreements. India has strived to be at the forefront of promoting human rights of its people. The Constitution of India strengthens the obligation of the state to uphold the rights and welfare of the people. Since India is a welfare state, the viewpoint of the people and their human rights has to be crucially incorporated while formulating the policies. Further, it must be ensured that policies are efficiently implemented, and their advantages reach to the intended beneficiaries. India, being a developing country still faces problems like poverty, unemployment, agricultural crisis in a rural based economy. Therefore, it is the responsibility of the state to take measures to eliminate poverty and provide welfare policies. The paper proposes to study and evaluate policy and human rights and its significance in contemporary times.

Key words: Human Rights, public policy, democracy, government, Magna Carta, United Nations of Organisation, participation, morality.



Regenerated cellulose nanofibers from cellulose acetate: Incorporating hydroxyapatite (HAp) and silver (Ag) nanoparticles (NPs), as a scaffold for tissue engineering applications

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ABSTRACT

Cellulose nanofibers, which are troublesome to spin into fibers, can be easily fabricated by post-regeneration of its acetate-derived threads. Cellulose is a natural polymer; it enjoys better biocompatibility, cellular mimicking, and hydrophilic properties than its proportionate analog. Herein, we regenerated acetate-free nanofibers by alkaline de-acetylation of as-spun nanofibers. The resultant cellulose nanofibers previously loaded with hydroxyapatite (HAp) were immobilized using silver (Ag) nanoparticles (NPs) by reduction of adsorbed Ag ions on using sodium borohydride. These amalgamated nanofibers were characterized for SEM, EDX, TEM, FTIR, and hydrophilicity tests revealing the existence of both HAp and Ag NPs in/on the nanofiber scaffolds. The de-acetylation of composite nanofibers resulted in spontaneous hydrophilicity. These nanofibers were cytocompatible, as resolved by MTT assay conducted on chicken embryo fibroblasts. The SEM of the samples after cell culture revealed that these composites allowed a proliferation of the fibroblasts over and within the nanofiber network, and increased concentration of HAp levitated the excessive of apatite formation as well as increased cell growth. The antimicrobial activity of these nanofibers was assessed on *E. coli* (BL21) and *S. aureus*, suggesting the potential of de-acetylated nanofibers to restrain bacterial growth. The degradation study for 10, 30, and 60 days indicated degradation of the fibers much is faster in enzymes as compared to degradation in PBS. The results certify that these nanofibers possess enormous potential for soft and hard tissue engineering besides their antimicrobial properties.

1. Introduction

In recent years, enormous progress has been achieved and is going on in the area of tissue engineering for the advancement of scaffolds used in various implants. In this regard, the diverse classes of polymers, especially natural and synthetic, have been fabricated to provide a short-term skeleton system for encouraging up the cell attachment, growth, migration, proliferation, differentiation, and finally, procreation to allow regeneration of the impaired tissues [1–3]. However, the natural polymers are ideal candidates in contrast to the counterpart scaffolds created from synthetic polymers [4]. Moreover, the natural polymers have tremendous potential in tissue engineering, regenerative medicine, drug delivery and stem cell-based research [5–7]. Cellulose is

an organic polymer with the formula of $(C_6H_{10}O_5)_n$ and is one of the most abundant polysaccharides present on earth. Besides, it is the primary structural component of the cell wall in plants, algae, oomycetes. Intriguingly, some of the bacterial strains are celebrated to synthesize 20–100 nm known as microbial cellulose [8,9]. The utilization of cellulose has been well-documented for various accomplishments in biomedical sciences. Among multiple facets of its application, cellulose as supporting structure in bone/cartilage restoration is accurately used [10,11], vascular tissue reconstruction [12], wound healing [13], diffusion controlling membranes [14] and coating material for drug releasing scaffold [15]. Due to the recent advances in technology, the cellulose is quickly processed into a diverse configuration (e.g., membranes, sponges, particles, and nanofibers) depending upon the method

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of fabrication and utility of the specific design [16]. These applications of cellulose are due to the strong intermolecular hydrogen bonding between the linear chains of $\beta(1 \rightarrow 4)$ linked to D-glucose units, and that accounts for its higher degree of crystallinity, mechanical strength, low solubility, hydrophilicity and slow degradation rate [17].

Currently, there are plenty of techniques available to fabricate desirable scaffold from cellulose for applications in hard and/or in soft tissue regeneration. Among the available methods, the electrospinning is considered as one of the most widely accepted processes to fabricate fibers with a diameter ranging from nano- to micro-scale and applied in various fields of technology [18–21]. The most important advantage of this technique is that it can produce nanofibers in a tailor-designed manner with controlled diameter, porosity and morphology that can mimic the extracellular matrix (ECM) of the native tissue present in the human body [22–24]. In addition to providing structural support as well as topographical and biochemical cues, the nanofiber supports in allowing the attachment, migration, proliferation and differentiation of the native cells, which are in dire need of regeneration. Regardless of the fact, cellulose is difficult to process into nanofibers by electrospinning owing its poor solubility in common organic solvents and its subservient nature to undergo thermal degradation before melting [25]. Furthermore, problems associated with fabricating the nanofibers by electrospinning are that the other solvents (e.g., *N*-methylmorpholine-*N*-oxide and lithium chloride) are either highly toxic and are difficult to remove during electrospinning or must be spun at elevated temperatures [26]. Moreover, the use of highly poisonous solvents places the additional risk of residual solvents in the final product, i.e., in nanofibers and the elevated temperatures during the electrospinning can lead to a reduction in mechanical properties and finally, all this adds up the additional burden in getting nanofibers deposited directly on collector surfaces. Therefore, only a few reports for the fabrication of pristine cellulose nanofibers by electrospinning are available in the literature [27].

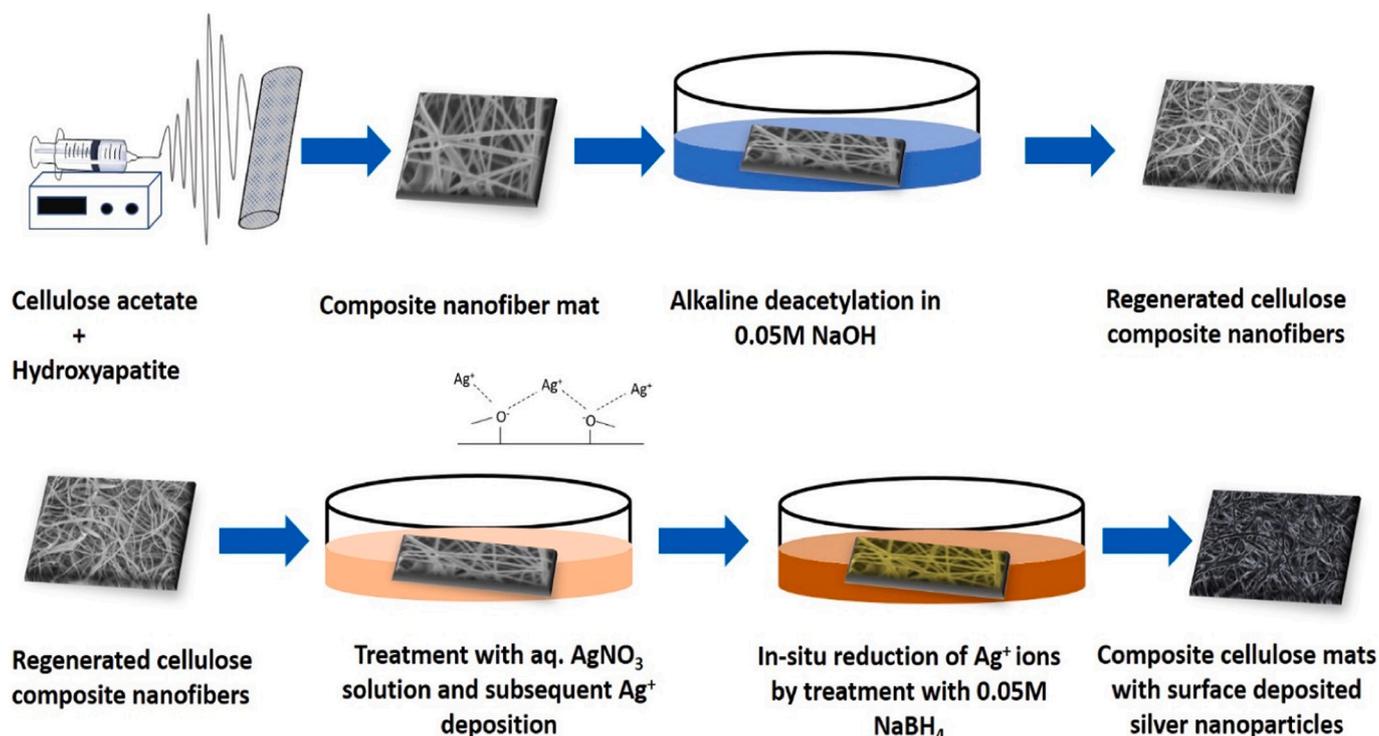
Moreover, bacterial cellulose fibers are extensively studied for biomedical applications, including hard and soft tissue engineering [28,29]. Nevertheless, the use of bacterial cellulose is limited because it is difficult to control the uneven growth of fiber morphology. Concurrently, synthesis by bacteria includes expensive fermentation process, also restricts its use compared to cellulose available from plant sources [29]. Therefore, a conveniently available approach is to exploit the use of cellulose acetate as a progenitor for the fabrication of pure cellulose nanofibers [30]. Cellulose acetate is the synthetic polymer and is commercially available. It is a derivative of acetate ester of cellulose and can be dissolved in most of the common solvents such as acetone, acetic acid, dimethylformamide, dimethylacetamide, methanol, chloroform, pyridine, etc. [31]. It is noteworthy to mention that cellulose acetate nanofibers are easily produced by electrospinning due to ease in choosing the aforementioned solvents. Subsequently, after the event of electrospinning the cellulose acetate nanofibers can be desirably regenerated using facilely alkaline de-acetylation process to eliminate acetate groups present on cellulose backbone [32]. In this work, we have attempted a resembling approach using acetone–water solvent system to disperse cellulose acetate and then de-acetylate the cellulose acetate nanofibers using NaOH to regenerate pure cellulose nanofibers.

The implantation of electrospun scaffolds into the host body depends upon the efficiency and strength of osteointegration between the bone and the surface of the implant in the case of bone tissue engineering [33]. One of the most commonly applied strategies is to induce osteointegration by surface coating the implanting fibrous scaffold with hydroxyapatite (HAp)-like minerals [34,35]. However, a fundamental problem associated with surface coating is the tendency of having improper and/or loose-fitting attachment resulting in detachment of bone inducing agents. Besides, after an event of cell proliferation on the implant surfaces, which is abundant in bone producing agents, the degradation takes place in the deeper sections of scaffolds

where no bone supporting agents are present. Furthermore, this can endanger the whole process, resulting in implant rejection at later stages of tissue growth.

Moreover, a conventional procedure to create a surface apatite layer on biomaterial scaffolds is to incubate them in the presence of simulated body fluid (SBF), which is an electrolyte solution that mimics the inorganic composition of the human blood plasma [36]. However, crystal growth is a complex process and requires the solution to develop into a beautiful crystal form the pre-existing crystal. Owing to this pre-request, it often requires precursors material to act as a nucleation site for the appropriate induction of HAp particles. For instance, the artificially introduced functional groups at the site of nucleation have shown the ability to induce the HAp layer on polymer scaffolds when treated with SBF solutions [37]. In another example, the functionalization of cellulose nanofibers with a carboxylic acid group was found to enhance the biomineralization process by prompting the apatite growth formation [36]. Other studies have reported that a pre-treatment of the scaffold with Ca^{2+} containing a solution, which is a prerequisite for the induction of Ca and P mineralization on the nanofiber framework during subsequent incubation in solutions of SBF [38]. However, our approach was to co-electrospun nano-HAp particles along with cellulose acetate to lodge these particles within and over the surface of the fibers, which probably will act as nucleating sites for further induction of apatite growth when treated with the SBF solution.

In present days, researchers focus on the advantage of possessing add-on properties of having antimicrobial attributes that are desirable when the nanofiber scaffolds are applied either externally and/or internally to avoid microbial infections near the area of its application [39,40]. Lately, due to the emergence of antibiotic resistance, by using organic antibiotics, the focus has been shifted towards use inorganic metals and their oxides against the hostile bacterial strains. In this connection, silver (Ag) has reported being effective against 650 bacterial species [41]. The ability of the Ag ions to act as a potential antibacterial agent is due to its ability to attach the cell membrane. Later on, to penetrate the respiratory chain of the cells leading to the shutdown of the oxidative phosphorylation pathway and hence causing the death of bacteria [42]. Compared to its pure metallic form, the Ag in its nanoparticle (NP) nature exhibits an enhanced antibacterial action due to its increased surface area, high-reaching potential for penetrating the multifaceted cell wall, and excellent release kinetics [43]. In the case of nanofibers incorporated with Ag NPs, the commonly preferred strategies can be classified into the following methods. One of the ways takes advantage of preparing the colloidal Ag NPs from its salts (e.g., AgNO_3 , Ag_2SO_4 , AgCl and $\text{C}_{10}\text{H}_9\text{AgN}_4\text{O}_2\text{S}$, etc.) by employing reducing agents like NaBH_4 or/or sugars [44,45]. Further on, this step can be followed by immersion of the samples into the already prepared colloidal dispersion for allowing the adsorption of the Ag NPs in/on the fiber by electrostatic interaction with the functional groups of the polymer present in the nanofiber scaffolds [46]. The alternative method exploits the in-situ preparation of the NPs by initially allowing the deposition of the Ag^+ ions on the nanofiber surface by electrostatic and/or by van der Waals interaction and the reduction of the Ag ions to Ag particles by a suitable reducing agent such as UV light [47], microwave [48], ultrasound [49], or chemical reduction [50]. Furthermore, a co-solvent method used in electrospinning such as *N,N*-dimethylformamide to reduce Ag salts and then to use the same solvent containing Ag NPs in preparing dispersions for electrospinning. However, the toxic nature of the solution limits its efficacy in the preparation of bio-scaffolds [39]. However, the only drawback of this system is that a higher concentration of AgNO_3 remains unreduced, which can instigate toxicity in the cultured fibroblasts [51]. In this paper, our approach includes in-situ methodology whereby the regenerated cellulose nanofibers containing different concentrations of the HAp are treated with AgNO_3 solutions, and then the deposited with Ag^+ ions by chemical reduction using NaBH_4 (Scheme 1). It is noteworthy to mention here that we cannot directly use the HAp and AgNO_3 solutions for electrospinning of



Scheme 1. Schematic representation of the fabrication of regenerated cellulose nanofiber mats containing HAp and Ag NPs. Sequential steps are shown to describe the various process involved in the fabrication process.

nanofibers, because solution forms unsuitable precipitate when the cellulose acetate is added to produce nanofibers by electrospinning. This work aimed to fabricate regenerated cellulose nanofiber scaffolds containing nano-HAp as nucleating agents and post-treatment with surface grafted Ag NPs to form scaffolds with antibacterial action. These scaffolds were investigated under physiological conditions in SBF to induce excessive apatite formation for further application to osteointegration. The antimicrobial activity of the fabricated nanofibers was assessed against *E. coli* and *S. aureus*. Furthermore, these scaffolds were investigated for biocompatibility and cell attachment against chicken embryo fibroblasts, in order to reveal the potential application in wound healing.

2. Experimental

2.1. Materials

Cellulose acetate (M_w 50,000; 39.7 wt% acetyl content), Silver nitrate, Sodium borohydride, glutaraldehyde (grade I, 50% in water), PBS tablets, TEM grids, and nano-HAp were purchased from Sigma-Aldrich, USA. Acetone (extra pure), Sodium chloride, Potassium chloride, Magnesium chloride, Calcium chloride, Potassium phosphate, Sodium sulphate, Tris buffer, Acetic acid, and Sodium hydroxide were purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Sodium bicarbonate and Hydrochloric acid were supplied by Avantor Performance Materials Ltd. Thane, India. Ethanol (absolute) was provided by Changshu Hongsheng Fine Chemical Co., Ltd., Jiangsu, China. All chemicals/solvents utilized were of analytical grade and were used without further purification throughout all experiments. The microbial strain *S. aureus* was a kind gift from the Department of Animal Biotechnology Sher-e-Kashmir University of Agricultural Sciences, Kashmir, India. The LB agar and LB broth were purchased from G-Biosciences®, St. Louis, MO, U.S.A. The chicken embryo fibroblasts (CEFs) were isolated from 9-day old chicken embryos and further maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Sigma, St.

Louis, Missouri, USA) supplemented with 10% fetal bovine serum (FBS) (Sigma, St. Louis, Missouri, USA) and 0.1% cocktail antibiotic composed of 10,000 U Penicillin and Streptomycin solution (HIMEDIA Lab, Nashik, India). Furthermore, the cell culture flasks, microplates, and all other reagent bottles necessary for microbial and tissue culture were purchased from BD Falcon®, USA.

2.2. Methods

2.2.1. Preparation of dispersions for electrospinning

Cellulose acetate (12 wt%) dissolved in acetone–water solvent system (90,10w/w%) was kept overnight on a magnetic stirrer (REMI, model 1MLH) at 500 rpm. The screw-top vials were tightly sealed by parafilm to avoid the loss of volatile solvents. The HAp NPs were prepared by following our previously developed strategy [52]. After preparation of HAp NPs, different concentrations of HAp NPs (i.e., 0.5%, 1.0% and 1.5%) concerning polymer weight % used for electrospinning were separately dispersed in 10 g of de-ionized water (10% of the solvent required for electrospinning) by using Jewelkleen™ Digital Ultrasonic Cleaner (Beijing Ultrasonic Co., Ltd., Yongda, China) operating at 50/60 Hz for 300 s. This was followed by completely dispersing the samples in Ultrasonic Probe Sonicator, Athena Technology, Mumbai, India (model ATP-750) having a 6 mm titanium probe for a period of 300 s (with a total on-time of 240 s and in between off-time of the 60s) at 70 Hz. The sonication of particles was performed in the presence of an ice bath to reduce the generated heat and/or for the disintegration of the present lumps due to the agglomeration of HAp NPs. The HAp NPs fully dispersed in water, was then mixed with the cellulose acetate polymer already dispersed in acetone. The mixture was kept on the magnetic stirrer until a homogenous dispersion of the HAp NPs was obtained. Different concentrations of the HAp NPs disseminated in the cellulose acetate were then fabricated into nanofibers using electrospinning.

2.2.2. Electrospinning procedure

The electrospinning equipment manufactured by Royal Enterprise, Chennai, India (model HD 30), was used to form nanofibers. In brief, the dispersions for cellulose acetate were into three plastic syringes (10 ml), fitted with an 18G needle having an internal diameter of 0.83 mm, at a temperature of 26 °C and with a relative humidity of 55%. The static voltage was set up at 25KV, and the working distance from the tip of the hypodermic needle to the collector was 10 cm apart. The flow rate of 0.8 ml/h was maintained while collecting the nanofibers on the collector (diameter of 5 cm and length of 15 cm) at 132 rpm. Overall, ~60 ml of the cellulose acetate dispersion was allowed to deposit on the aluminum foil-covered to the rotatory collector. Similarly, the electrospun mats of cellulose acetate containing highly dispersed HAp NPs (0.5, 1.0 and 1.5 wt%) were fabricated utilizing the aforementioned optimum conditions for electrospinning. In this case, also ~60 ml of dispersions containing different concentrations of HAp was deposited to form nanofibers. Finally, the as-spun fibers were dried in vacuum for 24 h to remove the residual solvent and closed in vacuum sealer bags for characterization and experimentation.

2.2.3. Regeneration of cellulose fibers from cellulose acetate fibers

A post-treatment approach invoked to regenerate cellulose nanofibers from cellulose acetate nanofibers by alkaline saponification as reported elsewhere [31,36,53]. Briefly, the fibers (cellulose acetate/cellulose acetate containing HAp NPs) were cut into 6 cm × 6 cm mats and treated with 5 ml of 0.05 M NaOH (prepared in 90% ethanol) solution in glass Petri dishes (100 × 20 mm) for 24 h. The fibers were then rinsed with a surplus amount of de-ionized water to remove traces of NaOH present. The rinsing continued until the pH of the solution was wholly neutralized to pH 7.4. The de-acetylated fibers were then air-dried and sealed in airtight Ziplock bags for further characterizations. The effect of saponification on the morphology of the nanofiber and content of de-acetylation was investigated by SEM, FTIR, and other techniques.

2.2.4. In-situ deposition of the Ag NPs on cellulose acetate/HAp fibers

To incorporate the Ag NPs on regenerated cellulose/HAp nanofibers, samples were treated with different molar ratios of AgNO₃ to synthesize and adsorb Ag NPs according to a previously reported procedure elsewhere [54]. In brief, regenerated cellulose nanofibers (4 × 4 cm) containing 0.5 wt%, 1.0 wt% and 1.5 wt% of HAp were treated with 3%, 5% and 7% AgNO₃ prepared in 2 ml solutions (equivalent to 0.15 M, 0.42 M and 0.70 M AgNO₃) for 4 h in Petri dishes to allow adsorption of Ag⁺ ions in/on the fibrous architecture of the regenerated mats. After treatment with AgNO₃ for the stipulated time, the excessive solution was carefully drained. Further on, the fibers in Petri dishes were treated with 1 ml of 0.05 M NaBH₄ to allow the reduction of deposited Ag⁺ ions. The reaction was allowed to proceed for 3 min before draining the solution. The tinged white color of the fibers changed to pale-yellow and finally to dark-brown, signifying reduction of Ag ions. The fibers were then air-dried for 24 h, observed under SEM and investigated for mineralization, antimicrobial and cell toxicity tests.

2.2.5. Mineralization of the regenerated cellulose/HAp/Ag nanofiber mats

The regenerated cellulose mats containing HAp and Ag NPs were subjected to simulate for in-vitro mineralization by incubating them in the presence of SBF. The fluid media was prepared according to the composition reported elsewhere [55]. The different inorganic components equivalent to that of human blood plasma were dissolved in de-ionized water, pH adjusted to 7.4 by Tris buffer (HANNA Instruments, Inc. USA) and stored at 4 °C until further usage. The nanofiber mats were carefully cut into small fiber strips of dimensions 3 cm × 3 cm and placed in the incubator (BenchTop Lab Equipment) with 2 ml of SBF at 37 °C for 15 days. After incubation, the samples were analyzed by SEM and FTIR to check the formation of apatite crystals on the fiber surface.

3. Characterization

The morphology of the pre- and post-treated nanofiber samples were analyzed by scanning electron microscopy (Hitachi S-3700H, Hitachi Ltd. Japan). The samples for analysis were placed on the double-sided adhesive carbon tape and sputter-coated by gold for a period of 50s using a discharge current of 10–15 Ma for consequent three cycles. Before scanning, the samples were dried in vacuum for half an hour, to remove the moisture. All the micrographs of the fibers were taken with the accelerating voltage 10 kV, and images were captured with magnifications of 1 K and 4 K. The average nanofiber diameter and distribution were measured by ImageJ software (NIH, USA) and data was plotted by using OriginPro 8 software. The field emission scanning electron microscope (JEOL JSM-7100F, Singapore) coupled with energy dispersive X-Ray spectroscopy (EDX) was used to investigate the presence of Ca and P on the regenerated nanofibers. The sample preparation was performed by using carbon tape and sputtering for 100 s to form a thin layer of gold. To figure out the presence of HAp and Ag NPs in the nanofibers, the transmission electron microscopy (JEOL JEM-2010, Ltd., Japan) was used. The operating voltage of 200 kV and final magnifications used to capture the images were 10 and 20 K, respectively. For sample preparation, 10 mg of nanofiber samples were suspended in 500 µl of absolute ethanol in a microfuge tube (2 ml). These tubes were sonicated in a bath sonicator for the 90s at a frequency of 42 Hz to allow disruption of the individual fibers on TEM grids. The resultant threads from its mat state present in the ethanol were then carefully deposited on 3 mm copper grids with a grid area (150 mesh × 165 µm pitch). All the samples were then dried at 37 °C for half an hour before viewing the samples under TEM. To determine the presence of HAp, shifts occurring in the functional group, the extent of de-acetylation process and effect due to post-mineralization using SBF of the regenerated nanofibers, the Fourier-Transform Infrared Spectroscopy (FTIR) of pre- and post-derivatized fibers was carried out using (Nicolet™ iS5, ART module, Thermo Fisher Scientific™, USA). The samples were scanned at a spectral range of 4000 to 400 cm⁻¹ with the resolution of 4 cm⁻¹ and the total number scans were 32 using an ATR module. The enhancement in the hydrophilic nature of the nanofiber mats was measured using a contact angle measuring instrument (HO-IAD-CAM-01). The contact angle assembly includes a digital camera (1.5 megapixels) and an optical lens (Computar, TV lens, Japan) with a focal distance of 50 mm that was placed in between the camera and the sample. For each sample analysis, three points were selected in a defined baseline along the surface of the drop. The images were captured using DuraCam software, whereas the contact angle was appraised using the software in ImageJ. 1.46 with extra plugin Drop Analysis extension [56]. Overall, the procedure for checking the contact angle involved taking images in triplicates against 50 µl Dulbecco's Modified Eagle Media (DMEM) on 3 cm × 3 cm fiber mats.

4. Antimicrobial assay

The most significant causes of implant rejection are due to microbial infections in the patients after an event of surgery. Therefore, it is highly imperative to have implant material with inherent antimicrobial activity. For this purpose, the samples were peeled out from aluminum foil and punched out in the form of circular discs (8 mm diameter) using biopsy punchers (Sterile Biopsy Punch, Mentok Healthcare, Jaipur, India). Before use, the samples were UV sterilized for half an hour on a clean bench. The antimicrobial activity was determined against *E. coli* and *S. aureus*. Both gram-negative and gram-positive microbes were efficiently reviewed in 10 ml LB broth in shaking incubator (Mini shaking Incubator ES-60) at 37 °C until optical density reached a maximum of 0.5–0.6 (approx. in 17 h). After raising the microbial populations, the LB agar plates were prepared under sterile conditions and spread uniformly by 300 µl of the reviving media containing bacterial strains. Post spreading of microbes, the sterilized nanofiber discs

from each of the nanofiber compositions were consistently placed at the surface of the agar plates. The plates were then incubated at 37 °C for 17 h and observed for zones of inhibition. The zones of inhibition were measured by Vernier Caliper (Sundershan Measuring and Eng. Ltd., India) and documented using a gel image system for further additional measurements using Photoshop 6.0 (data not shown). All the experiments were carried in triplicate and under aseptic conditions.

5. Biocompatibility assay

The biocompatibility of the nanofiber mats was evaluated by an indirect assay that can determine the toxicity of materials. The test is based on the reduction of 3-(4,5-Dimethyl 2-thiazolyl)-2,5-diphenyltetrazolium Bromide (MTT) into purple color formazan crystals by the enzymes present in the living cells. In brief, the CEFs were isolated from 9 days old chicken embryo after removing the embryo from the eggs. The study was approved by the ethical committee of the faculty of veterinary sciences and animal husbandry, SKUAST-K. The head and other developed extremities were aseptically cut and discarded. Further, the embryo was separated by chopping with the aid of a surgical blade. Then 1 ml warm trypsin (Sigma-Aldrich) was flushed over the chopped embryo and mixed with the cut tip, followed by incubation for 5 min at 37 °C. After trypsin treatment, the 1000 µl of culture media (DMEM + 10% FBS + 0.1% Penicillin and Streptomycin) from (Sigma, St. Louis, Missouri, USA) was carefully added and mixed well with the contents. Further, the materials were placed in the 15 ml tube and kept upright for 5 min to allow the coarse debris particles to settle down. Then the supernatant was collected and seeded in 25cm² flasks containing the culture media (DMEM + 10% FBS + 0.1% Penicillin and Streptomycin). In these studies, the second passage cells were used for all experiments. The processing of nanofiber samples involved punching of 8 mm circular discs (previously UV sterilized for 30 min) with the aid of sterile biopsy punchers and then seeding each of these mats with 100 µl culture media (DMEM + 10% FBS + 0.1% Penicillin and Streptomycin) containing chicken embryo fibroblasts (2×10^5 cells/ml) using sterile 96 well plates. The microplates were then incubated humidified chamber (Galaxy 170S, Eppendorf, Hamburg, Germany) at 37 °C in 5% CO₂ environment, and cell viability was observed on the 2nd, 4th and 6th day of incubation. During the incubation of fibroblast in the presence of nanofiber scaffolds, the 100 µl of the exhausted media was replaced with the addition of fresh media after every 2nd day of incubation. Post culturing of microplates for several days, the viability of the seeded cells was analyzed by the addition of 10 µl of MTT reagent in PBS (0.5 mg/ml) over the scaffolds before removing the media. Further on, the microplates were incubated for 4 h at 37 °C to allow the reduction of MTT solution into formazan by mitochondrial oxidases. The formed formazan crystals were treated with 100 µl of 10% SDS solution in 0.01 M HCL to solubilize the formazan crystals at 37 °C overnight. The resulting purple-blue colored solution was then collected and analyzed at 570 nm in a microplate reader. The cell viability was then calculated indirectly by using the following formula.

$$\text{cell viability (\%)} = \frac{\text{Sample absorbance} - \text{Blank absorbance}}{\text{Control absorbance} - \text{Blank absorbance}} \times 100$$

6. Cell attachment study

The attachment of the chicken embryo fibroblasts on the nanofiber mats was studied to investigate the ability of these fiber mats for cell attachment reflecting the growth, migration and proliferation of the cells. In this study, 100 µl of DMEM containing fibroblasts (2×10^5 cells/ml) were cultured over the 8 mm circular nanofiber mats placed in 96-well plates for 10 days. The media was replaced after every 2nd day to provide nutrition and removal of waste metabolites from the growing cells. After 10 days of incubation, the media was washed-out, and the

scaffolds containing cells were flush with 0.1 M PBS ($2 \times$). The cells on fiber mats stayed fixed with 200 µl of 4% glutaraldehyde (prepared in 0.1 M PBS and pre-warmed to 37 °C before use) solution for 2 h. After that, the fixation media was drained off and subjected to ethanol dehydration by serial dilution at room temperatures. The cells remained dehydrated by treating them with increasing concentrations of ethanol in PBS (25%, 50%, 75% and finally 100%). Treatment with each combination was given for a period of 20 min. Finally, after usage with 100% ethanol, the solution was removed and the samples in the wells were air-dried, and then these fixed cell samples were sputter-coated (SC7620 sputter coater, Quorum) with gold-palladium for 180 s. The gold coated samples were analyzed at an acceleration voltage 5-10 kV with the magnifications of 1–10 K for investigating the cell attachment using scanning electron microscopy (SEM) (EVO 18, Zeiss, Germany).

7. Degradation studies

The regenerated cellulose and counterpart nanofibers containing different concentrations of HAP and Ag NPs were subjected to degradation in pure PBS or PBS supplemented with cellulase enzyme (cellulase from *Aspergillus niger*, Sigma-Aldrich). The study was experimentally carried according to the previously reported protocols [57,58]. To investigate the degradation behavior, oven-dried and pre-weighed samples were cut into (2×2 cm) mats and immersed in 10 ml of the PBS and kept at 37 °C in a shaking incubator (Mini shaking Incubator ES-60) at 150 rpm. The samples retreated after 30 and 60 days, rinsed with distilled water ($3 \times$), dried under vacuum and weighed. For enzymatic degradation, a similar procedure was adopted, except the study was carried only for 10 days (owing to fast degradation by enzymes). In brief, for each nanofiber mat incubated with 10 ml PBS solution supplemented with cellulase enzyme (0.1 mg/ml) was utilized. After incubation, the samples were withdrawn from the incubator, dried under vacuum and weight was determined. Following is the equation used to calculate the weight loss due to degradation:

$$\text{Weight loss (\%)} = \frac{W^{\circ} - W}{W^{\circ}} \times 100$$

where W° = initial dry weight of the nanofiber samples and W = dry weight of the nanofiber mats after removing from the degradation media.

8. Results and discussion

Initially, several screening experiments carried to investigate the effect of the different parameters (e.g., voltage, flow rate and needle to collector distance, solution parameters polymer concentration, choice of solvents), which influenced the process of the electrospinning. During these experiments, parameters affecting the yield, physical mat appearance, fiber morphology diverse from beaded appearance and/or distorted forms were elucidated to have smooth and bead-free nanofibers. Our findings were almost correlating to the results obtained by other research groups [32,59,60]. Overall, the optimum conditions for fabricating the cellulose acetate nanofibers, as described in Section 2.2.2, were 12% (w/w) polymer concentration in acetone–water (90:10). This system resulted in bead-free with smooth-enriched fibrous morphology. Fig. 1A shows the morphology of the pristine cellulose acetate nanofibers. The formation of smooth nanofibers using the introduced solvent system is corroborated by other studies as well [61]. Furthermore, the morphology of the cellulose acetate nanofibers containing HAP NPs is presented in Fig. 1B, C, and D. The presence of HAP NPs in nanofibers did not affect the final morphology of nanofibers at lower concentrations. However, at a higher level of HAP (i.e., presented at Fig. 1D) shows the presence of a few beaded HAP NPs. This can be accounted for due to the maximum loading capacity of HAP particles in the used colloidal solution for electrospinning. In precise, it can be concluded that the incorporation of HAP changed the fiber morphology

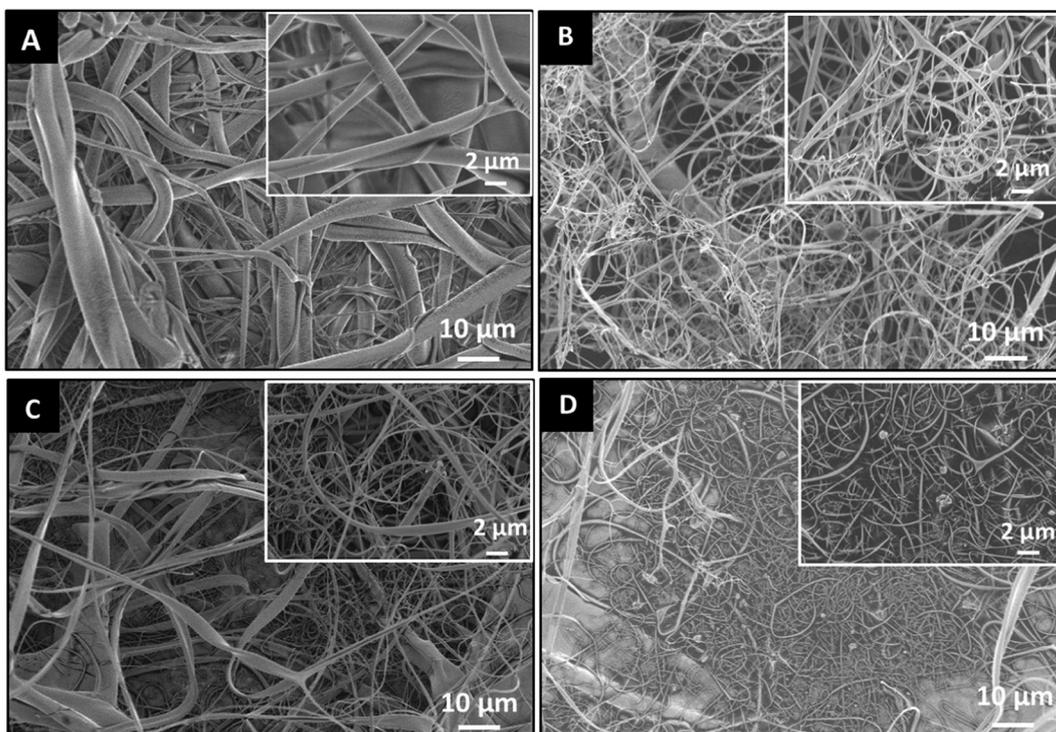


Fig. 1. SEM images of cellulose acetate nanofibers containing HAp at magnifications 1 k (and 4 k magnification for the inset micrograph). ‘A’ represents SEM images of cellulose acetate nanofibers only; B, C, and D represent SEM images of cellulose acetate nanofibers containing 0.5%, 1%, and 1.5% HAp respectively. The SEM images describe a smooth morphology of the nanofibers and a decrease in fiber diameter with a rise in HAp concentration.

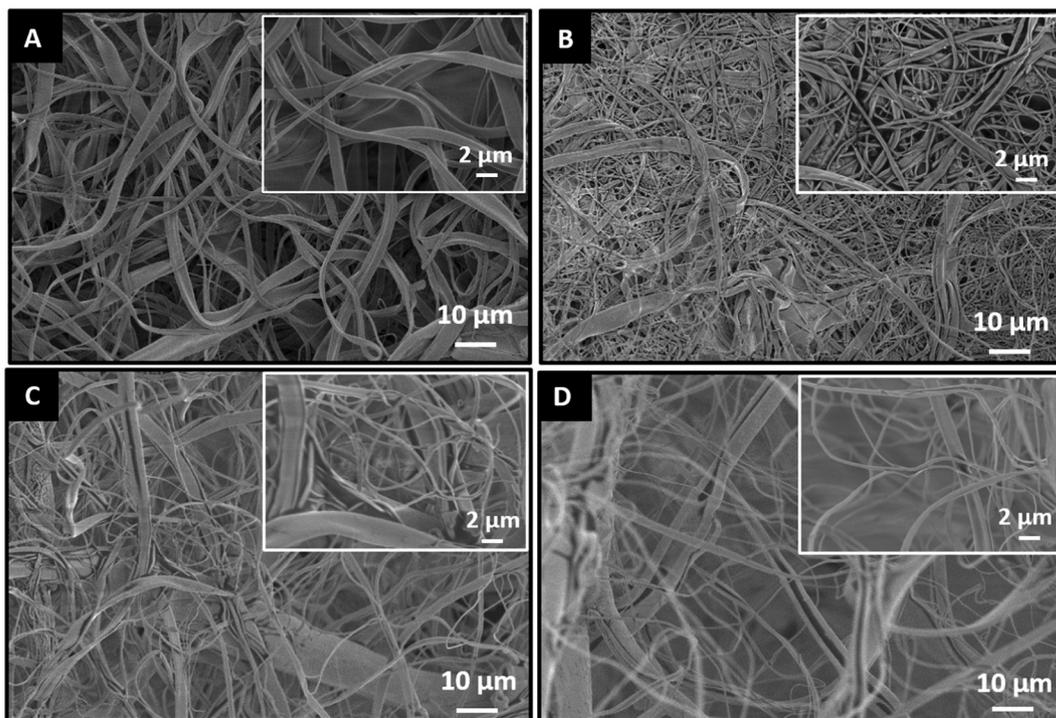


Fig. 2. SEM images of regenerated cellulose acetate nanofibers containing HAp at 1 k magnification, and the inset images at a magnification of 4 k. ‘A’ represents SEM image of pristine regenerated cellulose nanofibers; B, C, and D denote SEM images of nanofibers containing 0.5%, 1%, and 1.5% HAp, respectively.

from cylindrical to nanofibers with ribbon and/or belt-shaped morphology in Fig. 1C. Following the protocol of deacetylation [62,63], the morphological results of regenerated cellulose nanofibers from cellulose acetate mats prepared using 0.05 M NaOH ethanol solution at room temperature is indicated in Fig. 2. Due to the de-acetylation process, the

fiber morphology was not distorted and/or broken. Moreover, to get the exact confirmation about the presence of HAp NPs, which were supposed to be carried through the course of electrospinning and retained through the deacetylation process, we have performed FESEM equipped EDX analysis. The results of the EDX analysis are demonstrated in

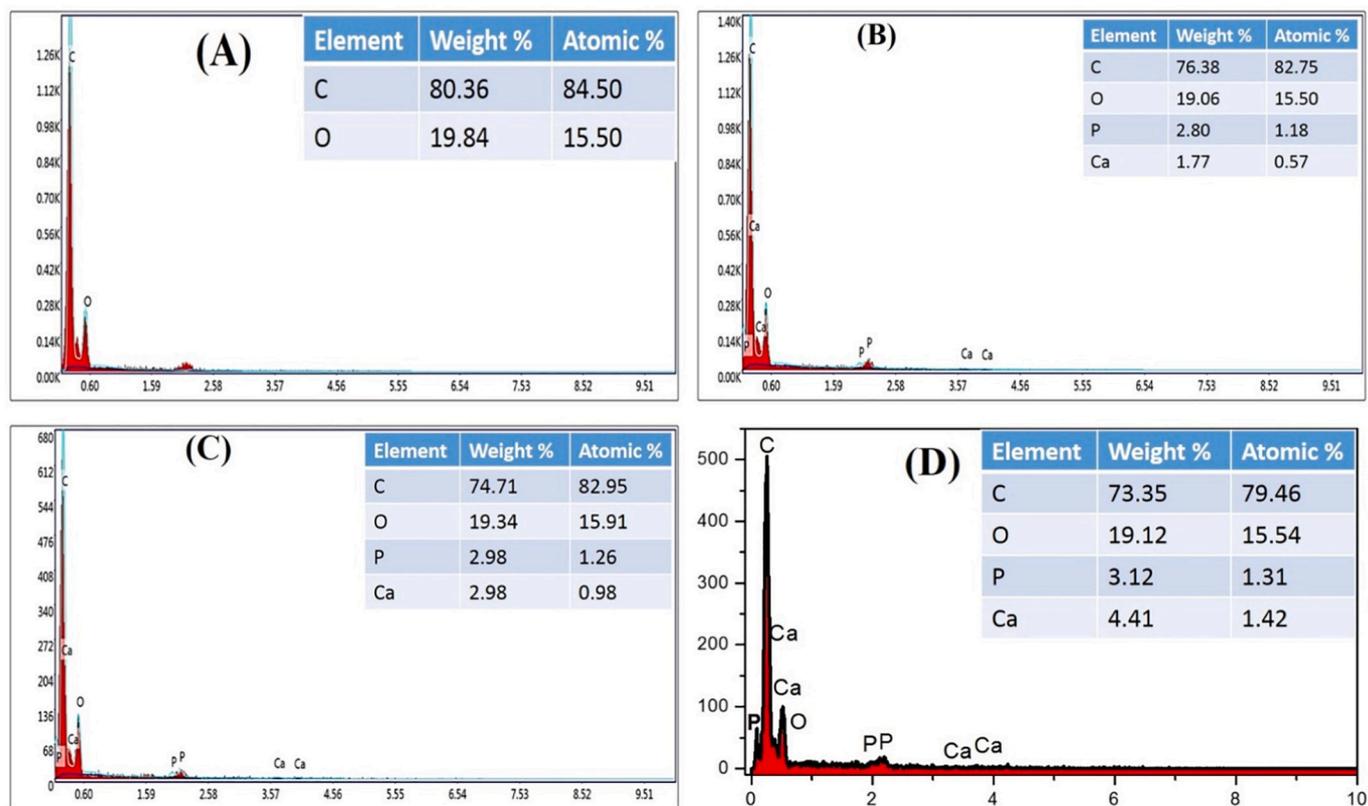


Fig. 3. FE-SEM coupled with EDX results of the nanofibers after deacetylation 'A' represents EDX results of pristine nanofibers; B, C, and D represent the EDX data of the deacetylated nanofibers containing 0.5%, 1%, and 1.5% HAP, respectively.

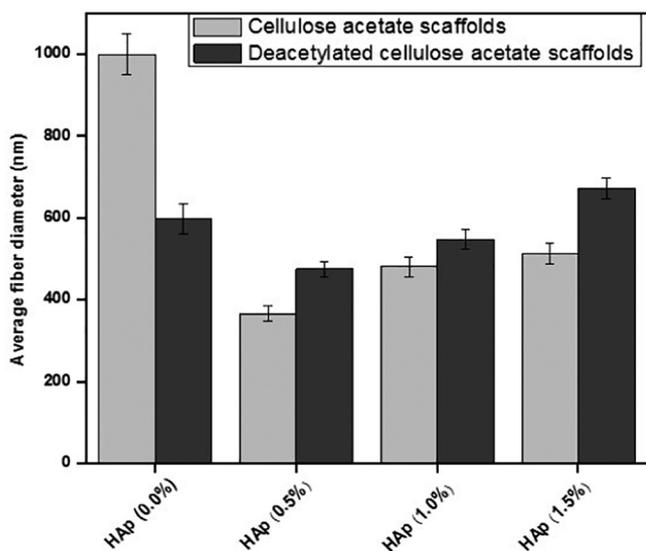


Fig. 4. Comparison of average fiber diameter (nm) of cellulose acetate fibers and regenerated (deacetylated) cellulose fibers, each containing different concentrations of HAP NPs. Fiber diameter showed a reduction due to deacetylation, whereas an increase in diameter can be observed due to the rise in HAP content. Statistical analysis performed by comparing the groups between one another using one-way Tukey's multiple comparison test (One Way ANOVA) indicates noteworthy differences ($P < 0.05$) in fiber diameter reduction (***) between cellulose acetate fibers and deacetylated fibers. Similarly, statistically significant differences were observed between each composition of acetylated and deacetylated fibers except between the CA and dCA fibers containing 1.0% HAP.

Fig. 3. In this figure, we can observe the pristine nanofibers, which are free of HAP NPs does not show any significant peak indicating the presence of Ca and P (Fig. 3A). In contrast, the nanofibers modified with HAP NPs indicate the presence of both Ca and P peaks, which further enriches the hypothesis about the beneficial attribute displayed by HAP NPs in the nanofibers. Furthermore, the stoichiometric Ca/P ratio is ~ 1.67 . However, the concentration of Ca and P after incubation on SBF is independent of the ratio of original loading of HAP NPs in the cellulose acetate solutions that were used for electrospinning (Fig. 3B, C, and D). In order to ascertain the changes that occurred in the diameters of the fiber after de-acetylation, the average was calculated. The nanofiber diameter decreased significantly in the case of de-acetylated nanofibers (Fig. 4). In more details, before de-acetylation, the nanofibers show the average diameter of 1000 ± 50 , 366 ± 18 , 481 ± 24 and 512 ± 25 nm for the nanofibers with 0, 0.5, 1.0 and 1.5% HAP content. The presence of HAP NPs reduced the average fiber diameter significantly (Fig. 4). The rationale may be due to the sequestration of the polymer threads by HAP particles, further enriching the fact about their presence within the fibers. Likewise, the higher concentration of the HAP over the surface of the fibers may promote the foundation of the crystals, which can lead to an intensification in the fiber diameter [36]. In comparison to nanofibers after de-acetylation and adsorption of Ag NPs, a parallel tendency is shadowed (Fig. 4). However, a drop in fiber diameter is observed for de-acetylated nanofibers. The reduction of fiber diameter in the case of de-acetylated nanofibers stemmed due to the deletion of the acetate groups from cellulose acetate nanofibers. Overall, the average nanofiber diameters calculated after de-acetylation are 597 ± 37 , 475 ± 18 , 548 ± 24 and 672 ± 25 nm for the nanofibers with 0, 0.5, 1.0 and 1.5% of HAP. These results portray that amalgamation of HAP NPs increases the fiber diameter, before and after de-acetylation of the nanofibers. The rationale would be owing to the effect of alkaline deacetylation HAP particles in the cellulose acetate, which might have effected in the surface aggregation of the HAP NPs

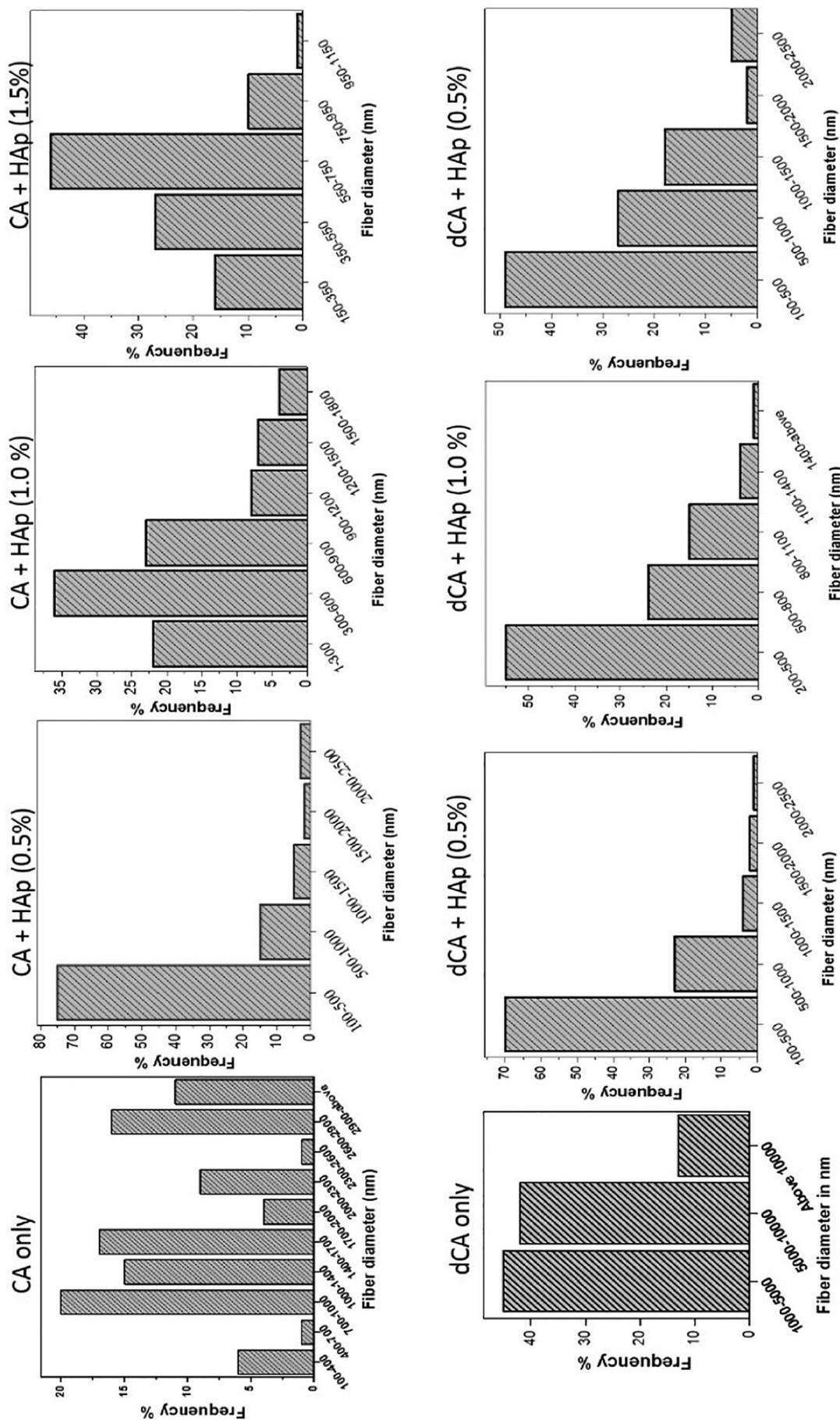


Fig. 5. Representative images of fiber diameter distribution of different scaffolds. Cellulose acetate (CA) fibers had a wide range of diameter distribution. In contrast, deacetylated/regenerated cellulose (dCA) nanofibers had a narrow range of diameter distribution. Regenerated cellulose fibers containing 1% of HAp had more than 50% of the fibers in the diameter range of 100-500 nm. All the experiments were carried in triplicates calculated using ImageJ software, plotted as average using Origin 8 pro software.

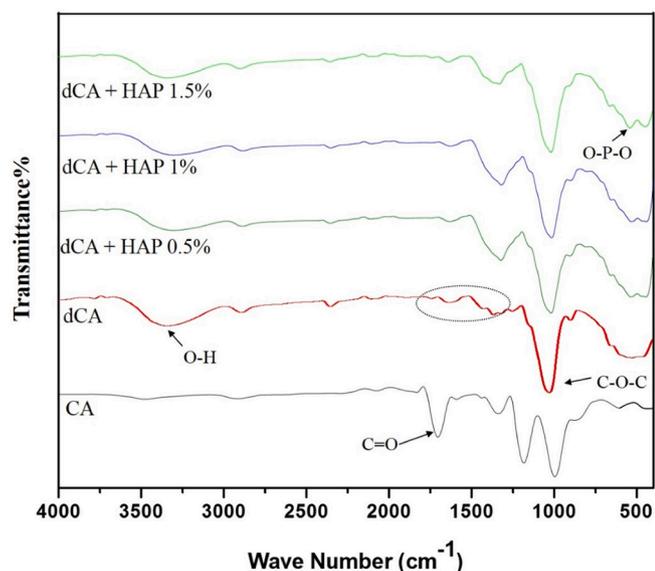


Fig. 6. FTIR spectra of the cellulose acetate (CA) fibers, regenerated cellulose fibers (dCA) and dCA containing various concentrations of HAP (0.5%, 1% and 1.5% HAP). The characteristic peaks of CA are 1737 cm^{-1} , 1222 cm^{-1} and 1367 cm^{-1} , corresponding to C=O stretching, C–O stretching and C–CH₃ stretching of the acetate group. The subsequent disappearance of these peaks in dCA and dCA containing HAP fibers shows deacetylation is achieved. The appearance of strong vibrations at 1021 cm^{-1} and 3480 cm^{-1} in the deacetylated (regenerated) fibers is due to the C–O stretching and O–H stretching of the alcoholic group of cellulose. The twin peaks at 559 cm^{-1} and 610 cm^{-1} occurring in HAP containing fibers are characteristics of O–P–O bending of the distorted PO_4^{3-} group of HAP.

[50,64]. These results further signify the fact of efficient accumulation of HAP NPs. The process of de-acetylation also had a significant effect on fiber diameter distribution (Fig. 5). It was found that due to the saponification of the fibers, the frequency fiber diameter changed from wide to narrow distribution. These results may be because of the effect of selective removal of the acetate group due to the alkaline saponification of the fibers. Furthermore, also due to the presence of the HAP particles in the nanofibers, it was observed that fiber diameter changed from wide to narrow distribution. Moreover, the nanofibers containing 0.5% HAP showed a narrow size distribution, with 75% of the fibers possessing diameter in the range of 100–500 nm (Fig. 5). Another proof of successful de-acetylation strategy is that the thickness of the dried nanofiber mat calculated was found to be $2.54 \pm 0.2\text{ mm}$ in all the nanofiber combinations and for the nanofibers after de-acetylation was $2.10 \pm 0.4\text{ mm}$. The reduction of nanofiber mat indicated efficient de-acetylation of nanofibers.

The regeneration of the cellulose nanofibers and presence of the HAP NPs in the fibrous mats was confirmed after analyzing the samples for FTIR spectroscopy. The FTIR spectra (Fig. 6) showed a sharp transmittance peak at 1737 cm^{-1} , corresponding to the carbonyl (C=O) stretching of the acetate group present nanofibers before de-acetylation. Interestingly, this peak disappears after the de-acetylation of nanofibers prepared by overnight treatment with ethanolic NaOH solutions. This indicates the successful removal of acetate groups from the de-acetylated nanofiber samples. The peak at 1222 cm^{-1} corresponds to the C–O stretching of the ether linkage present in the ester groups. A subsequent decrease in intensity of these peaks is observed in this group after chemical treatment. Moreover, the presence of strong vibrations occurring at 1367 cm^{-1} corresponding to the stretching modes of the C–CH₃ group in the cellulose acetate is disappearing in the de-acetylated nanofiber combinations. However, after treatment with alcoholic NaOH, there is a presence of strong stretching vibration occurring at 1021 cm^{-1} in the de-acetylated cellulose acetate nanofibers,

which are associated with C–O stretching frequency of the alcoholic group of de-acetylated cellulose acetate (Cellulose). The broadening of the peak around $3400\text{--}3600\text{ cm}^{-1}$, centering mostly at 3480 cm^{-1} , is due to the hydroxyl-stretch bands appearing due to substitution of the acetyl group by the hydroxyl groups [36,53,65]. In all the de-acetylated nanofibers containing different concentrations of HAP, firm transmittance peaks can be observed at wavenumbers of 559 cm^{-1} and 610 cm^{-1} . These peaks are the characteristics of the –O–P–O bending occurring in the PO_4^{3-} group of HAP. This doublet peak also suggests that the precursor phase of the HAP was octacalcium phosphate [66,67]. These FTIR results indicate that cellulose has been wholly regenerated from its acetate precursor. Overall, the FTIR results denote the presence of the HAP crystals in the nanofibers scaffolds. This is further supported by SEM studies, which show the presence of the HAP in the nanofiber scaffolds. However, it is worthwhile to mention that alkaline saponification in the ethanolic medium is an inconsistent process, and it is hard to portray the kinetics of this complex regime, where diverse electrospinning parameters influence the fiber diameter and morphology of the cellulose acetate fibers. However, to be precisely surer the de-acetylation process, the degree of de-acetylation needs to be ascertained.

The Ag NPs were deposited on the regenerated cellulose nanofibers by the in-situ method, as described in the earlier section [54]. We have implemented this method and have not co-electrospun AgNO_3 solutions with the cellulose acetate and HAP dispersions because the addition of Ag ions in this dispersion leads to increase in the dripping rate from the needle and this is associated with the decrease in viscosity of the polymeric solution due to the accumulation of the Ag ions [68]. Furthermore, the addition of the salts to the polymeric dispersions leads to loss of entanglement of the polymer threads and, as a result, leads to a decrease in the viscosity [68]. As a result, the polymer is difficult to spin into nanofibers by electrospinning. Consequently, we adopted the reduction procedure for adsorbing the Ag ions by NaBH_4 solution. The treatment was given for a shorter duration of 5 min to avert the undesired degradation of the regenerated cellulose by the in-situ generation of B_2H_6 , which can have toxic side-effects on cells [69]. The presence of the Ag NPs on the nanofiber mats was confirmed by the TEM. In this regard, the Fig. 7 depicts the results of TEM investigation of the nanofibers showing black-dotted Ag NPs/aggregates all through the surface. Moreover, the HAP clusters are also seen on the nanofiber surfaces. The size of the NPs, as calculated from the ImageJ software, varies in the range of 10–30 nm. The density of the Ag and HAP NPs on the fiber surface describes the extent of adsorption and/or reduction of Ag ions.

Regenerated cellulose scaffolds containing HAP particles as nucleating agents were allowed to incubate in the SBF solution to determine if these fibers can induce the formation of an apatite layer on the nanofiber surface by Ca–P crystallization [36]. In this regard, the Fig. 8 shows the SEM images of regenerated cellulose-containing different concentrations of the HAP after 15 days of incubation. This result suggests that regenerated cellulose mats encompassing HAP as a nucleating agent can preferably provoke excessive crystal induction under SBF treatment. Ca and P crystals were formed throughout the surface of the fibers serially as far as the HAP concentration is raised in the nanofibers (Fig. 8). Moreover, the Ca and P crystals of varying shapes and sizes can be observed within and over the nanofiber network, suggesting enhanced mineralization of the fibrous scaffold. In order to enrich the SEM results for induction of excessive Ca and P formation in nanofiber samples, the percent transmittance of the FTIR spectra of the regenerated fibers from pre and post SBF treatment is shown in Fig. 9. The FTIR spectra show the presence of sharp dual transmittance peaks occurring at 559 cm^{-1} and 610 cm^{-1} originating from O–P–O bending vibrations of PO_4^{3-} group describing enhanced crystal growth [66,67]. These peaks occur at a higher intensity than the peaks present in regenerated cellulose nanofibers (before SBF treatment) containing HAP as a nucleating agent.

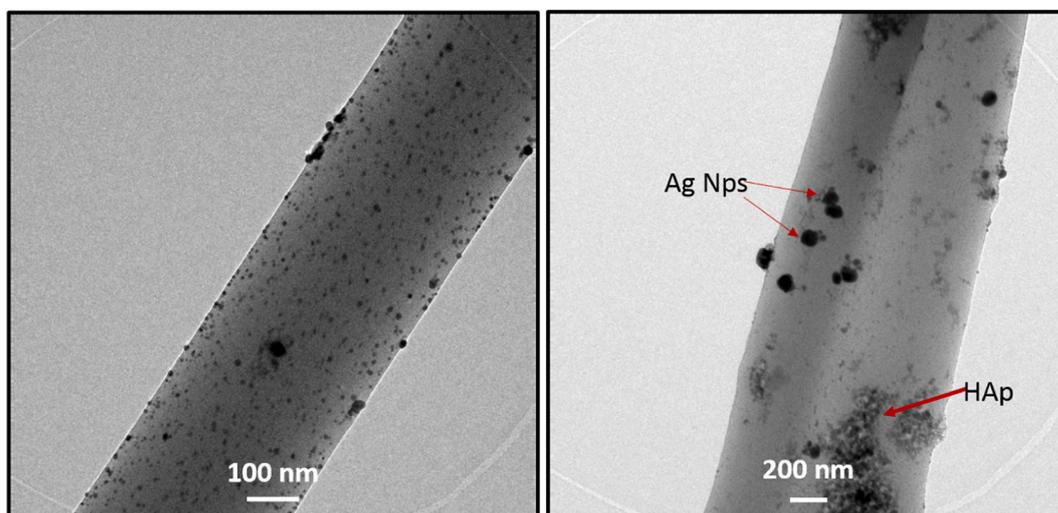


Fig. 7. Transmission electron microscopy (TEM) micrographs of regenerated cellulose nanofibers containing 1.5% HAp and treated with 7% AgNO_3 . The micrographs display the existence and distribution of Ag NPs throughout the fiber surface as black dots. The Ag NPs in size range of 10–30 nm are precisely seen and HAp particles are comprehended as aggregates. The images were captured at an operating voltage of 200 kV and a magnification of 10 K and 20 K.

For any in-vivo application, nanofibers must have a certain degree of hydrophilic character so that they can be used at the site of injury where they will be firmly attached to deliver the bioactive compounds. To infer the influence of de-acetylation and incorporation of HAp NPs on the surface wettability, we determined the water contact angle of each of the mats and analyzed the data using contact angle measurement system. The results are shown in Fig. 10A and B. From this figure; it is clear that due to the process of de-acetylation, the contact angle of the fiber mats decreases significantly, i.e., leads to an increase in hydrophilic character. The contact angle falls from $106.6 \pm 2^\circ$ in pristine cellulose acetate nanofiber mats to $42.3 \pm 3^\circ$ in pure de-acetylated

mats. On comparison of the contact angles between the nanofiber mats of cellulose acetate containing different concentrations of the HAp NPs, the differences are not significant. The result suggests that HAp had a non-significant contribution in imparting hydrophilicity to the nanofiber mats. However, after the de-acetylation of these mats, the contact angle decreases sharply as the concentration of the HAp NPs is increases. This is due to the reason that the de-acetylation process also leads to the crystallization of the HAp. These results are supported by previous studies where it was concluded that the chemical treatment of the cellulose acetate nanofibers leads to the formation of the Ca–P deposition when incubated with Ca salts [36]. The lowest contact angle

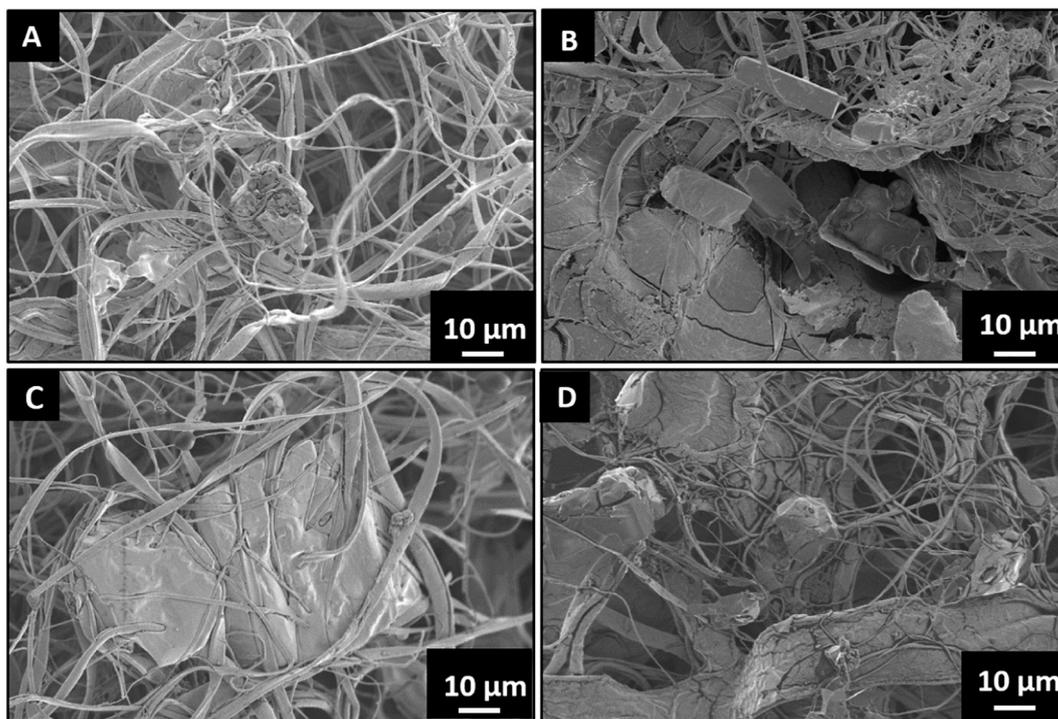


Fig. 8. SEM images of regenerated cellulose fibers containing HAp after the 15 days of mineralization in simulated body fluid. A, B and C represent SEM images of regenerated cellulose fibers containing 0%, 0.5%, 1% and 1.5% HAp, respectively. The images clearly show the growth of apatite crystals throughout the fiber network. Bigger crystals witnessed with an increase in the HAp concentration. All the images are taken at 1 k magnification, a working distance of 9.5 mm, and a voltage of 4 kV.

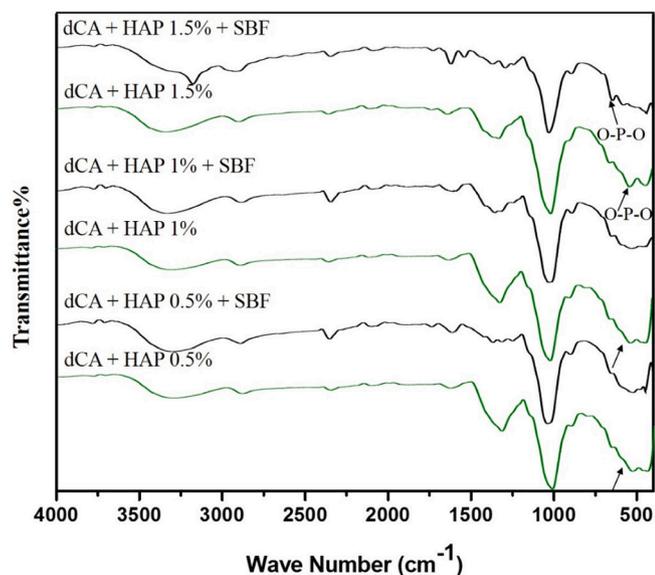


Fig. 9. Comparative FTIR spectra of regenerated cellulose fibers pre (green) and post (blue) mineralization with SBF after 15 days of treatment. The twin peaks at 559 cm^{-1} and 610 cm^{-1} occurring in HAp containing fibers are characteristics of O-P-O bending of the PO_4^{3-} group of HAp and are more intense than the pretreated fibers. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of $10.2 \pm 1^\circ$ was detected in the regenerated cellulose mats that contain 1.5% of HAp, signifying the very hydrophilic nature of the nanofibers with higher concentrations of HAp NPs. These results suggest that regenerated cellulose mats containing HAp could alter the wettability of the mats and can be used for different in-vivo applications.

The antimicrobial activity of the nanofiber mats was tested against *E. coli* and *S. aureus* using disk diffusion assay. The results from zones of inhibition (ZOI) due to different variants of the scaffolds containing Ag NPs against these two strains stand shown in Fig. 11. It can be perceived that pristine regenerated cellulose nanofibers could not inhibit the growth of any bacteria. In contrast, the Ag NPs containing mats were effective in suppressing the microbial growth of both the strains. In general, the Ag holding mats were more effective against *E. coli* than *S. aureus*. Moreover, the regenerated cellulose mats containing 1.5% HAp and 7% Ag NPs showed the highest ZOI of $2.79 \pm 0.23\text{ mm}$ against *E. coli* and $1.64 \pm 0.33\text{ mm}$ ZOI against *S. aureus*. The results suggest that these nanofiber mats containing nano-Ag are effective in controlling microbial growth and can be functional to different in-vivo systems, where antimicrobial attributes to suppress the microbial infections are necessary.

Biocompatibility of the cellulose acetate mats and those of the regenerated cellulose mats containing varying concentrations of the HAp and Ag NPs were assessed against chicken embryo fibroblasts by using MTT assay. The fibroblasts were preferred because these are the first type of cells that come in contact with the damaged tissue in order to have growth of tissue, which further is progressed with the growth of hard tissue [70]. The viability of the cultured cells is proportional to the absorbance of the formazan, which is created as a result of mitochondrial oxidase activity of the viable cells. Fig. 12 represents the viability of the chicken embryo fibroblasts when cultured on different types of mats. Effects of cell viability are shown for the 2nd, 4th and 6th day of post cell seeding. The overall results suggest that all the variants of the nanofiber mats supported the growth as well as the proliferation of the fibroblasts. From those results, it can be concluded that pure cellulose acetate and de-acetylated nanofibers with 0% Ag/HAp NPs do have the same cell growth behavior. However, the nanofiber mats containing the highest concentration of the Ag NPs (i.e., 7%) were toxic to the growth

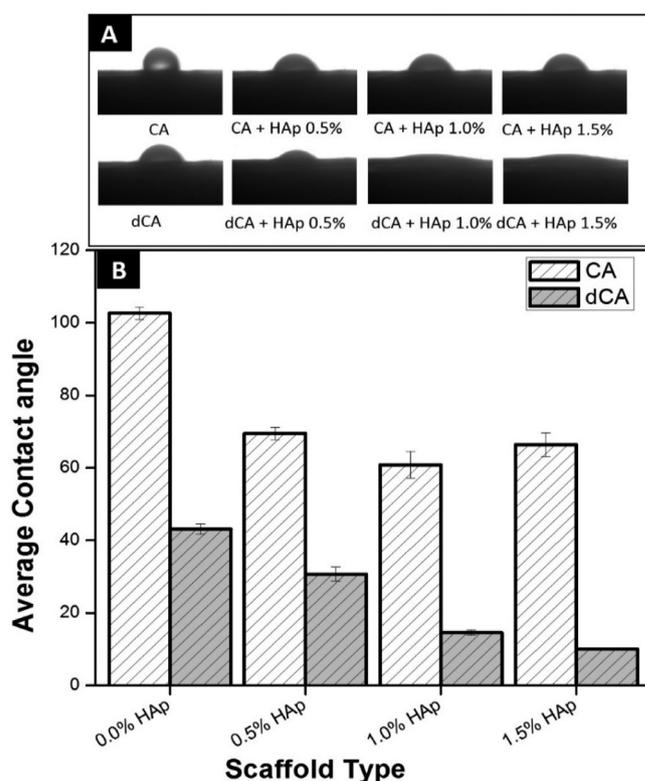


Fig. 10. (A) Contact angle images of DMEM drops on the cellulose acetate (CA) fiber mats/CA fiber mats containing different concentrations of HAp and regenerated cellulose (dCA) fiber mats/dCA mats captured using contact angle measuring equipment at a drop exposure time of 10s. (B) Contact angle values of cellulose acetate (CA) fiber mats/CA fiber mats containing different concentrations of HAp and regenerated cellulose (dCA) fiber mats/dCA mats containing different concentrations of HAp. Tukey's multiple comparison test (One-way ANOVA) was applied to test the groups with each other at a confidence level of 95% ($P < 0.05$). Statistical analysis revealed that significant differences (***) in contact angle was observed between pristine CA and dCA fiber mats and between each of their compositions.

and proliferation of the fibroblasts. This is due to the reason that Ag ions at a particular concentration can penetrate the respiratory chain of the cell leading to the shutdown of oxidative phosphorylation and causes cellular damage [41]. In supplement to this, the traces of unreduced AgNO_3 due to in-situ generation using NaBH_4 solution and formation of undesired B_2H_6 cannot preclude with nanofiber mat containing 7% of AgNO_3 . Moreover, among the various nanofiber combinations, the regenerated cellulose mats containing 1.0% HAp and 5% Ag were found to enhance the growth and proliferation of the fibroblasts at each day of the study. Furthermore, no difference in supporting cell growth and proliferation has been observed between the pristine cellulose acetate and de-acetylated cellulose nanofiber mats, suggesting that HAp is necessary for making cell contact to scaffold contact for growth and proliferation of the cells.

The cell attachment of the fibroblasts was demonstrated and supported by SEM. The cells after the 10th day of incubation were fixed using glutaraldehyde solution, serially dehydrated using ethanol, and then observed under SEM to evaluate the morphology of the fibroblast attached to the nanofiber mats. Fig. 13 describes the morphology of the chicken embryo fibroblasts on the regenerated cellulose scaffold containing different concentrations of HAp and Ag NPs. A very few fibroblasts can be observed on the unsullied regenerated cellulose mats (Fig. 13A). In contrast, the regenerated cellulose mats containing 1.5% HAp and 7% Ag NPs (Fig. 13D) are found to upkeep the determined growth and proliferation of the fibroblast due to superior hydrophilic character and this can be correlated to the generation of an apatite layer

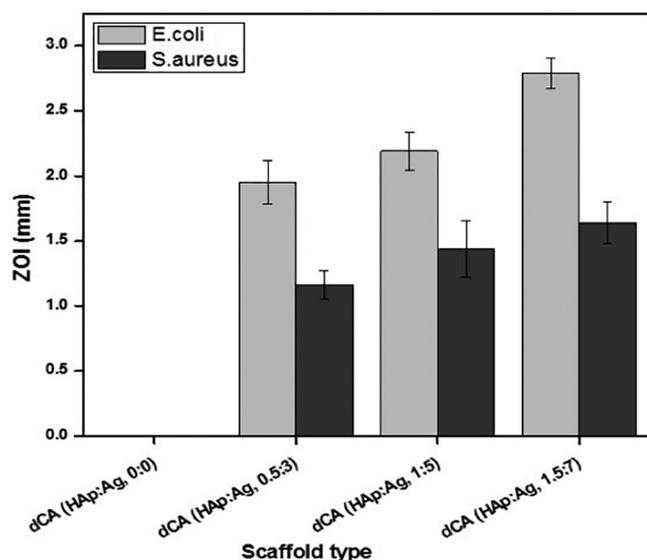


Fig. 11. Zones of inhibition in mm's occurring due to different variants of regenerated cellulose (dCA) or dCA adsorbed Ag NPs against *E. coli* and *S. aureus*. The pristine dCA fiber mats do not inhibit bacterial growth. In contrast, the dCA fiber mats containing the highest concentration of the adsorbed Ag NPs are most effective in inhibiting bacterial growth. The zones between the dCA and dCA composites were statistically compared using Tukey's multiple comparison one-way ANOVA test (Graph Prism®). Statistically highly significant differences (***) in zones of inhibition were observed by comparing each composition with the pristine dCA scaffold (both in *E. coli* and *S. aureus*). Moreover, there are no significant transformations observable between zones of inhibition in the scaffolds containing 3% and 5% Ag NPs for *E. coli*.

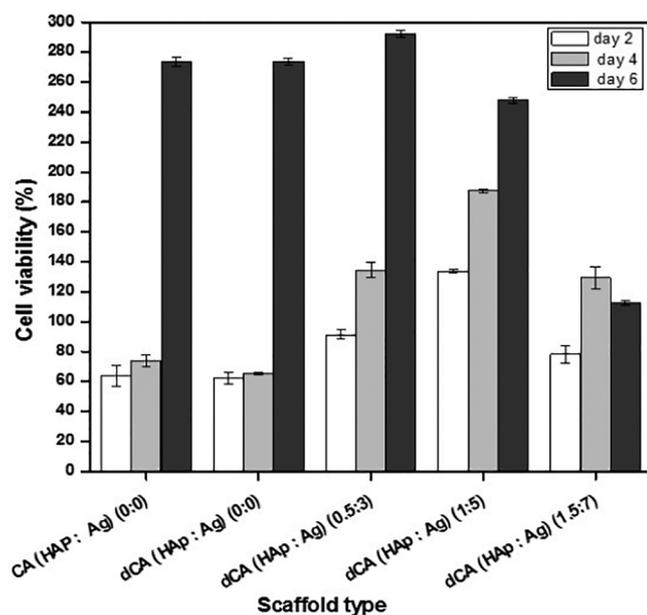


Fig. 12. The highest cell viability is observed on dCA fiber mats containing 1% HAp and 5% Ag for the 2nd and 4th days. Fiber mats containing 7% Ag were somewhat toxic to the growth as well as the proliferation of the cells. Statistical analysis was done by comparing the groups between one another using one-way Tukey's multiple comparison test (One Way ANOVA). A confidence level of 95% ($P < 0.05$) was set to determine the significance between groups. Significant differences (***) were observed in viability between 2nd or 4th vs. 6th. However, no significant difference was observed between viabilities in pristine CA and dCA scaffolds on the 2nd and 4th days. Also, no significant differences were found in the viabilities of pristine CA and dCA scaffolds on the 6th day.

on the fiber mats (Fig. 7 the SBF results). Moreover, the presence of fibroblasts can be seen throughout the fiber network. The fibroblasts can be seen occupying the porous system present in the nanofibers as well as some fibroblasts can be seen well-spread in the fiber direction. These results suggest that fibroblasts proliferated well on the fiber mats after the 10th day of incubation. The fiber mats containing both HAp and Ag NPs were found to provide native habitat for the growth and proliferation of the fibroblasts as compared to the pristine regenerated fiber mats. This has been accredited to the influence of HAp NPs to improve the cell-fiber contact.

The degradation study of the regenerated cellulose nanofibers and its variants containing different concentrations of HAp and Ag NPs was tested with and without enzyme (cellulase) supplemented in PBS. The degradation profiles of nanofibers after incubation are presented in Fig. 14. From these results, it is clear that the degradation of the nanofiber mats has accelerated with time (Fig. 14A). Moreover, it is observed that the deterioration of the nanofibers containing HAp and Ag NPs is comparatively higher than the natural counterparts. The presence of HAp and Ag NPs within the fiber network lowers the fiber density and, as a result, is easily susceptible to degradation due to the dissolution of the deposited HAp in the degradation medium. Also, the lower density of the fiber network allows for the natural disintegration of the fibers. As can be observed, ~18.6% of the weight loss resulted in the regenerated cellulose nanofibers containing 1.5% HAp after 60 days of incubation as compared to the only 6.1% in the pristine regenerated fibers. Although the cellulose nanofibers can primarily be degraded by the cellulase enzymes (not present in the humans) [71], the partial chemical hydrolysis of the cellulose backbone has reported leading to chain termination [72]. Furthermore, the changes in pH within the degradation medium with time have linked to cellulose chain scission [73]. The increase in hydrophilicity of the regenerated cellulose nanofibers on increasing the HAp and Ag NP content has known to accelerate the degradation process [74]. The results of the degradation studies augmented by the presence of the cellulase enzyme are shown in Fig. 14B. It can be detected from this study that the occurrence of the enzyme in the PBS accelerated the degradation process. Within 10 days of the experiment, 24.6% of the regenerated cellulose nanofibers containing 1.5% HAp degraded. The degradation of the pristine cellulose nanofibers is, however, still slower but at a higher rate than in the PBS media without enzyme.

8.1. Conclusion and future perspective

In this study, we report the fabrication of regenerated cellulose nanofiber mats containing both the HAp and Ag NPs. Cellulose acetate nanofibers fabricated using a highly volatile solvent (i.e., acetone) resulted in a ribbon-like morphology. In contrast, the existence of water in the solvent system helped to create the physical as well as the chemical bond between the fibers after the de-acetylation course. The regeneration of the cellulose acetate nanofibers carried out by alcoholic NaOH solution retained the original fiber morphology as the presence of Ag ions in the electrospinning solution leads to loss of spinning character. Therefore, we have opted for a two-step approach to incorporate HAp and Ag NPs in the nanofibers. Initially, the HAp nanoparticles were introduced in the spinning solution, while for adsorbing the Ag NPs on nanofibers, the post-fabrication method using a chemical reduction process was employed. Characterization of the fiber samples by different means revealed the presence of both HAp and Ag NPs. The HAp particles were responsible for mineralization of the fiber mats by acting as nucleating agents in the Ca–P crystal formation on the fibers during SBF treatment. The mineralization of the fiber mats provided the physiological environment for adhesion, growth, and proliferation of chicken embryo fibroblasts. However, moderately more cells proliferated with an increase in HAp concentration in the fibers.

Furthermore, these nanofiber mats containing Ag NPs showed excellent antibacterial properties against *S. aureus* and *E. coli*. However,

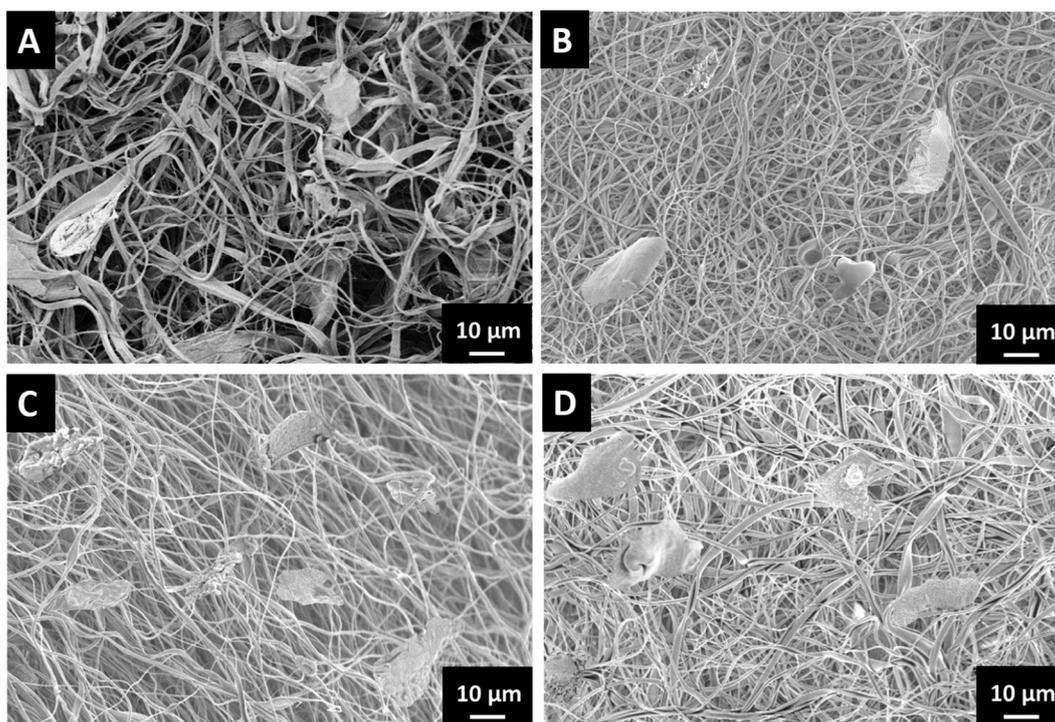


Fig. 13. SEM images of chicken embryo fibroblasts fixed by glutaraldehyde and dehydrated by ethanol on regenerated cellulose mats comprising of different HAp and Ag concentrations (A = 0% HAp, 0% Ag; B = 0.5% HAp, 3% Ag; C = 1% HAp, 5% Ag and D = 1.5% HAp, 7% Ag). An increase in cell adhesion, as well as proliferation, was observed with an increase in HAp content in the fibers.

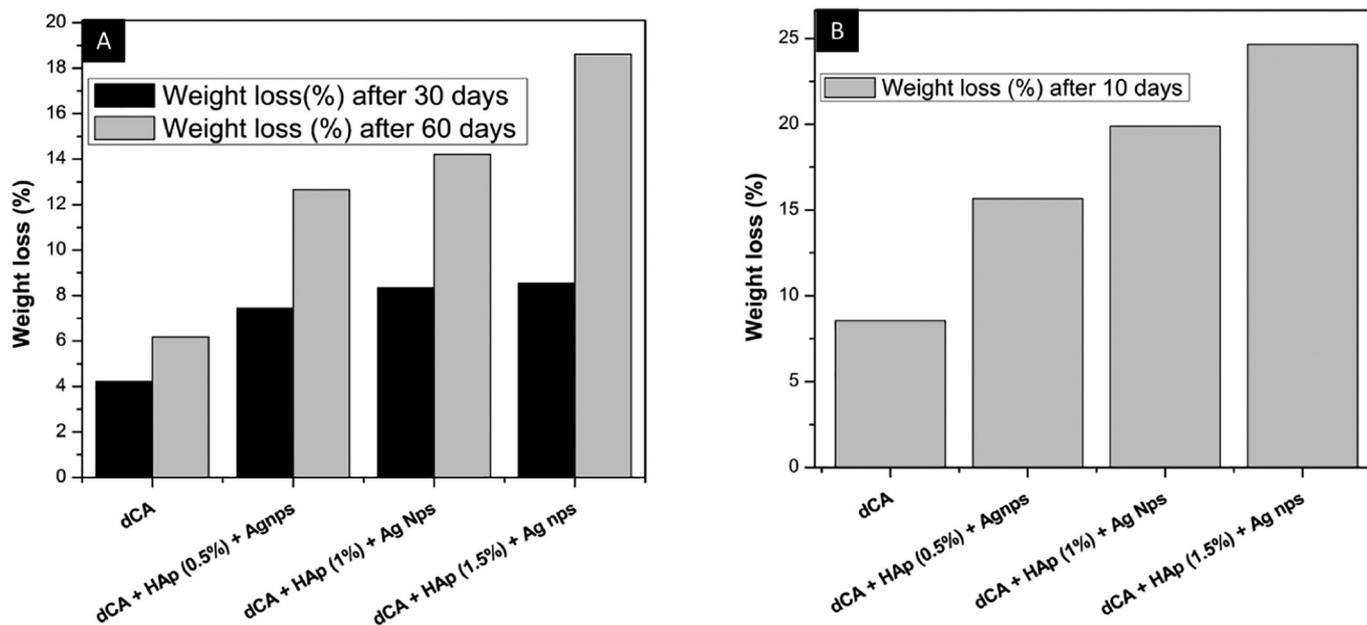


Fig. 14. Degradation of nanofiber scaffolds of regenerated cellulose (dCA) or its variants containing different concentrations of HAp and Ag NPs in PBS (A) or PBS supplemented with cellulase (0.1 mg/ml) (B). The rate of enzymatic degradation of the fibers is faster as compared to degradation in PBS without enzyme supplement. Tukey's multiple comparison test (One-way ANOVA) was applied to test the groups with each other at a confidence level of 95% ($P < 0.05$). Statistical analysis revealed that significant differences (***) were observed in different dCA composites as compared to the pristine dCA scaffold both in 30-day and 60-day groups. A similar trend was found in the enzymatic degradation study. However, no significant difference in degradation was observed for the 30-day study (PBS) in composite dCA scaffolds containing 0.5%, 1.0%, and 1.5% HAp.

the maximum level of the Ag NPs also depicted cytotoxicity against the proliferation of fibroblasts. It is, therefore, believed that these fiber mats holding both cell proliferation and antibacterial properties at an optimized concentration of HAp and Ag NPs could have potential applications in wound healing and bone tissue regeneration. In this study, we further plan to find the in-vivo use of these fiber mats in wound

restorative as well as the bone regeneration process. Suitable animal models and experimental protocols need to be assessed for checking applicability in bone tissue regeneration as well as wound healing processes. Histological analysis of the treated samples/controls needs to be conducted to ascertain the effectiveness of these fiber mats. However, before that these mats need to be probed by studies such as

controlling pore size in the mats for proper infiltration of the cells, optimizing the HAp/Ag concentration to mimic the native tissue niche and other in-vivo studies which will further promise the use of the mats in different areas of tissue regeneration.

CRedit authorship contribution statement

All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Research output on ICT applications and digital commons in LIS field: A bibliometrics study

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***Abstract** - Research Output would be the instrumental procedure and part of routine activity of the researcher. Hence the measurement Tool, Laws and Principles for research output have become crucial functions for the academic organization. Bibliometrics is the discipline where quantitative methods were employed to probe scientific communication process by measuring and analysing different aspects of written documents by various indicators. A research of “Alan Pritchard” (1969) by title “the applications of mathematical and statistical methods to books and other media” is illustrative. The study was conducted with specific objectives related to measures research contributions regards “ICT Application” and “Digital Common”. Entirety 727(100%) contributions from the respective three journals are analysed. It is noticeable that the highest research output in the LIS field of the selective journal The Electronic Library has contributed 322 articles (Out of 727), DESIDOC Journal of Library and Information Technology has 281 (Out of 727) while Science and Technology Libraries has 124 (Out of 727) research output observed from 2014 to 2018. It is traceable that the two selected source journals has similar periodicity is “Bimonthly” while one The Science and Technology Libraries has quarterly; it has obviously effected of the total research output, highest collaboration, authorship pattern, etc on the analysis of this study.*

Keyword: Digital Commons, ICT Application, DJLIT (DESIDOC Journal of Library and Information Technology), STL (Science and Technology Libraries), TEL (The Electronic Library), CAGR (Compound Annual Growth Rate), Collaboration.

Introduction

ICT application and Digital commons both are vast disciplines itself. At the same time, the library and information science is a field where it has applied in the library services, administered, and academic purpose. The use of ICT and the digital object can be more visible for shows research outputs by visualization effect and be more accurate reports and results with quickness. Since, the researches show that all disciplines of knowledge world have to be gotten more exposure towards practices, enable to approach to inter and multi-disciplinary subject, and proverbial with useful matrices after be applicative the ICT and digital commons.

Bibliometrics is indicating the parameters regard authors' contribution, content variety, scattered level of specific theme, term, or subject, collaboration etc. Collaboration science includes different methods for the extent of author and authorship. Identification of items/subject/theme especially in article is a first application and others are finding out the rank of journal. This paper shows the research output related ICT Application and Digital

commons by using different parameters of bibliometrics i.e. source of journals, authorship pattern, collaboration, used citation, etc. It is perceptible that the CAGR is implicate in the study for calculate the rate for the term “ICT Application” and “Digital Commons”

Background of the Study

During the digital era, all service sectors has been using ICT and digital commons in services, production and management, for making it more visible, usable, and user friendly. The library has a service sector, since the 20th century, it explored the ICT to enable functions and services automatically. Therefore to know the approaches and interest of library professional, researcher and academicians toward responsibility for being a part of developing the field of LIS the study is to conduct.

Composition of Journals

This paper has studied the bibliometrics of following three journals, many similarities and differentiation to be seen on their composition which is showed on the below mentioned table.

Particulars	Parameters	DJLIT	STL	TEL
Establishment Year		1981		1983
Types of Source	Printed/Electronic	Both	Electronic	Electronic
Periodicity	Monthly/quarterly/Half Yearly/Yearly	Bimonthly	Quarterly	Bimonthly
Publishing House			Taylor & Francis, Inc.	Emerald Publishing
Variety of Articles	Research paper, case study, Analytical study,			
Content Variety	Papers, Poster, Book Chapter Abstract, Conference Theme Abstract, Editor Message/Paper	Research Paper		Research Paper, Case Study, Book Abstract
Accesses Policy	OPEN ACCESS	Open Access		Hybrid

DJLIT = DESIDOC Journal of Library & Information Technology

STL = Science and Technology Libraries, TEL = The Electronic Library

Reviews of Literature

During 2004 to 2015 Garg, K.C. and Sharma, Chetan conducted the study on the Indian Citation Index, here they describe the research output of library and information science most prolific institutions i.e. university of Mysore, university of Delhi, CSIR-NISCAIR, etc as well as prolific authors as Indian LIS researchers with their citation impact by most preferred journal i.e. DESIDOC, SRELS, KELPRO, Information studies, etc. (Garg, K.C. and Sharma, Chetan). In 2013 an unpublished doctoral thesis had been submitted to the cited university. The research has been conducted with analysis of 107 titles of the journals related to the library and information science, it also analysed with different attributes i.e. categories of articles, core subject of LIS, richness of electronic form i.e. pdf, pot, Doc, and Google scholars with citation numeric of all 107 titles (Statute, Dattatraya Tukaram, 2013). The denotation and impact of bibliometrics, the measuring scientific communication from bibliometrics to cybermetrics in way of transformation is gracefully described by Nicola de Bellis in the book titled Bibliometrics and citation analysis from the science citation index to cybermetrics (2009). In medical science PTJ has been published in maximum research work

in different form i.e. research report, case report, technical, perspective, linkage evidence and practice, etc. Maximum work is published on collaboration, researcher also describe the details of subjects' variety in the Physiotherapy field i.e. musculoskeletal, rehabilitation, gynaecology, PT in education, standardized questionnaire, etc. (A.P, Minaxi, 2017).

Objectives

Research output can be showing the conceptual growth of the discipline, it's included concrete, procedures, place, tools, etc. ICT Application and Digital common are procedures simultaneously tools for the library and information science, hence it would be needed to know them research output with accepting some limitations. Here following objectives has to be consider with them different importance in the field of library and information science for the research.

- To determine the authorship pattern of selected journals from 2014 to 2018.
- To identify research outputs of the selected journal with the terms "ICT application" and "Digital Commons" in the discipline of LIS.
- To measures the collaboration by various level i.e. two author, three author, more than three for the selected journals.
- To measure the used citations of the selected journal for the period of 2014- 2018
- To calculate Compound Annual Growth Rate (CAGR) for the terms "ICT Application" and "Digital Commons" for known to scattering terms form 2014-2018 in selected journals.

Methodology

Comparative Bibliometrics has been applied in the methodology with detail bibliographical features of the articles and citations, analysis, pattern of authorship etc. A pertained data was taken from the following journals.

1. DESIDOC Journal of Library & Information Technology
2. Science & Technology Library
3. The Electronic Library

The period has been taken from 2014 to 2018. The SPSS software has been used for statistical analysis while tables and graphs forms are used for it presentation. A researcher has to be taken to calculate the Compound Annual Growth Rate (CAGR) for knowing the scattering rate of ICT Application and Digital Commons. It has to be used to know the growth rate of the investment in management discipline. A researcher can be convert the CAGR in the study for knowing year wise growth of the research output in term of "digital common" and "ICT Application" The primary equation of CAGR and converted it in for the two selective terms i.e. "ICT Application" and "Digital Commons" are as under.

CAGR = Ending Balance/Research output of "Digital Common

$$CAGR = \left(\frac{\text{Endingvalue}}{\text{beginingvalue}} \right)^{\left(\frac{1}{5} \right)} - 1$$

Convert the equation into LIS RESEARCH

$$\begin{aligned} \text{CAGR for Digital Common} \\ &= (\text{"digitalcommon" research output/total research output})^{(1/5)} \\ &- 1 \end{aligned}$$

$$\begin{aligned} \text{CAGR for ICT Application} \\ &= (\text{"ICT Application" research output/total research output})^{(1/5)} \\ &- 1 \end{aligned}$$

Data Analysis

Table 1. Research Output of the source journals

Name of the Journal	Year wise output					Total
	2014	2015	2016	2017	2018	
DESIDOC Journal of Library & Information Technology	60	53	49	58	61	281
%	21.35	18.86	17.44	20.64	21.71	100
Science and Technology Libraries	26	19	25	28	26	124
%	20.97	15.32	20.16	22.58	20.97	100
The Electronic Library	60	91	53	60	58	322
%	18.63	28.26	16.46	18.63	18.01	100
TOTAL	146	163	127	146	145	727
%	20.08	22.42	17.47	20.08	19.94	100

Table 1 shows the growth of the research output of selective three source journals for the period of 2014 to 2018. It seems from the data presented that the journal "Electronic Library" has 322 number of research output which is highest on the comparison with remain two journals. It also highlighted that the variation between the total research output of the tenure is dependence on the periodicity of the journal, i.e. Bimonthly, quarterly, yearly, and weekly.

Table 2. Year wise Authorship Pattern

Name of the Journal	Authorship Pattern	2014	2015	2016	2017	2018	Total
DESIDOC Journal of Library & Information Technology	One Author	21	17	18	16	15	87
	%	24.14	19.54	20.69	18.39	17.24	100
	Two Author	28	25	21	34	28	136
	%	20.59	18.38	15.44	25	20.59	100
	Three Author	9	10	9	6	13	47
	%	19.15	21.28	19.15	12.77	27.66	100
	More than Three Aauthor	5	2	3	3	5	18
	%	27.78	11.11	16.67	16.67	27.78	100
	Total	60	53	49	58	61	281
	%	21.35	18.86	17.44	20.64	21.71	100
Science and Technology Libraries	One Author	22	2	14	13	17	68
	%	32.35	2.94	20.59	19.12	25	100
	Two Author	3	7	6	7	4	27
	%	11.11	25.93	22.22	25.93	14.81	100
	Three Author		6	3	5	2	16
	%		37.5	18.75	31.25	12.5	100
	More than Three Author	1	4	2	3	3	13
	%	7.69	30.77	15.38	23.08	23.08	100
	Total	26	19	25	28	26	124
	%	20.97	15.32	20.16	22.58	20.97	100

The Electronic Library	One Author	34	66	24	25	35	184
	%	18.48	35.87	13.04	13.59	19.02	100
	Two Author	14	13	17	19	14	77
	%	18.18	16.88	22.08	24.68	18.18	100
	Three Author	8	8	7	9	5	37
	%	21.622	21.622	18.919	24.324	13.514	100
	More than Three Author	4	4	5	7	4	24
	%	16.67	16.67	20.83	29.17	16.67	100
	Total	60	91	53	60	58	322
	%	18.63	28.26	16.46	18.63	18.01	100

Above table revealed the authorship pattern for the researches tenure 2014 to 2018 for the selective source journals. It comes to the notice during data collection that the slot of more than three authors of researches include more than three authors' paper i.e. 5 authors, 7 up to 10 authors. It also found from the table that in slot of three authors contributions for the tenure of 2018 the DESIDOCJLIT has highest 13 contributions (27.66%) for the said slot, while TEL has 9 contributions (24.324%) STL has only 2 contributions (12.5%). It is much less in compared of other journal; it is also noticeable that STL do not have an contribution for three authors slot on year 2014.

Table-3 Strength of Collaboration

Authorship Pattern	2014 to 2018			Total
	DJLIT	STL	TEL	
Two Author	136	27	77	240
%	56.67	11.3	32.1	100
Three Author	47	16	37	100
%	47	16	37	100
More than Three Author	18	13	24	55
%	32.73	23.6	43.6	100

Table no. 3 shows the status of collaboration from 2014 to 2018 for all three source journals are as under.

- DJLIT journal have highest number of researches in three range of authorship pattern, due to the periodicity in compared of STL and TEL.
- It is noted that the TEL have maximum researches on the third range, with 24 papers (43.64%) out of 55 analyses, while STL have 13 papers (23.64%) and DJLIT 18 articles (32.73%). It seems here the total number of papers in the third range of collaboration for all selective journals is not more than 25. Hence, TEL has 24 the highest researches (43.64%) in compare with DJLIT and STL.
- STL have quarterly periodicity; hence, the contributions could not come out by equal and at least 25% of the level in compare to the DJLIT while it is not double by the first collaboration range of TEL.

Table-4 Used Citations in source journals

Range of Used citation	Up to 14				15≤25				More than 25			
	DJLIT	STL	TEL	Total	DJLIT	STL	TEL	Total	DJLIT	STL	TEL	Total
2014	27	17	42	86	9	11	29	49	7	11	26	44
%	31.4	19.8	48.8	100	18.37	22.5	59.2	100	15.91	25	59.1	100
2015	29	18	43	90	7	10	30	47	6	10	27	43
%	32.22	20	47.8	100	14.89	21.3	63.8	100	13.95	23.3	62.8	100
2016	20	19	61	100	8	11	47	66	7	9	44	60
%	20	19	61	100	12.12	16.7	71.2	100	11.67	15	73.3	100
2017	31	23	45	99	15	12	37	64	13	12	36	61
%	31.31	23.2	45.5	100	23.44	18.8	57.8	100	21.31	19.7	59	100
2018	39	21	38	98	24	11	30	65	19	11	27	57
%	39.8	21.4	38.8	100	36.92	16.9	46.2	100	33.33	19.3	47.4	100

Above table describe measures of used citations in researches from 2014 to 2018. It is bifurcate in three range i.e. up to 15, >15≤25, and More than 25 references from each research paper from all three journals. The data presentation highlighted amazing truth that the journal who has less research output for the selective tenure, has utilized less references in compared journals. For the first range a DJLIT have 27 references (31.40%), TEL have 42 references (48.84%), while STL has 17 (19.77%). The year wise total references columns on comparative mode of each range has showing fluctuation; while the year wise total references of first two slot is gradually increasing, and last one shows the fluctuation.

Table-5 Compound Annual Growth Rate

Name of the Journal	Digital Commons	Total Research Output	2014-2018	CAGR	ICT Application	Total Research Output	Year	Growth Ratio
DESIDOC Journal of Library & Information Technology	49	281	5	0.4	71	281	5	0.3
Science and Technology Libraries	7	124	5	0.8	4	124	5	1
The Electronic Library	111	322	5	0.2	104	322	5	0.3
Total	167	727	5	0.3	179	727	5	0.3

Table 5 show the Compound Annual Growth Rate of selected journals. The calculation of CAGR has to be done for knowing the scattered level of selective subjects are Digital Commons and ICT Application only for the period of 2014 to 2018.

CAGR for Digital Commons:

Table no. 5 shows the CAGR for “Digital Common” is cooperatively 0.34 for all three sources of journal. While split CAGR as per the total research outputs of all selected journal on ascending order is highest 0.78 for STL, 0.42 for DJLIT and 0.24 for TEL.

CAGR for ICT Application

A CAGR for “ICT Application” cooperatively 0.32, while Arranged split CAGR on ascending order is 0.32 to DJLIT, 0.99 to STL and 0.25 to ETL. It is explained from the analysis that where the numbers of contributions is decreases the CAGR is increase while it reciprocates when contributions of source journal is increase.

Findings

- First objective of the study is to determine the authorship pattern for the selected source journal from 2014 to 2018. Table. No.2 data is show the authorship pattern i.e. one, two, three and more than it. On the basis of the data a researcher get first, second and third rank to the source journal. It is finding out that for the selected five year DJLIT journal has contribute 136 highest with two author while 47 with three authors. ELT comes on second with respectively 77, 37 and 24 contributions. The STL comes third with 27, 16 and 13 contributions.
- As per the second objective of the study, it is find out that the term digital commons get 17.43% contribution from DJLIT, 5.66% from STL and 34.47% from the TEL. A term ICT Application gets 25.27% from DJLIT, 3.23% from STL and 32.30 from ETL.
- Third objective of the study is to measure the collaboration by various levels. As per entire study table no. 2 named year wise authorship pattern and table no. 4 named strength of collaboration describe the detail about the objective. It is seeming that two authors collaboration rate of DJLIT is highest with 56.67% in comparison of remain two. While TEL journal has 32.08% and STL has 11.25%. It is noticeable that the Electronic Library journal and DJLIT has similar periodicity, and it's highly contributes in both terms.
- As per the fourth objective of the study is to determine the cited references in source journals. Table no. 4 named Cited references in source journal. A researcher has split the cited references in three zones i.e. Up to 14, 15≤25 and more than 25. It shows that a TEL source journal has highest cited references due to them periodicity in compare of remain two sources. Journals.
- Fifth objective of the study is to calculate CAGR for both terms from 2014 to 2018. It is determine that both terms scattering gradually on LIS field. Table no. 5 named annual growth rate shows the calculation of CAGR. It is revealed from the table 5 that CAGR is highest for the source journals; has low contribution, while lower when highest contribution. It meant by the low CAGR is define highest scattering of the term or discipline. The data revealed that the Electronic Library journal has highest rate scatter.

Suggestions

All three journals are available on different platform on internet, with different presentation and service style. Here it is suggested by the researcher that all references used in each research paper should be highlighted by exact number on the content page. Therefore,

detailed bibliometric study will be possible. Users and scholars have to be understood the importance of references and their styles. Through the bibliometrics analysis by variables of references, may find out the variety of references by form of documents i.e. books, articles, thesis, newspaper, etc hence it will be helpful to educators to increase the variety of forms in the references.

Persistence research is the foundation of growth of all discipline. It seems from the analysis of this study that a researcher has to continue research at least toward one core subject; hence the rate of growth will be visible on discipline level as well as authorship pattern. Hence it needs to increase the collaboration on the research for more values, implication and applications of research.

Conclusion

Bibliometrics has many indicators i.e. publication, science and technology, authors, etc., all are using for determining the scattering level of contributions, disciplines, forms of research, etc. Its strength of research helps to recognise the related literature of subjects and an area of knowledge world where it is spread on relevant areas i.e. interdisciplinary, multidisciplinary and its own field. Here the study reveals that total 727 articles have been published in LIS field it's included the term "Digital commons" and "ICT Application".

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Role of Literary Traditions In Writing Translation History

*Dr. Vinai Kumar Donthula**

Abstract

Translation without literature does not make sense. Each language has its own literary tradition. To study about Indian translation tradition, one should study how literatures evolved in India. Being a multi-lingual country, India has multiple literary traditions. Therefore, it becomes very difficult to give a comprehensive history of the literature in India and thus its translation tradition.

The historiography of translation should comprehensively describe the different approaches of the translation traditions of various languages from antiquity to the present and then explain the emergence of these approaches in the context of their time and show how they reflect the socio-economic, cultural, political and religious developments of their time.

In the western world, from the ancient past to the late 19th century, expressions about translation theory entered into traditionally defined areas of thinking about language and culture, i.e., rhetoric, literary theory, philosophy. The issue of periodization is very important in history and no historian could do without it. Various translation scholars have different points of view and approaches regarding periodization.

Key words: literary tradition, translation tradition, historiography

One cannot think of translation without literature. Each language has its own literary tradition. To study about Indian translation

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tradition, one should study how literatures evolved in India. Being a multi-lingual country, India has multiple literary traditions. Therefore, it becomes very difficult to give a comprehensive history of the literature in India and thus its translation tradition.

The German historian Leopold von Ranke (1795 – 1886), who is often portrayed as the “founder” of scientific history embraced a vision of history as an impartial account of reality. Yet he brought together both the scientific history and Romantic history approaches in his works. He was influenced by his predecessor, Barthold Georg Niebuhr (1776 – 1831), who has applied the methods of textual criticism taken from philology to the study of Roman history. Ranke developed his interest in history as a schoolteacher of classical literature (Eileen Ka-May Cheng, 2012: 70).

E. V. Ramakrishnan (2006) in his guest editorial “Mapping Indian Traditions in Translation: Concepts, Categories and Contestations” in *Translation Today*, Vol. 3 Numbers 1 & 2 states that the political boundaries of the linguistic states in India do not coincide with their cultural boundaries, as India has a complex history of social and cultural formations. The Indian translational discourses have been rendered unintelligible in our institutional debates and dialogues. He further states that ‘translation studies as a discipline’ or as ‘a discipline at the interface of disciplines’ is yet to be conceptualized with reference to Indian literary history. The hegemonic role played by English has further complicated the relationships between Indian languages. It has sealed off a domain of interactive relationships and creative dialogues.

As regards Indian literary theory, E.V. Ramakrishnan opines that it is a complex network of texts travelling like a river which take a new form in different types and varieties of excerpts. He states that the Western theories and versions of translation are not enough to understand or clarify the ways of articulation, dissemination and acceptance of Indian literary theory. Therefore, he opines that translation studies needs a new paradigm, a new perspective of looking at translation as a deed in order to understand the complex network of textual and cultural relationships. Hence, there is a need for both diachronic and synchronic studies across several Indian languages to map the uncharted expanse of Indian translation tradition.

The historiography of translation should comprehensively describe the different approaches of the translation traditions of various languages from antiquity to the present and then explain the emergence of these approaches in the context of their time and show how they reflect the socio-economic, cultural, political and religious developments of their time. Translation scholars with good knowledge of history and historians wanting to contribute to the ‘discipline of translation studies’ should work in collaboration for this project. Multi-disciplinary approach is very much required for this. To put in Pym’s words: “the ultimate purpose of studying the past is to improve the present.”

As regards the difficulties in diachronic and synchronic studies, Christopher Rundle (2009) in “Historiography” in *The Routledge Encyclopaedia of Translation Studies (3rd edition)* states:

One of the difficulties that emerges in historical approaches to translation studies is finding a way to reconcile the diachronic perspective, which is natural in any historical inquiry, and the synchronic perspective which is apparent in the strongly felt need in translation studies to find definitions and categories that are applicable across different historical contexts, usually with the aim of allowing these to be compared to each other.

Referring to different types of sources Rundle (2009) further states:

Translation historians who work on the texts and para-texts, and who make less use of archival material, will tend to see their sources as fairly unambiguous from a historical point of view. Reconstructing ‘what happened’ is not particularly difficult. But when we are dealing with historical archival sources, there are usually a series of difficulties that mean that even a straightforward reconstruction of events can be quite complex.

As regards the ancient Indian literature, the “Vedas” are the earliest texts which are believed to have been composed around 1500 BC. “Natyashastra” is believed to have been written by sage Bharata between 400 BC and 400 CE. The epics, “Ramayana” and “Mahabharata” were orally passed from one generation to another till

they were recorded in writing. “Ramayana”, which is believed to have been composed by sage Valmiki, is believed to predate “Mahabharata”, which is believed to have been composed by sage Vyasa. It is very difficult to pinpoint exact dates of composition of these works.

All these works were written in Sanskrit, which was the lingo of the educated high society or upper caste. Drama was the only literary genre of those times. The characters in the play spoke Sanskrit, Prakrit and other vernacular dialects according to their hierarchy in the society. Educated upper class men conversed in Sanskrit, women conversed in Prakrit and the remaining subservient characters conversed in different of dialects like Magadhi, Pali and Sauraseni. Therefore, while watching a play, one had to do simultaneous translation in some sense.

In south Tamil was the prominent language in those days. Like “Natyashastra” in Sanskrit, “Tolkappiyam”, written by Tolkappiyar in Tamil, was the major work on Dravidian aesthetics. This work has parallels with “Natyashastra”. The ancient body of Tamil literature, called the Sangam literature shows influences of the Sanskrit epics, pointing to some form of cultural contacts.

Avadesh Kumar Singh (2006) expresses the following views about translations during Bhakti period in his paper “Translation in/and Hindi Literature” in Translation Today, Vol. 3 Numbers 1 & 2

The poets of the Bhakti period (1100 – 1700) were translators in a different and loose sense, as they strove to translate ancient Indian knowledge and wisdom manifested in different treatises through Sanskrit by appropriating it in various bhashas (native languages). The period from 1100 to 1700 was marked by the lokabhashikaran** of knowledge in Sanskrit. The Bhakti poets namely Nanak, Kabir, Sur, Tulsi Narsinh, Mira, Gyaneshvar democratized the knowledge in Sanskrit, by transferring it into dialects and lokbhashas (languages of ordinary people). Translation from non-Indian languages into Indian languages and vice versa was less than desired.

In medieval India during the Mughal period Persian was a courtly language. It was also a language of scholarship. During Akbar’s

**Lokabhashikaran means vernacularisation here.

time the epic ‘Mahabharata’ was translated into Persian. The Upanishads and ‘Bhagwat Gita’ were translated into Persian by Akbar’s great grandson, Prince Dara Shikoh.

During the British rule, the Britishers tried to colonize India politically and intellectually. Many European texts were translated into some local Indian language. Some ancient Sanskrit works like “Abhijnanasakuntalam” and “Bhagwat Gita” were translated into English. The Bible was translated into local Indian languages, as the aim of the British was to spread Christianity.

History of translation has very close link with the history of literary tradition of a geographical area. If there is no literature, then one cannot think of translation. Literature is further classified as ‘canonized’ and ‘non-canonized’. G.N. Devy (1997) in the abstract of his paper titled “Literary history and translation: An Indian view, says:

A discussion of the relations between canonized and non-canonized literary forms, between ‘self’ and the ‘other’, within the Indian context, leads to a differentiation between the Western tradition of a single dominant literary tradition and the more diverse, and inclusive, parallel and multiple traditions of India. At the origin of such traditions and holding them together long enough to permit cross fertilization, are acts of translation, merging sign systems and forming a community of ‘translating consciousness’ where several languages are used simultaneously and are a part of a larger, continuous spectrum. Translation in such a multilingual context plays a fundamental role, transforming and revitalizing original texts.

Further viewing translation through metaphysical perspective G.N. Devy (1999) in his paper “Translation and literary history: An Indian view” says:

Indian metaphysics believes in an unhindered migration of the soul from one body to another. Repeated birth is the very substance of all animate creations. When the soul passes from one body to another, it does not lose any of its essential significance. Indian philosophies of the relationship between form and essence, structure and significance are guided by this metaphysics. The soul, or significance, is not subject to the laws of temporality; and therefore significance, even literary significance, is ahistorical in Indian view. Elements of plot,

stories, characters, can be used again and again by new generations of writers because Indian literary theory does not lay undue emphasis on originality. If originality were made a criterion of literary excellence, a majority of Indian classics would fail the test. The true test is the writer's capacity to transform, to translate, to restate, to revitalize the original. And in that sense Indian literary traditions are essentially traditions of translation.

Western or European tradition of translation is very much different to Indian tradition of translation in comparison. Bh. Krishnamurti opines that India being a multi-lingual country is a linguistic area and thus a translation area. Being polyglots, Indians use more than one language while conversing and thinking. Hence, they are translation conscious. Indra Nath Choudhuri (2010) in "Towards an Indian theory of translation" opines that

The ancient Indian view that translation is nothing but repetition also means that translation is clarification, interpretation which is obtained by repetitive utterances and therefore to an Indian society, steeped in an oral literary tradition of smriti and shruti, differing versions were the norms, not exceptions. The method of producing the authentic and 'pure' text perpetuated in Europe particularly during the period of colonial domination by the Europeans was an alien notion for Indians. To an Indian mind translation is rebirth where 'atma' the invariant core remains constant but other things take a new form.

In the western world, from the ancient past to the late 19th century, expressions about translation theory entered into traditionally defined areas of thinking about language and culture, i.e., rhetoric, literary theory, philosophy. The issue of periodization is very important in history and no historian could do without it. Various translation scholars have different points of view and approaches regarding periodization. For example, Clara Foz's explanation of how Ljudskanov, Georg Steiner, Julio-Cesar Santayo and Michel Ballard have periodized the literature regarding the theory, practice and history of translation in her chapter "Translation, History and the Translation Scholar" is summarized as follows:

Ljudskanov proposed structuring the practice of translation

into four stages. *Word for word translation* during High Antiquity and Antiquity, *Sense translation* from the early first century AD to the fifteenth century, *Free translation* from the sixteenth to the eighteenth centuries, and finally *Adequate translation* from the nineteenth century to the end of the 1960s.

George Steiner's periodization also comprised of four phases. He situated all translational practices from Cicero to the end of the eighteenth century within the same category. He referred to this phase as empiricism. The second phase corresponds to the hermeneutic approach, where the theory and the reflections on what it means to understand a text predominate. It gives a clear philosophical aspect to translation and extends from Friedrich Schleiermacher (1813) to Valéry Larbaud (1946). The third phase marked by automatic (machine) translation and formalism, begins in the forties and ends in the seventies. Overlapping this period is the fourth phase, originating at the beginning of the sixties and marked by interdisciplinarity.

Julio-César Santoyo's periodization in 1987 also comprised of four phases. The first phase of oral translation extends from prehistory to 3000 years BC and the second phase of written translation is determined from 2400 BC to Cicero. In the third phase the reflections on translation stretches from Cicero to the end of the eighteenth century. In the fourth phase the "real" theorization begins with Tytler (1791) and Schleiermacher and lasts into the eighties.

Michel Ballard's periodization comes under the form of five general categories. The first phase, dubbed Sources, extends from Prehistory to the fourth century. The second phase, where translation appears as relay, extends from the fifth century to the fourteenth century. The third phase, when translation goes hand in hand with discovery and the opening of new horizons, covers the fifteenth and the sixteenth centuries and the fourth period is marked by the Belles infidèles in seventeenth-century France. The fifth phase begins in the seventeenth century and ends with Walter Benjamin (1892–1944).

As Indra Nath Choudhuri (2010) says, translation in the West in modern times has been subjected to scrutiny from a variety of perspectives such as pure literary discourse of Paul Valéry, Cultural

Studies of George Steiner, theoretical linguistics of Catford, psychoanalysis of Andrew Benjamin, structuralism of Jakobson, deconstruction of Derrida, gender study of Lori Chamberlin and post-colonial discourse of Lawrence Venuti.

The translation historian(s) should strive for a global history of translation. Translation traditions of more and more languages must be included in writing the translation history. Only then the history of translation can exhibit pluralism. The translation historian(s) can make it possible through collaborative research and team work.

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Role of Women Colleges in promoting women's Higher Education: A Case Study of University College for women Koti - Hyderabad

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Key words: Women, Higher Education, Access, women's Educational Institutions

(The Role of women's Institutions in providing access to Higher Education)

Abstract

Women's education is currently at the center of international development discourse. In the discourse on education and development, 'education' has major contribution towards women's emancipation. There are 233 women colleges in A.P and the issue of women's higher education has so far received modest attention due to women's Education Institutions. This paper focuses on the access and participation of women students and advantages and opportunities in Higher Education in women's colleges in the State of Andhra Pradesh. How are the women progressed by the women college's options offered by higher education? Do we have sufficient analyses on the trends and shifts in the modern education and role of women colleges in promoting the choices of women in higher education? It is possible only because of women colleges was fully started in order to arrest the doubts of parental insure towards teenage girl and social security of these institutions. However, their participation was enlightened their future and the role of higher education provides scope in decision making. Further, socio-cultural and economic factors acted as barriers to their ability to access higher education but these institutions met this demand to promote research in the gender studies and compete with masculine domains in other fields of research. The major findings of the study elucidates access to higher education, post collegiate outcomes, confidence levels, problem solving, decision making, and empowerment of women.

Introduction

Education system should make an individual better suited to the needs of the ever changing dynamic world. It should provide a new culture of work ethos and an array of young talented skilled and well equipped target groups who will be able to shoulder the developmental responsibilities in general and women's education in particular. Education of girls is not only vital on grounds of social justice but also because it accelerates social transformation. Promotion of gender equality in education is essential for human resource development. By educating women you educate the whole family, if you given a woman has the responsibility of the whole family on herself, an educated woman is better capable of taking care of the health, nutrition and education of her children and more so be an active agent in the social and economic development of the country. Moreover, no society has ever liberated itself economically, politically or socially without a base of educated women. Education has a direct impact on women empowerment as it creates awareness in them about their rights, their capabilities and the choices and opportunities available to them. Many studies have indicated that there is a positive correlation between female education and several developmental indicators such as increased in economic productivity, improvement in health, delayed age at marriage, lower fertility, increased political participation, and effective investments in the next generation.

There has been phenomenal expansion of educational opportunities for women in the field of higher education both general and technical. Women education at the university and college levels has been diversified and reoriented in tune with the changing requirements of the society, industry and trade. The proportion of women entering higher education 1950-51 was 10.9 percent and in 2008-09 it was 42.16 percent. Therefore, in this paper an attempt has been made to analyze the educational status of women in the university college for women, higher education for girls in India in general and in Osmania college for women in particular and disciplinary choices for women. The researchers have examined on various aspects of analyses on the trends and shifts in the modern education and role of women colleges in promoting the choices of women in higher education. However ,the major findings of the study elucidates access to higher education, post collegiate outcomes, confidence levels, problem solving, decision making, and empowerment of women through women's college.

Objectives of the Study:

- ❖ To find out the access and participation of women students in higher education in A.P
- ❖ To elucidate the role of women's colleges in promoting the choices of women in higher education
- ❖ To prove the women's colleges have advantages and opportunities in Higher Education for girls in A.P
- ❖ To examine the impacts and influences acquired from women's college in their course of study

Review of Literature

This study adds to the existing literature on women and education in higher education in general and in A.P in particular, by recognizing the important linkages on various aspects of women's higher education and the acquisition of female literacy in university and college education. In this we are addressing some of the important familial, socio-cultural, individual level, and institutional factors which promote female educational attainment. While our focus is on A.P state, findings also have the value for other women's colleges.

Chanana (1998) in her article 'Social change or Social Reform: The Education of women in India' highlights about the disciplinary choices for women in higher education, and the relationship between availability of disciplinary choices and women's ability to access them are not directly related, and nor they dependent on women's academic achievement. She cited main reasons for this are due to social ethics, and majority of women are deprived in selecting the subjects of their choice in school as well as in higher education. The major findings of the study are women from low social categories are the most affected by the stratification of disciplines, programs and institutions, and there is no link between academics and career choices.

Sandhya, Ranjanni&Neeraja (2011) in their article 'An analysis of Tribal Women' Education in India' explains about the importance of education to women and they opinioned that it is very vital as men. The influence of education on women and changes took place in the life of tribal women and how it is correlated to economic independence was highlighted. Through education

how they organize themselves to form strong groups in order to analyze the situations and conditions of living, and to understand their rights and responsibilities and to enable them to participate and contribute to the development of women and the entire society.

Jacob (1996) in his article 'Gender Inequality and Higher Education' pointed out about the gender inequality is prevails more in higher education than in others. He analysis talks much about access to higher education, college experiences in women's college, Women fare relatively well in the area of access, less well in terms of the college experience, and are particularlydisadvantaged with respect to the outcomes of schooling. Explanations of gender inequality in higher education should distinguish between these different aspects of education and should explain those contexts in which women have attainedparity as well as those in which they continue to lag behind men. He concluded in his study about the access, process, and outcomes of higher education and criticizes about the sociology of education neglected women's higher education and he explains gender often becomes a matter of variations on the main theme of socio-economic and racial inequality. Secondly, he mentions about the educational decision-making process needs to more attention. Thirdly, aspects of process and outcomes need to be linked to the educational analysis. Fourth, the relationship between gender and institutional development needs should be more concerned, and the role of institutions in promoting women's higher education.

Victoria (1998) in her article 'Women's Education in India' pointed out the relation between Infant mortality rates by mother's educational level. She explains the low level of literacy not only has a negative impact on women's lives but also on their families' lives and on their country's economic development. In India, girls are likely to be taken out of school when they reach puberty due to common schooling system which is co-education. She explained about the issues related to girl's education, such as barriers to girls education, gender gaps in university education, inadequate school facilities, gender bias in curriculum in developing countries, restricted choice of opting disciplines in higher education.

Halai (2011) in his article '*Equality or equity: Gender awareness issues in secondary schools in Pakistan*', focused on gender awareness and dimension of addressing the quality of education in

Pakistan from the social justice perspective. Beyond access there are several factors that compromise quality of education and raise issues of equity and social justice. He also examined on the issues policy and practice in school education and teacher education in the schools where minority sections are concentrated.

Methodology

In this study, we have taken the university college for women Koti, as the Study Area, which is very popular as women's college for higher education with various disciplines. The researcher carried out the study based on both Primary and Secondary sources. The Primary sources are mainly the field survey with a structured interview schedule. The data was collected from 120 respondents from various disciplines such as B.A. B.Sc, B.Com, M.A. M.Sc, MBA and the opinion about their women's college, advantages and their problems by the researcher. The Secondary sources are collected through a survey of literature both published and unpublished, books, articles, and vernacular newspaper essays. The study conducted between the months of January - February 2019. The data is analyzed by both qualitative and quantitative techniques; in the month of January we did pilot study to make them comfortable to give proper answers and their time in filling up the interview schedule. The researchers carried out some of the case studies related to the promotion of their education and getting jobs after completion of their courses from this college.

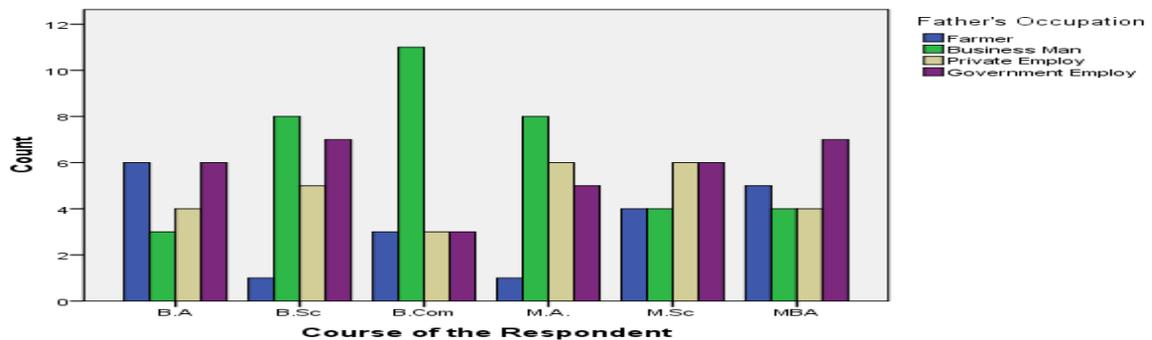
We have compared the system of co-education and women's education at collegiate level. The Study explored the difference between the institutions regarding access, process and outcomes. Major findings reveal that the positive impacts and influences, increasing women students' enrolment, and social security issues in women's college.

Data Analysis and Field Notes

Table- 1: Respondent’s Course and Father's Occupation

Course of the Respondent	Father's Occupation				Total
	Farmer	Business Man	Private Employ	Government Employ	
B.A	6 31.6%	3 15.8%	4 21.1%	6 31.6%	19 100.0%
B.Sc	1 4.8%	8 38.1%	5 23.8%	7 33.3%	21 100.0%
B.Com	3 15.0%	11 55.0%	3 15.0%	3 15.0%	20 100.0%
M.A.	1 5.0%	8 40.0%	6 30.0%	5 25.0%	20 100.0%
M.Sc	4 20.0%	4 20.0%	6 30.0%	6 30.0%	20 100.0%
MBA	5 25.0%	4 20.0%	4 20.0%	7 35.0%	20 100.0%
Total	20 16.7%	38 31.7%	28 23.3%	34 28.3%	120 100.0%

Bar Chart

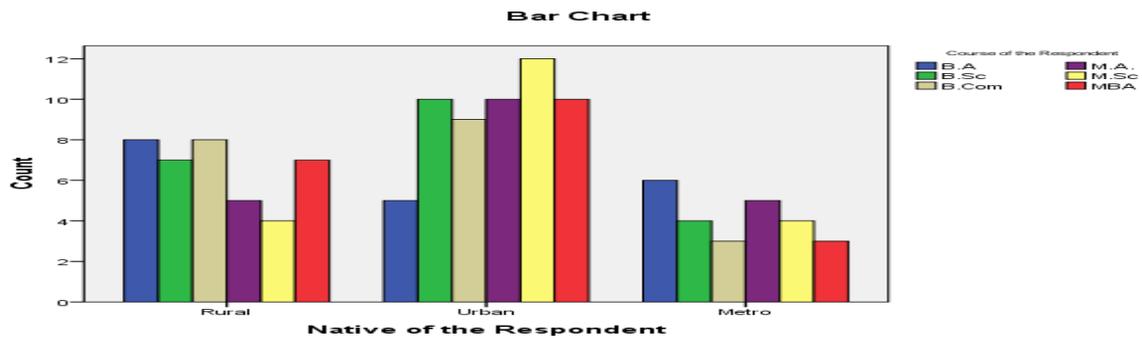


The above table explains about the course of the respondent and their father’s occupation which is correlated to the joining of a particular course. The girls whose father’s occupation if business

majority of 31% joined in these six courses followed by government employ daughters with 28.3% and with 23.3% are from private employ daughters. Lastly, 16.7% are from agrarian family background.

Table -2: Respondent’s Native and the course chosen

Native of the Respo ndent	Course of the Respondent						Total
	B.A	B.Sc	B.Com	M.A.	M.Sc	MBA	
Rural	8 20.5%	7 17.9%	8 20.5%	5 12.8%	4 10.3%	7 17.9%	39 100.0%
Urban	5 8.9%	10 17.9%	9 16.1%	10 17.9%	12 21.4%	10 17.9%	56 100.0%
Metro	6 24.0%	4 16.0%	3 12.0%	5 20.0%	4 16.0%	3 12.0%	25 100.0%
Total	19 15.8%	21 17.5%	20 16.7%	20 16.7%	20 16.7%	20 16.7%	120 100.0%

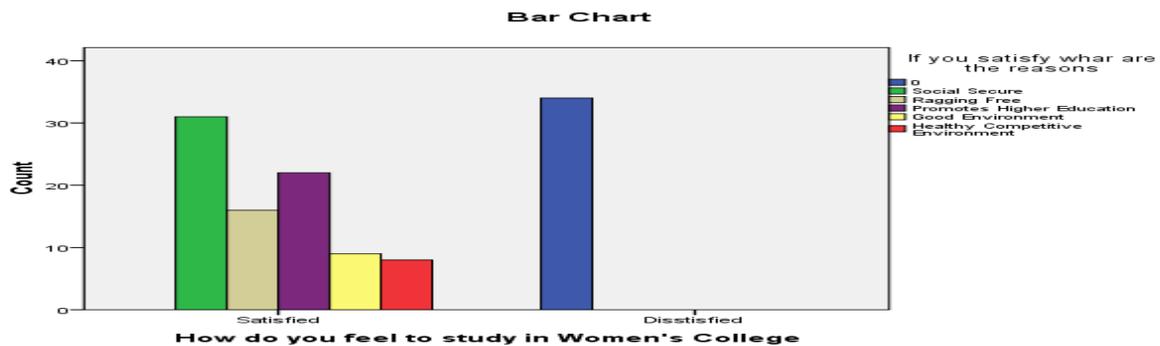


The table enumerates the background of the respondent and the course of study. Among rural, urban and metro background the urban has the lion’s share in all the courses from B.A to MBA. Among B.A and B.Com students rural background children with 20.5% followed by B.Sc and MBA with 17.9% and lastly M.A with 12.8% and 10.3% in M.Sc. The urban background students gave importance to M.Sc with 21.4%, followed by MBA, M.A and B.Sc with 17.9%. They have given least priority B.A and B.Com courses. The metro background students gave

more emphasis to B.A, M.A with 24% and 20% followed by B.Sc, M.Sc with 16% and B.Com MBA with 12%. In total 17.5% has highest priority to B.Sc and 15.8% with least priority to B.A.

Table -3: Respondent's perception in Women's College and reasons of satisfaction

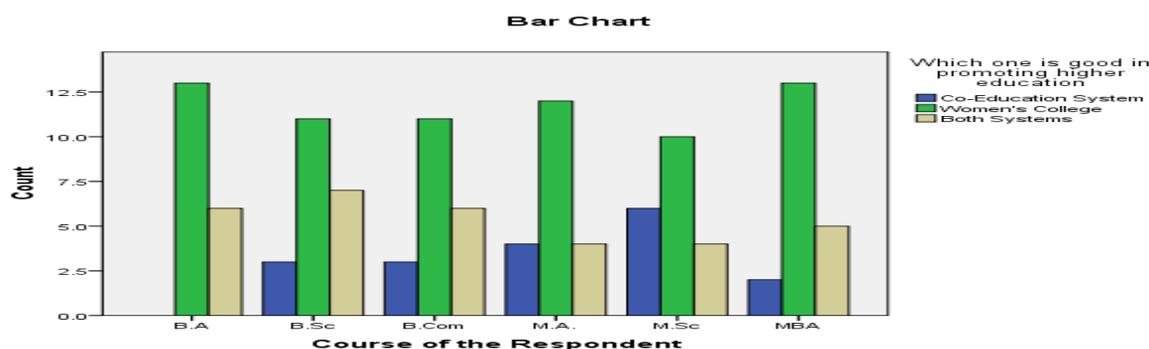
How do you feel to study in Women's College	If you satisfy what are the reasons						Total
	Not Applicable	Social Secure	Ragging Free	Promotes Higher Education	Good Environment	Healthy Competitive Environment	
Satisfied	0 .0%	31 36.0%	16 18.6%	22 25.6%	9 10.5%	8 9.3%	86 100.0%
Dissatisfied	34 100.0%	0 .0%	0 .0%	0 .0%	0 .0%	0 .0%	34 100.0%
Total	34 28.3%	31 25.8%	16 13.3%	22 18.3%	9 7.5%	8 6.7%	120 100.0%



The above table explains about their feeling in women's college and the reasons for their in women's college. Though 28% girls are dissatisfied with small reasons such as jealous and groupism, compare to co-education in women's college the competitive environment is low for few of them. The majority of 25.8% girls felt that they have social security in women's college, followed by 18.3% opinioned these institutions promoting higher education, without any tension to parents and relatives. Around 13% said that women colleges are ragging free, and 7.5% opinioned it provides good environment for education, and 6.7% pointed out in women's college there is a healthy and competitive environment for studies.

Table-4: Respondent’s Course and good one in promoting higher education

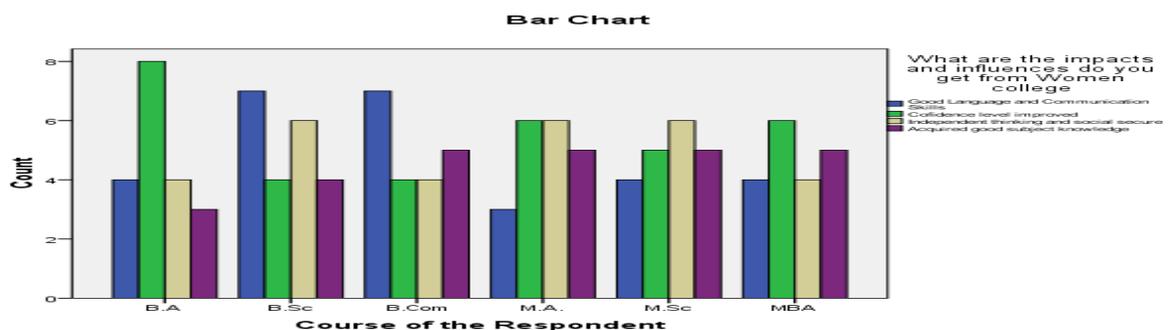
Course of the Respondent	Which one is good in promoting higher education			Total
	Co-Education System	Women's College	Both Systems	
B.A	0 .0%	13 68.4%	6 31.6%	19 100.0%
B.Sc	3 14.3%	11 52.4%	7 33.3%	21 100.0%
B.Com	3 15.0%	11 55.0%	6 30.0%	20 100.0%
M.A.	4 20.0%	12 60.0%	4 20.0%	20 100.0%
M.Sc	6 30.0%	10 50.0%	4 20.0%	20 100.0%
MBA	2 10.0%	13 65.0%	5 25.0%	20 100.0%
Total	18 15.0%	70 58.3%	32 26.7%	120 100.0%



From the above table, the course of the respondent is examined which type of system is good in promoting higher education for women. Majority of the respondents of 58% opinioned women’s colleges are best in providing good and safe education for girls. The remaining percentage women responded both the systems have great contribution towards women education and to some extent the co-education also promotes girls education with 27% and 15% respectively.

Table -5: Respondent’s Course and the positive impacts

Course of the Respondent	What are the impacts and influences do you get from Women college				Total
	Good Language and Communication Skills	Confidence level improved	Independent thinking and social secure	Acquired good subject knowledge	
B.A	4 21.1%	8 42.1%	4 21.1%	3 15.8%	19 100.0%
B.Sc	7 33.3%	4 19.0%	6 28.6%	4 19.0%	21 100.0%
B.Com	7 35.0%	4 20.0%	4 20.0%	5 25.0%	20 100.0%
M.A.	3 15.0%	6 30.0%	6 30.0%	5 25.0%	20 100.0%
M.Sc	4 20.0%	5 25.0%	6 30.0%	5 25.0%	20 100.0%
MBA	4 21.1%	6 31.6%	4 21.1%	5 26.3%	19 100.0%
Total	29 24.4%	33 27.7%	30 25.2%	27 22.7%	119 100.0%

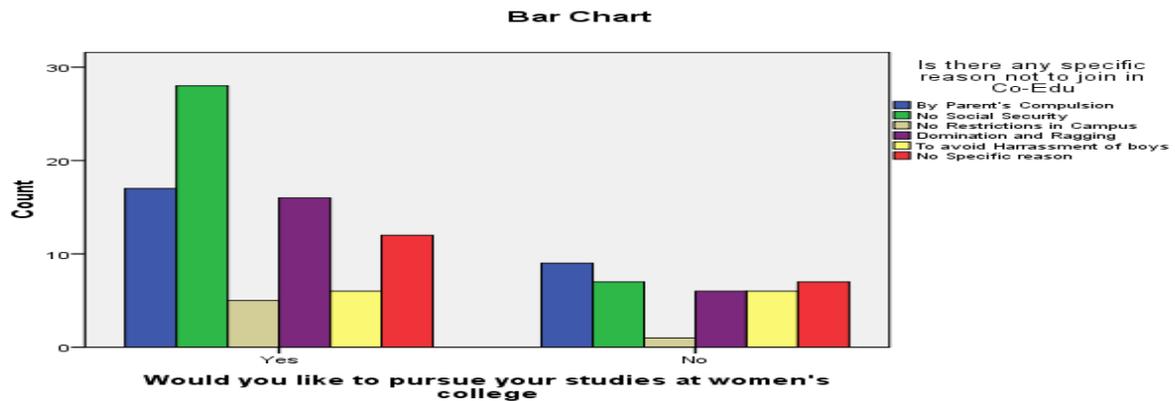


The above table examines the impacts and influences on the respondents based on their course of study. Majority of 28% mentioned their confidence level improved through women’s college, followed by 25% started independent thinking and overcome insecure feeling within them.

About 24% have stated there is a drastic change in their language and communication skills and lastly 23% feeling they have acquired good subject knowledge compare to co-education system.

Table -6: Pursuing Education at women's college and reasons for not to join in Co-Education

Would you like to pursue your studies at women's college	Is there any specific reason not to join in Co-Education						Total
	By Parent's Compulsion	No Social Security	No Restrictions in Campus	Domination and Ragging	To avoid Harassment of boys	No Specific reason	
Yes	17 20.2%	28 33.3%	5 6.0%	16 19.0%	6 7.1%	12 14.3%	84 100.0%
No	9 25.0%	7 19.4%	1 2.8%	6 16.7%	6 16.7%	7 19.4%	36 100.0%
Total	26 21.7%	35 29.2%	6 5.0%	22 18.3%	12 10.0%	19 15.8%	120 100.0%



The above table explains about the desire to continue their studies in women’s college and they have their own reasons regarding not to join in co-education system. Out of 120 respondents 84 girls are in favor to continue their studies in women’s college. Among these 33% felt this is very secure, followed by 20% joined by the compulsion of parents, and 19% are afraid of domination and ragging in co-education. Around 14% they does not have any specific reason to not to join in co-education, and 6% felt in co-education there is no control and restriction on boys and girls. The girls of 25% who dislike continuing in women’s college are joined only by the parents’ compulsion.

Major Findings of the Study

- ❖ Majority of the respondents (68%) have joined due to their parents compulsion in women's college
- ❖ Around 55% are responded that they were afraid of ragging, eve-teasing and harassment from boys in co-education institutions
- ❖ About 34% have gave their reply to join in women's college that their elder sisters are studying in the same college
- ❖ Though women's college is safe and secure, around 17% would like to do their studies in co-education. They felt co-education gives more opportunities to interact either male or female faculty or boys also helpful in resolving the classroom doubts.
- ❖ The girls from women's college about 72% felt that female teachers are more accessible and friendly compare to male teachers, and they felt female teachers are good in teaching compare to male.
- ❖ Majority of 72% responded they did not face any problems from male faculty, but so far the remaining percentage of 19% have suggested it is not good to appoint male teachers in women's college and around 9% have emphasized that male teachers are feeling shy to interact with them and behave has introvert.
- ❖ One important observation has made by the researchers that more girls from business community family are pursuing only B.Com and MBA, so that it will be helpful after their marriage also.
- ❖ Government employ and private employ daughters are very high in professional courses compare to rural background girls.
- ❖ The urban background girls have high incidence in all the courses, followed by rural and lastly metro background girls. The metro background girls are good in English and in communication so that they are opting jobs immediately after their graduation in various fields such as BPOs, in star hotel as receptionists and teaching in private schools.
- ❖ Final observation about the positive impacts and influences the girls acquired from women's college as their confidence level improved along with language and communication skills, they think independently, and they does not have insecure anywhere either in work or in society.

Conclusion

This paper examined the trends in women education in the state of Andhra Pradesh, the investments on education and infrastructural facilities supports and promotes women higher education through women's college. The study revealed that there had been significant progress in the performance of women in women educational institutions compare to co-education system of education revealed from female literacy levels and its change over time. It was also observed that the gaps between rural and urban female literacy rates are narrowing down.

To explore the influence of certain variables of interest such as advantages, social security, and parental insecureness, positive impacts and influences acquired from women's collegiate education. Regarding co-education, there are very serious issues such as ragging, comments; eve-teasing and violence against girls are also promoting the girls to join in women's college. It was observed that rural girls are performing better than urban girls in outcome aspects such as in pass percentage and class top mark etc. acts as a push factors for women's education rather than as an obstacle to women's education. The significant influence of urbanization on women's education implied that urbanization had been playing a beneficial role in the attainment of women's education through learning new skills such as language and communication, how to talk, how to behave and socialize themselves according to the situation and circumstances. At the same time, the drop-out rate had a negative effect on women's education. It revealed that that reduction of girl's drop-out rates is necessary for achieving women's education. The initiatives of the government through investment and infrastructure in developing education in India were developed by establishing and increasing the women's colleges in India. With regard to facilities in higher education, it had improved significantly, but a lot more need to be done.

In sum, the study revealed that there have been concerted efforts to encourage girls to attend higher education through women's colleges, which would lead to higher literacy in future. The study also revealed that there are several infrastructural barriers to women education in India which needs to be focused. The study calls for focused approach towards increasing women centered educational institutions so as the women education is essential to develop herself and society at large.

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Routledge Handbook of South Asian Criminology, K. Jaishankar (Eds.), *New York & London: Routledge Taylor and Francis Group, 2020, pp. 398, ISBN: 978-1-4822-4822-6045-8 (hb), £190*

South Asia, moving rapidly with its population and economic growth, leads to changes in behaviors of Individuals, Groups, Society as well as its Institutions. Thus, the behaviors, including that of criminality and victimization had also transformed. This calls for a new understanding of Criminology, which is much influenced by both cultural transition and social changes. Thus, this book is important for professional social workers and social work educators as there are greater intersections between social work practices and criminology in the five parts that this book is being divided such as: (a) crime and criminal justice process; (b) Historical perspectives of crime and Justice; (c) Politics of Crime and Justice; (d) Crime and Justice Policy; and (e) Victims and Victimology.

This book is intended as a key resource book of South Asian criminology, working in the area of criminal justice social work, criminology, Victimology, especially focusing on the eight south Asian countries, imbibing its local uniqueness and reflecting the larger connections across these eight south Asian countries in the areas of criminal justice and its functioning. Prof. K Jaishankar, had very eloquently selected the contributors from not only the eight south Asian countries, but also from USA, UK and Australia, that enriches with comparative understanding beyond the South Asian Criminology and further connecting the dots to the larger knowledge criminal justice and its practices across the globe.

Part I, Crime and Criminal Justice at the chapter one, discusses about the criminal justice system of Afghanistan and brings forth the idea that there is a need for coordination between state and non-state actors for effective delivery of justice. Chapter 2 discussed the issues of criminal justice system of Bangladesh with specific focuses on the police, courts and corrections. Some of the issues that had been brought out in this chapter by the author are, dissatisfaction of the public with the law enforcement agency, overcrowding in prisons, increasing incidences of domestic violence and judicial interventions. Chapter 3, discussed about Bhutanese criminal justice system and crime that are being reported there, that includes drug abuse and sex trafficking. The author points out numerous initiatives taken by the government to check corruption in the criminal justice system. Chapter 4 discusses the evolution

of criminal justice system in India, that includes the ancient, medieval and modern India. Even though there are few reforms that are initiated, in the context of criminal justice system in India, broadly it the colonial baggage's are still there, that needed to be re-invented and transformed. The author also mentions that there is a gradual shift in the nature of punishment from the earlier colonial retributive type to reformative and rehabilitative. Chapter 5 talks about the criminal justice system and crime situation of Maldives. Some of the most reported crimes at Maldives are illicit drug use and domestic violence. The author also discussed about the restructuring of criminal offences and punishments, initiatives for the creation of Maldives Police Service, reforms at the prisons, courts. Chapter 6 discussed about the criminal justice system of Nepal, with focus on policing, accountability, issues of trial process and legal aid. Chapter 7 discusses the criminal justice system of Pakistan and the issues related to policing, judiciary, crime situation and prisons. The author points out the concerns of gender disparity and inadequacy of modernization of the criminal justice system. Chapter 8 discusses on the criminal justice system of Sri Lanka, including its constitution and relevant statutes that controls the law enforcement agencies. The author points out at the recognition of rights guaranteed by the Sri Lankan constitution for the accused. It also discussed about the prison management system of Sri Lanka.

Part II discusses the Historical Perspectives of Crime and Justice with specific references to Manusmriti, witchcraft accusation and victimization in Nepal, contribution of Tamil literature (Thirukkural and Silappadikaram) into Justice and Administration of Tamil society, genesis of the Goondas Act at Calcutta, size and scope of opium production in three south Asian nations (India, Pakistan and Afghanistan) and linked up with criminological perspectives such as situational crime prevention and routine activity approach. Part III discusses the Politics of Crime and Justice in South Asian countries, with focus on terrorism and trafficking. Part IV discusses on south Asia and its regional cooperation, responses to human trafficking, rights of transgender, transnational justice process in Bangladesh, situational analysis in Nirbhaya incidence and Juvenile Justice policies in India as well as secondary victimization of Rohingyas in Myanmar and Bangladesh.

This book throws an overview of criminal justice system and it's functioning as well as the crime and Justice across South Asia countries, especially for those researchers and students interested to engage with criminal justice social work

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अलख को लखता लोक

प्रमोद कुमार तिवारी

गोरख भारतवर्ष के दुर्लभ चरित्र हैं। यह बात थोड़ी चकित करती है कि गोरख की भूमिका जितनी बड़ी है उसके अनुपात में उनकी चर्चा बहुत कम हुई है। गोरख लोगों को जगाते रहे, बहुत सारे रचनाकारों के लिए उन्होंने जमीन तैयार की, परन्तु गुमनामी की खाई में खोते चले गए। वे एक पंथ और मठ तक सीमित हो गए। यही नहीं 'गोरखधंधा' शब्द को घालमेल, भ्रष्टाचार आदि के अर्थ में प्रयुक्त कर उनकी अवमानना भी की गयी। ऐसा क्यों हुआ?

किसी भी बड़े चरित्र को समझने के दो रास्ते हैं—एक, जो शास्त्र से होकर गुजरता है और दूसरा लोक से होकर। शास्त्र और लोक में बड़ा फर्क यह है कि शास्त्र किसी चरित्र को स्थिरता देता है, वहीं लोक खास तरह की तरलता देता है। शास्त्र प्रामाणिक होता है इसलिए उसमें वर्णित चरित्र को बार-बार दोहराया जाता है, उसकी समृद्ध परंपरा बन जाती है, उसे सम्मान मिलता है। लोक अपने किस्से कहानियों और किंवदंतियों में, कर्मकांडों में व्यक्ति को सँजोता है, परन्तु समय के साथ, नये नायकों के आगमन के साथ चीजें धूमिल होती चली जाती हैं। शास्त्र चरित्र को व्यक्तिवाची बनाता है, लोक समूहवाची। शास्त्र एकआयामी बनाता है, लोक बहुआयामी। गोरख 40 ग्रंथों के रचयिता और विविध परंपराओं के निर्माता होकर भी मूलतः लोक के रह गए।

ऐतिहासिक दृष्टि से गोरखनाथ इतने भी पुराने नहीं हैं कि वे मिथकीय चरित्र में बदल जाएँ, उनसे काफी पुराने संतों, कवियों आदि का व्यक्तित्व स्थापित नजर आता है जबकि गोरख का व्यक्तित्व एक खास तरह की रहस्यमयता के आवरण में लिपटा दिखता है। वे ढेर सारी काल्पनिक कथाओं के नायक बनते हैं और ऐतिहासिक होते हुए भी उनके साथ एक पौराणिक चरित्र की तरह बर्ताव किया जाता है।

यह महत्वपूर्ण है कि जो व्यक्ति भारतीय चिंतन-धारा की दिशा बदल देता है, जो



Safe, efficient, and economically beneficial remediation of arsenic-contaminated soil: possible strategies for increasing arsenic tolerance and accumulation in non-edible economically important native plants

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Abstract

Anthropogenic activities, geological processes, and biogenic sources have led to the enhanced concentration of arsenic (As), a toxic metalloid in water and soil. Non-edible, economically important plants can be employed for safe As phytoremediation in addition to generating extra income. However, these plants may get affected by stressful local environmental conditions. Native plant species are adapted to local environmental conditions and hence overcome this problem. Native non-edible economic plant species which show high As tolerance and accumulation are promising candidate for safe, efficient, and economically beneficial phytoremediation of As-contaminated sites. The current review discusses the potential of native economic plant species that can be used in As phytoremediation programme. However, since their phytoremediation potential is moderate, possible strategies for increasing As tolerance and accumulation, especially genetic modification, have been discussed in detail. Knowledge gained from the review can be used for the development of As tolerance and accumulation in non-edible economic native plants.

Keywords Hyperaccumulation · Phytoremediation · Genetic manipulation · Non-edible plants · Native plant species

Introduction

Arsenic (As), a redox-active metalloid, finds its way to soil and water through geogenic, biogenic, and anthropogenic sources (Fig. 1). Rapid industrialization and globalization have resulted in uncontrolled increase of As contamination. Over past centuries, extensive studies have been done on the hazardous effect of As and successfully grabbed the attention of scientific communities and environmental protection agencies. Owing to its toxic nature, International Agency of Research on Cancer (IARC) has classified it as class I category of carcinogen (Cohen et al. 2019) and also ranked as first

among the most hazardous substance list (ATSDR 2019). Consumption of As-contaminated water and plants has resulted in increased health issues of humans and animals. Furthermore, plant establishment mostly in As-polluted land gets seriously affected (Sharma et al. 2018). To overcome these problems, numerous studies have been conducted to acquire knowledge regarding arsenic speciation, uptake mechanism of As in plant parts, their toxic behaviour, and ultimately cope up and tolerance processes in plants (Meharg and Hartley-Whitaker 2002; Srivastava et al. 2007, 2010; Rai et al. 2011; Tripathi et al. 2012; Sharma et al. 2014; Kumar et al. 2019; Singh et al. 2019a; Singh et al. 2020; Singh et al. 2021).

In recent years, the usefulness of plants in the removal of As from soil and revegetation of As-contaminated land has been recognized. Remediation and utilization of contaminated lands using plants provide economic, social, and environmental solution. Phytoremediation is considered eco-friendly and cost-effective over conventional methods. Therefore, research has been directed towards the use of plants to remediate and revegetate As-contaminated lands. Arsenic tolerance and phytoremediation efficiency of plants can be enhanced by

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शिकार बड़ी संख्या में कला-साहित्य-संस्कृति के मूर्धन्य हुए हैं। दिल्ली के सादतपुर में किसी ऋषि की तरह रहते 87 वर्षीय कवि, संपादक, आठ अनुवादक और जीवनी-लेखक विष्णुचंद्र शर्मा भी इसकी चपेट में आ गये और 2 नवम्बर 2020 को उनका देहावसान हो गया।

प्रमोद कुमार दिवारी ने विष्णुचंद्र शर्मा को याद करते हुए बड़ी ही ऊष्मा और आत्मीयता के साथ यह स्मरण-आलेख लिखा है, उनकी महत्ता का आकलन किया गया है।

प्रस्तुत है:

विष्णुचंद्र शर्मा वह एक अकेला कर्मशील प्रमोद कुमार दिवारी

और विष्णु जी चले गए, उसी ठसक के साथ, जिसमें उनका होना निहित था; उसी सादगी और नामालूमपन के साथ जो आखिरी जन की ा हुआ करती है; उन्हीं शतों के साथ जिसके बगैर उनकी कल्पना नहीं की जा सकती थी। 2 नवंबर की दोपहर में उनके पड़ोसी और बेहतरीन क. महेश दत्तजी का संदेश मिला कि विष्णुजी को दिल्ली के सेंट स्टीफेन अस्पताल लेकर जा रहे हैं, शायद वे कोविड संक्रमित हैं और फिर २ देर बाद ही दूसरा संदेश मिला कि नहीं रहे।

हिन्दी के युवा अध्येताओं की तरह बहुत लोप पूछेंगे कौन विष्णु जी? वही विष्णु जी, जिन्होंने अपने जीवन के घनघोर मेहनत भरे 80 वर्ष भाषा और साहित्य को दिये थे; वही विष्णु जी जिन्होंने मुक्तिबोध, राहुल सांकृत्यायन और काजी नजरूल इस्लाम की जीवनी लिखने के लिए जुड़े इलाकों से सामग्री पाने और उनके भावबोध के बनने की प्रक्रिया को समझने के लिए अपने देर सारे खूबसूरत वर्ष खपा दिए थे; वही वि. जिनके नाना रामनारायण मिश्र नागरी प्रचारिणी सभा के संस्थापकों में से थे, और जिनके पिता कांग्रेस के इतने बड़े नेताओं में से थे कि जवाहर नेहरू अपने भतीजे विष्णु के कहने पर कॉलेज के कार्यक्रमें में न केवल पहुंच जाते थे बल्कि अव्यवस्था होने पर खुद ही भीड़ को संभालते वही विष्णु जी जिन्होंने विरासत के नाम पर एक जित पायी थी और साहित्य के लिए अलग ही तरह का समर्पण पाया था जिन्होंने नौकरी से दूसरी तमाम सुविधाओं को लेने से इसलिए इनकार कर दिया था कि भरे पिता ने आज्ञादी की लड़ाई किसी प्रतिदान या बदले के लिए नह थी; कि उन सुविधाओं को स्वीकार करना पिता की परंपरा को शर्मिंदा करना होगा; वही विष्णु जी जो दिल्ली पहुंचने वाले हिंदी के बहुत श्रुः साहित्यकारों में से थे परंतु जो दिल्ली के कभी ना हो सके; भारत के केंद्र दिल्ली में रहकर भी वे सादतपुर नामक हाशिये के नागरिक रहे दिल्ली मुहावरे वाले अर्थ में हमेशा दूर ही रही।

कोई चाहे तो पूछ सकता है कि जो जीवन उन्होंने जिया वह उनका वचन था, इस बात पर इतनी शिकायत क्यों? सराहना से ज्यादा नाराज्ज लेने वाले विष्णु जी पर लोगों ने स्याही नहीं खर्च की तो क्या हो गया? पूरा जीवन सिर्फ साहित्य को समर्पित करने वाले विष्णु जी प्रक साहित्यकारों आदि से खुद ही उलझते रहे तो यह होना स्वाभाविक ही था; आप ही बताइए भला ऐसा भी कोई होता है कि अपने ही सम्मान र में, सम्मानित करने वाले लोगों को जम के सुना दे (लहक सम्मान), व्यावहारिकता भी कोई चीज होती है कि नहीं?

सब में दिनों दिन हम उस ओर बढ़ रहे हैं जब हमें साहित्यकारों और सब्जे लोगों की बजाय कुछ भैजनों की जरूरत रह जायगी; जो सब व करना जानते होंगे, जो लोगों को साधना जानते होंगे और अनेक स्तरों पर विपरीत ध्रुवों में भी जैसे जैसे संतुलन खिलना जानते होंगे; विष्णुः बिल्कुल नहीं थे किसी भी बात को गलत महसूस करने पर तड़ाक से वहीं हिसाब बराबर कर देने वाले और फिर अपनी धुन में रम जाने वाले

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Scholars' Perceptions and Practices on Research Data Management in the Universities of Gujarat: A Survey

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A B S T R A C T

Research Data and its management process is a foundation practice of scholars. The research data included collected raw data from the selected samples for the research as well as the output of research in form of suggestions and analysis. Therefore to manage and preserve the raw data of research, one electronic platform would be needed that takes good care of data as well as enabled to serve it as per the requisites of the scholars during the research. Research Data Management, is a platform to conscious in taking care, authenticity, preservation and services of it. Collected Data is the backbone of any research. So it's much needed to organize, store and preserve the collected data during research in a proper way. Research Data Management (RDM) is that approach which overcomes all the obstacles of data collection and proper application. When data is collected in organized form then it's very easy to apply any statistical tools and techniques. Institutes can easily archive them so it helps the prospective researchers to use them in the future. It increases the usability and longevity of the data. In this digital era, data generation is exponential. To organize this huge amount of data there should be an effective management plane. In this study researcher uses the survey method of data collection through the questionnaire. The questionnaire has been filled by research scholars of the universities of Gujarat. After analyzing the responses received, get a pattern of scholars' perceptions and practices. The core objective of this study is to make scholars aware of the use of RDM and its effect on the ease of doing research. The limitation of this study only conducted on only research scholars of universities of Gujarat. Research scholars of the total of 25 universities of Gujarat i.e. state and central level are taken as a sample. This helps know the actual status of awareness and practices of research data management among research scholars. To find out, the areas that need to improve. By knowing the actual problem institute or RDM planner can plan the solution efficiently. Well structured strategy or resources like software and institutional repositories helps universities to improve Research Data Management (RDM). RDM services influence the research level. Good research always adds gems to the institution.

Keywords: Research Data Management, Research Data, Collected Data, Data Archiving, Data Preservation

Single-Step Fabrication of Core–Shell Microgels for the Controlled Release of rhBMP-2 and Simvastatin to Induce Osteogenesis

Lalit Kumar Meena, Hilal Ahmad Rather, and Rajesh Vasita*

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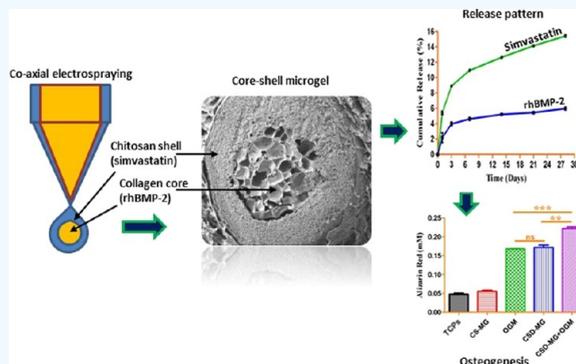
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ABSTRACT: Dual delivery of bioactive molecules (drugs and growth factors) has been attempted to enhance multiple processes during tissue regeneration. For bone tissue engineering, many attempts have been made to enhance osteogenesis coupled angiogenesis, which plays a major role during the bone regeneration process. In this study, core–shell microgels were fabricated for controlled release of recombinant human bone morphogenetic protein 2 (rhBMP-2) and simvastatin from the core and shell, respectively. The microgels were formed with a discrete core and shell structure. The Fourier transform infrared analysis demonstrated the composition of microgel, whereas swelling behavior demonstrated its rapid swelling property. Thermal properties demonstrated the ionic gelation in microgels, which minimizes the thermal degradation of polymers. The degradation study demonstrates that the core–shell structure of microgels was intact until 49 days under physiological conditions. The release profile demonstrates the sequential and controlled release of rhBMP-2 from the core and simvastatin from the shell of the microgels, respectively. The bioactivity of rhBMP-2 and simvastatin released from microgels was preserved as indicated by the alkaline phosphatase (ALP) activity assay. The cell proliferation of mouse preosteoblast (MC3T3-E1) cells and the live–dead staining assay demonstrated cytocompatibility of the microgels. Scanning electron microscopy images demonstrate that the microgels support adhesion of cells on the surface and promote extracellular matrix (ECM) production. The osteogenic differentiation of MC3T3-E1 cells demonstrated the synergistic effect of drugs and growth factors up to 21 days. The controlled and sustained release of simvastatin and rhBMP-2 induced higher mRNA and protein expressions of RUNX2, osteocalcin, and VEGF. The overall results demonstrate the effect of controlled release of rhBMP-2 and simvastatin from core–shell microgels to promote osteogenesis and angiogenesis.

KEYWORDS: core–shell microgels, chitosan, collagen, rhBMP-2, simvastatin, dual-drug delivery, osteogenesis



1. INTRODUCTION

Bone is the second highest transplanted tissue globally with over 2 million surgeries annually. Although bone has innate potential to repair the defects, however, when the size of defect is beyond the innate potential of self-healing, then the conventional surgical bone grafting methods are most preferred. However, grafting has major limitations such as death of autologous tissue, higher cost, donor site morbidity, and immune rejection.^{1,2} Therefore, bone tissue engineering remains a matter of hope and has grown substantially in the last few years.^{3–6} Because bone fracture healing is a complex event of temporally regulated phases, regulating angiogenesis and osteogenesis in a coupled manner using scaffolding technology is attempted to improve the scope of bone regeneration.^{5,6} Hence, delivery of dual factors that could enhance angiogenesis and osteogenesis has been frequently utilized. During the fracture healing process, the level of BMP-2 protein is maintained for approximately 4 weeks.⁷ BMP-2 has higher osteoinductive potential; however, its shorter systemic

half-life (only 6–7 min) and inadequate temporal presentation at defect sites remain a major limitation.⁸ Therefore, sustained release of BMP-2 from carriers over a long time period may be an effective approach to boost osteogenic differentiation. Moreover, early-stage angiogenesis is also equally required in critical size defects for successful bone regeneration. To mimic the presentation of angiogenic and osteogenic signaling molecules at the fracture site, various hybrid scaffolds have been reported to deliver the vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) at an early stage, whereas bone morphogenetic proteins (BMPs) at a later stage.^{9,10} An alternative drug molecule with the equal potential

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of neovascularization would be desirable for fabrication and delivery via scaffolding. Simvastatin, an FDA-approved drug traditionally prescribed to reduce blood cholesterol,¹¹ is reported to enhance angiogenesis by augmenting VEGF expression in osteoblastic cells.¹² It is also reported to enhance osteogenesis by increasing the BMP-2 levels in osteoblasts both in vitro and in vivo.^{13,14} Sequential delivery of osteogenic and angiogenic factors would be desirable and can be achieved via a dual-drug delivering scaffold. In one study, a core-shell hydrogel scaffold was designed with BMP-2 in the core and cobalt ions (as an angiogenic factor) in the shell, where cobalt was released within 7 days while the release of BMP-2 continued for 3–4 weeks.¹⁵ Furthermore, local controlled release of simvastatin and platelet-derived growth factor from core-shell microspheres promotes bone regeneration.¹⁶ A recent study reported by our research group demonstrated that the dual delivery of simvastatin along with dexamethasone from the bioinspired scaffold has promoted early osteogenesis and angiogenesis.¹⁷ Therefore, biomaterial-based core-shell microgels were fabricated in the present study in order to control the release of simvastatin and BMP-2 in a sequential manner. Use of biomaterials is very well known for regeneration of bone defects because of their biodegradability and biocompatibility.¹⁸ Nowadays, many different bone substituting materials either derived from biological products or polymer-based substitutes are chosen depending on the purpose of their function.¹⁹

In the present study, we have fabricated core-shell microgels using collagen as the candidate material for the core and chitosan for the shell, which act as BMP-2 and simvastatin carriers, respectively. Collagen is the most abundant protein constituent of the bone extracellular matrix (ECM). It is extensively used in BMP-2 delivery because of its binding affinity,²⁰ which may be crucial for sustained release of BMP-2. Known for the property of ionic gelation, chitosan is extensively used for scaffolding.²¹ Both collagen and chitosan are biocompatible and biodegradable, which provides an additional advantage for their use in bone tissue engineering.^{17,22,23} The rapid gelation of chitosan allows the encapsulation of collagen during the process of coaxial electrospraying. This bioactive core-shell microgel was investigated for its release, degradation, and osteogenic and angiogenic properties.

2. MATERIALS AND METHODS

2.1. Materials. Chitosan powder (medium molecular weight), sodium tripolyphosphate (STPP), and simvastatin were purchased from Sigma-Aldrich, USA. Collagen type I was extracted from rat tails. The recombinant human bone morphogenetic protein 2 (rhBMP-2) and Quantikine ELISA Kit were purchased from R&D Systems, USA. Acetic acid was obtained from SRL, India. α -MEM and fetal bovine serum were purchased from HiMedia, India, and Gibco, USA, respectively.

2.2. Methods. **2.2.1. Microgel Fabrication.** Chitosan-collagen core-shell microgels (CS-MG) were fabricated using electrospraying technique with the ionotropic gelation method. Chitosan (3% (w/v)) and collagen type I (0.5% (w/v)) solutions were prepared in glacial acetic acid. The solutions were filled in two different syringes and set on syringe pumps, which were connected to a coaxial module setup. The flow rate for core and shell solutions was adjusted to 30 μ L/min using a 26G needle and 100 μ L/min using an 18G needle, respectively. The potential difference between the electrodes was kept at 10 kV at a distance of 15 cm. The microgels were collected in 5% (w/v) STPP solution, rinsed with water and freeze-dried for further use. Furthermore, loaded core-shell microgels (CSD-MG)

were fabricated by adding rhBMP-2 (1 μ g/mL) into collagen and simvastatin (446 μ g/mL) into chitosan solutions, respectively. The control microgels (C-MG) were fabricated by electrospraying chitosan (3%) using an 18G needle.

2.2.2. Physical Characterization of CS-MG. The surface morphology and internal microstructure of core-shell microgels were analyzed using scanning electron microscopy (SEM) (EVO 18; Zeiss, Germany). The working distance was 10 mm with an acceleration voltage of 5–10 kV. For internal microstructure analysis, microgels were sliced in two equal halves. The chemical composition of microgel was characterized by attenuated total reflection Fourier transform infrared (ATR FT-IR) spectroscopy (Nicolet iS5; Thermo Scientific, USA). All samples were scanned in the range of 4000 to 600 cm^{-1} with a resolution of 4 cm^{-1} at room temperature.

Thermogravimetric analysis (TGA) was performed using a thermogravimetric analyzer (EXSTAR TG/DTA7300, USA) as reported previously.¹⁷ Briefly, 5 mg of each sample was analyzed with a heating rate of 10 $^{\circ}\text{C min}^{-1}$ under a nitrogen (N_2) atmosphere. The denaturing temperature was increased from 48 to 800 $^{\circ}\text{C}$.

2.2.3. In Vitro Swelling Study. The gravimetric method was utilized to study the swelling behavior of microgels in 1X phosphate-buffered saline (PBS) and deionized water as reported.²⁴ The microgels ($n = 3$) were weighed for dry weight (W_d) and immersed in PBS and deionized water separately at 37 $^{\circ}\text{C}$. After the predetermined time points, the microgels were removed to measure the swollen weight (W_s). To calculate the swelling ratio, the following equation was used:

$$\text{swelling ratio} = \frac{(W_s - W_d)}{W_d} \quad (1)$$

2.2.4. In Vitro Degradation Study. In vitro degradation of microgels was performed in the presence of lysozyme enzyme (1.5 μ g/mL in PBS). Briefly, dry weight (W_0) of microgels ($n = 3$) was measured and immersed in a 2 mL PBS medium. The samples were incubated in PBS with 60 rpm continuous agitation at 37 $^{\circ}\text{C}$. At the predetermined time points, microgels were removed from PBS, washed with water, freeze-dried, and weighed (W_t). Then, the degree of degradation (%) was calculated using the difference between the dry weight before and after incubation using the following equation:

$$\text{degree of degradation (DD)\%} = \frac{W_0 - W_t}{W_0} \times 100 \quad (2)$$

The change in the pH of degradation media was measured at the predetermined time points. The change in morphology and microstructure during degradation was observed using SEM analysis.

2.2.5. Encapsulation and Release of rhBMP-2 and Simvastatin. Immediately after electrospraying of CSD-MG, the STPP solution was filtered out and centrifuged at 1000 rpm (at 4 $^{\circ}\text{C}$) for 5 min. The supernatant was analyzed for nonencapsulated rhBMP-2 using a commercially available Quantikine ELISA Kit following the manufacturer's protocol, whereas free simvastatin was analyzed using a UV-vis spectrophotometer (Hitachi U-2900) at 238 nm. The encapsulation efficiency and percent yield were calculated using the following equations:²⁵

$$\begin{aligned} \text{encapsulation efficiency (\%)} \\ = \frac{\text{mass of total drug} - \text{mass of free drug in STPP}}{\text{mass of total drug}} \times 100 \end{aligned} \quad (3)$$

$$\begin{aligned} \text{yield (\%)} \\ = \frac{\text{dry wt of total core-shell microgels}}{\text{wt of used simva + rhBMP-2 + chitosan + collagen + STPP}} \times 100 \end{aligned} \quad (4)$$

For the release study, approximately 40 mg of CS-MG and CSD-MG ($n = 3$) were immersed in the 1.5 mL volume of PBS. The samples were incubated in a shaking water bath at 37 $^{\circ}\text{C}$. At the predetermined time points, an 800 μ L release medium was withdrawn

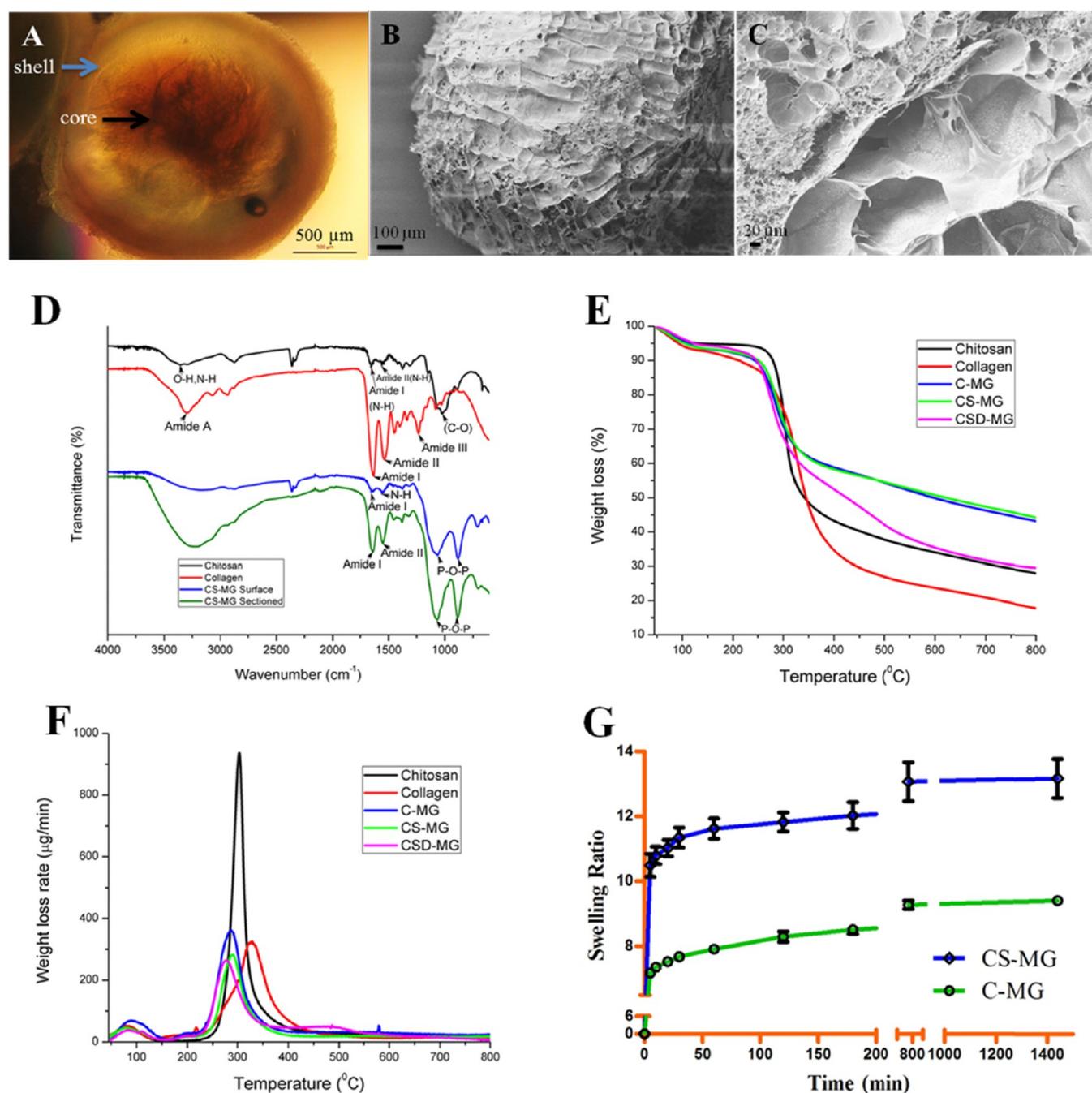


Figure 1. (A) Light microscopic image, scale bar = 500 μm . Scanning electron micrographs showing (B) surface (scale bar = 100 μm) and (C) internal morphology of a lyophilized core-shell microgel, scale bar = 20 μm . (D) FT-IR spectra of chitosan powder (black), collagen type I (red), core-shell microgel (blue), and sectioned core-shell microgel (green); (E) thermogravimetric analysis; and derivatives of thermal analysis curves of (F) weight loss of polymers and microgels and (G) swelling ratio of CS-MG and C-MG in 1X PBS at 37 $^{\circ}\text{C}$ ($n = 3$).

and the same volume of fresh PBS was added. Then, rhBMP-2 and simvastatin were quantified as mentioned above.

2.2.6. In Vitro Bioactivity Assessment of Released rhBMP-2 and Simvastatin. The bioactivity of released rhBMP-2 and simvastatin from CSD-MG was assessed using alkaline phosphatase (ALP) activity assay. Briefly, 3×10^4 mouse preosteoblast (MC3T3-E1) cells were seeded in each well of a 24-well plate. After 24 h, the cells were treated with releasates of day 3. The filtered released solution of rhBMP-2 and simvastatin was diluted with the α -MEM culture medium to a concentration of ~ 100 pg/mL and 346 ng/mL, respectively. An equal amount of free rhBMP-2 (~ 100 pg/mL) and simvastatin (346 ng/mL) alone, and in combination, was used as a positive control. The released medium of CS-MG was used as a

negative control. Cells in a normal expansion medium were also used as a control, and the medium was changed every alternate day. After 10 days, the medium was removed from the wells and washed with PBS. After that cells were lysed by adding 50 μL lysis buffer and subsequent freeze-thaw cycles. Then, the 200 μL substrate solution was added into each well and incubated in dark at 37 $^{\circ}\text{C}$ for 30 min. Then, the enzymatic reaction was terminated by adding the 50 μL ice-cooled 3 N NaOH solution. Afterwards, the 100 μL reaction mixture was taken in a 96-well plate and the absorbance was measured at 405 nm using a UV-Vis spectrophotometer.

2.2.7. Cytocompatibility Assessment. Cytocompatibility assays were performed using three different test procedures suggested by ISO 10993-5, 2009, for medical devices: First by indirect contact:

where the microgels were incubated on the MC3T3-E1 cell monolayer. Second by a leached medium: where the microgel-leached culture medium was used. Third by a direct contact test: where cells were grown on the surface of microgels.

For the first and second assay, 0.5×10^4 cells/well of a 96-well plate were seeded and grown for 24 h before treatment. The cell proliferation was measured using Alamar Blue assay, 24, 48, 72, and 96 h after treatment as reported by our group previously.²⁶ While for the third test, microgels were placed in a 96-well plate, precoated with agarose and soaked with α -MEM for 1 h. Afterward, 2.5×10^4 cells/well were seeded on the surface of microgels. The cell proliferation was measured at 1, 3, 7, 10, 14, and 21 days using Alamar Blue assay.

The cytocompatibility of microgels was also assessed using fluorescence-based Live/Dead staining (Molecular Probes; Invitrogen, USA) as reported previously.^{26,27} The morphology of cells on core-shell microgels was analyzed using SEM. The microgels were removed from the culture medium and washed with 1X PBS solution. The cells were fixed with 2% glutaraldehyde in sodium cacodylate buffer (0.1 M) for 1 h. After that the microgels were serially dehydrated using ethanol gradient. Then, the samples were processed as mentioned in section 2.2.2.

2.2.8. Alkaline Phosphatase Activity Assay. In order to assess the differentiation of MC3T3-E1 cells into osteoblasts, the expression of ALP as an early osteogenic marker was quantified; 3×10^4 MC3T3-E1 cells were seeded per well of a 24-well plate and grown for 21 days with CS-MG, CSD-MG, osteogenic media (OGM), and CSD-MG + OGM. Tissue culture plates (TCPs) were taken as cell culture controls. Afterward, the ALP activity assay was performed as mentioned in Section 2.2.5.

2.2.9. Calcium Quantification by Alizarin Red S (ARS) Staining. The mineralization in presence of microgels by differentiated MC3T3-E1 cells was quantified through ARS staining. After culturing of cells for 21 days, with and without microgels, media were removed and the cells were washed gently with PBS. The cells were fixed with 2% paraformaldehyde for 30 min, and ARS solution (40 mM, pH 4.1) was added to each well and incubated for another 30 min. After incubation, the cells were washed and imaged using a light microscope (Primovert; Zeiss, Germany). The cells were then air-dried, and the dye was extracted using 10% acetic acid overnight at room temperature. Afterward, the extracted dye was heated at 85 °C for 10 min, incubated on ice for 5 min, and centrifuged at 20,000g for 15 min. The supernatant was collected in fresh tubes, and the pH was adjusted to 4.1 using 10% ammonium hydroxide. Finally, the 200 μ L supernatant was transferred into a 96-well plate and the absorbance was measured at 405 nm using an ELISA plate reader.

2.2.10. von Kossa Staining. Calcium deposition by differentiated MC3T3-E1 cells was observed using von Kossa staining. After 21 days of cell culture, the cells were fixed with 10% formaldehyde for 30 min. After that formaldehyde was removed and washed using ddH₂O followed by incubation with the 5% silver nitrate solution in the ultraviolet light for 1 h. Then, the silver nitrate solution was removed and the cells were washed twice using ddH₂O to remove the unbound stain and observed using a light microscope.

2.2.11. Gene Expression by sqRT-PCR. For RNA isolation, 1.5×10^5 MC3T3-E1 cells were seeded in a 60 mm culture plate with and without microgels. After 21 days of cell culture, RNA extraction was carried out using the RNA isolation kit (NucleoSpin RNA; MACHEREY-NAGEL, Germany) according to the manufacturer's protocol. cDNA synthesis was performed using the cDNA synthesis kit (PrimeScript 1st strand cDNA synthesis kit; TAKARA) according to the manufacturer's protocol. The primers and polymerase chain reaction (PCR) conditions used for the study are mentioned in Table S1. The PCR products were analyzed using agarose gel electrophoresis, and the images were captured using a GelDoc imaging system (SYNGENE; G:BOX).

2.2.12. Immunocytochemistry. For immunocytochemistry, the samples were prepared as reported previously.^{17,22} Briefly, after 21 days, the samples were washed using PBS and fixed with paraformaldehyde. The cells were permeabilized using 0.5% Triton X followed by blocking (0.5% bovine serum albumin and 0.15%

glycine in 1X PBS) for 1 h. The cells were then incubated with the fluorochrome-conjugated primary antibody (with 1:200 dilution) overnight at 4 °C. Finally, the cells were incubated with DAPI (50 ng/mL) for 5 min. The images were observed and captured using a confocal microscope (LSM780; Zeiss, Germany).

2.2.13. Statistical Analysis. All the statistical analyses were performed using GraphPad Prism 5 software. All the experimental data obtained were statistically analyzed in triplicates unless otherwise mentioned. The statistically significant differences between two groups were determined with $P < 0.05$ by Student's t-test. All quantitative data are expressed as mean \pm standard error of mean.

3. RESULTS AND DISCUSSION

3.1. Morphological and Chemical Analysis of Core-Shell Microgels. The CS-MG were fabricated by a customized coaxial electrospinning process as demonstrated in the Supporting Information Figures S1–S3 and Table S2. The optimized concentration of polymers for a shell was 3% (w/v) chitosan, whereas for a core, it was 0.5% (w/v) collagen type I. The result demonstrated an intact and spherical microgel with the darker zone as collagen and the glassy zone as chitosan shell (Figure 1A). The reduced collagen concentration minimizes the viscosity of the entrapped collagen solution. Hence, the chitosan solution of the shell was able to bear the less viscous collagen force to the shell.

The SEM image analysis (Figure 1B) demonstrated the spherical shape of microgels with a rough surface. The roughness enhances the surface area of the microgel, which could be desirable for drug release and surface attachment of cells. Figure 1C shows the internal microstructure of sectioned core-shell microgels. The SEM images demonstrated the macroporous core structure of the microgel, which is alike cryogels. Since the microgel was freeze-dried as soon as it was fabricated, cross-linking in collagen occurred at a slower pace as the core temperature kept decreasing. Therefore, a cryogel-like microstructure was obtained. The structure of the shell was found to be highly dense due to greater concentration and cross-linking of chitosan. The difference in the rate of cross-linking between the chitosan shell and collagen core possibly results in micro/nanoporous structure formation. The small pores in the shell region could be helpful in controlled release of bioactive molecules. The microgel fabrication through the ionic gelation method in a mild environment could help protect the bioactivity of loaded bioactive molecules. Furthermore, there was no change in the microstructure of core-shell microgels observed after loading of rhBMP-2 and simvastatin. The C-MG were spherical with rough surface topography, whereas the internal structure was uniformly microporous and densely cross-linked without any discrete core-shell structure.

The CS-MG were characterized for its composition and cross-linking through FT-IR analysis (Figure 1D). The FT-IR spectrum of core-shell microgels shows appearance of duplet with peak shift for amide I at 1650 and 1635 cm^{-1} and amide II at 1557 cm^{-1} (toward lower wavenumber) with a reduced peak intensity due to electrostatic interactions between the protonated amino group of chitosan and the phosphoric group of STPP. It directly confirms participation of the protonated amino ($-\text{NH}_3^+$) group in a cross-linking reaction with the phosphate group of STPP, and these results were in agreement with previously reported studies.^{28–30} The broadening and flattening of the absorption peak of chitosan at 3357 cm^{-1} in the core-shell microgel spectrum confirms enhanced hydrogen bonding, and it was found to be consistent with previously

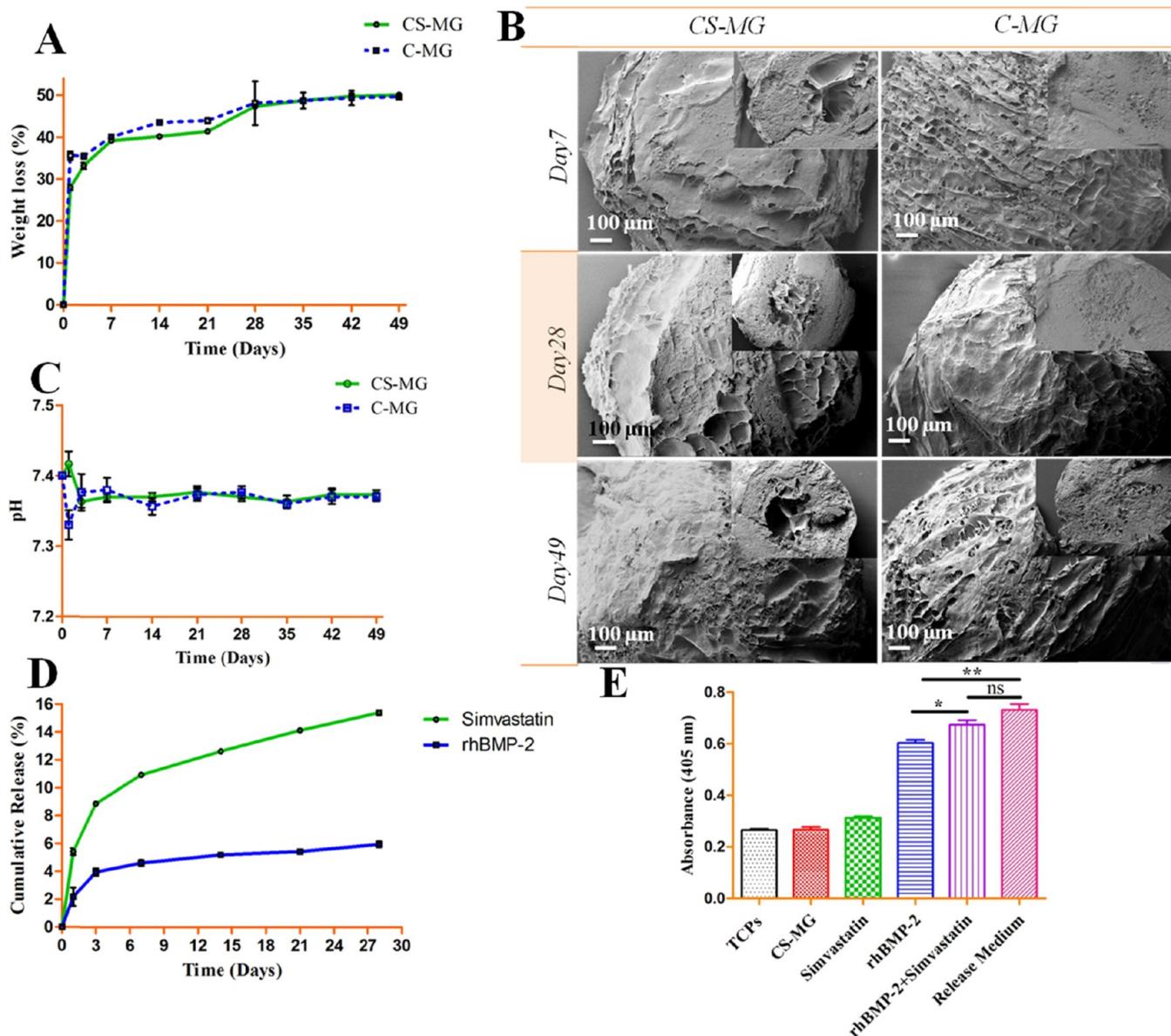


Figure 2. (A) In vitro degradation measured as weight loss in presence of lysozyme. (B) SEM images of degraded CS-MG and C-MG exhibit surface degradation; inset SEM images show the internal structure of degraded CS-MG and C-MG (scale bar = 100 μm). (C) Effect of the degraded byproducts of microgels in a medium pH. (D) In vitro cumulative release of rhBMP-2 and simvastatin from CSD-MG at different time points. (E) ALP activity of MC3T3-E1 cells cultured with growth media containing free simvastatin (346 ng/mL), rhBMP-2 (100 pg/mL), rhBMP-2 + simvastatin, and released rhBMP-2 + simvastatin.

reported results.²⁸ The appearance of new absorption peaks at 1067 and 883 cm^{-1} can be assigned to P–O–P asymmetric stretching, and it confirms the role of STPP in an ionic cross-linking reaction with the amine group of chitosan.^{31–34} The spectrum of sectioned CS-MG showed characteristic absorption peaks of collagen at 1640 and 1545 cm^{-1} for amide I and amide II, respectively, and it confirms the presence of collagen in the core. In conclusion, the results confirm ionic crosslinking of chitosan (shell) with STPP and collagen as core materials.

The thermal stability of the microgels was determined by TGA. Figure 1E shows pyrolytic patterns of chitosan, collagen, C-MG, CS-MG, and CSD-MG. Initial weight loss was observed in all samples from 5 to 9.42% at 48–200 $^{\circ}\text{C}$ (the Supporting Information Table S3) due to loss of absorbed and structural bound water molecules. The weight loss in the

second phase at 200–400 $^{\circ}\text{C}$ could be attributed to the thermal degradation of polymers. Chitosan and collagen exhibited 51.38 and 55.96% weight loss, respectively. This weight loss for chitosan could be associated with depolymerization and pyrolytic degradation of the polysaccharide structure.^{35,36} In collagen, the degradation could be associated with protein chain and peptide bond breakage.³⁷ The weight loss in C-MG, CS-MG, and CSD-MG was 33.38, 34.14, and 40.70%, respectively. These results indicate that ionic gelation in microgels minimize the thermal degradation of polymers. It may be possible due to STPP cross-linking of chitosan and stronger interactions between chitosan and collagen. In the third phase (400–800 $^{\circ}\text{C}$), all samples exhibited weight loss from 14.02 to 22.91%. Figure 1F shows the derivative TGA, representing the maximum weight loss at particular temperature. Chitosan and collagen demonstrate the maximum

degradation rate at 303.38 and 328.21 °C, respectively, whereas for C-MG and CS-MG, it was observed at 288.18 and 291.44 °C, respectively. The peak intensity of CS-MG was reduced compared with chitosan and collagen, which indicates a less thermal degradation rate and more thermal stability. The thermal stability is desirable for prolonged degradation and sustained release of bioactive molecules from the delivery device.¹⁷

The swelling behavior of CS-MG in a PBS medium was studied through swelling behavior analysis. Figure 1G shows that the initial swelling ratio of CS-MG and C-MG was recorded to be 10.48 and 7.17, respectively, after 5 min. The ratio increased to 11.82 and 8.29, respectively, after 2 h. The equilibrium swelling ratio after 24 h for CS-MG and C-MG was measured to be 13.17 and 9.41, respectively. The higher swelling ratio of CS-MG shows the potential of a less self-cross-linked collagen core to bind with more water molecules due to some free –OH groups. Furthermore, the macroporous structure facilitates accommodating a higher volume of PBS compared with C-MG. The controlled swelling of microgels would lead to controlled release of encapsulated bioactive molecules, which is desirable for controlled drug delivery applications.

3.2. In Vitro Degradation and Release Study of Core–Shell Microgels. In drug delivery devices, the controlled degradation is associated with the controlled drug release. The results (Figure 2A) demonstrated 35.55 and 28.02% weight loss after 24 h for C-MG and CS-MG, respectively. The initial weight loss could be anticipated due to release of uncross-linked polymeric chains after swelling, salt leaching, and degradation. However, the morphology and microstructure were not significantly changed (data not shown). After 7 days, the weight loss observed was 40.01 and 39.14% for C-MG and CS-MG, respectively. After 28 days, the weight loss was 48.12 and 47.36% for C-MG and CS-MG, respectively. After 49 days, the weight loss of C-MG and CS-MG was 49.60 and 50.12%, respectively. The average degradation rate for C-MG and CS-MG was 1.01 and 1.02% per day, respectively. CS-MG and C-MG exhibited a similar degradation rate because the outer shell in both is made up of STPP cross-linked chitosan. The constant and controlled degradation rate will be helpful in controlled drug release. The results exhibited the biodegradability of microgels, which is a prime requirement for any implantable drug delivery device.

Figure 2B shows the surface and the bulk structure of degraded microgels. At day 7, both microgels exhibited mild erosion and porous structures on the surface, whereas the bulk structure did not alter significantly. On day 28, formation of tiny fissures on the surface confirms rapid erosion compared with day 7. At day 49, the emergence of new minute pores and fissures on the surface indicates erosion-induced porosity on the microgel. Due to poor solubility in water, the shell polymer chitosan did not degrade rapidly in the initial days. The slow erosion of chitosan protects the leaching and degradation of the core collagen. Even after 49 days with 52.12% weight loss, the CS-MG had maintained its round shape and intactness of the internal microstructure. The controlled degradation of CS-MG while maintaining the structural intactness is a desirable feature for long-term controlled drug delivery applications. The results confirmed the biodegradability of microgels, which is required for bone regeneration applications. The effects of the degraded byproducts of core–shell microgels in a medium pH were also studied to assure nontoxicity of microgels as a dual

drug delivery device for bone regeneration (Figure 2C and Supporting Information Figure S5).

The encapsulation efficiency of core–shell microgels was 54.36% for rhBMP-2 and 62.60% for simvastatin (the Supporting Information Figure S6A). The *in vitro* release pattern of rhBMP-2 and simvastatin from core–shell microgels is shown in Figure 2D. The pattern shows controlled and sustained release of rhBMP-2 initially. At day 1, 3, and 7, rhBMP-2 was released as 2.18, 3.97, and 4.61%, respectively. The sustained release continued until day 28 with 5.97% release. The Supporting Information Figure S6B shows the released rhBMP-2 concentration at different time points. It demonstrates the release of rhBMP-2, which was obtained to be 2408.11, 1988.11, 721.44, 645.88, 268.11, and 580.33 pg/mL, at day 1, 3, 7, 14, 21, and 28, respectively. The released concentration of rhBMP-2 from CSD-MG at each time point was above the normal physiological concentrations of BMP-2 (18.8–21.9 pg/mL) at a bone defect site.^{38–42} However, the released concentration of rhBMP-2 was below the supra-physiological doses of BMPs (BMP-2 and BMP-7; 2.1 to 12 mg) used in the human clinical treatments.^{40,41} Simultaneously, the release pattern of simvastatin showed initial slow release with 5.41 and 8.89% release at day 1 and 3, respectively. At day 28, 15.42% was released, and the release pattern demonstrated controlled and sustained release over a longer period. The Supporting Information Figure S6C demonstrates the release concentration of simvastatin at day 1, which was recorded to be 10.84 μg/mL followed by a constant decrease for 28 days. In the entire study, the simvastatin concentration was maintained between 10.84 and 2.56 μg/mL. This released concentration of simvastatin was above its reported therapeutic concentration.⁴³

The core–shell microstructures have a definite role in the release pattern of rhBMP-2 and simvastatin. Previously, collagen has been reported as a carrier for rhBMP-2 delivery. However, it exhibits 80% release of BMP-2 within 6 days.^{44,45} The fast release of BMP-2 may cause its accumulation that could further lead to side effects such as ectopic bone formation and immunological reactions.⁴¹ Therefore, in the present study, rhBMP-2 was encapsulated in the core of the microgel. The encapsulated rhBMP-2 was released in a controlled and sustained manner for 28 days. Simvastatin was also released in a sustained manner for longer time. The compact shell structure did not allow the diffusion of drug immediately. A mild diffusion followed by surface erosion could be a potential reason for the sustained release of drug. The early diffusion of simvastatin will enhance the possibility for early angiogenesis. The release of rhBMP-2 controlled by a shell microstructure barrier may slow its diffusion, while simvastatin loaded in the shell also exhibits controlled release because of the cross-linked network of chitosan that controls swelling of microgels. The concentration of both BMP-2 and simvastatin release was above the therapeutic level and, therefore, can induce osteoblast differentiation and enhance the expression of angiogenesis-related markers.^{12,43,46} In summary, this study demonstrated the fabrication of core–shell microgels with potential to deliver two different bioactive therapeutic molecules with different release rates.

Since bioactivity maintenance of rhBMP-2 and simvastatin released from CSD-MG is crucial to achieve the biological effects, the ALP activity of MC3T3-E1 cells was assessed as their differentiation into osteoblasts is corroborated with high ALP activity.^{47,48} Figure 2E shows that the ALP activity of

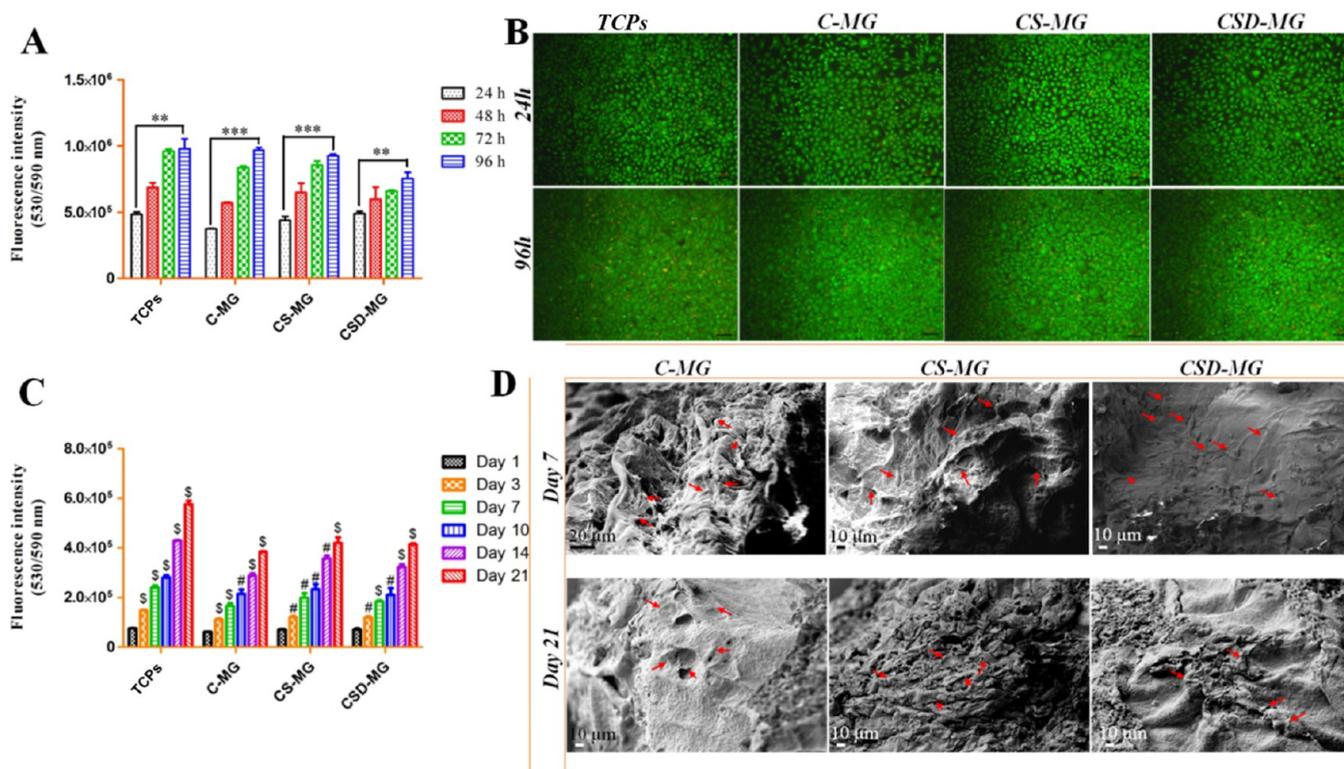


Figure 3. Cytocompatibility of microgels measured by the indirect method using (A) Alamar blue assay and (B) Live/Dead assay using MC3T3-E1 cells. Cytocompatibility analysis of microgels measured by the direct method using (C) Alamar blue assay ($\#p < 0.01$, $\$p < 0.001$) and (D) SEM, scale bar = 10 μm ; red arrows indicate adhered cells on the surface of microgels ($**p < 0.01$ and $***p < 0.001$).

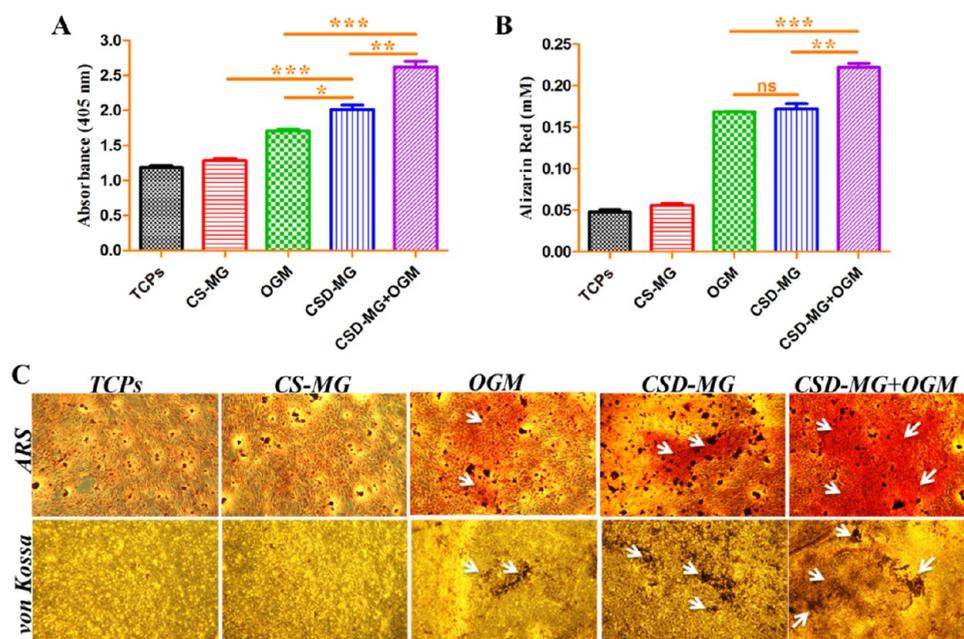


Figure 4. ALP activity of MC3T3-E1 cells after (A) 21 days of osteogenic differentiation ($*p < 0.05$, $**p < 0.005$, and $***p < 0.001$) and (B) ARS quantification ($**p < 0.005$, $***p < 0.0005$, and ns: not significant). (C) Upper panel representing the images for ARS staining and lower panel depicting von Kossa staining of MC3T3-E1 cells for TCPs, CS-MG, OGM, CSD-MG, and CSD-MG + OGM. White arrows denote mineral deposits. Images were captured at 10X magnification.

MC3T3-E1 cells increased 2.2-fold in free rhBMP-2-treated cells and 1.1-fold in simvastatin-treated cells, respectively, compared with the control group. The cells treated with free rhBMP-2 and simvastatin in combination demonstrated a 2.5-fold increase in the ALP activity, whereas in presence of a

release medium from CSD-MG, a 2.7-fold increase was observed. The difference in ALP activity between the release medium and free rhBMP-2 + simvastatin was statistically nonsignificant. The results further indicate that the release medium was equally able to induce the osteogenic differ-

entiation of MC3T3-E1 as free rhBMP-2 + simvastatin. The results also indicate that the electrospraying fabrication technique along with the ionic gelation method was a compatible and mild process to encapsulate the stress-labile growth factor and drug molecules.

3.3. In Vitro Cytocompatibility and Cell Proliferation Study. The cytocompatibility of microgels was assessed using the indirect method, leached media method, and direct method. Figure 3A shows the result of cell proliferation by the indirect method. A significant increase in fluorescence intensity was observed from 24 to 96 h for C-MG, CS-MG ($p < 0.001$), and CSD-MG ($p < 0.01$). The increase in fluorescence intensity indicates the increasing number of metabolically active cells. All groups showed a similar trend of cell proliferation as shown by TCPs (no microgels). The result confirms cytocompatibility of core-shell microgels. The viability of cells was also assessed by the Live/Dead assay (Figure 3B). In C-MG, CS-MG, and CSD-MG, cells exhibited green fluorescence that indicated live cells. All groups exhibited similar viability of cells. Although very few red fluorescence cells were observed after 96 h in all groups that may be due to the stress of overcrowding cell population. The fluorescence images clearly showed confluency of cells after 96 h in all groups. The results indicate cytocompatibility of microgels and support Alamar Blue assay results. The leached medium method (the Supporting Information Figure S7) also confirms that the microgels are cytocompatible and can be used as an implantable drug delivery device for bone regeneration.

The cytotoxicity of core-shell microgels was further assessed using the direct contact method. In the direct contact method, the MC3T3-E1 cells were grown on microgels. Figure 3C shows the result of the direct contact method at different time points. In TCPs, C-MG, CS-MG, and CSD-MG, the significant increase in fluorescence intensity was observed from day 1 to day 21 ($p < 0.001$). The increase in fluorescence intensity from day 1 to day 21 depicts the cell proliferation in all groups, which confirms the cytocompatibility of microgels. After cytocompatibility, cell adhesion on microgels was observed using SEM analysis. Figure 3D shows SEM images of cell attachment on the C-MG, CS-MG, and CSD-MG surface at different time points. After 7 days, ECM secretion by cells was observed and the cells were buried into their own ECM. After day 21, the SEM images exhibit that a layer of cells covered the microgels and formed a layer of ECM. These observations demonstrate that the microgels are cytocompatible and support cells adhesion as well as proliferation.

3.4. In Vitro Assessment of Core-Shell Microgels as an Osteoinductive Delivery Device. The osteoinductive potential of CSD-MG was assessed by osteoblastic differentiation of MC3T3-E1 cells. ALP is an early osteogenic marker; it hydrolyzes phosphate esters and enhances matrix mineralization.^{23,49,50} Figure 4A shows the ALP activity of MC3T3-E1 cells after 21 days. CS-MG exhibited ALP activity was slightly higher than TCPs due to the osteoconductive nature of its constituent polymers. The OGM treatment exhibited a 1.4-fold increase in the ALP activity, whereas CSD-MG demonstrated a 1.7-fold increase in the ALP activity compared with TCPs, CS-MG, and OGM. The increase in ALP activity could be due to the controlled release of rhBMP-2 and simvastatin from CSD-MG, which could further lead to osteoblastic differentiation of MC3T3-E1 cells. Because BMP-2 is a well-known osteoinductive molecule, its controlled release from scaffold enhances ALP activity.⁵¹ The sustained

release of simvastatin is also reported to induce the BMP-2 production via Ras-activated Akt signaling and mitogen-activated protein kinase (MAPK) pathways.^{14,48,52,53} In CSD-MG + OGM, the ALP activity was increased 2.2-fold, which shows a synergistic effect on osteoblastic differentiation of MC3T3-E1 cells.

The bone matrix is composed of 50–70% inorganic part, and the major portion is hydroxyapatite. It is a crystalline form of calcium and phosphate that is deposited on the surface of collagen fibrils.⁵⁴ The ARS stain selectively binds to calcium and produces red color that indicates osteoblastic maturation and mineral secretion by osteoblast cells.^{17,23} Figure 4B shows the ARS stain quantification, and the Figure 4C upper panel shows the representative images in which white arrows indicate calcium deposition stained by ARS staining after 21 days of cell culture. Cells on TCPs exhibited very less (0.048 mM) ARS binding. It could be due to absence of osteoinductive signals, whereas for CS-MG, ARS quantification was slightly higher (0.055 mM). The OGM treatment increased ARS (0.168 mM) 3.5-folds. CSD-MG demonstrated a 3.6-fold (0.172 mM) increase in ARS. CSD-MG + OGM demonstrated a 4.6-fold (0.222 mM) increase in ARS. The enhanced calcium deposition could be due to the synergistic effect of OGM and controlled release of rhBMP-2 and simvastatin. It has been reported that the controlled release of simvastatin over longer time enhances the ARS staining.⁴³ These results further support ALP activity assay results and confirm that the osteoinductive core-shell microgels could be a potential dual drug delivery device to enhance bone regeneration.

The viability of MC3T3-E1 cells during the osteogenic differentiation study was also confirmed by Live/Dead assay as shown in the Supporting Information Figure S8.

von Kossa staining is used to identify the bone nodule that indicates osteogenic differentiation. The treatment of silver nitrate followed by an exposure to strong light reduced calcium and replaced it with silver deposits (silver ions bind with phosphate). These black and metallic silver deposits indicate calcium phosphate deposition.⁵⁵ The Figure 4C lower panel shows representative images of von Kossa staining for TCPs, CS-MG, OGM, CSD-MG, and CSD-MG + OGM at 21 days. No staining was observed on TCPs. However, very few staining patches were observed in CS-MG. For OGM, von Kossa staining was clearly observed with black patches (indicated by white arrows) that indicate mineral deposition by differentiated osteoblasts cells. More prominent staining patches were observed in the case of CSD-MG. A further increase was observed in CSD-MG + OGM, which support the synergistic effect of CSD-MG and OGM on osteoblastic differentiation of MC3T3-E1 cells. These von Kossa staining results corroborate with ALP activity and ARS staining results. In conclusion, the CSD-MG induce calcium and phosphate deposition by the controlled and sustained release of osteoinductive molecules.

3.5. Controlled Release of rhBMP-2 and Simvastatin Induces mRNA and Protein Expression of Osteogenesis and Angiogenesis Markers. The osteoinductive potential of CSD-MG was evaluated at a molecular level by evaluating runt-related transcription factor 2 (Runx2) and osteocalcin (OCN) mRNA expressions using semiquantitative reverse transcription (sqRT-PCR). The osteoblastic differentiation at transcription levels is tightly regulated by the Cbfa1/Runx2 pathway.^{56,57} The growth factor such as BMP-2 upregulates the Runx2 expression.^{58,59} Osteocalcin is the second most

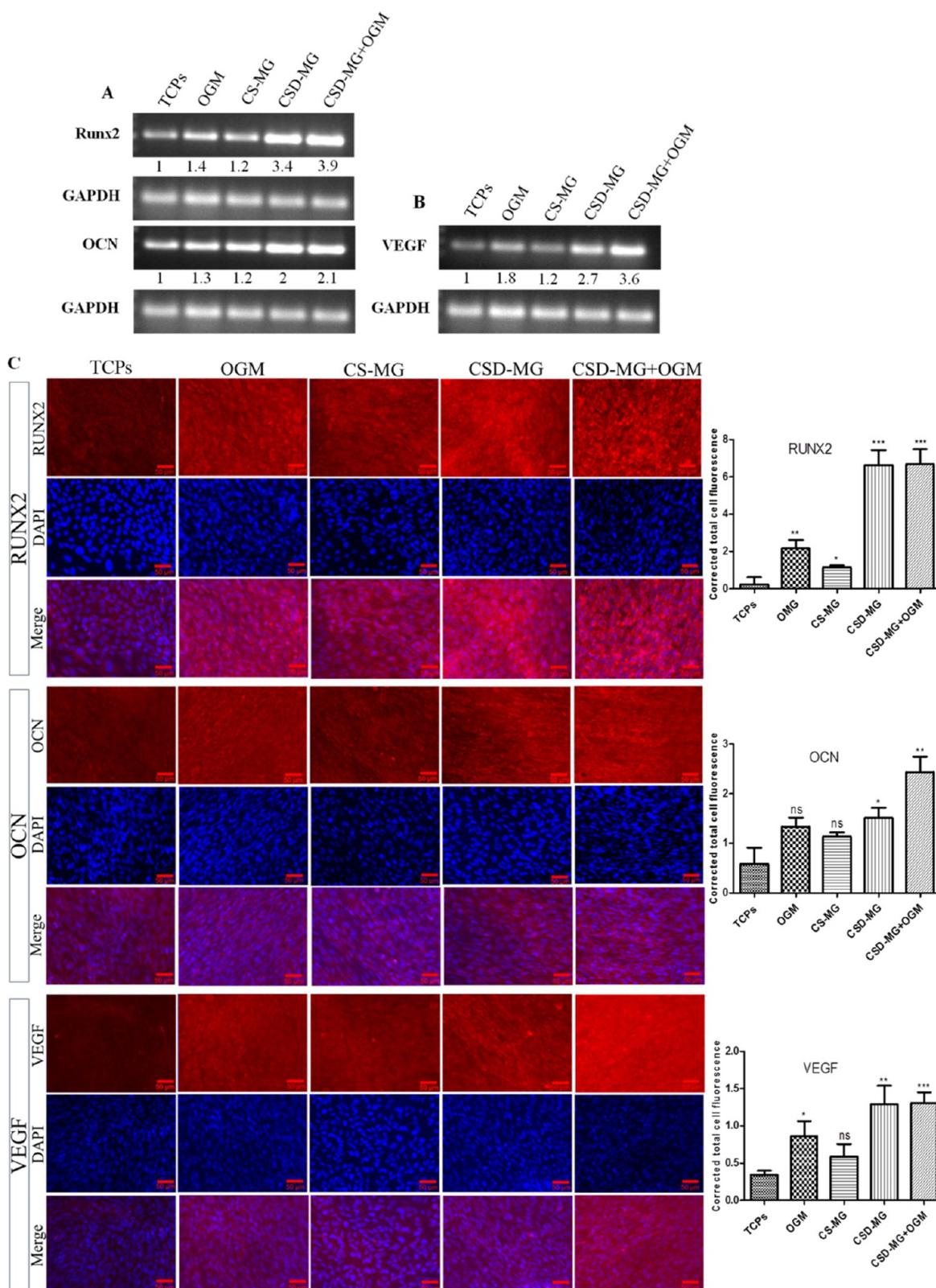


Figure 5. Representative images of (A) osteogenesis-related Runx2 and OCN mRNA expressions and (B) angiogenesis-related VEGF mRNA expression in MC3T3-E1 cells after 21 days. (C) Representative immunofluorescence images of Runx2, OCN, and VEGF protein expressions (Scale bar = 50 μm) and their respective corrected total cell fluorescence. Each bar represents mean \pm SD ($n = 3$), and p values < 0.05 (* p < 0.05, ** p < 0.005 and *** p < 0.0005).

abundant noncollagenous protein in the bone matrix. It is considered as a late-stage marker of osteoblast differentiation and maturation.⁶⁰ OCN regulates mineralization in bone.⁶¹

Figure 5A shows Runx2 and OCN mRNA expressions after 21 days. The OGM exhibited a 1.4-fold and 1.3-fold increase in Runx2 and OCN expressions, respectively, which could be due

to differentiation factors. The CSD-MG exhibited a 3.4-fold and 2-fold increase in the expression of Runx2 and OCN, respectively. It indicates that controlled release of rhBMP-2 and simvastatin induces a higher expression of Runx2, which confirms the BMP-2 regulation of the Cbfa1/Runx2 pathway. The CSD-MG + OGM showed a 3.9-fold and 2.1-fold increase in the expression of Runx2 and OCN, respectively. It was higher than that of CSD-MG, which demonstrates the synergistic role of differentiation factors with rhBMP-2 and simvastatin. Overall, the osteoinductive CSD-MG could enhance the osteogenic differentiation through the controlled release of rhBMP-2 and simvastatin, which could accelerate bone regeneration.

Angiogenesis and osteogenesis are the two critical and interdependent events in bone healing and regeneration.^{5,10} Angiogenesis is an early-stage event in bone fracture healing that further enhances the new bone formation.⁶² It ensures exchange of gases and supply of nutrients at the bone regenerating site. To induce angiogenesis, simvastatin has been used in many studies for bone regeneration.^{12,17,63,64} The VEGF is the key regulator of neovascularization in bone fracture healing.⁶² In this study, the effect of controlled release of simvastatin on VEGF mRNA expressions was assessed. Figure 5B shows that OGM exhibited 1.8-fold higher VEGF expressions compared with TCPs. It shows that osteogenic differentiation factors could also induce angiogenesis.¹⁷ The CSD-MG demonstrated 2.7-fold higher VEGF expressions. The controlled and sustained release of simvastatin and rhBMP-2 induces the higher expression of VEGF. In CSD-MG + OGM, the expression was further increased to 3.6-folds. The results reveal that rhBMP-2, simvastatin, and osteogenic differentiation factors are showing synergistic effects to induce angiogenesis as well. The maximum release of simvastatin (12.62%) within 14 days was a desirable pattern to induce the secretion of VEGF and the new blood vessel formation. The initial release of VEGF within 10 days and sustained release of rhBMP-2 have been used for blood vessel network formation and bone tissue repair.¹⁰ In conclusion, the osteoinductive core-shell microgels were able to induce angiogenesis. The controlled and sustained release of rhBMP-2 and simvastatin were a critical pattern to induce the higher VEGF expression. The osteoinductive core-shell microgels are potential osteogenic and angiogenic drug-releasing scaffolds that can augment the bone regeneration process. As demonstrated in our previous studies, the coupling of osteogenesis and angiogenesis during bone regeneration has the synergistic effect on functional bone formation.^{5,17}

The osteoinductive and angiogenic potential of CSD-MG were further supported by the immunofluorescence study for the expression of RUNX2, OCN, and VEGF by MC3T3E1 cells after 21 days. Figure 5C shows the results of RUNX2, OCN, and VEGF expressions. TCPs showed the least expression of RUNX2, OCN, and VEGF. The OGM and CS-MG demonstrated a slight increase in the corrected total cell fluorescence (CTCF). The reason could be the presence of osteoinductive molecules in OGM, which could induce the osteogenic differentiation. CSD-MG demonstrated a 30-, 2.5-, and 3.7-fold increase in the expression of RUNX2, OCN, and VEGF, respectively. CSD-MG + OGM demonstrated a further increase by 30-, 4-, and 3.8-folds in the expression of RUNX2, OCN, and VEGF, respectively. The results are in corroboration with the mRNA expression results. These results of osteogenic molecular marker expressions support osteogenic

staining results. Hence, the osteoinductive core-shell microgels will be a potential device that can deliver two different bioactive molecules in a controlled manner to enhance osteogenesis coupled angiogenesis that could further lead to enhanced bone regeneration.

4. CONCLUSIONS

Growth factor delivery systems for prolonged release are meant to provide the sustained release of growth factors. In this study, core-shell microgels were fabricated from ECM-mimicking biomacromolecules. The physicochemical characterization depicts the discrete core made up of collagen and the shell made of chitosan. The distinguished core and shell enable the dual loading of drugs or bioactive molecules. The compact shell structure could not allow the diffusion of drugs immediately. The affinity of the collagen core with rhBMP-2 would enable the improved stability and delayed release of the growth factor. The release of rhBMP-2 was sustained for 28 days, which could enhance bone regeneration. The early diffusion of simvastatin from the shell could enhance the possibility for early angiogenesis. The fabricated core-shell microgels are cytocompatible and biodegradable. Drug-delivering and growth factor-delivering microgels enhanced the osteogenic differentiation of MC3T3-E1 cells in a synergistic manner by increasing the ALP activity, mineral deposition, and higher expression of RUNX2 and OCN. The microgels also enhanced the expression of angiogenesis-related (VEGF) gene and the protein expression. The overall results demonstrate the use of core-shell microgels as a dual-drug delivery system to enhance osteogenesis coupled angiogenesis for bone tissue regeneration applications.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsapm.0c00815>.

Primers sequence and PCR condition details; customized CS-MG fabrication setup details; polymer concentration optimization for CS-MG fabrication; swelling ratio of CS-MG and C-MG in deionized water; thermogravimetric analysis of chitosan, collagen, C-MG, CS-MG, and CSD-MG; in vitro pH alteration study; encapsulation efficiency, yield, and drug release studies; cytocompatibility study by the leached medium method; and study of cytocompatibility of cells during osteogenic differentiation (PDF).

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Author Contributions

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Notes

The authors declare no competing financial interest.

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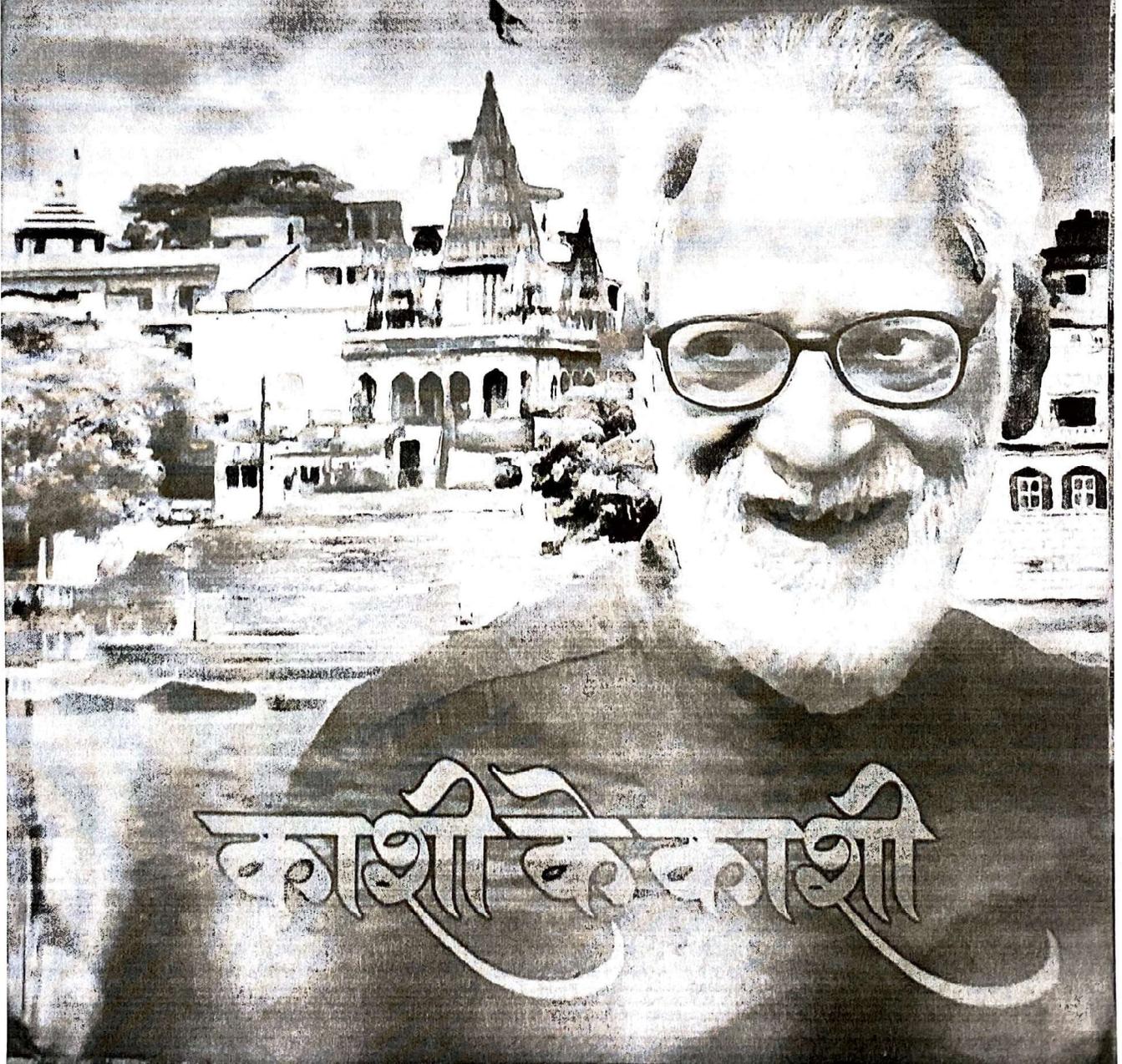
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सौच विचार

साहित्यिक पारिवारिक मासिकी



काशी के काशी

टूटते मूल्यों और बिखरते संबंधों के आख्यान

संजीव कुमार दुबे

आजादी के बाद बदलते गाँवों की शिनाख्त हिन्दी कथा साहित्य की प्रमुख प्रवृत्ति रही है। इन उपन्यासों में बदलावों के लिए सृष्टि करते पात्रों की रचना स्वाधीनता आंदोलन से विकसित हुई थी। अस्सी के दशक के उत्तरार्ध में, गाँवों में जो परिवर्तन घटित हुए उसे भूमंडलीकरण की प्रक्रिया ने और तेज कर दिया। गाँवों के विकास के स्थानीय स्वशासन को मजबूती देने की शुरुआत राजीव गांधी के प्रधानमंत्रित्व काल में हुई थी। 1985 में अकाल और भुखमरी से जूझते उड़ीसा के 'कालाहांडी' की सभा में राजीव गांधी ने स्वीकार किया था कि गाँवों के विकास के लिए जारी किये जाने वाले एक रूपये में से सिर्फ 15 पैसे ही अस्मरतमंदो तक पहुँचता है। इसके बाद से ही गाँवों के विकास के लिए जवाहर रोजगार योजना आदि के माध्यम से ग्राम पंचायतों को विकास कार्यों के लिए सीधे अनुदान राशि आबंटित करने की शुरुआत हुई, जिसने ग्रामीणों में ग्राम पंचायतों के प्रति उदासीनता को तोड़ा और एक नई राजनीतिक जागृति आई। इसी दशक के अंत तक विवादित बाबरी मस्जिद पर राम मंदिर निर्माण आंदोलन ने तेजी पकड़ी और गाँव भी सांप्रदायिकता की जट में आने लगे। नब्बे के दशक की शुरुआत में मंडल आयोग की सिफारिशों को लागू करने की घोषणा ने गाँवों के पिछड़े वर्ग की आतियों में नई उत्तेजना और उत्साह का संचार किया तो दूसरी ओर गाँव अगढ़ी और पिछड़ों में बटने लगे। 1992 में ही संविधान के 73 वें संशोधन के माध्यम से ग्राम पंचायत से जिला पंचायत तक जाति के आधार पर सीटों के आरक्षण की शुरुआत हुई। परिणामतः स्थानीय स्वशासन पर सदियों से चला आ रहा सवर्ण वर्चस्व टूटा। इन सबके बीच भारत के विश्व व्यापार संगठन की शर्तों के आगे झुकने, वैश्विक उत्पादों को भारत के बाजारों में फैलाने और बहुराष्ट्रीय कंपनियों के दरवाजे भारतीय युवाओं के लिए खुलने के साथ अमेरिका और यूरोप के बेचैन करने वाले सपने ने क्या गाँव, क्या नगर-महानगर, सभी के राजनीतिक, सामाजिक, आर्थिक एवं धार्मिक-सांस्कृतिक जीवन में आमूल-चूल परिवर्तन घटित किये। 'महुआचरित' के एक प्रसंग में काशीनाथ सिंह लिखते हैं, कि: 'ये दस साल बहुत होते हैं बदलाव के लिए। दस साल में वसुधा भूमंडल हो जाती है और प्यार कूड़ेदान का कचरा। उसकी जगह इस्टेब्लिशमेंट में हो जाती है दिल में नहीं।'

इतनी तेज गति से हो रहे बदलावों को साहित्यिक कृतियों में दर्ज करना इतना आसान काम नहीं है। इसे मौजूदा फैशन की तरह दर्ज करने की जल्दबाजी में कथा कृतियों में देशकाल अथवा वातावरण का तत्व इतना प्रधान होता जा रहा है कि बाकी सारे तत्व गौण हो गए हैं। उपन्यास के शास्त्रीय विधान में देशकाल - वातावरण का महत्व है। पर वह एक मात्र प्रधानतत्व नहीं है। कथा वस्तु, पात्र सृष्टि एवं भाषा को विश्वसनीयता प्रदान करने में उसकी भूमिका होती है। आज की उपन्यास सृजना और आलोचना में देशकाल एवं वातावरण का वृत्तान्त महत्वपूर्ण हो गया है। कथा वस्तु एवं चरित्र सृष्टि सहायक की भूमिका में आ गए हैं। आज चरित्र गायब हैं, कथा के स्थान पर कुछ ब्योरे हैं, सतह पर तैरते यथार्थ के कुछ विवरण हैं तो कुछ आश्चर्याक्तियों, जिनके माध्यम से एक रचना प्रस्तुत कर दी जाती है। यह सीमाएं उन रचनाओं की हैं जिनकी भूमंडलीकरण के संदर्भ में बड़े जोर-शोर से चर्चा सुनाई पड़ी है। उपन्यास

हमारे समय के दस्तावेज भर नहीं हैं। काल को रचना में निबद्ध करने की जैसी प्रवृत्ति दिखाई पड़ रही है वह रचनाओं की कालजयिता के लिए बड़ा संकट है। इसका दुष्परिणाम यह है कि रचनाएं बहुत जल्दी कालबाह्य हो रही हैं। दुनिया के पुरानी पढ़ने के पहले रचनाएं पुरानी पड़ती जा रही हैं। रीतिकाल में जैसे लक्षण ग्रन्थ लिखे जाते थे, उसी प्रकार भूमंडलीकरण को केंद्र में रखकर उपन्यास लिखे जा रहे हैं। विद्याओं में आवाजाही पर मुघ होने का एक चलन चल पड़ा है। इस तरह रचना किसी एक विद्या की नहीं रह जाती और फिर अंततः उपन्यास बन कर बाजार में आ जाती है। हम सब जानते हैं कि साहित्य के बाजार में उपन्यासों की धूम ज्यादा है। आरंभ में दलित आत्मकथाओं को भी उपन्यास अथवा आत्मकथात्मक उपन्यास कह कर बाजार में उतारा गया था। दलित लेखकों - चिंतकों के विरोध के बाद उसे आत्मकथा के रूप में प्रकाशित किया जाने लगा। कथा तत्व से संपृक्त गद्य रचना को उपन्यास साबित कर देने का जो चलन प्रकाशकों की साजिश से चल रहा है, उस पर भी ध्यान दिया जाना जरूरी है।

'कहन' का अलहदापन काशीनाथ सिंह को अपने समकालीनों से अलग करता है, तो 'पौत्रवय' पीढ़ी के युवा कहानीकारों के लिए चुनौती भी पेश करता है। जीवन के बेस्वाद यथार्थ को 'सुस्वादु' कलाकृति के रूप में पेश करने के लिए उन्होंने, पारंपरिक किस्सागोई में अपने पूर्ववर्ती कहानीकारों द्वारा ईजाद किये गये नुस्खों का अभिनव इस्तेमाल किया है। काशीनाथ सिंह की लेखकीय पाकशाला में निर्मित सुस्वादु पकवानों का राज सही ताव और समुचित ताप है। समुचित ताप पर पकती रचना को सही ताव पर उतारने का हुनर उन्होंने अपने गांव, शहर, विश्वविद्यालय और विद्यार्थियों के सुदीर्घ संग साथ से अर्जित किया है। सही ताव पर रचना के उतरने के बाद विद्वान आलोचकगण प्रस्तुत व्यंजन के नामकरण को लेकर बहसतलब हो सकते हैं पर उसके स्वाद की शिकायत नहीं कर पाते।

काशीनाथ सिंह लगभग छह दशकों से रचनारत हैं। उनकी पहली कहानी 'संकट' 1960 में कृति पत्रिका में प्रकाशित हुई थी और पहला संग्रह 'लोग बिस्तरों पर' 1968 में साया हुआ। यूं तो 1972 में उनका पहला उपन्यास 'अपना मोर्चा' प्रकाशित हो गया था, परंतु पिछली सदी तक उनकी ख्याति कहानीकार और संस्मरणकार के रूप में रही। नई सदी में उन्होंने एक उपन्यासकार के रूप में अपने को स्थापित करने का सफल प्रयास किया है। इस कड़ी में 'काशी का अस्सी', 'रेहन पर रघू', 'महुआचरित' और 'उपसंहार' उल्लेखनीय हैं। काशी का अस्सी मूलतः संस्मरणों की शृंखला के रूप में हंस मासिक में प्रकाशित हुआ था। पहली कड़ी के मंगलाचरण में ही काशीनाथ सिंह ने पाठकों को संबोधित करते हुए लिखा है- 'मित्रों यह संस्मरण वयस्कों के लिए है, बच्चों और बूढ़ों के लिए नहीं और उनके लिए भी नहीं जो यह नहीं जानते कि अस्सी और भाषा के बीच ननद भौजाई और साली बहनोई का रिश्ता है।' (काशी का अस्सी, पृष्ठ-11) संस्मरणों की ये पाँच कड़ियां 2002 में उपन्यास के रूप में प्रकाशित होने के पूर्व ही खासी चर्चित और विवादित हो चुकी थीं।



SOCIAL CAPITAL MOBILISATION THROUGH COMMUNITY BASED ORGANISATIONS FOR THE EFFECTIVE MANAGEMENT OF RURAL-COMMONS: A STUDY ON 'KUDUMBASHREE'

Jasmy Anto * and Litty Denis**

ABSTRACT

Commons is a self-organised social system for managing common-pool resources which refers to natural or man-made common goods like fishing grounds, forest, irrigation systems, etc. For the effective management of Commons, the participants must have mutual trust and communication that can lead to collective action. Hence, social capital is important whereby community members communicate, cooperate and network to resolve any difficulties that knock their door in the way for the common upright. The cost associated with this collective action in managing rural commons can be reduced by social capital. Therefore, finding ways to strengthen the social capital of a community may be one key to solve the complications of the commons. The potential of microfinance enables the incorporation of the community by collective action and creation of more sustainable community-based organisations. This paper seeks to draw the connection between group-based microfinance programmes, social capital and commons. It also pursues to empirically analyse how microfinance contributes to managing rural commons with reference to 'Kudumbashree', microfinance-led financial security model of local economic development and women empowerment in Kerala. Neighbourhood groups of Kudumbashree act as a meaningful agent in the creation and development of social capital and managing resources towards the objective of economic development and women empowerment. The study extensively used secondary sources of information like reports from governments, public agencies, international organisations and the academic literature in the field.

Keywords: Social Capital, Commons, Community-Based Organisations, Microfinance, Kudumbashree.

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Introduction

Over the past few years, the concept of commons gained wider appreciation around the world. Commons is a self-organised social system for managing common-pool resources which refers to natural or manmade common goods like fishing grounds, forest, irrigation systems, etc. 'Commons' arises whenever a specific community agrees to manage a resource in a collective mode, concerned with equitable access, use and sustainability. The commons is not the resource itself, but includes a particular community, and the management of these resources with the conceived values, norms and practices in the community. For the effective management of Commons, the participants must have mutual trust and communication that can lead to collective action. Collective action can be made possible only if there is a great amount of cooperation and mutual trust. These stocks of trust, norms and networks, arising from the social relationship are often known as social capital. Thus, social capital becomes important, whereby community members communicate, cooperate and network to resolve any difficulties that come in the way for the common upright. The associated cost with this collective action in managing rural commons can be reduced by social capital. Therefore, finding ways to strengthen the social capital of a community may be a key to solve complications of the commons. Microfinance with their group lending techniques can

strengthen the social capital of a community. They also can create new social capital. The potential of microfinance enables the incorporation of the community by collective action and creation of more sustainable community-based organisations. This paper seeks to draw the connection between group-based microfinance programmes, social capital and commons. It also pursues to empirically analyse how microfinance contributes to managing rural commons with reference to 'Kudumbashree', microfinance-led financial security model of local economic development and women empowerment. The study extensively used secondary sources of information like reports from governments, public agencies, international organisations and the academic literature in the field.

Addressing Commons

Human beings rely on some common resources for survival. The system for managing common-pool resources like water, forests and fisheries is described as 'commons'. The resources are used and managed collectively according to certain rules. It is generally governed by 'unofficial' norms, institutions and procedures. Commons allow the individuals to consume common resources without any restriction. This feature of 'commons' may create an issue i.e., availability for one group of users can be depleted by others. This is popularly known as the tragedy of commons. Garrett Hardin (1968) coined this phrase where selfish people who cares about themselves

only and don't consider others ultimately may drain a common pool resource. Hardin states freedom in commons may lead to the collapse of the system (Hardin, 1968). He explains that when the population expands beyond some point where resources are limited, self-centred individuals will consume excessively and eventually deplete a common resource having limited availability. In his book 'The Tragedy of the Commons', Hardin put forward the idea of 'mutual coercion mutually agreed upon' for conserving the resource. He is of the opinion that the coercion of individuals by society is necessary. The opponents argue that social values are too diverse to allow the negotiation of mutual coercion and in consequence eradicating it from being Commons.

Elinor Ostrom challenged this approach of Hardin towards "Tragedy of the Commons" stating that ordinary people can generate mutual coercion. She demonstrated a group of individuals in 'Commons' could manage their shared resources. Ostrom argues that the tragedy of commons may be due to the collective action problem and it can be solved by cooperation and mutual trust (Ostrom, 1990). In her own words, this commons dilemma may be due to the fact that "participants may simply have no capacity to communicate with one another, no way to develop trust, and no sense that they must share a common future" (Ostrom, 1990). Through empirical evidences, Ostrom debates that individuals could achieve better results than being "rational" by making conditions where "reciprocity, reputation and

trust" can aid to overcome the self-interest (Cosmides & Tooby, 1994; Ostrom, 1998). Sirianni and Friedland define this "Stocks of social trust, norms and networks that people can draw upon in order to solve common problems", as social capital (Sirianni & Friedland, 2001). Hence, finding ways to strengthen the social capital of a community may be one key to solve the complexities of the commons (Anderson, Locker, & Nugen, 2002).

Social Capital and Community

During the 1990s, there was a growing concern regarding the detachment of individuals and communities from political and social engagement. Hence, for the same reason, social capital which refers to the benefits that arise from the development of social relationships and networks of people becomes an important theme in the academic debates of that decade. Many studies and articles have been published on social capital (Putnam, 1993, Coleman, 1988; Bourdieu, 1986). They highlighted the impact of social bonds and collective norms for welfare and economic efficiency (Wiesinger, 2007).

In development studies, it is a paradox that the unsatisfactory developmental outcome of many rural regions, even after spending huge financial resources and various other measures while in other regions could achieve better socio-economic settings despite much support from outside. Social capital is considered to be the missing link that can explain why there exist these differences in economic performance

(Guiso, Sapienza, & Zingales, 2010). Putnam considers it a fundamental ingredient for democracy maintenance and institutional performance (Putnam, 1993). He inter-related the notions of social capital to the importance of having associations of public and voluntary community organisations. He also stressed on the positive effects of social control (Wiesinger, 2007). Social capital is presumed to be created by networks and flows of information and resources (Wiesinger, 2007). The positive effects of social capital create solidarity among the members of a community, which in turn helps in easy communication of each other, early recognition of problems if any for bringing out solutions to the problems. These activities ultimately complement to the formulation of economic capital and accomplishing overall development of the entire community.

Microfinance Perspective

Despite the fact that the concept of microfinance is one of the most discussed topics in development studies, its connection with the social capital generation by group-based activities is explored very less. Like microfinance, many other credit arrangements such as trade credit, rotating credit and saving associations (ROSCAs), local money lenders, etc., are serving the credit requirements of the poor using social relationships and networks among them (Bastelaer, 1999). From this, microfinance had a great acceptance all over the world due to its long standing and more sustainable and long-standing approach to

serve the underprivileged and poor people. Microfinance denotes financial services for poor and low-income people. Initially, it concentrated on borrowing or microcredit alone. Eventually, according to the increasing needs of the members, it extended to include many other financial services like insurance, savings and money transfer, etc., for the benefit of the low-income people. These enhanced services benefit the poor to improve their standard of living by facilitating them to start more viable businesses, possessing more assets, balancing consumption and protecting themselves from risks.

In spite of a long informal history, microfinance became popular with the works of Grameen Bank in Bangladesh by Mohammed Yunus, Nobel Prize laureate. As it observed low potential profitability and imperfect information as the most imperative problems in rural credit history (Stiglitz, 1990), Grameen Bank made a new approach in lending to the poor which designed specific methods to overcome this information asymmetries and low profitability, resulting in a remarkable programme performance. From the previous experiences with the poor, Yunus found that they are creditworthy. He formed a group lending methodology to ensure the repayment of small non-collateral loans, which depends on social interactions and social bonds among the borrowers (Khandker, 1998). They conducted business openly in the villages and put in place simple procedures to avail loans by ensuring the participation of

the poor and women. Majority of the groups lower the transaction cost, which was one of the concerns for formal banking institutions. Simultaneously, they insisted to repay the small non-collateral loans in weekly instalment. The frequent payments and peer group pressure facilitated consistent repayment rates of an excess of 95% (Todd, 1996).

Group-based Microfinance and Social Capital

Microfinance, through group lending is one of the most effective forms of financial services for the poor. Instead of physical collateral, group-based microfinance programmes used social collateral. The group techniques of microfinance were framed by utilising the relationships and trust among the people in the villages i.e., the existing social capital. It employs a joint liability and contingent renewal of loans. This is about potential borrowers having to form a group by selecting reliable group members by themselves. The members have to act as a guarantor for each other and receive further loans contingent on other members in the group paying their loans back (Bastelaer, 1999). Besides the use of existing social capital, microfinance also generates new social capital. They conduct regular meetings and training to the borrowers, which in turn reinforces the group dynamics thereby reducing the costs of information uncertainties. The unique idea of using social collateral in the place of physical collateral for allocating loans to the poor to overcome the negatives of this segment like

relatively 'low potential profitability' and the 'imperfect information' is the major reason for the success of microfinance.

It is evident from the research that adequate institutional arrangements to communicate will help the participants to use the opportunity to find out, coordinate and implement strategies to improve their income levels and also to deal with the non-conforming players (Ostrom, Gardner, & Walker, 1994). The creation of social capital involves great effort and dynamism. Group lending microfinance minimises the efforts of continuous evaluation and helps in imposing the rules and regulations existed in the group, and also reduces the cost of creating new rules. Studies had consistently put forth that face-to-face communication helps in achieving substantial increase in the levels of cooperation (Ostrom, 1998). Microfinance can facilitate communication through frequent meetings and required peer group interaction to meet the shared goal of credit repayment. Communication encourages collective action as it enables to gather information about other group members to develop mutual understanding and reliability among members to create awareness about the group incentive formats (Ostrom et al., 1994).

Microfinance and Commons

Microfinance programmes provide institutional structure for resource management that is critical for sustaining the Commons through their group and group-based activities. Thierry Van Bastelaer (1999)

had projected 'social' aspects of group liability model microfinance programmes as "self-selected, small, and homogeneous borrowers' groups in densely populated areas are jointly liable for loans" and "denying access to future credit to all group members in the case of default by any member is the most effective and least expensive way of enforcing joint liability" (Bastelaer, 1999). Joint liability benefits to lower the risk of delinquency and transaction costs by shifting the screening, selection and monitoring of borrowers from the institution to fellow group members. The group of individuals sharing similar views on the economic status can ensure mutual trust and cooperation (Bastelaer, 1999).

According to Schneider, microfinance enables collective action, harmony of the members and more enduring community-based organisations (Schneider, 1999). This collective action enables to endue in the development programmes of acquiring skills and education, in turn, helps the members of the community to establish specific community-based projects according to the local requirements (Schneider, 1999). Communication among members of microfinance groups significantly promotes the success of collective action in managing Commons. It also can reduce the cost of the collective action. Frequent group meetings which are a condition of their loan in microfinance help to reduce any further expenses of collective action for any other community projects, most likely based on

common pool resource.

Women are known for their skills to promote social cohesion with their social networks and informal communications (Inglehart & Norris, 2003). Majority of the clients of microfinance are women who are the main users of common pool resources. For that reason, group activities of microfinance programmes may deliver a space for mediating the operational rules and norms among themselves for the effective governing of common pool resources.

Case Study: Kudumbashree

Kudumbashree is a revolutionary experiment initiated by the Kerala government for empowering poor women and enhancing their participation in the public sphere. It was launched in 1998 as the Kerala State Poverty Eradication Mission which aimed to eliminate absolute poverty within a time frame of ten years. It strives for a holistic outlook to alleviate poverty through an integrated approach of demand-led convergence of resources and actions. The conversion of Kudumbashree from a 'microfinance-led financial security model' into a 'more inclusive model of local economic development' with the support of local government bodies makes it different. Here, they implemented a different method to eradicate poverty. The women from low-income families in the State are organised under the community-based organisations namely Neighbourhood Groups (NHGs). Economic empowerment is one of the important strategic domains of Kudumbashree

in which programmes are formulated and rolled out through community network. It involves programmes which support women members to start and run enterprises. Neighbourhood groups of Kudumbashree act as a meaningful agency in the formation and accumulation of social capital towards the final goal of economic development and women empowerment. Major programmes of Kudumbashree comprise of microfinance, micro enterprises, collective farming, livestock farming, market development, training programmes and women empowerment initiatives. Kudumbashree serves as a pertinent model as many government microfinance programmes have not been as effective and either just been existent on paper or closed down prematurely. Therefore, the role of governance of these neighbourhood groups as an institution to build social capital and consequently economic development is substantial.

Kudumbashree – Community-based Organisational Structure

Kudumbashree focuses on formation and capacity building through a three-tiered organisational structure of Community-based Organisations (CBOs) of poor women – NHGs at the grassroots, Area Development Societies at the ward level and Community Development Societies at the Municipal/ Panchayat level, which facilitates collective action. The grassroot level organisations of Kudumbashree system are the neighbourhood

groups (NHGs) comprising of 10-20 adult women members where eligible women can join themselves as members, each from a different family of the area concerned. Following this, the Neighbourhood Groups (NHGs) are affiliated into ward level as Area Development Society (ADS) and in Municipal/ Panchayat level as Community Development Society (CDS). This three-tier organisational system of the poor women functions as a platform for congregating various State and Central government-sponsored poverty-eradication programmes. These community-based organisations consisting of Neighbourhood Groups, Area Development Societies and Community Development Societies are the core of Kudumbashree. The community-based organisations of the Kudumbashree stimulate self-help and mutual help, and it delivers opportunities for public action. The administrative power of these organisations that functions as a three level system is vested in the hands of the women chosen democratically from the poor families. Another significant element of Kudumbashree structure is its link with and support from local self-governments which is designed and empowered by the Constitutional Amendments of 73 and 74, and bureaucracy, both at the Ward and Panchayat/Municipal level.

Effective Management of Resources

Microfinance programmes, through their group lending model activities provide

institutional structure for collective resource management which is vital for sustaining the Commons (Jetti, 2011). Kudumbashree, since its inception, considered microenterprises as a valuable tool for sustainable regional and rural economic growth. The selection of microenterprises involves strategies for right selection of creative and innovative designs, appropriate for specific local environment in which they are living and ideas to solve the problems faced by a particular community by using the technological expertise that is already locally available (Johnkutty, 2012). Kudumbashree tries to build the awareness of the society's changing needs and the environmental, political and cultural practices within which its members have to function. Kudumbashree promotes microenterprises by proposing solutions to the problems and threats faced by the people in their day-to-day life under the prevailing socio-economic systems of the society. The idea of solutions to the existing problems will lead to an opportunity for setting up an enterprise. There are many illustrations to explain these community-based solutions.

Collective Farming is such an initiative taken by Kudumbashree to encourage cultivation among neighbourhood groups. For the last several decades, Kerala has witnessed huge depletion of agricultural land. Majority of its food consumption requirements was fulfilled by the neighbouring States. Collective farming initiatives helped to improve the agricultural production in the State by fetching

uncultivated wasteland into agricultural use. Through collective farming, women started providing safe and nutritious food to the neighbourhoods after forming joint liability groups of women farmers. As on 2018, Kudumbashree supports almost 60,000 joint liability groups in cultivation. They cultivate in an approximate area of 55,000 hectares of land. Crops like vegetables, paddy and banana are mainly cultivated under the programme. The programme facilitates training, interest subsidies, incentives and many other supporting measures to the groups of women to encourage livelihood promotion. This brought substantial changes in the lives of the poor household women by increasing their returns from farming and also works as an effective tool for food security.

Kudumbashree converted 'waste disposal', one of the life-threatening issues of Kerala, into a means for income generation. Existing waste disposal system was rather unhealthy and a threat to the environment. Kudumbashree had formed group micro enterprises for the collection and safe disposal of solid waste from houses, hotels, shops, etc.

Another important initiative is rainwater harvesting. Although Kerala has always been projected as a water surplus State, it is currently facing a big issue of lack of drinking water. The drinking and irrigation water of the State has been severely depleted. Rainwater harvesting and artificial well recharge have been recognised as cost-effective methods

for enhancing groundwater resources and for raising the groundwater levels. 'Sujalam' is a community-based well recharge programme initiated by Kudumbashree, which makes a livelihood opportunity for its members. Through convergence with local governing bodies and with the strength of community network, Kudumbashree has committed to the cause of water security. Rainwater harvesting, the technological solution for sustainable water management, has been identified as a livelihood opportunity. Through technical guidance in developing rainwater harvesting micro enterprises units, Kudumbashree is contributing to the national effort to conserve water resources for the future.

Like collective farming and Sujalam, many other initiatives in capacity building, women empowerment, local economic development and social development have been introduced by Kudumbashree. Group activities of Kudumbashree help in attaining great cohesion among the members. During the crisis, it serves as a social safety net for reducing vulnerabilities. This is empirically evident from the rescue and relief operations done by Kudumbashree members during the recent floods in Kerala. They volunteered for cleaning of houses and public places. They took initiatives to provide temporary shelter for affected people, to gather food and other materials for relief camps and in counselling for instilling mental strength and courage in children and families.

Conclusion

Social capital constitutes a critical element in the sustainable development of a community. It could act as a channel to connect the resources available with the poor. It enables coordination and cooperation for collective benefit. The effectiveness of leveraging social capital in engaging low income segments of society is already evident in literature (Reficco & Marquez, 2009). It is easy to work together in a community blessed with extensive social capital (Putnam, 1993). The practical implications of this insight for many issues can be perceived from the successful initiatives of Kudumbashree. It could achieve capability building and diffusion in less privileged women by connecting them with resources through collective action. This leads to a result of an improved standard of living and socio-economic empowerment of the poor women in the society. From the initiatives like Sujalam and collective farming, it is evident that neighbourhood groups of Kudumbashree act as a meaningful agent in the formation and accrual of social capital and managing resources towards the final goal of economic development and women empowerment. The role of Kudumbashree in building reciprocity, mutual trust and collective action among their members will enable them to adopt plans for effective management of common pool resources, whenever required.

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Review Paper

Status of seawater intrusion in coastal aquifer of Gujarat, India: a review



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Abstract

Seawater intrusion (SWI) is a universal concern, intensified and aggravated due to over-exploitation of groundwater, sea-level rise, and climate change in the coastal province. According to the Ghyben–Herzberg principle, the stability between freshwater and saltwater is caused by the density difference between the two, until the pressure equalizes. The keystone of India's geography, economy, biodiversity, and the environment are its extended and vast coastline, which occupies the most precious as well as potential aquifer system. This review mainly fascinated on the status, knowledge, and vulnerability of SWI within the aquifers of the coastal region of Gujarat, India. Foregoing investigations reveal that the meteoric intensification in the salinity of coastal regions of Gujarat is the chief provenance of socioeconomic development and environmental degradation. Different studies are performed to analyze and mitigate the SWI problem. In this region, groundwater over-exploitation is the foremost driver of SWI. There are miscellaneous developmental and management skills and techniques to diminish the SWI in coastal regions of the world. Numerous models are fabricated and established to analyze and epitomize the complications associated with it. Numerical models suggest some valuable techniques to manage groundwater-related problems. This study also delivers some advanced management strategies such as dilution of salinewater by artificial recharge techniques, construction of physical barriers in the subsurface to reduce the intrusion etc. feasible to the coast of Gujarat.

Keywords Seawater intrusion · Ghyben–Herzberg principle · Salinity contamination · Gujarat · Artificial recharge

1 Introduction

On the planet earth, groundwater is the enormous source of freshwater. It has been utilized to a great extent to meet the demands of agricultural, industrial, and municipal water supply schemes all over the world including coastal regions [1]. Coastal belts encompass some of the highest densely populated zones with an average population density of almost 80 individuals per sq. km, which is estimated as twice of the world's average population density [2]. Along with rampant increase in the population growth, uninterrupted progress in standards of living is additionally enhanced the groundwater requirement in coastal

regions [3]. But seawater intrusion (SWI) in the coastal region is such a menace that not only contaminates this precious groundwater resource but also affects vegetation, soil conditions and the sustainability of groundwater that contributes to the economic improvement of the coastal communities. Therefore, SWI is considered as a global coastal threat. Furthermore, the key elements contributing to SWI comprise changes in land use patterns which might influence the recharge of aquifer and rise in sea-level owing to climate changes. Degradation of groundwater quality is influenced by the intrusion of seawater into the coastal aquifers where less than 1% of

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Swertisin, a novel SGLT2 inhibitor, with improved glucose homeostasis for effective diabetes therapy

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ABSTRACT

Failing pancreas and subsequent loss of pancreatic β cells worsen diabetic conditions which are further alleviated by the mounting up of glucose levels. Inhibition of sodium glucose cotransporter 2 (SGLT2) in the kidney responsible for glucose reabsorption strikingly reduces blood glucose levels. Bioactive swertisin showed a promising glucose-lowering effect. Hence, we aimed to mechanistically dissect the glucose lowering property of swertisin. A systematic *in silico*, *in vitro*, and *in vivo* approach was directed for target analysis of swertisin. Molecular docking was performed with Swertisin-hSGLT2 complex. Glucose uptake assay and protein expression for SGLT2 and regulatory proteins were performed under swertisin effect. Various physiological and metabolic parameters were evaluated in STZ induced BALB/c mice using swertisin treatment. SGLT2 expression was evaluated in the kidney tissue of mice. Swertisin-hSGLT2 molecularly docked complex showed similar binding energy compared to the Canagliflozin-hSGLT2 complex. Swertisin inhibited glucose uptake and decreased expression of SGLT2 in HEK293 cells. Swertisin does not affect GLUT mediated glucose transport. Swertisin treated diabetic mice demonstrated remarkable improvement in overall glucose homeostasis. Reduced expression of SGLT2 was found in kidney tissue along with reduced PKC expression which is one of the key regulators of SGLT2. Our study explored SGLT2 as a selective target of swertisin for its swift glucose-lowering action which not only inhibits SGLT2 but also reduces its expression in diabetic condition. Thus, the potential property of swertisin as a glucose-lowering agent is remarkable which points towards the likelihood of a wider avenue of diabetes therapy.

1. Introduction

A state of hyperglycemia is a hallmark characteristic of diabetes mellitus. Sustaining good glycaemic control is a pre-requisite of any effective diabetes therapy. It has been demonstrated that the kidney apart from other organs plays a key role in maintaining normoglycemia in blood. 97% of reabsorption of glucose occurs via Sodium glucose co-

transporter 2 (SGLT2) and 3% by SGLT1. But this mechanism becomes malabsorptive in diabetes [1] and hence hyperglycemia persists. Hyperglycemia also affects certain protein kinases that are involved in the regulation of SGLT [2,3].

SGLT2 inhibitors are highly explored current class of drugs not only in type 2 diabetes but also in type 1 diabetes as its mechanism is independent of circulating insulin or insulin sensitivity [1]. Gliflozins are

Abbreviations: SGLT2, Sodium glucose co-transporter 2; SGLT1, Sodium glucose co-transporter 1; EL, *Enicostemma Littorale*; HEK293, Human embryonic kidney 293 cell line; Caco2, Cancer coli-2 (human colorectal adenocarcinoma cell line); 2-NBDG, (2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)-2-Deoxyglucose); GLUT, Glucose transporter; PKC, Protein kinase C; pp38MAPK, Mitogen-activated protein kinase; ERK1/2, Extracellular signal-regulated kinase 1/2; OGTT, Oral glucose tolerance test; STZ, Streptozotocin; MIN6, Mouse Insulinoma 6; T1DM, Type 1 diabetes mellitus; T2DM, Type 2 diabetes mellitus.

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commercially available SGLT2 inhibitors for the management of high glucose levels. Gliflozins like canagliflozin, dapagliflozin, empagliflozin inhibit the reabsorption of glucose in the kidney, thereby causing excretion of glucose in the urine (glucosuria) [4]. Apart from SGLT2 inhibition, other potential actions have also been attributed to this class of drugs. One of the SGLT2 inhibitors, empagliflozin treatment was found to be related to improved β -cell function in type 2 diabetic patients and preserving β -cell mass in type 1 diabetes in mice model studies [5,6]. On the other side, canagliflozin also improves β -cell function in patients with type 2 diabetes [7]. Hamamatsu et al. demonstrated that diabetic mice treated with canagliflozin significantly preserved β -cell mass [8]. So gliflozins have beneficial effects on pancreatic β -cells apart from inhibiting SGLT2. The important role of the natural compound phlorizin in the development of SGLT inhibitors has been extensively reported. Besides phlorizin, various flavonoids and especially flavonoid enriched plant extracts have been explored for their glucose lowering effect targeting SGLT2. Phlorizin and naturally available flavonoid glycosides have paved the way for the development of various C glycosides and their analogs [9–11].

One such bioactive, swertisin, from a medicinal plant *Enicostemma littorale* (EL) has been explored as a potent islet neogenic agent by our group [12,13]. SGLT inhibitors have demonstrated protection against failing pancreas [14]. Swertisin being a C-glucosyl flavone has been explored for SGLT2 inhibition using the target prediction tool [15] and explored various metabolic alterations associated with the application of swertisin in both *in vitro* and *in vivo* diabetic models. We have elucidated the role of swertisin in establishing a link between its glucose lowering action and SGLT2 inhibition and proved it an excellent antidiabetic drug.

2. Methods

2.1. *In silico* studies

In silico target prediction of swertisin was done by swiss target prediction tool [15]. We then proceeded for homology modelling of SGLT2 using the I-TASSER server [16]. The model was evaluated by the Ramachandran Plot predicted by the Rampage server [17]. The coordinates for Swertisin (CID: 124034) and Canagliflozin (CID: 24812758) were downloaded from the PubChem database [18]. The ligands were minimized with CHARMM forcefield using Discovery Studio v.20 for 2000 max steps and with Momany-Rone partial charge estimation. The ligands (Swertisin and Canagliflozin) and receptor (hSGLT2) used for molecular docking were prepared using AutoDock Tools 4.2 graphical interface [19]. The PDB coordinates were converted to PDBQT format which includes added charges if necessary, merge non-polar hydrogens, and assign appropriate atom types. Affinity (grid) maps were generated by placing the center of the grid near active site residues. For molecular docking, the AutoDock Vina program [20] was used. The docked complexes were analysed using Discovery Studio Visualizer.

2.2. *In vitro* studies

2.2.1. Chemicals

Swertisin was previously isolated and stored from the whole dried plant of *Enicostemma littorale* as reported [12,13,21]. Canagliflozin was commercially purchased as INVOKANA® (Janssen Pharmaceuticals, Inc.).

2.2.2. Cell culture

HEK293 and Caco2 cell lines were propagated at 37 °C in 5% CO₂ in DMEM high glucose (Gibco#12100-046) supplemented with 1.0% of penicillin-streptomycin (Gibco#15140-122) and 10% FBS (Gibco#10270-106).

2.2.3. Sodium dependent glucose uptake assay

2.2.3.1. Fluorescence microplate analysis. 10,000 cells of HEK 293 or Caco2 were plated in 96-well plates. After 24 h preincubation, cells were washed with sodium free buffer (140 mM choline chloride, 5 mM KCl, 2.5 mM CaCl₂, 1 mM MgSO₄, 1 mM KH₂PO₄, and 10 mM HEPES (pH 7.4, adjusted with 2.5 M Tris). Cells were serum starved and then preincubated with 0, 7.5, 15, 30 and 40 μ g/ml swertisin for 15 min to which was added 2-NBDG (Invitrogen#N13195) in sodium buffer (Sodium buffer contained 140 mM NaCl instead of choline chloride), sodium free buffer, and sodium buffer with 10 μ M cytochalasin B (MP Biomedical#195119) (GLUT inhibitor) [22] for 60 min. After 60 min, the buffers were removed and the cells were rinsed in sodium free buffer and lysed with cold lysis buffer (1% Nonidet P-40, 1% sodium deoxycholate, 40 mM KCl, 20 mM Tris, pH 7.4). The fluorescence intensity was detected on Biotek Synergy HT (USA) microplate reader (Excitation: 485/20, Emission: 528/20). To measure DNA, Hoechst (Himedia#TC266) was added and fluorescence intensity was measured (Excitation: 360/40, Emission: 460/40). 30 μ M canagliflozin was taken as a positive control [23].

2.2.3.2. Fluorescence microscopic analysis. 0.5 \times 10⁶ cells of HEK293 cell line was seeded in 35 mm cell culture dish was grown about 80% confluent. Cells were washed with sodium free buffer thrice after aspiration of cell culture media. Cells were then preincubated for 15 min with and without 7.5 μ g/ml swertisin in sodium buffer with 10 μ M cytochalasin B. 100 μ M of 2-NBDG was added and Live cell imaging was performed with “Objective Lens” “UPLSAPO 20X”, “Objective Lens Mag.” “20.0X” and “Objective Lens NA” “0.75” under a confocal microscope (FV3000 Olympus, USA) with incubation at 37 °C and 5% CO₂. Cells without swertisin treatment were considered as control.

2.3. Protein extraction and western blotting

HEK293 cells or Kidney tissues were harvested and kept on ice. Minced tissue powder or harvested cell lysate were lysed in Laemmli lysis buffer. Total protein concentration was estimated by Bradford's method and 20 μ g or 15 μ g of total protein were resolved and transferred to nitrocellulose membrane for tissues and cells respectively. After blocking was performed for 1 h, blots were subsequently probed with SGLT2 (Abcam#37296), PKC (Millipore#07-264), pp38MAPK (Cell Signalling#9216), ERK1/2 (Cell Signalling#9102), and Beta-actin (BD bioscience#612657) primary antibodies overnight at 4 °C. Blots were then incubated with respective secondary antibodies conjugated with HRP for 1 h at RT. Specific bands of proteins were visualized using enhanced chemiluminescence (ECL) reagent (Bio-Rad) and images were captured on Alliance 4.7 UVI Tec Chemidoc (Uvitech, Cambridge) gel documentation system. Densitometric analysis was carried out by Image J software.

2.4. *In vivo* studies

2.4.1. Animal selection and induction of diabetes and *in vivo* experimental design

6–8 weeks old adult male BALB/c mice were kept at the animal house with 12 h light and dark cycle with water and pellet diet *ad libitum*. These studies were carried out in strict accordance as per the guidelines and approval of the institutional Committee for the Purpose of Control and Supervision on Experiments on Animals, India (CPCSEA) (Protocol no. MSU/BC/IAEC/2016/04). After successfully inducing diabetes with STZ injection (65 mg/kg body weight) for 5 days, the Fasting Blood Glucose of animals was confirmed using Accu-check Performa glucometer (Accu-check, Roche, USA) at regular intervals to monitor their diabetic status. After establishing the STZ induced diabetic mice model, mice were divided into four groups control, diabetic, swertisin, and

canagliflozin treatment. Each group had 8 to 12 mice each. STZ diabetic mice were treated with swertisin (2.5 mg/kg body weight) and other group treated with canagliflozin (10 mg/kg body weight) from 0 day of experiment till the 15th day. Swertisin was administered with saline intraperitoneally. On the penultimate day of the study, mice from each

experimental group were individually housed in metabolic cages by gradual acclimatization with 12 h light and dark cycle with water and pellet diet *ad libitum*. Data were collected in the morning (07:00 h). Animal weight, water, chow consumption, and urine volume were recorded and urine specimens were taken for analysis. Proteinuria was

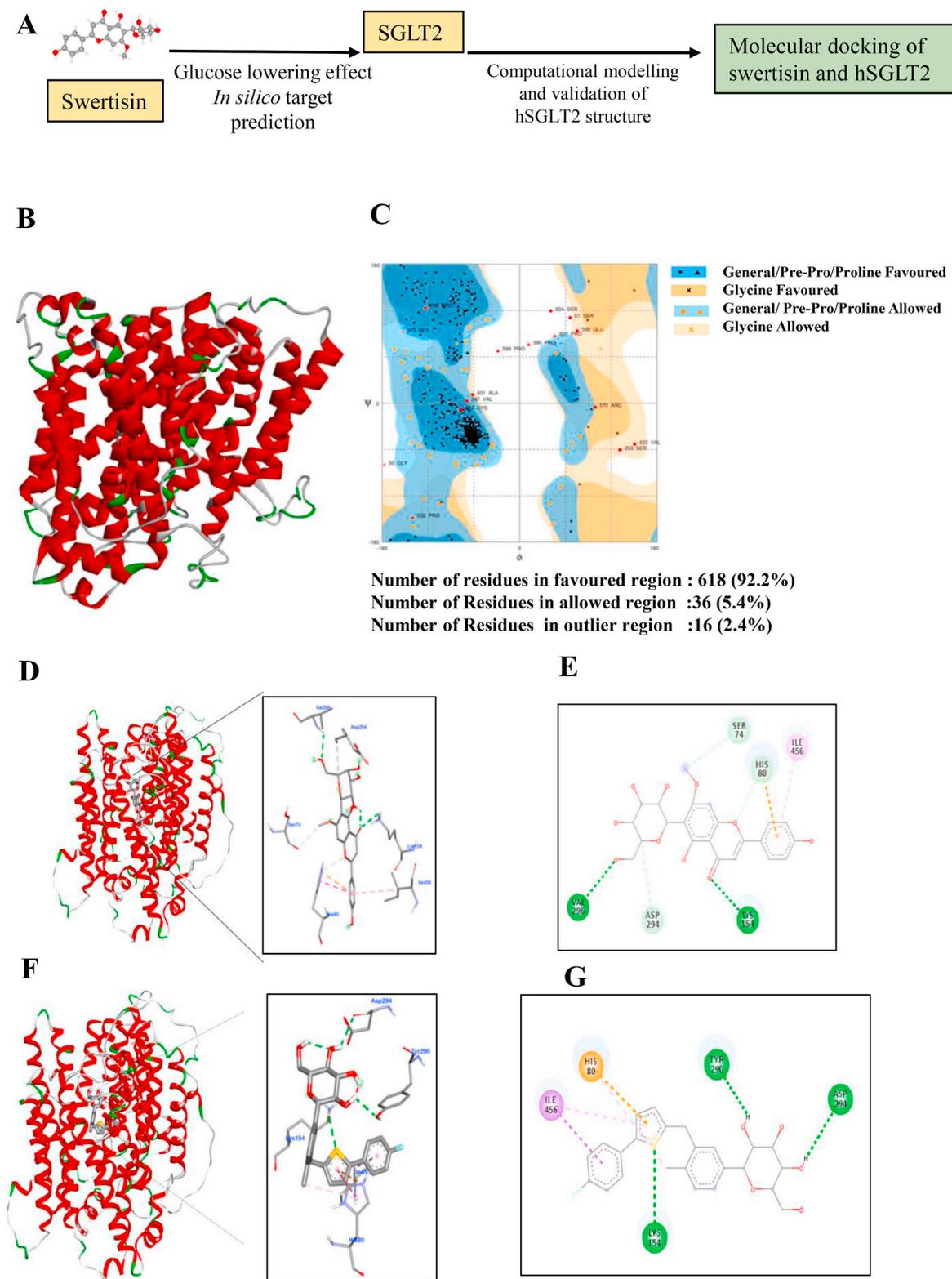


Fig. 1. Swertisin interacts with key residues within the active site of hSGLT2 by molecular docking. (A) *In silico* plan of work (B) Homology model of hSGLT2 (C) Ramachandran plot analysis of the constructed model (D–E) 3D and 2D diagram of molecular docking interaction of Swertisin-hSGLT2 (F–G) 3D and 2D diagram of molecular docking interaction of Canagliflozin-hSGLT2.

analysed. Serum and urine samples were analysed for urea and creatinine using Reckon diagnostics P. LTD.(India) kits. For the endpoint oral glucose tolerance test, glucose tolerance was monitored by taking blood glucose reading at regular intervals until 2 h.

2.5. Statistical analysis

The data is represented as Mean \pm SEM. The significance of difference was evaluated by the Student's t-test or ANOVA by Graphpad prism 7.

3. Results

3.1. Swertisin- SGLT2 docked complex explains SGLT2 inhibitory action of swertisin

Sodium glucose cotransporter 2 was one of the targets of swertisin by the target prediction tool. We used computational docking studies to understand structural complexities and binding affinities between the target (SGLT2) and ligand (swertisin). So, we computationally modelled the human SGLT2 structure and proceeded with molecular docking (Fig. 1A). The homology model of hSGLT2 constructed using I-TASSER was having an acceptable C-score of -0.68 [range: -2 to 5]. I-TASSER uses a multi-template threading approach and for construction of the hSGLT2 model, PDB: 3DH4 and PDB: 2XQ2 (Crystal structure of Na/Sugar symporter from *Vibrio parahaemolyticus* were used as major templates (Fig. 1B). The hSGLT2 model was evaluated using the Ramachandran Plot with 92.2% residues in the favoured region, 5.4% residues in the allowed region, and 2.4% residues in the outlier region (Fig. 1C). The minimized ligands had final potential energy of 39.62 from initial 90.90 kcal/mol for Swertisin and 20.72 from initial 62.21 kcal/mol for Canagliflozin. Affinity (grid) maps of $15.01 \times 21.51 \times 18.98$ Å grid points with 29.48, -41.04 , and 54.97 x, y, and z spacing respectively were generated for molecular docking covering important residues in the active site region. For molecular docking of Swertisin with hSGLT2, SGLT2 inhibitor canagliflozin was also used for docking studies. The docking score for the Swertisin-hSGLT2-interaction and Canagliflozin-hSGLT2-interaction were determined to be -8.5 and -8.7 kcal/mol respectively. In the case of Swertisin, S74, H80, K154, D294, and V296, formed h-bond with C15, O, O1, C10, and O3 atoms of swertisin respectively. The I456 and H80 of hSGLT2 formed a Pi-Alkyl and Pi-Cation interaction with the benzene ring of swertisin respectively (Fig. 1D and E). Two hydrogen atoms of canagliflozin (H9 & H11) formed hydrogen bonds with T290 and D294, whereas K154 formed an h-bond with S1 atom. H80 of hSGLT2 formed a Pi-Cation interaction with a five-carbon ring of canagliflozin and Pi-Alkyl interaction with C14 atom. The I456 of hSGLT2 formed a Pi-Sigma interaction with a benzene ring and Pi-Alkyl interaction with a five-carbon ring of canagliflozin (Fig. 1F and G). The overall interaction pattern indicates a stable interaction of swertisin within the active binding site of hSGLT2 which is also demonstrated by canagliflozin. Thus *in silico* data gives a similar activity of swertisin with canagliflozin suggesting swertisin a potential candidate for SGLT2 inhibitor.

3.2. Swertisin suppresses sodium dependent glucose uptake by selectively inhibiting SGLT2

SGLT2 and GLUT2 are major glucose transporters present in the kidney which facilitates glucose reabsorption in the blood which is sodium dependent and independent respectively [9]. Since the computational analysis affirmed the interaction of swertisin to that of SGLT2 which is mainly expressed in the kidney; *in vitro* investigation was performed to assess the sodium dependent and independent 2-NBDG uptake by swertisin in the HEK293 cell line. To probe whether swertisin interacts with GLUT transporter, uptake was performed in sodium free buffer. SGLT2 dependent uptake was again followed by sodium buffer

with cytochalasin B respectively (Fig. 2A). Cytochalasin B is well characterized for inhibition of glucose transport by GLUTs [24,25]. It inhibited 2-NBDG uptake in pancreatic [26] and endothelial cells [27]. Some biochemical studies have demonstrated that cytochalasin B binds at or near to the sugar export site of GLUT1 in RBCs [28].

These tests revealed that 7.5 $\mu\text{g/ml}$ concentration of swertisin strongly inhibited sodium dependent glucose uptake, reducing 2-NBDG from 100% to 51.4% and 30.4% in the absence or presence of cytochalasin B respectively compared to control. Uptake inhibition was consistent even at higher concentrations of swertisin (Fig. 2B and D). Whereas canagliflozin demonstrated reduced 2-NBDG by 34.36% from 100% at 13 $\mu\text{g/ml}$ in sodium buffer with cytochalasin B compared to control. These results point out almost similar degree of glucose uptake inhibition by swertisin at a much lower dose than canagliflozin. As anticipated our sodium independent uptake of 2-NBDG experiment demonstrated that it was unaffected by swertisin (Fig. 2C).

Further, fluorescence microscopy analysis demonstrated time-dependent intracellular accumulation of 2-NBDG in HEK293 cells in control where it preferentially localizes inside the cell. Whereas striking aggregation of 2-NBDG on HEK293 cell membrane in swertisin treated cells was evident where 2-NBDG can be observed as confined to the HEK293 cell membrane (Fig. 2E). (See the supplemental material for live confocal imaging video). These results further strengthen our conviction about the SGLT2 inhibition property of swertisin.

Supplementary video related to this article can be found at <https://doi.org/10.1016/j.ceramint.2019.04.220>

To study the effect of swertisin on SGLT1, we performed similar sodium dependent and independent 2-NBDG uptake in Caco2 cell line (Supplementary Fig. 1 A-D). We observed unaltered and non-significant inhibition of glucose uptake even at a higher concentration of swertisin. Thus, swertisin displayed higher selectivity of SGLT2 rather than SGLT1 at lower inhibitory concentration compared to canagliflozin.

3.3. Swertisin regulates key SGLT2 related proteins

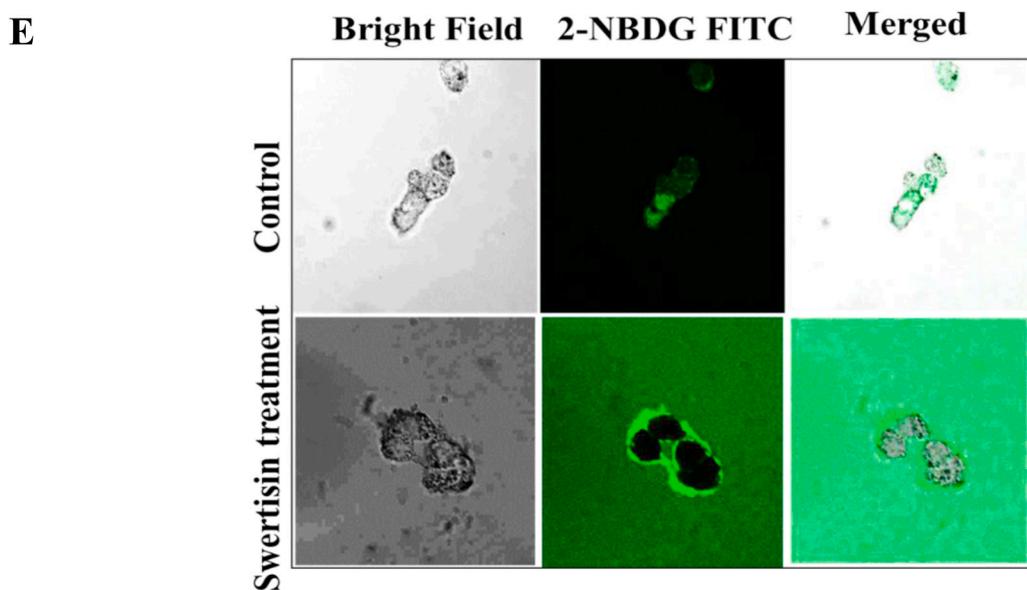
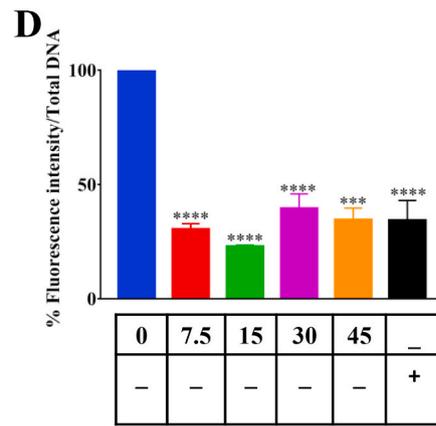
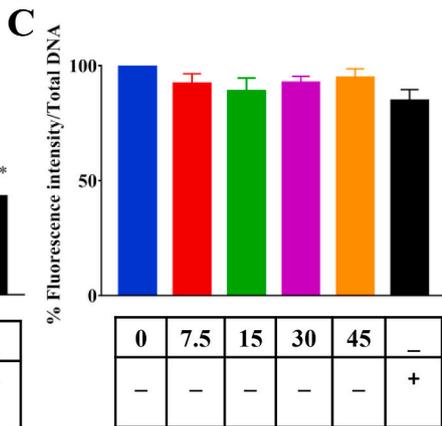
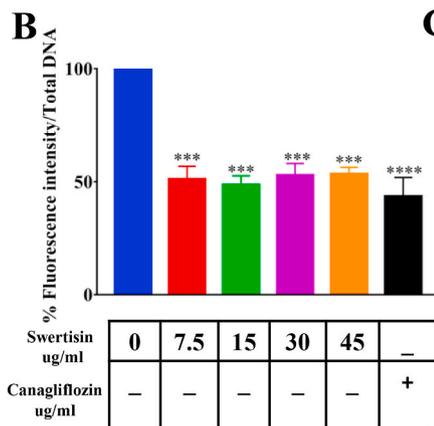
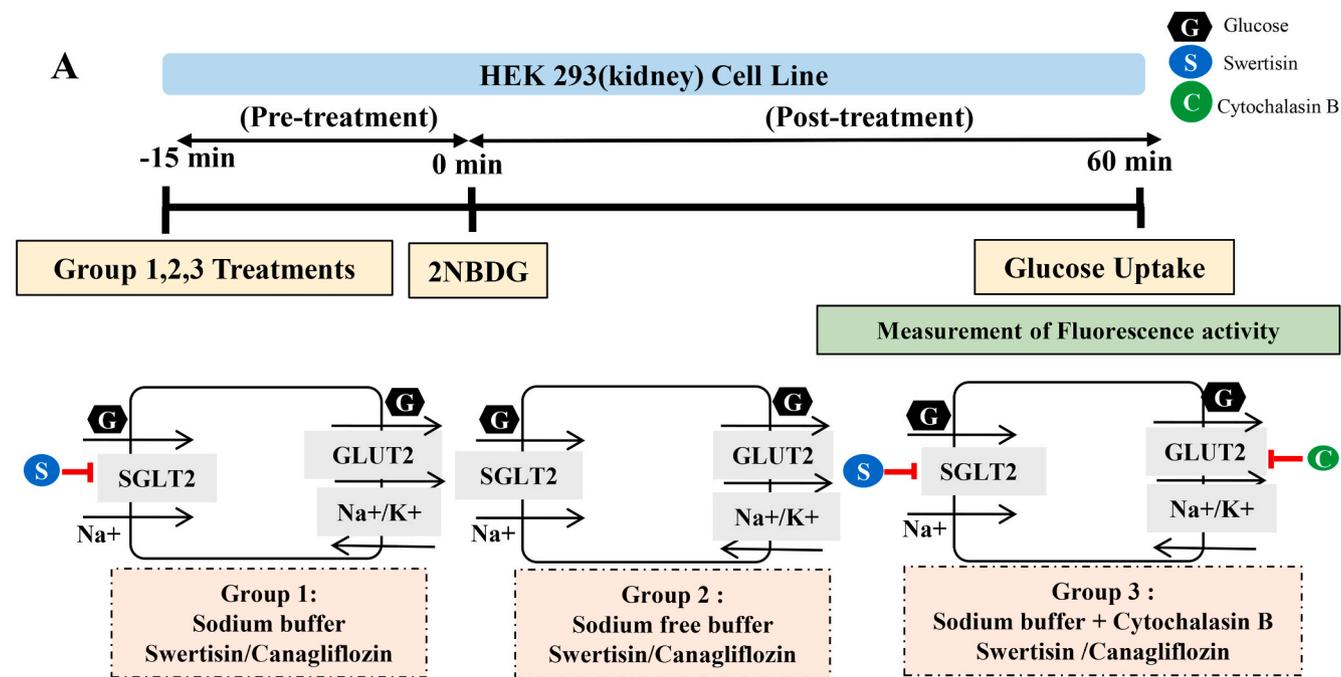
One of the contributing factors in uptake of glucose by the transporters in the cells depends on the expression of the transporter protein which in turn is regulated by many factors. So we wanted to determine whether swertisin contributed to the regulation of SGLT2 expression, we examined the time-dependent expression of SGLT2 in presence of swertisin. As expected, incubation of the HEK293 cell line with 7.5 $\mu\text{g/ml}$ swertisin abolished the induction of SGLT2 expression in a time dependent manner (Fig. 3D). Evident downregulation of SGLT2 protein expression was persisted till 12 h incubation with swertisin compared to control.

It led us to explore the protein expression of kinases which contributes to the regulation of SGLT2. Stimulation of Protein kinase C is known to regulate SGLT2. pp38 MAPK and *Erk1/2* also plays role in regulating the expression of the SGLT2 [2,3] In the present study, we observed downregulation of SGLT2 with the upregulation of PKC (Fig. 3A) from 4 h till 12 h of post swertisin treatment incubation.

SGLT2 regulation by PKC involves pp38 MAPK (Fig. 3B) which also demonstrated increased expression than control at 12 h. Another important kinase is ERK1/2 (Fig. 3C) and non-significant change was observed until 12 h. Thus, temporal analysis of SGLT2 levels with swertisin treatment demonstrated overall downregulation of SGLT2 (Fig. 3D) with differential regulation of regulating proteins.

3.4. Swertisin improves glycaemic control in STZ induced diabetic mice: an *in vivo* study

In silico and *in vitro* results established the association of swertisin to that of SGLT2. To get a deeper understanding of SGLT2 inhibition by swertisin, we then proceeded for *in vivo* model to get insight of swertisin SGLT2 inhibitory action at a physiological level by monitoring the efficacy of swertisin in STZ induced diabetic Balb/c mice. The effect of



(caption on next page)

Fig. 2. SGLT2 specific inhibition by swertisin affects sodium dependent glucose uptake. (A) *In vitro* plan of work. Sodium dependent glucose uptake assay was performed in the HEK293 cell line. Swertisin treatment was given at varying doses and uptake inhibition of 2-NBDG was performed in (B) sodium buffer (C) sodium free buffer and (D) sodium buffer with 10 μ M cytochalasin B (GLUT inhibitor) for 60 min. Canagliflozin was taken as a positive control. Results are represented as % Fluorescence intensity per total DNA \pm SEM, N = 3. Significance is expressed as p-value *** <0.001, **** <0.0001 control vs treatment groups. (E) Representative time dependent fluorescence imaging of uptake was performed HEK293 cells were incubated in sodium buffer in the absence (Control) and presence of 7.5 μ g/ml swertisin with 10 μ M cytochalasin B for 10 min in presence of 2-NBDG (green) (Magnification:20X) G = Glucose, S=Swertisin, C=Cytochalasin B. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article). (See the supplemental material for live confocal imaging video)

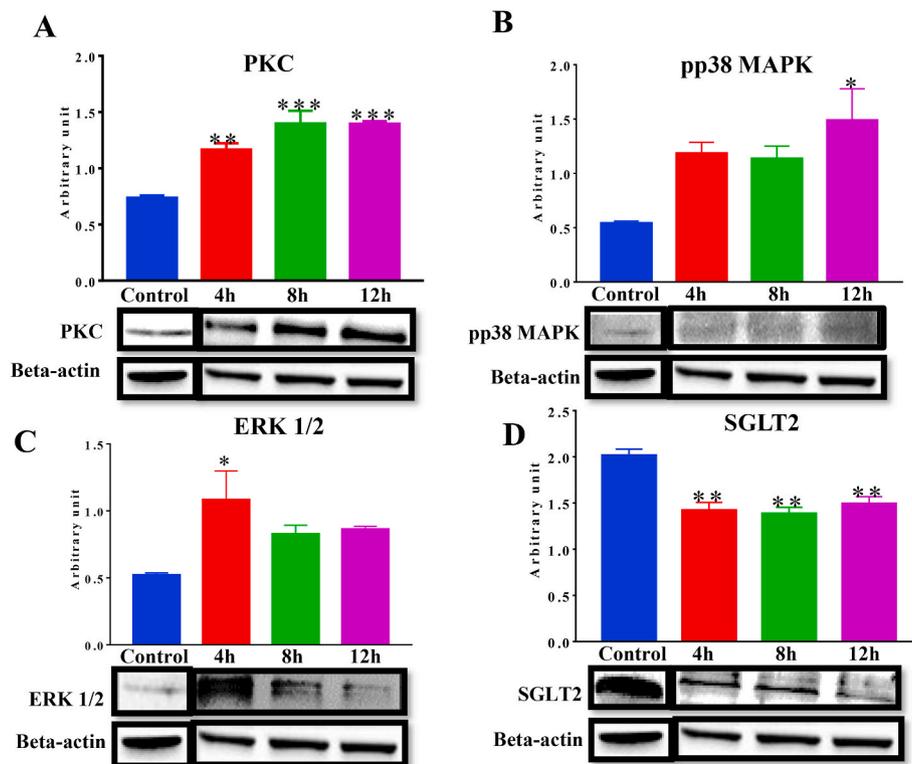


Fig. 3. Swertisin selectively regulates SGLT2 expression. Time dependent protein expression of SGLT2 and regulating factors in the HEK293 cell line were studied. Western Blot analysis of proteins (A) PKC, (B) pp38 MAPK (C) ERK1/2, and (D) SGLT2 along with densitometric analysis normalized to Beta-actin are expressed as arbitrary unit \pm S.E.M. N = 3, Significance is expressed as p-value * <0.05, ** <0.01, *** <0.001 control vs treatment groups.

swertisin and canagliflozin was monitored over 15 days in diabetic mice. Swertisin dose was initiated at day 0 (326 ± 46.11 mg/dl) of treatment and reduced levels of fasting blood glucose was evident right from day 5th of treatment (253 ± 30.72 mg/dl), day 10th (230 ± 61.75 mg/dl) and persisted till day 15th (124 ± 19.08 mg/dl) (Fig. 4A). Remarkably the extent of glucose lowering effect of swertisin was observed at a much lower dose (2.5 mg/kg body weight) compared to positive control drug canagliflozin (10 mg/kg body weight). These results prove a higher potency of swertisin than canagliflozin for demonstrating sustainable and consistent glucose lowering effect. An oral glucose tolerance test (OGTT) was performed on Day 15th on overnight fasted mice which demonstrated controlled glycemia over the period of 2 h (Fig. 4B). As predicted, OGTT results were also comparable with canagliflozin treated mice.

3.5. Swertisin affects physiological and metabolic parameters in STZ induced diabetic mice

In silico and *in vitro* results established the association of swertisin to that of SGLT2. We then examined several physiological parameters in all the treatment groups of mice. Inhibition of SGLT2 is expressed as changes in various metabolic parameters ultimately affecting glucose homeostasis. STZ induced weight loss in mice is commonly observed and

was persistently detected in swertisin and canagliflozin treated mice (Fig. 5A). Chow intake of the Diabetic and Canagliflozin group was significantly increased compared to control mice (Fig. 5B). On the contrary, swertisin and canagliflozin groups demonstrated decreased water intake compared to the diabetic (Fig. 5C). Urine output was significantly higher in diabetic, swertisin, and canagliflozin groups with respect to control (Fig. 5D).

Glucosuria and proteinuria are a few of the important characteristics of diabetes [9,29]. Swertisin and the canagliflozin group demonstrated lesser proteinuria compared to a diabetic with the least proteinuria in the swertisin group (Fig. 5E). Glucosuria was higher in swertisin and canagliflozin groups compared to the diabetic group with the swertisin group showing the highest glucosuria (Fig. 5F).

SGLT2 inhibition affects renal functions and pertains to intrarenal and extrarenal effects [4]. Serum (Fig. 5G) and urine (Fig. 5H) creatinine levels were increased in diabetic mice compared to control mice. Creatinine clearance (Fig. 5I) was higher in a diabetic with respect to control mice. Serum urea decreased in swertisin and canagliflozin treatment compared to the diabetic group (Fig. 5J). Urine urea increased in swertisin treatment compared to the diabetic group (Fig. 5K). Urea clearance increased in swertisin treatment and canagliflozin treatment concerning diabetic (Fig. 5M).

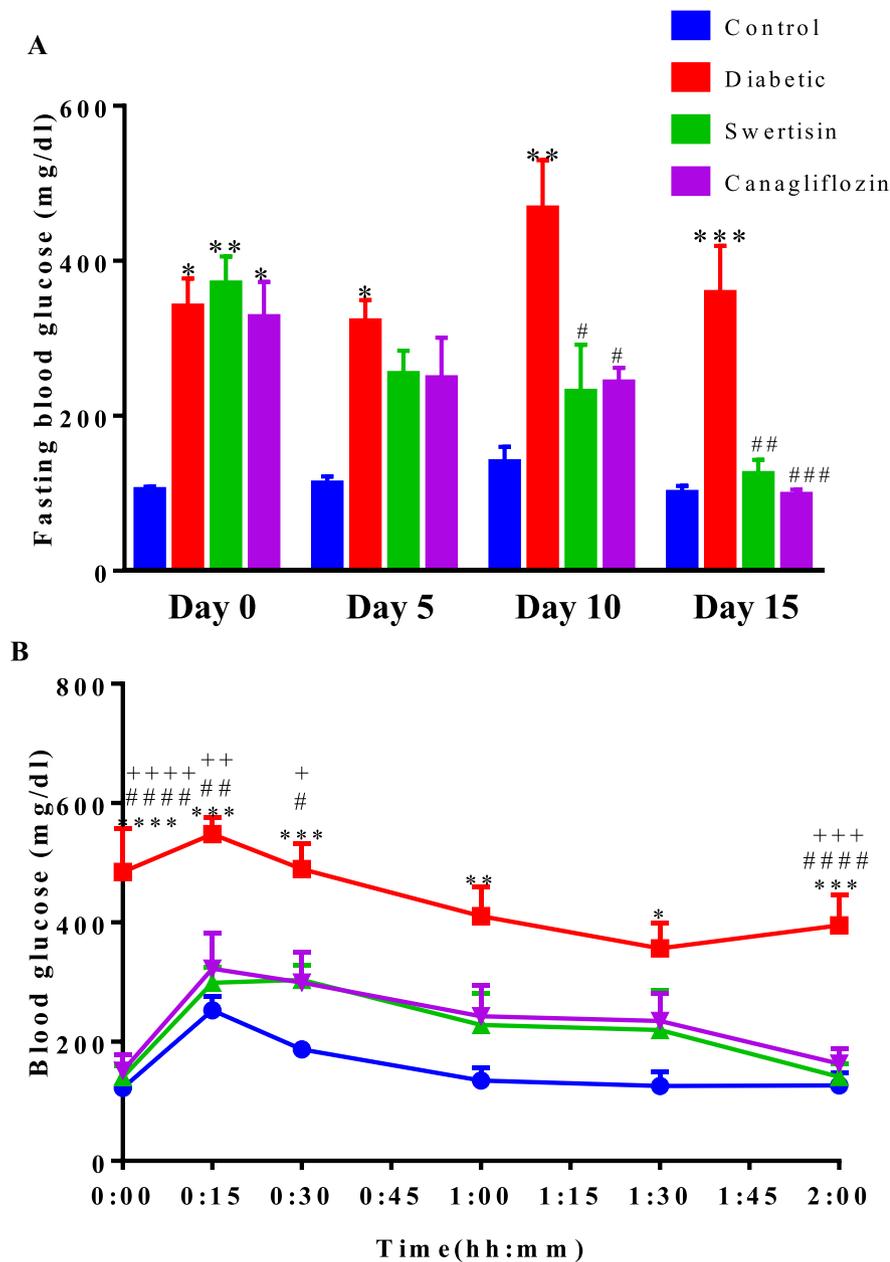


Fig. 4. Swertisin ameliorates glycaemic control in STZ induced diabetic mice. (A) Graph representing fasting blood glucose at different days of treatment for control, diabetic, swertisin and canagliflozin treated STZ diabetic BALB/c mice groups. Data are represented as mean \pm SEM. * <0.05 , ** <0.01 , *** <0.001 Control vs treatment groups # <0.05 , ## <0.01 , ### <0.001 Diabetic vs treatment groups (N = 8-12) (B) Graph representing blood glucose levels for oral glucose tolerance test over 2 h for control, diabetic, swertisin and canagliflozin treated STZ diabetic BALB/c mice. Data are represented as mean \pm SEM. * <0.05 , ** <0.01 , *** <0.001 , **** <0.0001 Diabetic vs control, # <0.05 , ## <0.01 , ### <0.001 , #### <0.0001 Diabetic vs swertisin treatment. Diabetic vs canagliflozin treatment + <0.05 , ++ <0.01 , +++ <0.001 , ++++ <0.0001 (N = 8).

3.6. Swertisin reduces expression of SGLT2 along with inhibition in kidney tissue of mice

Reduction in SGLT2 expression in diabetic condition has been demonstrated to improve the overall glucose homeostasis of the body by reducing the reabsorption of glucose in the blood by the kidney [30]. We next addressed the question of whether swertisin affects the expression of SGLT2 in the kidney. After 15 days of treatment increased expression of SGLT2 in the diabetic and canagliflozin treated group were observed compared to control. However, the most striking result to emerge from the data is the critically reduced expression of SGLT2 which was observed in the swertisin group as compared to diabetic and canagliflozin treated mice (Fig. 6B). We also assessed PKC expression under swertisin action and favorably found that swertisin treatment not only reduced SGLT2 expression but also reduced PKC expression as well compared to diabetic (Fig. 6A). PKC was found upregulated in diabetic, swertisin, and canagliflozin groups. PKC was also found upregulated in the canagliflozin group compared to diabetic and swertisin groups.

These results offer indisputable evidence that swertisin action involves the reduction in SGLT2 expression along with inhibition of SGLT2 action which clearly shows an advantage over canagliflozin which only inhibits the action of SGLT2 with unaltered SGLT2 expression.

4. Discussion

The primary goal of this study was to establish the glucose lowering action of swertisin to its SGLT2 inhibition property. Our *in silico* data demonstrated stable molecular binding of swertisin with SGLT2 by molecular docking. Marketed SGLT2 inhibitor canagliflozin served as a comparator for our studies [31]. Appreciably, the docking scores for Canagliflozin-hSGLT2 (-8.7 kcal/mol) and Swertisin-hSGLT2-interactions (-8.5 kcal/mol) were similar. This result strongly pointed to the likelihood of steady binding of swertisin with SGLT2.

Asp 294 of SGLT-2 is important for sugar-binding. Both luteolin and orientin, bioactive flavonoids, indicated *in silico* interaction with Asp 294 [32]. Similarly, our structural docking study demonstrated that C10

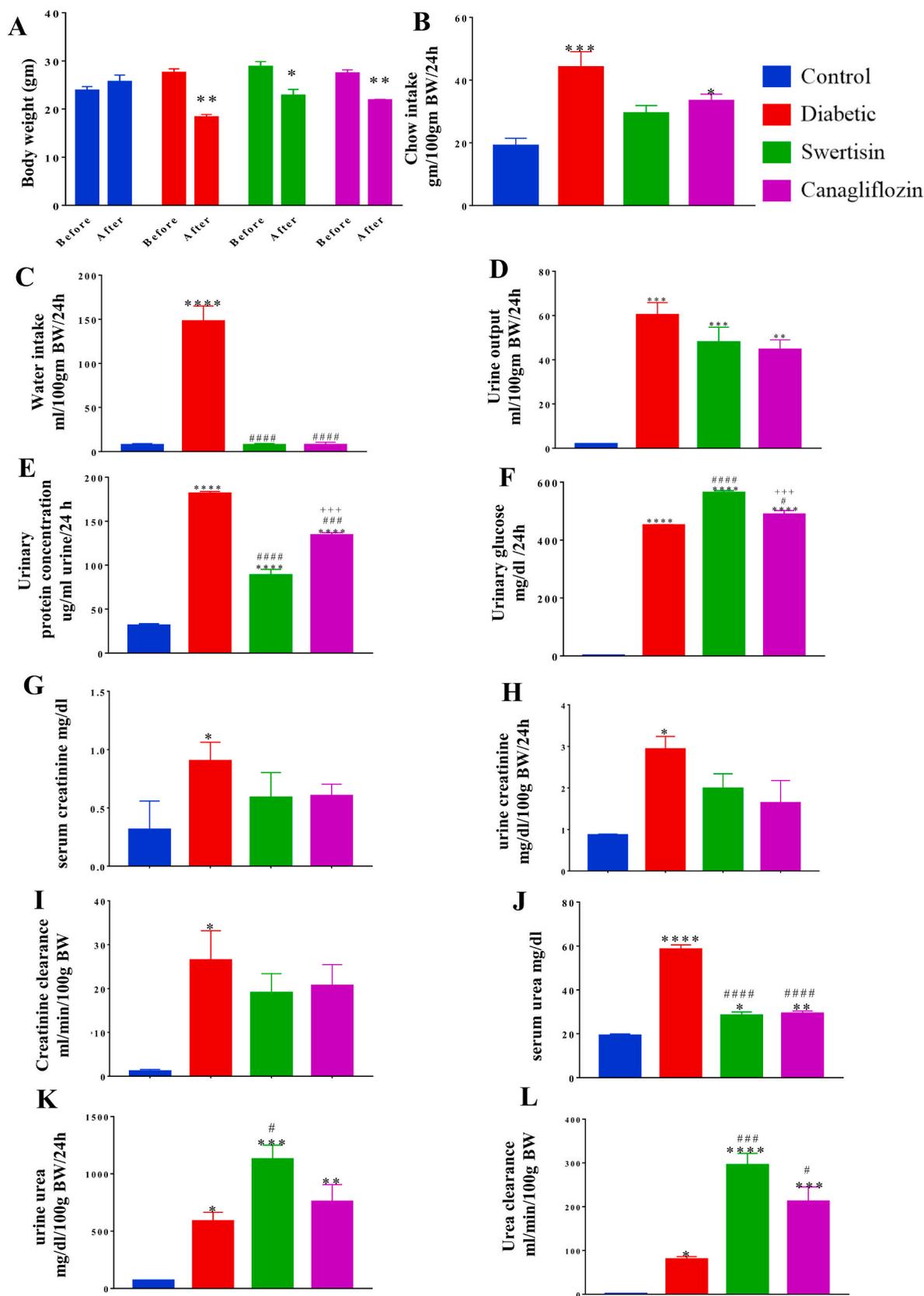


Fig. 5. Swertisin impacts physiological and metabolic parameters in STZ induced diabetic mice. Graphs representing different parameters (A) Body weight (B) Chow intake (C) water intake (D) urine output (E) proteinuria (F) glucosuria (G) serum creatinine (H) urine creatinine (I) creatinine clearance (J) serum urea (K) urine urea (L) urea clearance for control, diabetic control, swertisin and canagliflozin treated STZ diabetic BALB/c mice groups. Data are represented as mean \pm SEM. * <0.05 , ** <0.01 , *** <0.001 , **** <0.0001 Control vs treatment groups # <0.05 , ### <0.001 , #### <0.0001 Diabetic control vs treatment groups. Swertisin treatment vs canagliflozin treatment +++ <0.001 (N = 8).

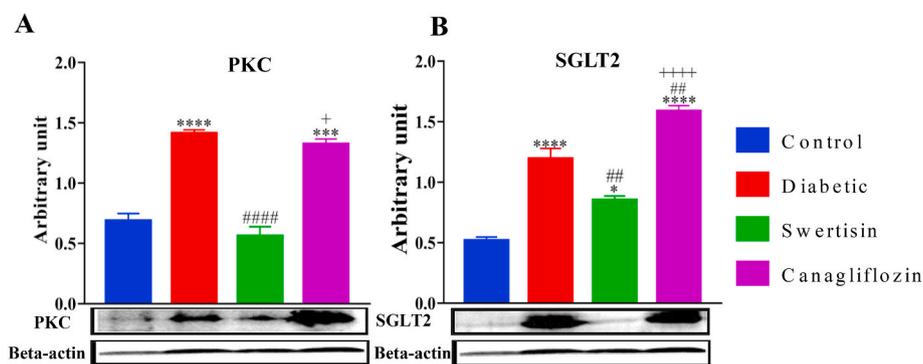


Fig. 6. SGLT2 and PKC expression is reduced by swertisin in mice kidney. Western Blot analysis of proteins PKC and SGLT2 along with densitometric analysis normalized to respective internal control Beta-actin. Data are represented as mean \pm SEM. $p < 0.05$, $*** < 0.001$, $**** < 0.0001$ Control vs treatment groups $## < 0.01$, $#### < 0.001$ Diabetic control vs treatment groups. Swertisin treatment vs canagliflozin treatment $+ < 0.05$, $++++ < 0.0001$ (N = 3).

atom of swertisin formed h-bond with Asp 294 which was a conserved pattern followed by canagliflozin as well.

The marked observation that emerged from our *in vitro* sodium dependent glucose uptake experiment was that the potency of SGLT2 inhibition by swertisin was significantly effective at a lower dose as against canagliflozin.

The selectivity for SGLT was confirmed by no inhibition of glucose uptake by swertisin in sodium free buffer. Inhibition of GLUT transporters leads to undesirable and adverse consequences due to the central key role of GLUT transporters in glucose homeostasis and metabolism [10]. Our findings confirm that swertisin does not interfere with non-sodium dependent GLUT transporters and further substantiates the selective inhibition for SGLT2 as against GLUT2 transporter.

In agreement with uptake results, aggregation of 2-NBDG outside the cell membrane of the kidney cell line critically confirms inhibition of SGLT2 by swertisin. This observation correlates favorably well with Ishihara et al. who reported the uptake of 3-O-methyl-D-glucose which is rapid and equilibration is 80% complete in 1 min in MIN6 cells [33].

Further, additional support for the selectivity of swertisin was demonstrated using the Caco2 (intestinal) cell line having SGLT1 as a major glucose transporter. SGLT1 inhibition can result in gastrointestinal side effects such as dehydration, diarrhea, and malabsorption since it is mainly expressed in the small intestine and helps in the absorption of glucose and galactose [34]. As expected, we observed a non-significant change in sodium dependent glucose uptake even at higher doses of swertisin. This can be highly attributed to the C glycosylation of the swertisin structure. Compared to phlorizin and O-glucosyl analogs, C-glycosylation enables achieving higher selectivity for SGLT2 over SGLT1 and GLUT [10]. Panchapakesan et al. reported that SGLT1 and GLUT2 expression are unaffected by hyperglycemia and also unaffected by inhibition of SGLT2 [35].

Further we investigated regulation of SGLT2 expression under the effect of swertisin with differential expression of some protein kinases. An important observation was the reduced expression of SGLT2 protein. PKC and MAPK pathways play a major role in regulating SGLT2 under hyperglycaemic conditions [3]. Upregulated PKC expression is often correlated with upregulated SGLT2 expression [1]. Although hyperglycemia-induced high expression of PKC was observed by swertisin treatment with downregulation of SGLT2 in *in vitro* study, it can be interpreted that swertisin does not affect PKC expression in acute treatment duration. Similarly, the expression of pp38MAPK and ERK1/2 was found to be higher. Haneda et al. lend support to high levels of ERK via hyperglycemia-induced PKC in the HEK293 cell line [2] which summarizes the differential regulation of PKC-MAPK pathway in the kidney by swertisin.

We then moved to a pre-clinical *in vivo* animal study with some inexplicable insights. Observation of a striking reduction in fasting blood glucose of STZ treated mice by swertisin treatment substantiated

our findings of improved glycaemic index. This is in good agreement with Liang et al. where canagliflozin improved glycaemic control in terms of blood glucose. Canagliflozin has also reported improved oral glucose tolerance test [31] which was evident for swertisin. Our finding highlights the fact that the improvement in glycaemic control by swertisin is strikingly at par with canagliflozin.

Body parameters are important for evaluating diabetogenic and metabolic parameters. Bodyweight loss in STZ induced swertisin treatment group was remarkably evident before and after treatment. Further weight loss is also aided by canagliflozin. Ji et al. further reported canagliflozin treatment increases body weight loss in diabetic mice [36]. A similar contribution of swertisin for weight management is noteworthy in our *in vivo* study.

Chow intake did not alter significantly in the swertisin group compared to the diabetic but intake was higher in the canagliflozin treated group compared to control which is also evident by Matsuba et al. who also observed that despite the increase of the calorie intake, weight loss was evident with better glycaemic control [37]. Water intake was significantly lowered but an increase in urine volume in swertisin and canagliflozin groups compared to diabetic was observed as a contrast to Tanaka et al. who found that canagliflozin did not significantly inhibit water intake but also observed increase in urine volume as exhibited by diabetic patients [38]. The most striking observation of SGLT2 inhibition is glucosuria along with proteinuria which correlates favorably well with reports [9,29]. Though there were differences among the control and diabetic group, the creatinine and urea levels were within normal physiological range and thus rule out the possibility of kidney dysfunction so to generate a strong possibility of effects emerging from exclusive SGLT2 inhibition which is the major focus of the study and not from kidney damage which is usually seen in chronic diabetic nephropathy models [29].

Renal SGLT2 expression was found upregulated in untreated diabetes in humans as well as T1DM and T2DM murine models. Higher PKC expression is found in the kidney in diabetes [1]. Rahmoune et al. have also observed high glucose uptake and upregulated SGLT2 expression in diabetic patients [30].

The most remarkable observation to emerge from these data was that swertisin was able to downregulate the protein expression of SGLT2 in contrast to canagliflozin. This is in good agreement with Maki et al. where they found that high glucose-induced increased expression of SGLT2 was not affected significantly by canagliflozin [39]. On the contrary, swertisin reduced SGLT2 expression which makes swertisin a better option than canagliflozin in diabetes therapeutics.

Diabetes being a multiorgan multitarget metabolic syndrome encompasses various receptors, transporters and proteins which play a key role in management of diabetes [40]. Having demonstrated the functional property of swertisin as SGLT2 inhibitor, another interesting aspect of SGLT2 as demonstrated by Lee et al. is its antagonistic role to

adenosine A1 receptor (A1AR). They demonstrated the ameliorating effects of swertisin on scopolamine-induced memory impairment via the involvement of adenosine (ADO)/A1AR signalling [41]. This signalling also contributes to insulin-controlled glucose homeostasis [42]. Though to delimit the scope of current study, cross talk of A1AR and SGLT2 inhibitor has not been addressed, but it would be intriguing and important to document link between swertisin, ADO/A1AR signalling and improvement in glucose homeostasis as future prospects.

5. Conclusion

Based on *in silico*, *in vitro*, and *in vivo* studies our research has highlighted the importance of SGLT2 inhibition and its reduced expression by swertisin. The direct role of swertisin in controlling hyperglycemia makes it an excellent pharmacophore agent that can ease the burden of diabetes healthcare management by providing holistic treatment and a foremost candidate for SGLT2 inhibitors.

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Authors contribution

GB Conceptualization, Methodology, Investigation, Software, Data curation, Formal analysis, Writing- Original draft preparation MV Supervision, Methodology, Validation, Reviewing and Editing AS Supervision, Methodology DP, AP, PM, RS software and *in silico*, HS Methodology, Investigation, Data curation SG Conceptualization, Visualization, and Supervision.

Declarations of competing interest

The Authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.abb.2021.108995>.

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THE IMPACT OF COVID-19 PANDEMIC ON THE STUDENTS IN HIGHER EDUCATIONAL INSTITUTIONS IN INDIA: TRENDS, CHALLENGES AND POSSIBILITIES

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Abstract

The proposed paper intends to elucidate the impact of Covid-19 pandemic on the lives of students who are pursuing their studies in different universities in the country. Thus, the paper is divided into two sections. First section intends to rely upon secondary sources and second section intends to evaluate the impact of Covid on the students who are pursuing education in the Universities based on the responses received through Google questionnaire. The paper section emphasises on Covid and its impact and spread across the world and eventually discusses negative impact on higher education. The second section deals with data presentation and analysis with the support of tables. Third section emphasises on findings and conclusions. In the light of this, it essential to understand Covid and its spread in the world. The main research questions framed that are what type of problems students have been facing during online classes? What is the impact of online classes on students' academic performance? What is the impact of Covid-19 on lifestyles of students? The research methodology that adopted to study the impact of Covid-19 pandemic on students in the universities for the current research project, online survey was carried out using an online Google form. The questionnaire was distributed online to students across selected universities in the country.

Introduction

COVID-19 Pandemic had tremendous impact on the economy, health, employment, state, non-state actors, urban, rural, organised, unorganized labour, etc., In other words, entire society implicitly or explicitly effected adversely due to this unprecedented pandemic. A pandemic is generally defined as a new disease that rapidly spreads in a number of countries and continents. Even a mild pandemic can kill several millions of people. Over the past hundred years, world witnessed and seen three deadly pandemics, namely, in 1918, 1957 and 1968. The most recent



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Introduction

This Essay aims to sketch a brief history of disarmament debates within the United Nations. It also traces and examines the transformation that occurred in the post-Cold War era and aims to analyse the structural and institutional challenges of dealing with this issue within the UN system. It argues that the Charter of the United Nations was preatomic, and therefore, was neither created nor equipped to deal with the challenge of nuclear weapons-related issues. Therefore, the 1945 modelled UN will have to address the inherent structural deficiencies in its machinery and invest more in multilateral outcomes if it wants to deal with the issue of nuclear weapons. No doubt, the UN has to play an important role, but it will have to reinvent, restructure, and reform itself to make itself relevant in today's world.

The issue of disarmament has been on the agenda of the United Nations (UN) since its inception. As we know, the Charter of the UN was signed on June 25, 1945, three weeks before the United States exploded the atomic bomb. Since the Manhattan Project, which produced the first bomb, was a secret project, the Charter did not have any reference to either nuclear energy or the bomb. Nonetheless, the scope and the character of this San Francisco document did provide the basic set of reference points to deal with the problem of international peace and security. The advent of weapons of mass destruction or the WMDs posed a threat of annihilation to the entire mankind, eventually transforming global politics and the rules of conduct in international affairs. To deal with this challenge, the UN was empowered to deliberate any issue deemed to be relating to international peace and security. Within the broad parameters, the UN started deliberations on disarmament.

Etymologically, disarmament is defined as an 'act of reducing, limiting, or abolishing weapons'. In a nuclear context, it means the total elimination of WMDs. The United Nations General Assembly (UNGA) defined General and Complete Disarmament (GCD) as the elimination of all WMDs, coupled with 'balanced reduction of armed forces and conventional armaments, based on the principle of undiminished security of the parties'. However, even within the UN system, the issue of disarmament was conditioned and constrained by the ideological confrontation, which marked the Cold War period. The UN, too, became a prisoner of the power rivalries between the US and the USSR. The collapse of the Soviet Union did not completely alter the discourse of

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To Assess the Impact of Government Public Health Services in Gujarat: A Case Study of Dang District

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Abstract: The paper examines the objectives and attainments towards major issue of awareness of households, economic conditions, economic and social groups, illiteracy rate of households, and problems in the government service system in the Gujarat State particularly, in the Dang District. These facts need detailed investigation and to find out various reasons behind it. The contemporary study has tried to focus on ignore aspects and study has measured effectiveness of health spending on marginalized section with focusing public policies target to take major enhancement in child health and literacy other indicators. The present study concluded the importance to contribute to improve the health care level services system and finds drawbacks in health delivery system.

Index Terms: Public health services, sanitation, drinking water, hospital facilities,

I Introduction

Health is an actual wealth of the country, Health improvement is one of the leading goal of the states. Health dependance on different aspects such as mental level, emotional as well social health and most important factor is economic conditions. In Gujarat particularly in Dang District is having maximum poor families with low income; the involvement of households in progress is highly poor so, definite terms that here arise the research problems throughout this investigation in the study would observe that in different ways government trying to improve the health conditions. Hence, it requirements a very serious consideration with a wide range and highly investigation to examination into numerous factors which highly concerned with public expenditure and health facilities with scientific investigation will positively convey all the probable conclusions in mentioned complications. Therefore, mentioned would positively concrete the tactic for carrying an elaborate with the operative forecasting and strategic schemes; it would benefit the rural households. It would produce an actual with permanent achievement for carrying socio-economic freedom to which lead to good health and highly living standard. In the context, of the above importance of the study we are going into a detail investigation of the Dang district as our primary survey. The study is going to investigate the living and economic standard, the literacy rate of these households, medical facility, medical services and the current study also focused on effectiveness of public spending. The study includes a brief introduction, and review of literature, basic demographic indicators and statistics of sample area of Dang District, the analysis results. Finally, study explained the findings and conclusions.

II Review of Literature

James, J.O., Ojanpinwa T.V, Yussuff R.O, The effect of health effects on 'public health costs and articles', is governed by the influence of public health spending control in Nigeria primarily in Nigeria. A Gani (2009) 'Health care financing and health outcomes in Pacific Island countries' researchers have integrated income and public health expenditure per person, in which the other three measures such as infant mortality rate are rare. J Nixon, P Ulmann (2006), 'The relationship between health care expenditure and health outcomes' T Kuen Kim, S. R. Lane (2013), 'Government Health Expenditure and Public Health Outcomes: A Comparative Study among 17 Countries and Implications for US Health Care Reform' Stephan Klasen (2007), their studies have examined the relationship between development, poverty, weakening, childhood and child mortality in developing countries under nutrition. Sundar Ramamani and A Sharma (2002) examined the patterns of morbidity and health care utilization by the urban poor living in slums and resettlement colonies in Delhi and Chennai



Two distinct monopartite begomovirus-betasatellite complexes in western India cause tomato leaf curl disease

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ABSTRACT

In India, begomovirus infection causing tomato leaf curl disease (ToLCD) is a major constraint for tomato productivity. Here, we have identified two distinct monopartite begomovirus and betasatellite complexes causing ToLCD in the western part of India. A new monopartite begomovirus (Tomato leaf curl Mumbai virus, ToLCMumV) and betasatellite (Tomato leaf curl Mumbai betasatellite, ToLCMumB) were isolated from the Mumbai sample. A distinct Tomato leaf curl Gandhinagar virus (ToLCGanV) and Tomato leaf curl Gandhinagar betasatellite (ToLCGanB) were identified from the Gandhinagar sample. Both of the cloned begomoviruses were recombinants. The demonstration of systemic infection caused by begomovirus (ToLCGanV or ToLCMumV) alone in *N. benthamiana* and tomato (a virus resistant variety) emphasizes that they were monopartite begomoviruses. Co-inoculation of cognate begomovirus and betasatellite reduces the incubation period and increases symptom severity. Thus, Koch's postulates were satisfied for these virus complexes. Further, an enhanced accumulation of ToLCGanV was detected in the presence of cognate ToLCGanB, however ToLCMumB did not influence the level of ToLCMumV in the agro-inoculated tomato plants. Our results indicate that the cloned viruses form potential virus resistance breaking disease complexes in India. This necessitates to investigate the spread of these disease complexes to major tomato growing regions in the country.

The GenBank accession numbers of the sequences reported in this paper are KC952005, KC952006, MH577022 and MH577037.

1. Introduction

Family *Geminiviridae*, is one of the largest family of plant viruses, causing severe crop loss in monocots and dicots (Varma and Malathi, 2003). It is categorized into nine genera based on pairwise sequence identities, genome arrangement, host range and insect vector (Zerbini et al., 2017). Insect-transmitted geminiviruses are characterized by their incomplete icosahedral particles which encapsidate circular, ssDNA of ~2.5–3.1 kb in size (Brown et al., 2012). As geminiviruses possess

limited coding potential, they have evolved to co-opt with several plant and insect proteins to complete its life cycle (Kumar, 2019; Kumar and Shivaprasad, 2020). *Begomovirus* is the largest genus in this family comprising >440 virus species and is sub-classified into bipartite (with DNA-A and DNA-B) or monopartite (containing DNA-A like genome) (Brown et al., 2012; Zerbini et al., 2017). Majority of the monopartite begomoviruses were reported from the 'Old World' (OW; Africa, Asia and Europe) whereas bipartite begomoviruses were prevalent in the 'New World' (NW; the Americas) with a few exceptions of monopartite begomoviruses (Brown et al., 2012; 2015). Monopartite begomoviruses are often found to be in association with circular, ssDNA satellites of ~1.3 kb, namely, betasatellites (Family: *Toleucusatellitidae*) and

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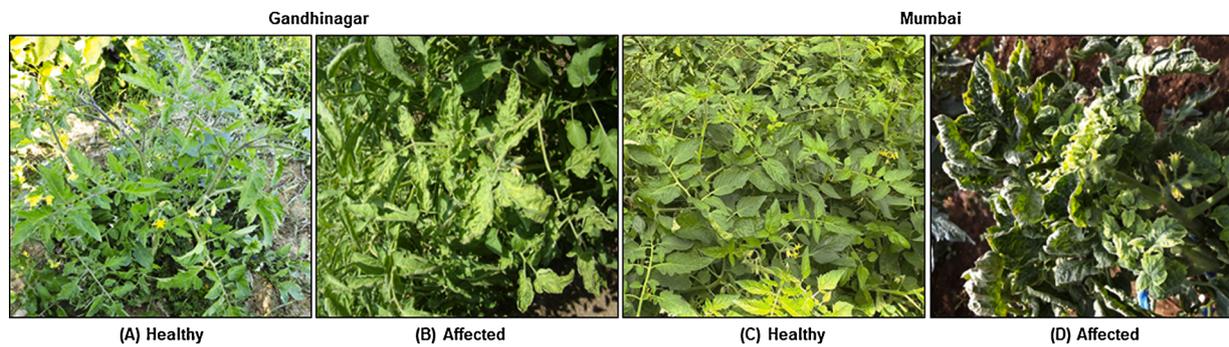


Fig. 1. Tomato plants exhibiting symptoms of tomato leaf curl disease. Asymptomatic tomato plants in the Gandhinagar (A) and Mumbai (C) regions. Tomato plants with the symptoms of leaf curl disease in the Gandhinagar (B) and Mumbai (D) agricultural fields.

alphasatellites (Family: *Alphasatellitidae*) (Briddon et al., 2016, 2018; Chahwala et al., 2020; Kumar et al., 2017a, 2020). These satellite DNAs contain a satellite conserved region (SCR), an adenine rich region and code for a single ORF (Briddon and Stanley, 2006; Briddon et al., 2018; Gnanasekaran et al., 2019). Betasatellites depend on helper begomovirus for their replication and movement, whereas the autonomously replicating alphasatellites require begomovirus for their movement (Briddon and Stanley, 2006; Ranjan et al., 2014; Prabu and Chakraborty, 2018; Gnanasekaran et al., 2019). Betasatellite-encoded β C1 protein functions as a pathogenicity determinant and plays multifaceted roles in the disease development (Gnanasekaran et al., 2019; Kumar, 2019).

Tomato (*Solanum lycopersicum* L.) of the Solanaceae family is an important food crop cultivated in temperate and tropical countries. India ranks second in the world tomato production; however, crop production has been hindered by whitefly-transmitted begomoviruses causing tomato leaf curl disease (ToLCD) (Chakraborty, 2008; Chakraborty and Kumar, 2020; Sivalingam and Varma, 2012). The diseased tomato plants display symptoms such as leaf curling, vein enation, leaf crinkling and puckering. At a severe stage, inadequate fruit setting, reduced fruit number, stunted plant growth and sterility was observed. In the past decade, ToLCD has been spread to all major tomato growing regions in the country causing severe yield loss (Chakraborty, 2008). Several monopartite begomoviruses such as Tomato leaf curl virus-ToLCV, Tomato leaf curl Bangalore virus-ToLCBaV, Tomato leaf curl Gujarat virus-ToLCGuV, Tomato leaf curl Joydebpur virus-ToLCJV, Tomato leaf curl Kerala virus-ToLCKeV, Tomato leaf curl Patna virus-ToLCPatV, Tomato leaf curl Pune virus-ToLCPuV, Tomato leaf curl Rajasthan virus-ToLCRaV and Tomato leaf curl Ranchi virus-ToLCRnV (Chakraborty et al., 2003; Chatchawankanphanich and Maxwell, 2002; Kumari et al., 2010, 2011; Muniyappa et al., 2000; Reddy et al., 2005; Tiwari et al., 2013) and two bipartite begomoviruses such as Tomato leaf curl Palampur virus-ToLCPaIV and Tomato leaf curl New Delhi virus-ToLCNDV (Kumar et al., 2008; Padidam et al., 1995) have been found to be associated with ToLCD in India. In addition, the importance of ToLCD-associated betasatellites in modulating symptom severity and helper begomovirus accumulation has been reported (Kumari et al., 2010, 2011; Ranjan et al., 2013, 2014; Sivalingam and Varma, 2012).

The present study characterizes the association of two distinct begomovirus-betasatellite complexes with ToLCD in India. Further, Koch's postulates were satisfied for these cloned virus complexes in the *N benthamiana* and tomato plants. The effect of cognate betasatellites on the helper begomovirus accumulation and disease development were discussed.

2. Methods

2.1. Isolation and cloning of viral genomes

During 2012 and 2014, tissues of six tomato plants displaying

symptoms such as leaf curling, leaf rolling, leaf crinkling, vein banding and stunting, were collected from the agricultural fields of Gandhinagar (23.2643°N, 72.6721°E) and Mumbai (19.5042°N, 73.3252°E), respectively (Fig. 1). Crop loss in these fields were found to be noticed between 40% and 50%. Total DNA was extracted from the symptomatic and asymptomatic plants of both regions (Dellaporta et al., 1983). Begomovirus infection in these samples was verified by PCR using abutting primers i.e., PAR1c715 and PAL1v1978 (Rojas et al., 1993). Subsequently, total DNA was used for rolling circle amplification using Φ 29 DNA polymerase (GE Healthcare, Waukesha, WI, USA). Highly enriched viral concatemers were independently digested with *Bam*HI, and the resulted ~2.7 kb fragments from each sample were cloned into *Bam*HI-digested pBluescript SK+ vector (Stratagene, La Jolla, CA, USA). Full-length betasatellites from each sample were PCR-amplified using universal beta primers (Briddon et al., 2002) and ligated into pTZ57R/T vector (Thermo Fisher Scientific, Waltham, MA, USA). Amplification of full-length alphasatellites was attempted using alpha primers as described (Kumar et al., 2017a). Genetic variability among the cloned viral sequences were analyzed by digesting them with various restriction endonucleases. One of the representative clones of begomovirus and betasatellite from each sample were sequenced commercially (Chromous Biotech, Bengaluru, Karnataka, India).

2.2. Sequence analysis of the isolated viral genomes

Pairwise sequence comparisons and phylogenetic analyses of the cloned viral genomes were performed using MUSCLE method available in SDT v. 1.0 (Muhire et al., 2014) and MEGA-X (Kumar et al., 2018), respectively. Recombination breakpoints and potential parental sequences of the cloned viral genomes were identified using RDP v.4.97 (Martin et al., 2015) as described by George et al. (2015).

2.3. Construction of partial tandem repeat viral constructs

Partial tandem repeat DNA of the cloned viral genomes were constructed to satisfy Koch's postulates. A 1116 bp fragment of ToLCGanV [*Bam*HI (149 bp)-*Eco*RI (1793 bp)] containing intergenic region (IR) was cloned into pCambia2300 vector linearized with *Bam*HI and *Eco*RI. The full-length monomer linearized with *Bam*HI was then ligated to generate tandem repeat construct of ToLCGanV (referred as GA). For ToLCGan β , a 434 bp *Hind*III (1301 bp)-*Xba*I (370 bp) fragment was cloned into pCambia2300, followed by insertion of the full-length monomer (1.3 kb) linearized with *Hind*III was then ligated to generate tandem repeat construct of ToLCGanB (referred as G β).

A *Bam*HI (172 bp)-*Eco*RI (1792 bp) fragment (1151 bp) encompassing IR of ToLCMumV was cloned into pCambia2300 vector followed by mobilization of full-length monomer (2.7 kb) at *Bam*HI site to generate ToLCMumV infectious construct (referred as MA). Similarly, a 1166 bp *Bam*HI (1148 bp)-*Sac*I (1352 bp) fragment of ToLCMumB was cloned into pCambia2300 vector followed by mobilization of full-

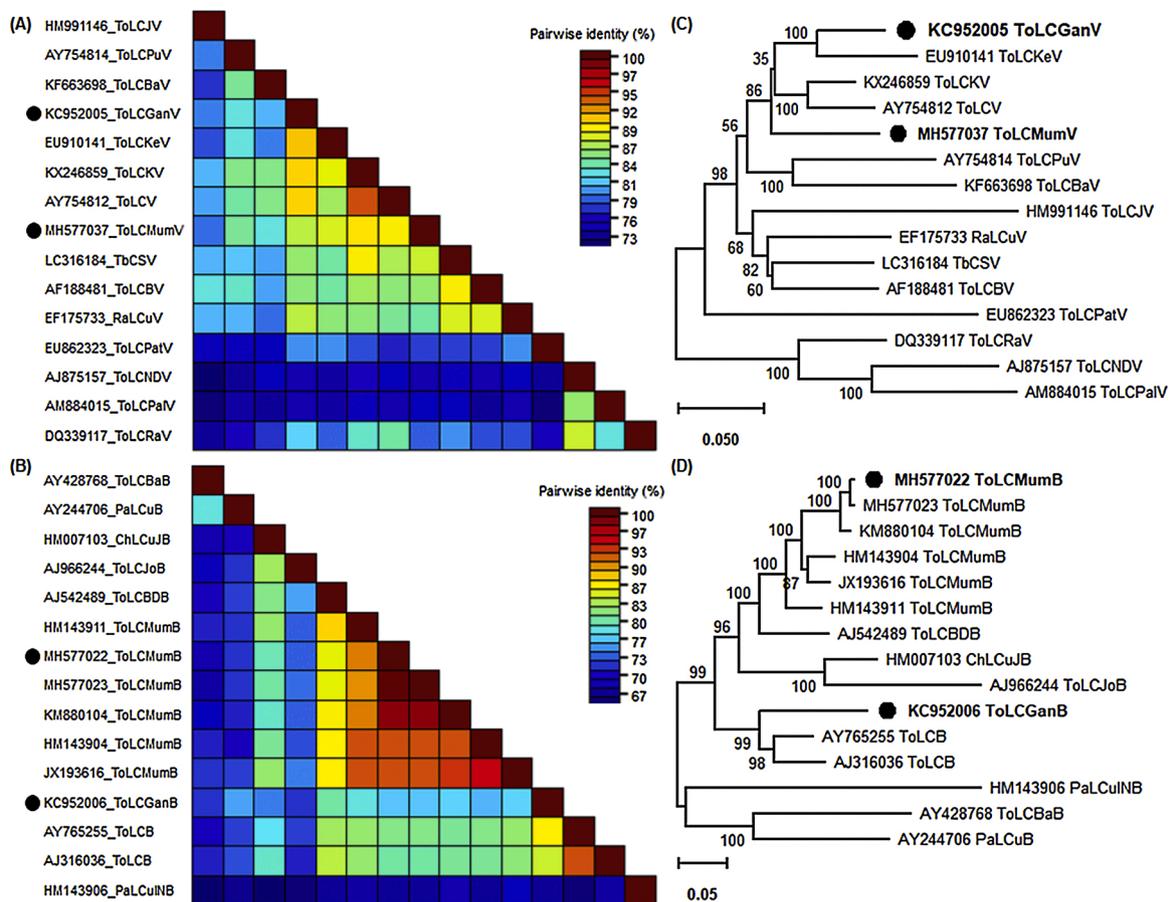


Fig. 2. Characterization of begomoviruses and betasatellites associated with tomato leaf curl disease in western India. Pairwise nucleotide identity matrices of begomoviruses (A) and betasatellites (B). Neighbor-joining phylogenetic dendrograms of begomoviruses (C) and betasatellites (D). The numbers at the nodes represent the per cent bootstrap values of 1000 replicates. The scale bars indicate the genetic distance. Viral genomes identified in this study are marked with dark circles.

length monomer (1.3 kb) at *Bam*HI site to generate ToLCMumB infectious construct (referred as M β).

2.4. Plant inoculation and detection of viral DNAs

All these infectious constructs were transformed individually into *A. tumefaciens* (strain EHA105) cells. Infection of the cloned components was performed on *N. benthamiana* and tomato cv. Kashi Vishesh (a moderate ToLCV-resistant variety) plants through *Agrobacterium*-based inoculation method (Singh et al., 2012). Inoculation with *A. tumefaciens* harbouring empty pCAMBIA2300 was considered as mock-inoculation. The presence of begomovirus and betasatellite in the agro-inoculated plants was confirmed by PCR using degenerate primers (Rojas et al., 1993; Briddon et al., 2002).

The SYBR Green dye-based qPCR was employed to quantify the viral copies in the agro-inoculated plants using specific primers for GA (F 5' TTGACGGTGATGATGTCTTG3', R 5' AGCAGAGAATGGCGT GTTTAT3'), G β (F 5' ACTGGAAGAAACGTGAGAGAAA3', R 5' AGT-CAGCTTGTCCCTCCATAAC3'), MA (F 5' CCACATAGTCTCCCGTCC3', R 5' GAAGAACTTGAGGAGTGGGCT3') and M β (F 5' CCTCTGT CGAAACTCCCAA3', R 5' CACCAGCCCTTGCAAAGAAGA3'). The qPCR assays were carried out in triplicate as described by Singh et al. (2021). Individual standard curves for viral full-length monomers were prepared by making a 100-fold serial dilution from 10⁹ to 10² copy. The calculated cycle threshold values (C_T) were converted to absolute quantification of viral molecules by comparing C_T values of recombinant clones of respective viral components on the standard curve (Shafiq et al., 2017).

3. Results

3.1. Association of two distinct begomovirus-betasatellite complexes with ToLCD in India

The begomovirus sequence identified from Gandhinagar (KC952005; 2760 nt) shared a maximum nucleotide identity of 90.8 % with Tomato leaf curl Kerala virus (ToLCKeV; EU910141). Similarly, a begomovirus sequence isolated from Mumbai (MH577037; 2771 nt) showed highest sequence identity of 89.7 % with Tomato leaf curl Karnataka virus (ToLCKarV; KX246859) (Fig. 2A). Further, they shared 87.4 % sequence identity with each other. As per begomovirus species demarcation threshold of 91 % (Brown et al., 2015), they can be considered as two distinct begomovirus species for which we propose the names, Tomato leaf curl Gandhinagar virus (ToLCGanV) and Tomato leaf curl Mumbai virus (ToLCMumV), respectively. In the phylogenetic dendrogram, ToLCGanV was found to be clustered with ToLCKeV whereas ToLCMumV was positioned in a separate clade (Fig. 2C).

One betasatellite sequence was cloned from each of these regions. However, we failed to isolate an alphasatellite from these samples. Sequence analysis of a betasatellite (KC952006) identified from Gandhinagar sample has highest nucleotide identity of 86.6 % with Tomato leaf curl betasatellite (ToLCB; AY765255) (Fig. 2B). According to betasatellite demarcation threshold of 91 % (Briddon et al., 2016), this sequence represents a new betasatellite group, which is named as Tomato leaf curl Gandhinagar betasatellite (ToLCGanB). A betasatellite (MH577022) cloned from the Mumbai sample showed nucleotide sequence identities between 90.4 % and 99.2 % with a group of

Table 1
Recombination events and the putative parental sequences of the cloned virus sequences.

Recombinant sequences	Recombination breakpoints in the genome	Putative minor parent ^a	Putative major parent ^a	Recombination analysis	
				Detection methods ^b	P-value [#]
KC952005; ToLCGanV	2047–2370 (AC1, AC4)	KF663698; ToLCBaV(?)	AF188481; ToLCBV	RGBMCS	3.23×10^{-09}
MH577037; ToLCMumV	2050–59 (AC1, AC4, IR)	LC316184; TbCSV	EU910141; ToLCKeV	RGBMCST	1.53×10^{-22}
KC952006; ToLCGanB	188–759 (β C1)	AJ316036; ToLCB	AY244706; PaLCuB	RGBMCST	1.09×10^{-03}
	1241–75 (SCR)	AY765255; ToLCB	JX193616; ToLCMumB	RGBMCST	1.18×10^{-04}

^a The names of viral genome abbreviated are Tomato leaf curl Bangladesh virus-ToLCBV, Tomato leaf curl Bangalore virus-ToLCBaV, Tomato leaf curl Kerala virus-ToLCKeV, Tobacco curly shoot virus-TbCSV, Tomato leaf curl betasatellite-ToLCB, Tomato leaf curl Mumbai betasatellite-ToLCMumB and Papaya leaf curl betasatellite-PaLCuB.

^b Recombination detection methods used are R-RDP; G-GENCONV; B-BOOTSCAN; M-MAXCHI; C-CHIMERA; S-SISCAN; T-3SEQ.

[#] The lowest p-value detected by the underlined method mentioned in the previous column.

unnamed betasatellites (Fig. S1). Further, they shared a maximum nucleotide identity of 87.7 % with Tomato leaf curl Bangladesh betasatellite (ToLCBDB; AJ542489). Therefore, these unnamed betasatellites can be placed in a newly proposed Tomato leaf curl Mumbai betasatellite (ToLCMumB) group. In the phylogenetic dendrogram, all ToLCMumB isolates were clustered together whereas ToLCGanB was found to be segregated with other isolates of ToLCBs (Fig. 2D).

3.2. Cloned virus genomes are recombinant in nature

Pairwise sequence identities of viral ORFs predicted the possibility of recombination events in the cloned begomoviruses. So, recombination analysis was performed to identify the recombined fragments and their putative parental sequences. The RDP analysis of ToLCGanV detected one recombination event around AC1/AC4 regions (2047–2370 nt) which could be contributed by ToLCBV and ToLCBaV (Table 1). Similarly, one recombinant event at 2051–59 nt (AC1/AC4/IR regions) was detected in the ToLCMumV of which ToLCKeV and TbCSV were identified as possible parents. Further, the isolates of ToLCB have been contributed to the emergence of ToLCGanB (Table 1).

3.3. Infectivity studies of the viral infectious constructs

Tomato and *N. benthamiana* plants were agro-inoculated with GA alone and in combination with its cognate betasatellite (G β). The GA alone inoculated *N. benthamiana* plants were capable of producing mild symptoms at 8 dpi, whereas in the presence of G β , symptoms were appeared earlier at 7 dpi (Fig. 3A, Table 2). At 28 dpi, an increased symptom severity (such as downward leaf curling, vein enation, vein banding, twisting of petiole, stunted plant growth) were observed in the systemic leaves of co-inoculated GA and G β plants than GA alone inoculated plants (Fig. 3A). In tomato plants, agro-inoculation of GA alone exhibited mild and delayed leaf curling symptom at 17 dpi (Fig. 3A). Co-inoculation of GA and G β in tomato reduces the incubation period and developed severe symptoms such as small leaves, twisting of petiole, leaf curling and rolling (Table 2).

The MA alone was capable of developing severe symptoms on agro-inoculated plants, whereas in MA + M β inoculated *N. benthamiana* plants, enhanced symptom severity was observed (Table 2). Mild symptoms of leaf curling and twisting of petioles were noticed in the tomato plants inoculated with MA alone. In the co-inoculated (MA + M β) tomato plants, severe symptoms such as leaf curling, twisting of petiole, yellow patches on leaves and stunted growth were noticed (Fig. 3A). The presence of M β has reduced the incubation period in both *N. benthamiana* and tomato (Table 2).

3.4. Viral DNA accumulation in the agro-inoculated plants

The presence of begomoviruses and betasatellites in the agro-inoculated *N. benthamiana* and tomato plants were verified by conventional PCR using degenerate primers (Fig. S2). No viral DNA was detected from the mock-inoculated plants (Table 2). Further, begomovirus and betasatellite titre in the inoculated plants was quantified by qPCR using begomovirus and betasatellite-specific primers (Fig. 3B). Begomovirus (GA) was detected from the systemic leaves of GA alone inoculated tomato plants at 28 dpi. Further, the accumulation of GA was found to be elevated in the tomato plants co-inoculated with GA and G β than in the plants inoculated with GA alone (Fig. 3B). Similarly, GA was detected in the newly emerged leaves of *N. benthamiana* plants inoculated with either GA or GA + G β . However, an increased level of GA was found when the plants were co-inoculated with GA and G β (Fig. 3B).

Higher accumulation of begomovirus (MA) was found when *N. benthamiana* plants were inoculated with the cognate betasatellite (MA + M β) than MA alone inoculated plants (Fig. 3B). However, the level of begomovirus was found to be comparable in the systemic leaves of tomato plants inoculated with either MA alone or MA + M β (Fig. 3B). Cognate betasatellites (G β or M β) were detected only when they were co-inoculated with their respective begomoviruses (GA or MA) (Table 2).

4. Discussion

Among plant viruses, geminiviruses cause severe diseases in numerous economically important crops resulting in significant crop loss in productivity (Varma and Malathi, 2003; Brown et al., 2012). One such disease is leaf curl disease of tomato caused by begomoviruses in the 'OW' countries. Several monopartite begomoviruses and few bipartite begomoviruses along with betasatellites have been reported to cause ToLCD in the Indian sub-continent (Chakraborty et al., 2003; Chatchawankanphanich and Maxwell, 2002; Kirthi et al., 2002; Kumar et al., 2008; Kumari et al., 2010, 2011; Muniyappa et al., 2000; Padidam et al., 1995; Reddy et al., 2005). The present study reports the characterization of two new monopartite begomovirus-betasatellite complexes associated with ToLCD in western India. Infectivity results demonstrated that these cloned components are the causal agents of ToLCD.

Tissues from ToLCD-affected tomato plants were collected from Gandhinagar and Mumbai regions where ToLCD-associated virus complex is yet to be investigated. Sequence analysis of the cloned genomes suggested the association of two new begomovirus-betasatellite complexes with ToLCD in western India. According to virus nomenclature guidelines, these cloned components are named as ToLCGanV and ToLCGanB (from Gandhinagar), and ToLCMumV and ToLCMumB (from Mumbai). Despite repeated attempts, the DNA B component could not be identified from these samples indicating that they are monopartite begomoviruses. The nature of begomoviruses identified here were found

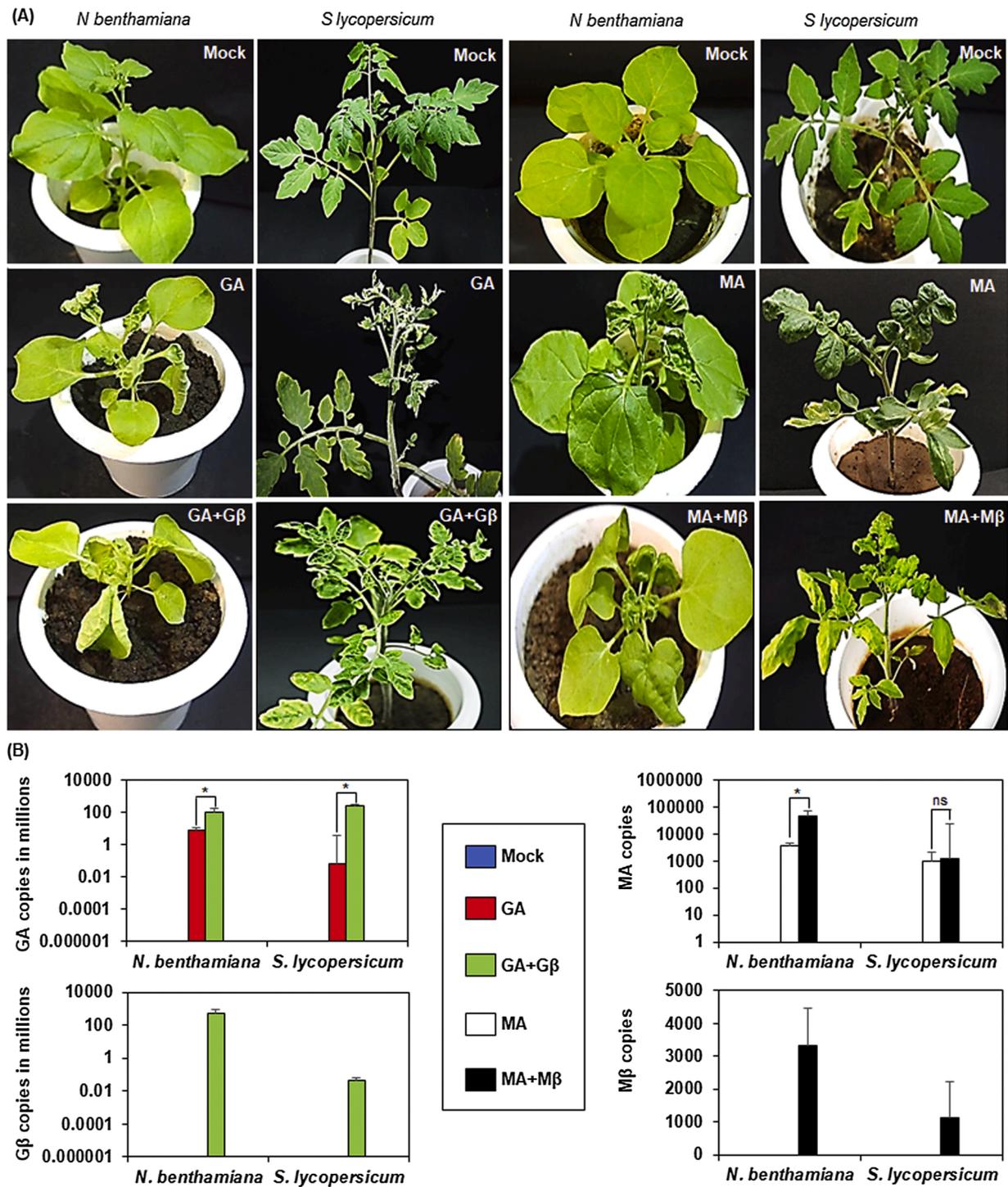


Fig. 3. Symptom appearance and viral DNA accumulation in the agro-inoculated *N. benthamiana* and tomato plants. Symptoms (A) and viral DNA accumulation (B) in the agro-inoculated *N. benthamiana* and tomato plants at 28 dpi. The virus component specific primers used for quantifying viral DNAs are provided in each graph. Each bar represents mean of three individual replicates \pm standard error. Asterisk indicates statistical significance according to Duncan's multiple range test. The infectious viral constructs abbreviated are GA-ToLCGanV, Gβ-ToLCGanB, MA-ToLCMumV and Mβ-ToLCMumB.

Table 2Infectivity analysis of the cloned infectious viral constructs on *N. benthamiana* and tomato plants.

Host	Inoculum ^a	Latent period (in days post-inoculation)	Plants showed symptoms/Plants inoculated	Symptom appearance ^b	Virus detection by PCR ^c	
					DNA A	DNA β
Tomato	Mock	–	0/16	–	–	–
	GA	17	10/16	LC, SL	+	–
	GA + Gβ	12	14/16	LC, SL, Tw, LR	+	+
	MA	12	14/16	DLC, YP	+	–
	MA + Mβ	10	16/16	DLC, YP, Tw, St	+	+
	Mock	–	0/15	–	–	–
<i>N. benthamiana</i>	GA	8	15/15	LC, LR, SL, St	+	–
	GA + Gβ	7	15/15	DLC, VE, St, Tw, LR	+	+
	MA	6	14/15	DLC, St, SL	+	–
	MA + Mβ	5	15/15	DLC, St, SL, Tw	+	+
	Mock	–	0/15	–	–	–

^a The infectious viral constructs abbreviated are GA-ToLCGanV, Gβ-ToLCGanB, MA-ToLCMumV and Mβ-ToLCMumB.

^b Type of symptoms abbreviated are DLC- downward leaf curling, LC- leaf curling, LR- leaf rolling, SL- small leaves, St- stunted plant growth, Tw- twisting of petiole, VE- vein enation, YP- yellow patches.

^c + and – indicate the presence and absence of specific viral genome, respectively.

to be similar to other ToLCD-associated monopartite begomoviruses reported from India. These monopartite begomoviruses were predominantly distributed in the eastern (ToLCJV, ToLCPatV and ToLCRnV), southern (ToLCBaV and ToLCKeV) and western (ToLCGanV, ToLCMumV, ToLCPuV and ToLCRaV) part of the country (Fig. 4). Both bipartite begomoviruses (ToLCNDV and ToLCGuV) were prevalent in northern India (Chakraborty and Kumar, 2020). The frequent association of Cotton leaf curl Multan betasatellite or Luffa leaf distortion betasatellite, and Tomato yellow leaf curl Thailand betasatellite with ToLCNDV and ToLCGuV, respectively, have been documented (Jyothisna et al., 2013; Ranjan et al., 2013, 2014; Sivalingam and Varma, 2012). Moreover, several recently identified monopartite begomoviruses (ToLCGanV, ToLCMumV, ToLCPatV and ToLCRnV) have been identified along with betasatellites that belong to distinct groups (This study; Kumari et al., 2010, 2011). These studies highlight the richness of ToLCD-associated begomovirus/betasatellite diversity than they presumed (Fig. 4). Nonetheless, a more focused and comprehensive survey on the diversity of ToLCD-associated disease complexes across the country is necessary to validate it.

Mixed infection, recombination, high mutation and nucleotide substitution rates are the key factors contributing to the emergence and evolution of begomovirus species (Duffy and Holmes, 2008; George et al., 2015; Padidam et al., 1999). Recombination analysis of the cloned begomoviruses identified recombination breakpoints around AC1 and/or IR regions. This result corroborates previous findings of these regions as viral recombination hotspots (George et al., 2015; Lefeuvre et al., 2007; Kumar et al., 2015, 2017b; Prasanna and Rai, 2007). The ToLCD-associated begomoviruses (ToLCBV, ToLCBaV and ToLCKeV) have been identified as putative parental sequences emphasizing that the emergence of these new begomoviruses could be via interspecies recombination between these begomoviruses.

The Koch's postulates were satisfied for these distinct virus complexes on *N. benthamiana* and tomato plants. Inoculation studies demonstrated that ToLCGanV alone can replicate autonomously in tomato, but at low titer, with mild and delayed symptoms. Further, the presence of cognate ToLCGanB had increased the symptom severity and enhanced helper virus accumulation. This is in agreement with several studies demonstrating that betasatellites are required for begomoviruses to cause severe disease symptoms in okra, chilli, cotton, radish and tomato (Briddon et al., 2001; Jose and Usha, 2003; Jyothisna et al., 2013; Kumar et al., 2015; Singh et al., 2012, 2021). On the contrary, no effect of cognate ToLCMumB on the accumulation of helper ToLCMumV was found in the agro-inoculated tomato plants. This result is similar to that of the infectivity nature of other ToLCD-associated

begomovirus-betasatellite complexes demonstrated from India (Kumari et al., 2010, 2011; Ranjan et al., 2013). Betasatellite-encoded βC1 proteins have been widely demonstrated as pathogenicity determinants by interfering with various host-mediated defense responses (Gnanasekaran et al., 2019; Kumar, 2019). The βC1 proteins are responsible for selective suppression of jasmonic acid biosynthesis and defense responsive genes for the successful disease development (Hu et al., 2019; Jia et al., 2016; Yang et al., 2008). Hence, it can be speculated that ToLCMumB-encoded βC1 protein potentially functions as a pathogenicity determinant leading to increased symptom severity without elevating the level of ToLCMumV. Furthermore, tomato cultivar used for the infectivity study has been found to be moderately resistant to ToLCV (Singh, 2014). The infection of this cultivar by ToLCGanV and ToLCMumV highlights that virus resistance of this cultivar is broken. This necessitates further study on the spread of these virus resistance breaking disease complexes to tomato growing regions in the country.

The present study reports the characterization of two new monopartite begomovirus-betasatellite complexes with ToLCD in western India. Further, Koch's postulates were satisfied for these virus complexes in the *N. benthamiana* and ToLCV-resistant tomato plants. Both cognate betasatellites (ToLCGanB and ToLCMumB) could enhance symptom severity, but only ToLCGanB influences the accumulation of its helper begomovirus (ToLCGanV) in tomato. This study has identified two potential virus resistance breaking disease complex in India.

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CRediT authorship contribution statement

Investigation, Data curation, Writing - original draft, Formal analysis. **Punam Ranjan:** Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **R. Vinoth Kumar:** Data curation, Writing - original draft, Writing - review & editing. **Bhavin S. Bhatt:** Investigation. **Fenisha D. Chahwala:** Validation. **Brijesh K. Yadav:** Validation. **Sunita Patel:** Methodology, Resources. **Bijendra Singh:** Methodology, Resources. **Achuit K. Singh:** Conceptualization, Funding acquisition, Supervision, Writing - original draft, Writing - review & editing.

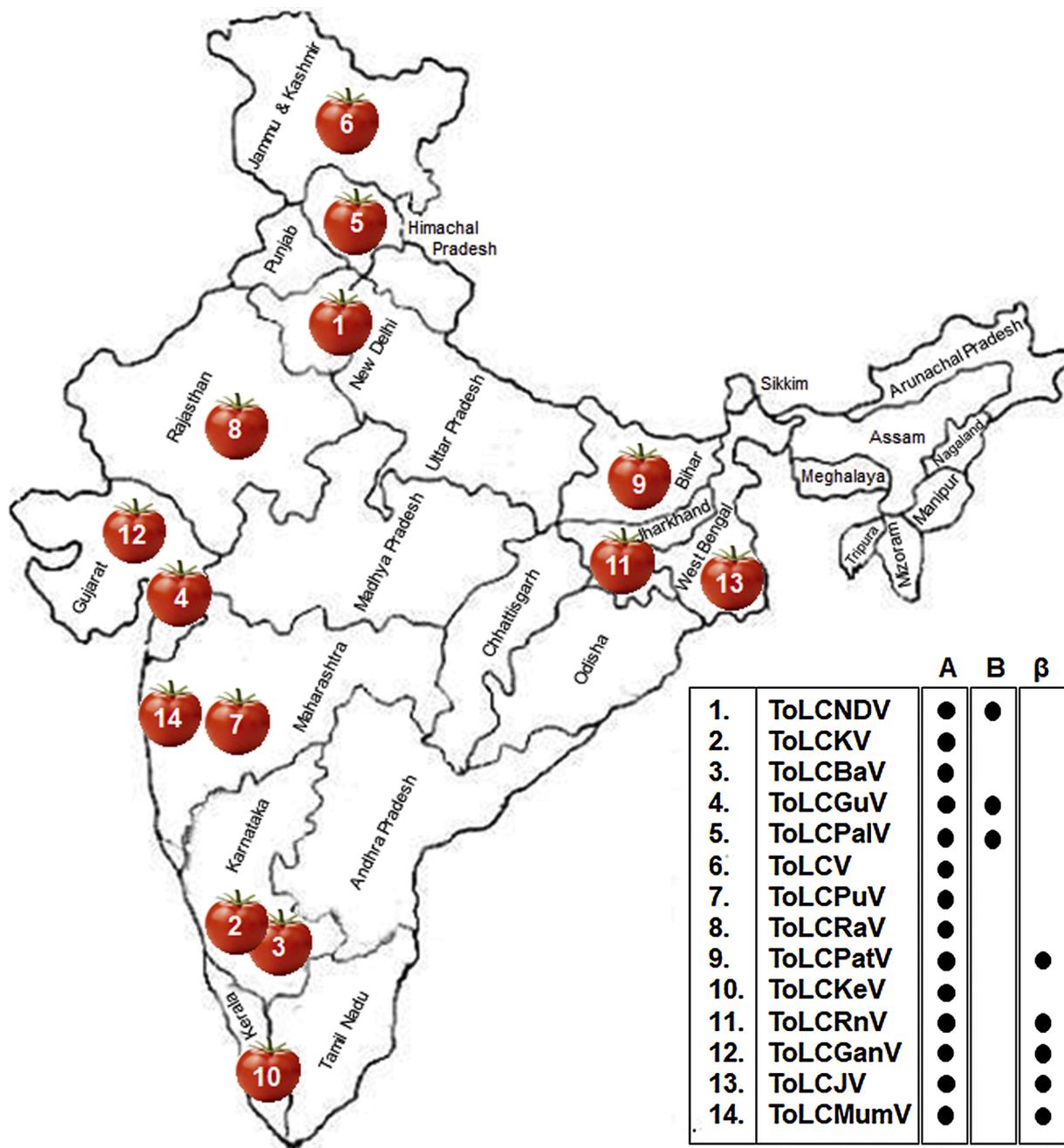


Fig. 4. Distribution of distinct begomovirus species associated with ToLCD in India. The abbreviated begomovirus names for the samples numbered are provided at the right-hand side. Dark circle indicates the presence of specific virus genome (A-DNA A, B-DNA B and β -betasatellite).

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.virusres.2021.198319>.

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2017 Gujarat Vidhan Sabha (VS) polls. In 2017, Congress put up its best performance in last three decades, clinching 77 assembly seats, 20 seats above its previous tally in 182 seats in Gujarat Legislative Assembly. No wonder, since 2014, Gujarat had witnessed political and social turmoil including the brunt of demonetisation and the goods and services tax (GST) on its industries. Therefore, it becomes interesting to understand how politics played out in Gujarat. Despite all of it, the BJP once again could manage to win all 26 LS seats in 2019 elections. It is not that BJP emerged victorious all of a sudden in 2014 followed by its repeat in 2019.

The state, which has experienced a two-party contest, has been ruled by BJP uninterrupted since 1998. After Narendra Modi became the Chief Minister (C.M.) in 2001, BJP never faced a serious challenge from the Congress. What explains the BJP's rise to power in Gujarat? More importantly, how does one explain BJP's 2019 victory in the backdrop of its best performance in last parliamentary elections but not so encouraging performance in last state assembly elections in 2017? To understand, one needs to first go into the political history of modern Gujarat. The transition of the BJP in the political context of Gujarat attains an even greater significance post-2014 as the party formed government at the Centre and enables us to understand changes in its ideology that is reflected through the course of state action adopted.

Demographic and Social Profile

At the time of independence, in 1947, the present day Gujarat was part of the Bombay state. It came into existence on 01 May 1960, after a prolonged *Maha Gujarat* (Greater Gujarat) movement which called for a separate state from Bombay on linguistic grounds. The present day Gujarat has 33 administrative districts and 250 talukas, divided into distinct regions - North Gujarat, Central Gujarat, South Gujarat and the peninsular Gujarat comprising of Saurashtra (Kathiawad) and Kutch.

The development in the state is, however, uneven. On the basis of statistical data, the population of Gujarat as per 2011 census is

Understanding Gujarat Polls-2019

Manish &
Vijendra Singh

This paper explains 2019 Lok Sabha verdict in Gujarat in the backdrop of last parliamentary and state assembly elections. It argues that after its best performance in 2014 parliamentary elections in the state, BJP faced serious debacle in 2017 state assembly elections. As a consequence, it recalibrated its strategy and adopted multipronged strategy during 2019. In 2019, BJP won many of the lost communities and castes, and improved its performance across the regions. Also, BJP's narrative during election campaign was much more targeted and focused, and thereby had much more resonance among the electorate than that of the opposition. The paper also argues that BJP's resounding victory in 2019 was also because of voters making distinction between the state and the national elections with Narendra Modi as the prime ministerial candidate.

Introduction

This article attempts to understand Bharatiya Janata Party's (BJP) electoral victory in the 2019 Lok Sabha (LS) elections in Gujarat. The 2019 Lok Sabha elections took many by surprise, particularly against the backdrop of resurgent Congress, which made significant gains in

***Usnea baileyi* (Stirt.) Zahlbr, a new record for Karnataka, India**

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ABSTRACT

Usnea baileyi (Stirt.) Zahlbr, a new record for Karnataka, India, is described. This is characterized by the corticolous thallus with isidia, hollow central axis and red pigmented medulla.

INTRODUCTION

Lichens are known to humans for hundreds of years finding a place in their daily life either being consumed as food materials or sources of drugs to cure diseases (Malhotra *et al.* 2007; Upreti *et al.* 2005; Zambare and Christopher 2012). One such lichen genus *Usnea* belongs to family Parmeliaceae, which is a popular fruiticose lichen represented by almost 300 species around the globe (Ohmura 2012). According to Singh and Sinha, 60 species of *Usnea* has been reported from India (Singh and Sinha 2010). Temperate and high altitude regions in Northern India, such as Eastern and Western Himalaya along with the Western Ghats are lichen biodiversity hotspots in the country (Upreti *et al.* 2005). *Usnea* is the fifth largest lichen genera in India which is mostly found growing on varieties of trees (corticolous) and rarely on rocks (saxicolous). First Monograph on this genus was published by Motyka in 1938, where genus *Usnea* was divided into 6 subgenera (*Lethariella* Motyka, *Chlorea* (Nyl.) Motyka, *Protousnea* Motyka, *Neuropogon* (Nees & Flot.) Motyka, *Euusnea* Jatta. and *Eumitria* (Stirt.) Motyka), later most of which became independent genera (Motyka 1938). The presence of the central axis is one of the most important identifying anatomical features of this genus. Anticancer compound usnic acid is universally found in *Usnea* species (Trease and Evans 1983; Zugic *et al.* 2016). In our search for *Usnea*

samples for an ongoing bioprospection study in Karnataka, we found *U. baileyi*, which turned out to be a first report of the species from the state. Previously *U. sinensis* Mot., *U. eumitrioides* Mot. and *U. aciculifera* Vain has been reported from Karnataka (Vinayaka *et al.*, 2012). This article gives an account of a new distributional report on *U. baileyi*.

MATERIAL AND METHODS

The lichen thalli were collected in the month of June 2018 from Western Ghats region of Karnataka (Muthodi Road, Chikmagalur, Karnataka, India, Altitude: 1076 ± 5.7 mt, GPS: 13° 22.997' N, 75° 39.162' E). Thalli were found hanging from a toddy palm (*Borassus flabellifer*) growing on the roadside. For identification of lichen sample, morphological and anatomical features were analyzed using a stereo-zoom microscope (Leica S8 APO, Germany) and compound microscope (Leica DM 500, Germany), respectively. In addition to morpho-anatomical features, chemical characteristics of thallus were tested by spot tests and thin-layer chromatography (TLC) employing solvent system A, i.e., toluene: dioxane: acetic acid (180:45:5) (Orange *et al.* 2001). TLC plate was also observed under UV to check the presence of fluorescent compounds if any. Horizontal and longitudinal sections were examined to calculate C:M:A (cortex: medulla: axis) ratio. The detailed taxonomic analysis was carried out at CSIR-National Botanical Research Institute (NBRI), Lucknow, following

Awasthi (Awasthi 2007). Identified samples were preserved in the herbarium of CSIR-NBRI, Lucknow (LWG).

Taxonomic Treatment

Thallus fruticose, corticolous, suberect to pendulous, to 13 cm in length, greenish grey, dichotomously branched, diam. 1.32 mm; % C:M:A: 1.6:1.5:6.8; transversally cracked at intervals; fibrils, pseudocyphallae and isidia present; soredia absent; central axis tubular (centrally hollow) (Figure1); medulla red-pigmented; apothecia absent.

Chemistry

Cortex and medulla K⁺ red, C⁻, P⁺ orange, UV-salazinic, norstictic, usnic acid, and unknown substance at R_f class 5 detected in TLC.

U. baileyi is a rich source of various phytochemicals including several secondary metabolites like depsides, depsidones, and xanthenes (Din *et al.* 2010; Ramesh *et al.* 1994; Yang *et al.* 1973). Recently, two new compounds bailexanthone and bailesidone have also been reported from *U. baileyi* (Van Nguyen *et al.* 2018).

Remarks

Being corticolous in nature, *U. baileyi* is parallel to most of the other *Usnea* sp. in its habitat. Similar to *U. baileyi*, other *Usnea* sp. from tropical Andes and Galapagos islands region have been reported to contain red-orange pigmentation in the medullar region (Truong *et al.* 2011). The invariable presence of red pigmentation has also been observed in *Usnea* sp. like *U. fragilis* Stirt. and *U. austro-indica* G. Awasthi (Shukla 2015). As mentioned by Alix *et al.* (1984), pigment imparting molecules in *U. baileyi* are bisxanthenes (like eumitrin). A thicker and hollow axis is present in *U. baileyi* (present investigation); similar observation has been made in a study by Truong *et al.* (2013), where % CMA of 3.5/1.5/90 in case of *U. antillarum* (Vain.) Zahlbr confirms the presence of wider axis in *Usnea* spp. Salazinic and norstictic acid have been traced in the medulla of eumitrioid species *U. subflaveola* (Truong *et al.* 2013), while usnic acid which imparts the yellow-green color to thallus is present in all the *Usnea* species e.g., *U. baileyi* (this study) and *U. implicita* (Stirt.) Zahlbr (Ohmura, 2001).

Ecology and Distribution

This species was found growing on the trunk of the toddy palm tree from the Chikmagalur district of Karnataka. Earlier, the species was known from Arunachal Pradesh, Assam, Kerala, Manipur, Meghalaya, Nagaland, Sikkim, Tamil Nadu (Nilgiri and Palni Hills), West Bengal (Darjeeling district) states of India, China, Indonesia, Japan, Malaysia, Papua New Guinea, Taiwan, Thailand, Nepal, Central and South America, Africa, Philippines, and Australia (Singh and Sinha 2010). This is a new report for Karnataka.

Specimen examined

India, Karnataka, Chikamagalur, elev. 1076 m, N13°22.997'; E75°39.162', the bark of toddy palm, 07 June 2018, Swati Joshi s.n. Acc. No. 35532 (LWG).

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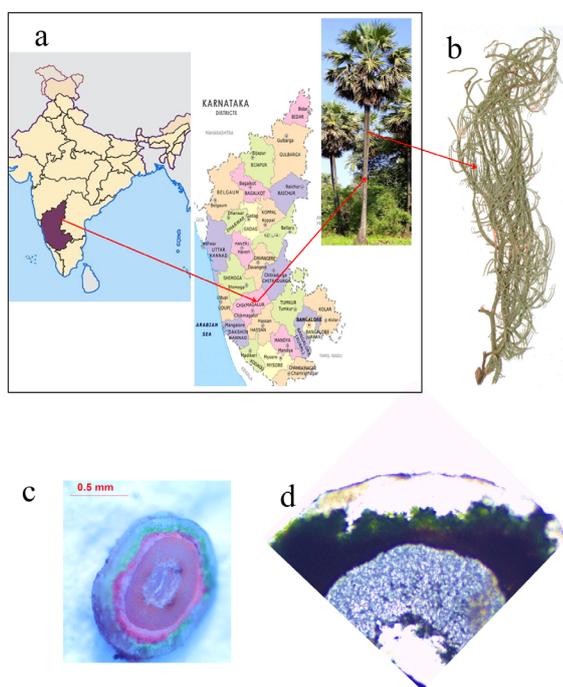


Figure 1 *U. baileyi* (Stirt.) Zahlbr. **a.** map location of the toddy palm *B. flabellifer* from which *Usnea baileyi* (Stirt.) Zahlbr was collected, **b.** *U. baileyi* (Stirt.) Zahlbr habit, **c.** cross section in stereoscopic zoom in (scale 0.5 mm), **d.** microscopic (10X) view of the thallus showing green algal layer within cortex in close association with red pigmented layer in medullar region and hollow central axis

Usnea baileyi (Stirt.) Zahlbr, a new record for Karnataka, India

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પ્રસિદ્ધ ૧૫ ઓક્ટોબર, ૨૦૨૧

શિક્ષાશાને સમર્પિત સામયિક

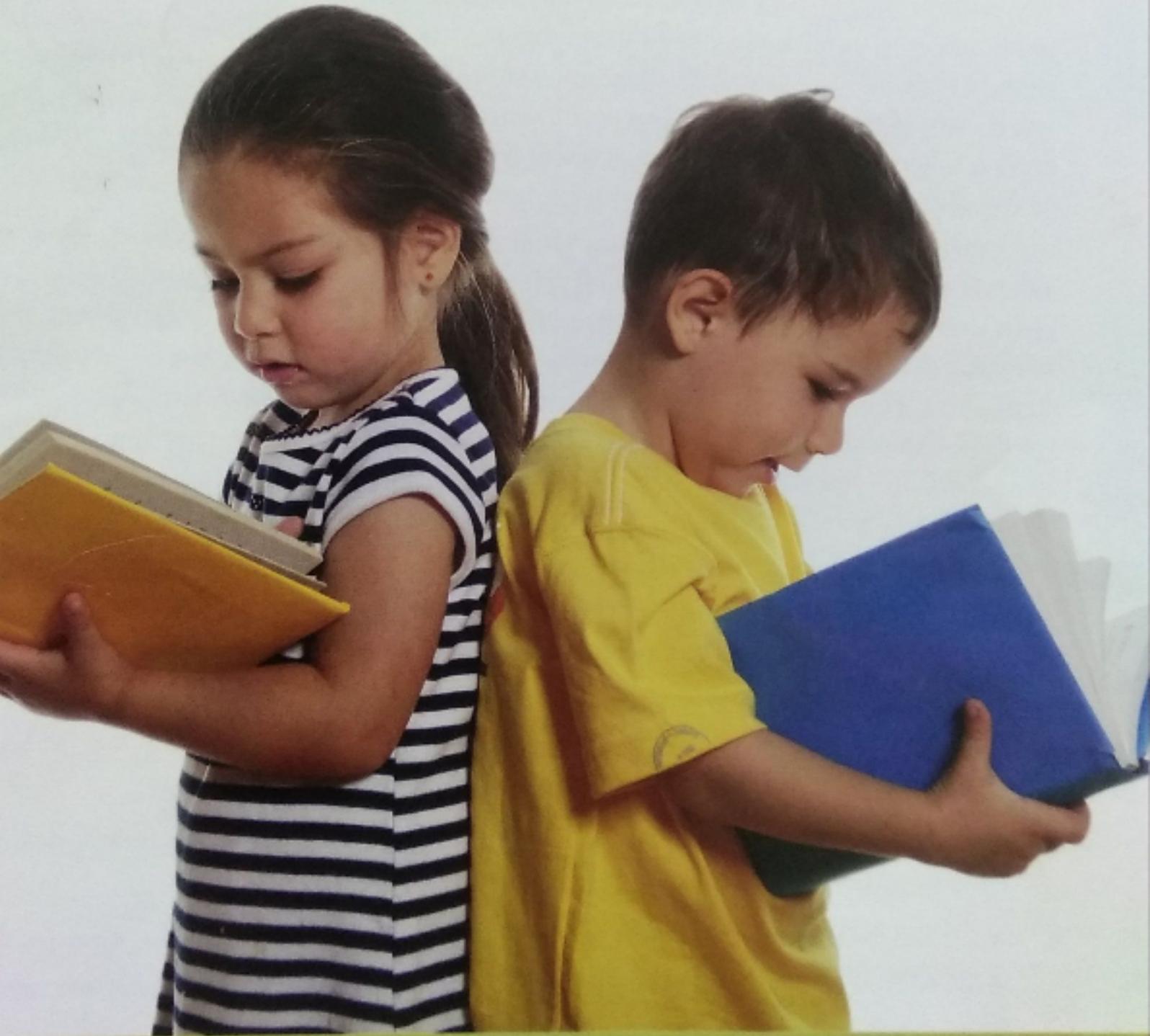
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શિક્ષણ જગતનું માર્ગદર્શક તથા પ્રેરણાદાયી સામયિક

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વિદ્યાર્થીઓનો અંતર્નદિ એટલે 'મારે કંઈક કહેવું છે !'

✍ ડૉ. બળદેવ પ્રજાપતિ

આજે શિક્ષણના નામે પ્રશ્નો અને નિરાશાજનક વાતાવરણ ઊભુ થયું છે. પણ 'શિક્ષક કભી સાધારણ નહીં હોતા' આ વાક્ય આપણે ખૂબ સાંભળ્યું છે. સાચો શિક્ષક કોઈની પરવા કર્યા સિવાય પોતાના કાર્યને લક્ષ્ય બનાવીને ગંતવ્ય સ્થાને પહોંચતો હોય છે. જેમને વિદ્યાર્થીના જીવનમાં અજવાળા કર્યા, નવી કેડી કંડારી એમની વાચાને શબ્દદેહ આપ્યો એવા એક શિક્ષક એટલે ડૉ. રાકેશ રાવત. આવા શિક્ષક સાથે સાક્ષાત્કાર થાય અને વર્ગની વાત એમના સ્વમુખે સાંભળવી પણ એક લહાવો છે. ઉત્તર ગુજરાતના તળપ્રદેશમાં જન્મી, ચરોતરને પોતાની કર્મભૂમિ બનાવી, આણંદ-વિદ્યાનગરમાં વર્ષ-૨૦૦૯થી રામકૃષ્ણ સેવા મંડળ સંચાલિત આણંદ હાઈસ્કૂલના ઉ.મા. વિભાગમાં ભાષા શિક્ષક તરીકે જોડાયા. જોતજોતામાં એક દસકો પસાર થઈ ગયો. અજાણ્યા વિસ્તારમાં અજાણી વ્યક્તિ સાથે નાતો બાંધવો એ શિક્ષક જ કરી શકે અને કરી બતાવ્યું છે. 'વર્ગમાં ઓગળ્યા વગર સ્વર્ગ ના બને !' જીવનમાં કંઈક પ્રાપ્ત કરવું હોયતો પોતાની જાતને શૂન્ય કરવી પડે, એ રાકેશભાઈએ કર્યું છે. આજનો વિદ્યાર્થી આવતી કાલનું ભવિષ્ય છે. આ ભવિષ્યના ભીતરમાં આપાર વેદના-સંવેદના, ભાવો, પ્રેમ, હુંફ, લાગણી વગેરે ધરબાયેલું પડ્યું છે, એને બહાર લાવવાનું કામ રાકેશભાઈએ કર્યું છે. બાળકના વિચારોને વાંચી, હૈયાની લાગણીઓને બહાર લાવવું ખૂબ અઘરું છે, પણ 'અડગ મનના મુસાફરને હિમાલય નડતો નથી'. શિક્ષકનો વ્યવસાય પૈસા કમાવવાનું સાધન નથી પણ એક વિચાર છે, જે રાષ્ટ્રને સુદ્રઢ બનાવી શકે. એ વિચાર સાથે કામ કરતાં શિક્ષક મિત્રો વર્ગખંડથી વાલી સુધી પહોંચી, બાળકના હૃદયના ભાવોને પરિવારના ભાવો સાથે જોડી, તે બાળક માટે યોગ્ય નિર્ણય લે છે. 'શિક્ષકની સાચી સંપત્તિ એ તો કેળવાયેલો વિદ્યાર્થી છે.' જીવનમાં એનાથી વિશેષ સંતોષ કયો હોઈ શકે ?

રાકેશભાઈએ આ માર્ગ પસંદ કરી વિદ્યાર્થીને વર્ગખંડમાં તો વાંચ્યા, પણ એના પરિવાર સુધી પહોંચી

એના ભાવ સંવેદનો જાણ્યા, અને એના હૃદયની લાગણીઓને શબ્દદેહ આપવાનું પુણ્ય પ્રાપ્ત કર્યું. નીત-નવીન પ્રયોગો દ્વારા વર્ગમાં વિદ્યાર્થીઓને પ્રોત્સાહન આપવાનું કાર્ય કરતાં-કરતાં રાકેશભાઈને વિચાર આવ્યો કે કંઈક નવીન કરવું. આ પત્રો કેવી રીતે લખાય એ સંદર્ભે ડૉ. રાકેશ રાવત પોતાની વાત 'ભીતર ભીનો સંવાદ'માં જણાવે છે કે, 'એક દિવસ વાત-વાતમાં વર્ગમાં કહ્યું કે જેને પણ જીવનમાં સુખ કે દુઃખ, તમારા મનની વાત, કે તમારી કલ્પના જે પણ લખવું હોય એ તમે લખી શકો છો. શરૂઆતમાં સંકોચ સાથે અને પછી તો નિઃસંકોચ પણ બાળકો લખવા લાગ્યા. આ સિલસિલો એક દસકા સુધી ચાલ્યો. દરેક બાળક એની કાલી-ઘેલી ભાષામાં એવું લખે કે મને પણ મારું બાળપણ યાદ આવી જતું તો ક્યારેક એવું વંચાતું કે અંદરથી હલબલી જવાતું. હવે હું મૂંઝાયો કે મારે શું કરવું ? મારા પ્રશ્નનું સમાધાન કરવા વડીલ શિક્ષકો સાથે ચર્ચા વિચારણા કરી મનસુખ સલ્લાનું 'અનુભવની એરણ પર' તેમજ મોહન પંચાલનું 'સાહેબ! મને સાંભળો તો ખરા' પુસ્તક મારા પથદર્શક બન્યાં.' બાળકોને નિહાળ્યા; એમાં કેટલાય બાળકો ભણતરના ભારમાં સુમ-સામ બેસી રહેતા; તો કેટલાક વિદ્યાર્થીઓ ઘરના પ્રસંગો કે ઘટનાઓમાં દબાયેલા હતા; એમના હૃદય વાંચી હૃદયના દ્વાર ખોલવા મહેનત કરી; અંતે વિદ્યાર્થીઓએ પોતાની વેદના, ભાવો, વિચાર, લાગણી કાગળમાં કંડારવાનો પ્રયત્ન કર્યો. રાકેશ ભાઈને ખબર પડી કે વિદ્યાર્થીઓના હૃદયમાં અપાર સંવેદનો ઝીલાયેલા છે, એના ફળસ્વરૂપે 'મારે કંઈક કહેવું છે !' એ પત્ર પુસ્તક પ્રાપ્ત થયું. આ પુસ્તકમાં કુલ ઓગણચાલીસ પત્રોને સમાવવામાં આવ્યા છે. આમ તો ધોરણ-૯થી ૧૨ના કેટલાય વિદ્યાર્થીઓના પત્રો રાકેશભાઈ પાસે હતા, પણ એમાંથી સંકલિત કરી ને ઓગણચાલીસ પત્રોનો સમાવેશ આ પુસ્તકમાં કર્યો છે. એમાં કોઈ સાહિત્ય કે ઇતિહાસની વાત નથી, એમાં રાષ્ટ્રના ભાવિના હૃદયની લાગણીઓ પ્રગટી છે, એટલે શિક્ષણ જગતમાં આ પુસ્તક શિક્ષકો

અને વિદ્યાર્થીઓ માટે એક પ્રેરકબળ પૂરું પાડશે. જેમાં એકત્રાસ પત્રોમાં બહેનોએ અને આઠ પત્રોમાં ભાઈઓએ પોતાના સંવેદન વિશ્વને પ્રગટ કર્યું છે. વિદ્યાર્થીઓએ પોતાની મૌલિક શૈલીમાં સાંપ્રત સ્થિતિ-ગતિની અભિવ્યક્તિ કરી છે. તરુણ અને યુવાવસ્થાના ઉબરે આવીને ઊભેલા આ વિદ્યાર્થીઓના સ્પંદનો ખરા અર્થમાં આપણને સ્પર્શી જાય છે. આ વિદ્યાર્થીઓ પોતાના હૃદયમાં ઉઠતાં પ્રશ્નો, સમસ્યાઓ અને સાંપ્રત પરિસ્થિતિની અકળાવતી વ્યથા-કથાને અહીં પોતાના પ્રિય શિક્ષક સમક્ષ પ્રસ્તુત કરે છે. પોતાના સ્વજનો સમક્ષ મૌન રહેલા અને આ વ્યથાનો ભાર વહન કરતા વિદ્યાર્થીઓ હૃદયમાં ઉઠતી ઉલ્લસનો વ્યક્ત કરતા હળવાફૂલ થઈ જાય છે. આ પત્રના પ્રસંગો વિશે પુસ્તકની પ્રસ્તાવનામાં દીપક તેરૈયા જણાવે છે કે, 'જીવાતા જીવનમાં બનતા પ્રસંગો, સ્વજનનું મૃત્યુ, ક્યારેક ઘરમાં કોઈ ખાટો-મીઠો પ્રસંગ, બાળપણમાં કરેલા મસ્તી તોફાનો, કોઈ મજાના પ્રવાસના સરસ મજાના સંસ્મરણો કે પછી મારાથી ખોટું બોલાઈ ગયું અને ઘરના વ્યક્તિએ કે મારા સ્વજને મને સરસ રીતે ક્ષમા આપી. આપણી હૃદય નોટના પ્રત્યેક પાનામાં અંકાયેલા જે પ્રસંગો છે. વર્ગથી સ્વર્ગ સુધીનો રસ્તો કદાચ વિદ્યાર્થીના ઘર સુધીનો હોઈ શકે. પત્રો વાંચીને સમસ્યાના સમાધાન માટે એક શિક્ષકની વિદ્યાર્થીના ઘર સુધીની સફર એ કાંઈ નાની-સૂની ના કહેવાય !'

સંયુક્ત પરિવારમાં ગેસ દુર્ઘટના ઘટે અને પંખીનો માળો વિખાઈ જાય. પરિવારના ફોઈ, દાદા, દાદી, મમ્મીનું અવસાન થાય તેની અસર બાળમાનસ પર કેવી થાય તે હૃદયદ્રાવક ઘટનાથી પત્રની શરૂઆત થાય છે. બાકીના પત્રોને દાદા-દાદી, માતા-પિતા, ભાઈ, ગૃહકલેશ (આર્થિક સંકડામણ, ઝગડા કે પ્રસંગ) મિત્ર-બહેનપણી, બાળમસ્તી, પ્રાણીપ્રેમ જેવા વિષયો પર પત્ર લખાયા છે. વિવિધ વિષયોના પત્રો સંદર્ભે 'ભીતર ભીનો સંવાદ'માં ડૉ. રાકેશ રાવત જણાવે છે કે, 'વિદ્યાર્થીઓના આ પત્રો આપણને વર્ગમાં વહેતા ઝરણાં જેવા લાગે છે. તેમના હૃદયના અવાજમાં ક્યાંક વતનનો તલસાટ તો ક્યાંક મુખ્ય બાળપણ ! મિત્ર સાથે મધુર મિલન તો ક્યાંક મિત્રની અણધારી વિદાય ! ક્યાંક પાલવનો પ્રેમ તો ક્યાંક

માને જોવા ટળવળતી આંખો ! ક્યાંક પપ્પા સાથે દુનિયાદારીનું ભાન તો ક્યાંક પપ્પા વિનાનો ખાલીપો ! ક્યાંક દાદા દાદી સાથે રમવું તો ક્યાંક એમનો સ્નેહ મેળવવા ભમવું ! એક સિક્કાની બે બાજુ, જાણે કે બેન અને ભાઈ એક ઝાટકે કાપી નાખી કોણે આ સગાઈ ! તો ક્યારેક એમાં આવે છે માનવતાની વાતો કલ્પનાઓનો એ કારોબાર એના હૈયામાં ક્યાં સમાતો ! આ પુસ્તકમાં વિદ્યાર્થીઓના આ પત્રોમાં ભાષાશુદ્ધિમાં ફેરફાર કર્યો છે તો ક્યારેક પદક્રમ અને પદસંવાદ જાળવ્યો છે. દરેક પત્રમાં સરસ મજાનું ચિત્ર અને કાવ્યપંક્તિ મૂકીને એના ભાવને ઉજાગર કરવાનો પ્રયત્ન કર્યો છે.'

આ પત્ર પુસ્તકના મહત્વ વિશે જણાવતા મનસુખ સલ્લા નોંધે છે કે, 'રાકેશભાઈએ એ અર્થમાં એક યોગ્ય દિશા ખોલી છે કે વિદ્યાર્થીઓને કેમ સમજવા અને તેમને અભિવ્યક્ત થવાની તક કેમ આપવી. વાચકો એનો પણ અનુભવ કરશે કે વિદ્યાર્થીઓની હૃદયપોથીમાંથી ઉપસેલું આ ભાવજગત પણ કેટલું આકર્ષક અને રસિક છે. જે શિક્ષકો 'મારે પણ કંઈક કહેવું છે' ના આ ભાવજગત સુધી પહોંચશે તો તેઓ અનુભવશે કે તેઓ પણ આવું ઘણુંબધું કરી શકે એમ છે.' આમ આ પુસ્તકમાંથી પસાર થવાનું થયું. જેમાં વિદ્યાર્થીઓને લગતી પાંત્રીસ ફિલ્મોના નામો, શિક્ષણ સંદર્ભના ઓગણચાલીસ પુસ્તકોના નામ, ગુજરાતી ભાષાની સાધના કરતા શિક્ષકોના નામ સરનામા, અંતે રાકેશભાઈની કવિતા કલગી સમાન સાબિત થાય છે. આ પુસ્તકની પ્રસ્તાવના મનસુખ સલ્લા અને દિપક તેરૈયાએ આપી છે. અંદર દરેક પત્ર પછી 'હૈયાનું તોરણ' નામે વિષયને સચોટ કરતી કવિઓની પંક્તિઓ મૂકીને પુસ્તકની શોભા વધારી છે. પૂરા પુસ્તકમાં બાળકોએ અનુભવેલ ઘટનાઓ ને સ્થાન આપ્યું છે. એટલે શિક્ષણ જગતમાં આ પુસ્તક ખૂબ ઉપયોગી સાબિત થશે. ■

સંદર્ભ પુસ્તક : મારે કંઈક કહેવું છે ! સંપા. ડૉ. રાકેશ રાવત, પ્રકાશક-ભરત રાજ્યગુરુ, પ્રથમ આવૃત્તિ ૨૦૨૧. (કિંમત-૧૫૦)

— આસિસ્ટન્ટ પ્રોફેસર, ગુજરાતી ભાષા સાહિત્ય કેન્દ્ર, સેન્ટ્રલ યુનિવર્સિટી ઓફ ગુજરાત, સેક્ટર-૨૯, ગાંધીનગર.

WOMEN ENTREPRENEURSHIP THROUGH MICROFINANCE: A CASE STUDY OF PRAYAS JAN VIKAS BHANDOL - GANDHINAGAR

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Abstract

Microfinance is emerging as a powerful instrument and dominating force for poverty alleviation and providing linkage between Self Help Group-Bank as a cost effective mechanism for providing financial services to the marginalized families by women entrepreneurship; which has been successful not only in meeting financial needs of the rural poor women but also strengthen collective self-help capacities of the poor women. The female entrepreneurs are getting support through Prayas Microfinance Wing (Prayas Jan Vikas Bhandol) results in women's ability to influence or make decision, increased self-confidence, better status and role in household etc.

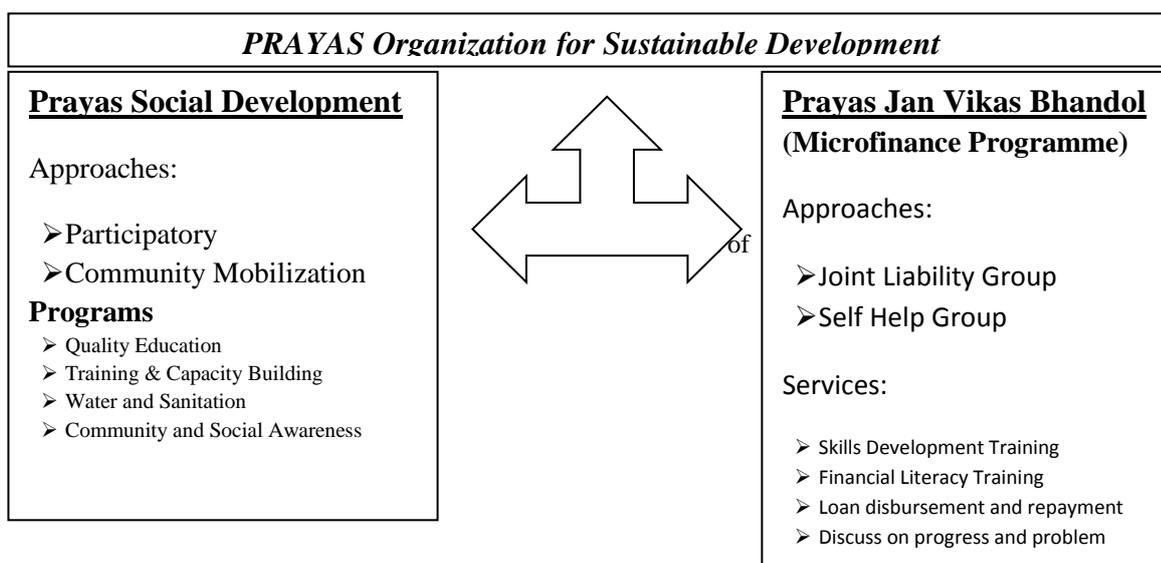
This paper puts forward how Prayas Jan Vikas Bhandol (PJVB) micro finance wing has received extensive recognition as a strategy for economic empowerment of women. This research examined the role of Prayas with respect to economic empowerment and poverty alleviation of rural women through entrepreneurship. An effort is also made to suggest the ways to increase women empowerment. The research used both quantitative and qualitative data collected from both primary and secondary sources. A sample of 30 women entrepreneurs from PJVB have been collected for the study. The Prayas Microfinance Wing was approached through questionnaire to collect primary data. Secondary data was collected from relevant books, journals, government publications, research reports, and various web sites. Analysis of data employed descriptive statistic. Further, the study established that Prayas microfinance wing provides economic support and have boosted women's self-esteem of the marginalized women through the services of microfinance.

Key words: Women Entrepreneurship, Empowerment, Microfinance, Prayas

Introduction

The seeds of Prayas were aimed at the gross root level empowerment, motivation and handholding support for deprived communities. The interventions of Prayas included in social development and microfinance enterprises over the period of last 17 years in Gujarat, Madhya Pradesh and Assam. They work especially with the needy women and children to bring positive changes in their life with the congregation of community members to enable them to avail government facilities as well as increase their knowledge and capacity for ensuring good health, education and financial security. Prayas vision envisions a society where people have better social and economic opportunities, accessibilities for financial services and awareness of rights and duties.

Prayas – Organization for Sustainable development emphasized on the activities which assembled towards finding solutions for the core development issues confronting the poor and marginalized women of needy community viz. entrepreneurship development and financial support to vulnerable families through microfinance, HIV/AIDS awareness, quality education, Adolescents health and hygiene, youth empowerment, water and sanitation.



*Source: Prayas – Annual Report 2019-20.

Prayas Microfinance Wing - Prayas Jan Vikas Bhandol (PJVB): It was set up in the year 2006 as a separate wing of PRAYAS with a commitment to enriching the lives of marginalized families through loans for income generation. PJVB is the second wing of the organization which provides economic support to the marginalized women through the services of microfinance. Its initiatives have cumulatively touched the lives of over 17000 members across the states of Gujarat and Madhya Pradesh. It has separate structure and employees from the social program of the organization are active in more than 92 villages and 47 urban localities. The implementation strategies of PJVB is SHG model in Bank partnership with YES bank and using Joint Liability Group (JLG) model for own portfolio. Prayas is presently focused to support to needy families through their female members only, which means to provide loan amount to the needy women microenterprises. The objective of providing microfinance services to the members are for their – Income generation; Help in the incidental economic crisis; House Construction.

The overall execution of PJVB program has been done by two teams: A core team at head office and a unit team at filed offices. The core team of microfinance is responsible for planning of routine tasks such as the goal of program can be achieved. The core team planning is included with financial planning, monitoring and controlling of microfinance operations. While the unit team works at the field level with group members as per decided planning and policies and are responsible for executing all field operations within time frame.

Loan Products through PJVB

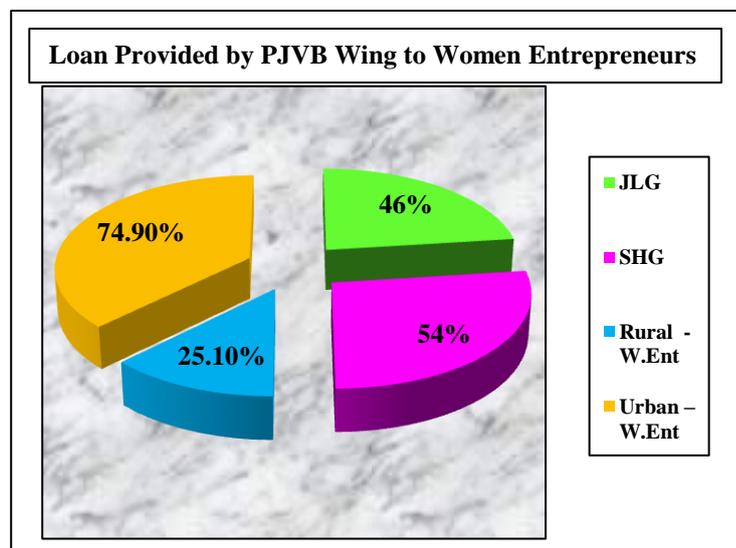
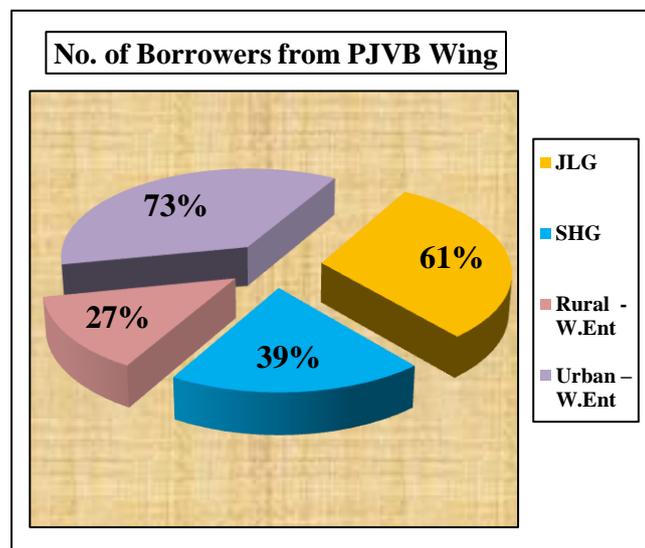
Features	JLG Monthly	SHG Monthly
Purpose	Agriculture, Live stock, Debt Redemption, Health, Business (Trading, Manufacturing/Service)	Agriculture, Live stock, Debt Redemption, Health, Business (Trading, Manufacturing/Service)
Group Size	5 to 20 members	8 to 20 members
Client Profile	Rural and urban poor women	Rural and urban women entrepreneurs
Loan Size in Rupees	5000 – 15000	10,000 – 30,000
Loan Tenure	12 months	12 months

*Source: PRAYAS – Annual Report 2019-20.

Present Scenario of PJVB

Product	No. of Borrowers	Percent (%)	Loan in Rupees.	Percent (%)
JLG	10534	61%	72,047,748	46%
SHG	6685	39%	87,791,876	54%
Rural - W.Ent	4657	27%	4,07,18,629	25.1%
Urban – W.Ent	12562	73%	12,13,59,060	74.9%

*Source: PRAYAS – Annual Report 2019-20.



Objectives of the Study

- To elucidate the approach of Prayas Jan Vikas Bhandol (PJVB) for women entrepreneurship.
- To study the kind of microenterprises and the impact of PJVB Microfinance Program.
- To find out the Loan drawn from PJVB program and Monthly Earnings among women entrepreneurs
- To identify the specific role of PJVB Microfinance program in developing women entrepreneurs and some successful beneficiaries case studies.

Literature Review

Manju & Mohan (2014) in their study “Role of NGO in Empowering Women through Microfinance: A Conceptual Study”, emphasis on women empowerment in a multi-dimensional ways and which helps women to gain self realization, financial security, and access to equal opportunities through microfinance. They found out the microfinance sector is the supporting system for the economic empowerment of women in both rural and urban poor and marginalized women. The study divulge that NGOs plays a key role in creating a feasible alternative platform for these women through microfinance program with the banking partnership by creating SHG models and awaring about the various government welfare schemes. Microfinance programmes are currently being promoted as a key strategy for simultaneously addressing both poverty alleviation and women's empowerment; whereas the financial service provision leads to the setting up or expansion of their micro-enterprises within the vicinity.

Richard, Ernest & Edith (2015) in the article entitled ‘Microfinance and Entrepreneurial Empowerment of Women: The Ugandan Context’, explained the role of microfinance in the entrepreneurial empowerment of women by adopting an exploratory cross sectional research design involving 150 women in Eastern and Central Uganda. The study revealed that access to credit is the major benefit from the microfinance services and the overriding benefit in the groups was observed to be social capital, networking superseding trust and reciprocity. Considering services received by women from the MFIs, there is need to support sensitization and technical skills such as marketing and financial literacy in order to enable women entrepreneurs put the borrowed finances to good use. Results reveal that microfinance and social capital have a significant relationship with entrepreneurial empowerment. This implies that Microfinance is an important tool towards the entrepreneurial empowerment of women in a resource perspective.

Sabiha (2014) in her study ‘Women Entrepreneurship as the Way for Economic Development’ analyzed the role of women entrepreneurs with respect to the banking institutions. This article studies the four aspects, such as the growth of women entrepreneurs in India; Studies tapping this growth in financing women entrepreneurs which is a major problem of failure in their businesses; third deals with the role of self help groups (SHGs) in promoting finances and empowering them through capacity building programmes; and the fourth aspect deals with future policies of the government providing conducive environment for their business and for their livelihood. The study critically analyzed many obstacles of women entrepreneurs, specifically in knowledge in accessibility to loans, funding agencies, awareness of government welfare programs, motivation, technical training and support from organizations, marketing their products and financial literacy as well as entry of rural women entrepreneurs in micro- enterprises by their effectual and competent involvement in entrepreneurial activities by strengthening entrepreneurial network.

Isidore, Norsiah & Razli (2010) in their work ‘The Effect of Microfinance Factors on Women Entrepreneurs’ performance in Nigeria: A Conceptual Framework’, discussed about the obstacles such as poverty, unemployment, low household income and societal discriminations mostly in developing countries have hindered their effective performance. It is discovered that women entrepreneurship could be an effective strategy to support their families as well as for poverty reduction in a country. However, women entrepreneurs do not have easy access to microfinance for their entrepreneurial activity than their men counterparts. The study also highlighted by examining the effect of credit, savings, training and social capital on women entrepreneurs’ performance in Nigeria by analyzing data with Structural Equation Modeling.

Shaik & Nikhat (2011) in their article ‘Women Entrepreneurship Development through Microfinance: A Study Focused on Hyderabad’, emphasized on entrepreneurship represents an appropriate opportunity for women and responds flexibly to entry, change and innovation. Women’s entrepreneurial activities are not only a means for economic survival but also have positive social repercussions and the objective of the study focused on the impact of Microfinance programs on entrepreneurial skills and socio-economic development of Muslim minority women entrepreneurs. A sample of 157 Muslim minority women confined to Hyderabad District financed by APSMFC is taken to test the hypothesis of the research work. The study found that Microfinance scheme of APSMFC is useful in development of women entrepreneurship. The authors conclude that, women can benefit from microfinance in increased income levels, empowerment, sustainability and self reliance which are the positive points in entrepreneurship development and if the constraints be removed, the micro finance sector is overall improving Entrepreneurship Development of Women.

Bernard (2015) in his work entitled ‘Microfinance Services: Facilitating Entrepreneurial Success of Poor Women’, was based on the review of literature on microfinance and the influence of microfinance services (factors of microfinance) on entrepreneurial success of poor women and understand weaknesses of such services in the process of women achieving entrepreneurial success with specific reference to Sri Lanka. The paper argues that the microfinance services play a major role in achieving entrepreneurial success of women, and also highlights drawbacks of microfinance as a strategy for developing entrepreneurship of poor women. The research identifies some important areas for empirical research and the findings would facilitate microfinance service providers to design their service offers to enhance entrepreneurial outcomes of their clients. Hence, there is a clear need for studies to explore as to how far poor women in Sri Lanka achieve entrepreneurial success through microfinance services and the relative importance of each of these services.

Rashid, John, Consolatta & Stephen (2015) the study ‘Impact of Microfinance Institutions on Economic Empowerment of Women Entrepreneurs in Developing Countries’, mirrored out with the Descriptive research design and assessed the extent to which Women economic empowerment co-relates with Microfinance Institutions services. The study found that there was an improvement in advisory services and savings services besides the microcredit services. Therefore the MFIs have assisted the

women entrepreneurs get more empowered economically than before. The data indicated that there was increased women responded that their standard of living had improved, participating in decision making in their houses, control over resources, self employment and increase in savings insurance services. The findings are highlighted that the policy proposals of MFIs meet the economic empowerment needs of women Entrepreneurs to make developing countries progress as Kenya prepares to achieve vision 2030.

Naeem, Khan, Sheik, Ali & Faqir (2015) in their article ‘The Impact of Microfinance on Women Micro-Enterprises “A Case Study of District Quetta, Pakistan”’, analyzed that lack of financial resources are the major constraints for women participation in economic development.

The microfinance is considered as one of the important tool for resolving this problem. This study also explored the impact of BRAC, Pakistan microfinance program on women microenterprises. Results of the study show that microfinance has positive impact on creation of microenterprises such as trading, business and self employment. It has significant impact on beneficiaries’ enterprise working capital and fixed assets as compare to non-beneficiaries. Therefore it is concluded from this research study that overall microfinance services are playing positive role in women entrepreneurial performance.

Methodology

PRAYAS is a Gujarat based Organization for Sustainable Development work with a vision to bring positive changes in the life of women. With the help of survey design; the primary data is collected through structured questionnaires covering a sample size of 30 women entrepreneurs who availed microfinance loans from the Prayas Jan Vikas Bandhol (Microfinance Program) with banking partnership of YES and IDBI banks. The sample population was women entrepreneurs from PJVB Wing within Gandhinagar selected who are the actual owners of the microenterprises in order to draw impact of microfinance on enterprise accurately. The stratified random sample is used for selection of members from both groups of JLG (Joint Liability Group) and SHG (Self Help Groups). The interview was conducted by visiting the clients on their enterprise and at the regional office where the clients have visited to pay their installments. Data collected was presented by descriptive statistics with univariate and bi-variate analysis by using pie charts and graphs. The collected data analyzed by using the Statistical Package for Social Scientists (SPSS) version 19.

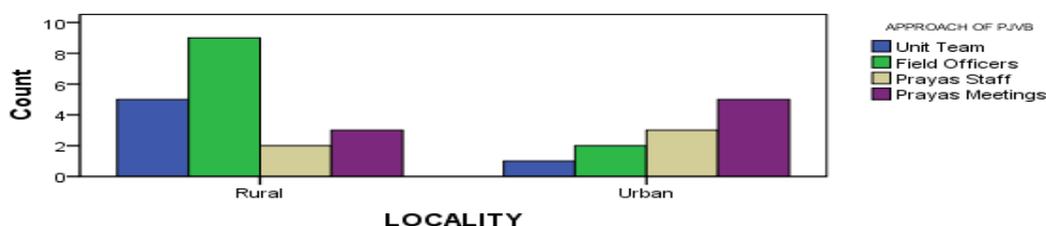
DATA ANALYSIS

Table: 1

LOCALITY * APPROACH OF PJVB

LOCALITY	APPROACH OF PJVB				Total
	Unit Team	Field Officers	Prayas Staff	Prayas Meetings	
Rural	5 26.3%	9 47.4%	2 10.5%	3 15.8%	19 100.0%
Urban	1 9.1%	2 18.2%	3 27.3%	5 45.5%	11 100.0%
Total	6 20.0%	11 36.7%	5 16.7%	8 26.7%	30 100.0%

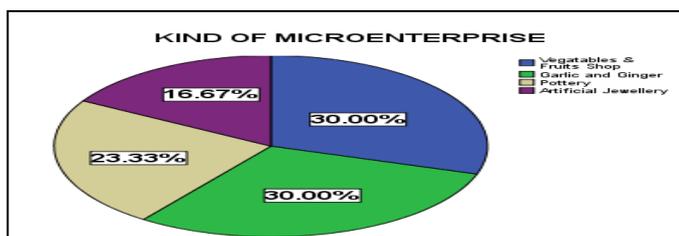
*Source – Field Survey



The above table explains about the approaches/strategies of PJVB Microfinance Program for the women entrepreneurship in both and urban localities of Gandhinagar. The respondents shared their opinion about the awareness of PJVB program through the services of unit teams by (20%), Field Officers (36.7%), by Prayas staff (16.7%) and through Prayas meetings (26.7%).

Table: 2 (A)

Kind of Microenterprise	Frequency	Percent
Vegetables & Fruits Shop	9	30.0
Garlic and Ginger	9	30.0
Pottery	7	23.3
Artificial Jewellery	5	16.7
Total	30	100.0

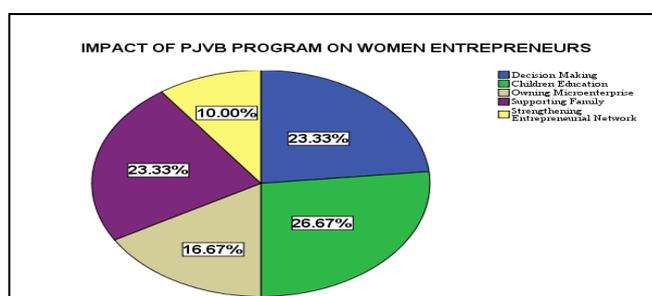


The table 2(A) enumerates about the kind of Microenterprise owning by the women entrepreneurs with the regard to PGVB Microfinance program. The equal percentage of women entrepreneurs with 30% own vegetable and fruits shops and Garlic and ginger business, followed by 23.3% of the respondents are with pottery business and around 16.7% are having their artificial jeweler business.

Table: 2(B)

IMPACT AND BENEFITS OF PJVB PROGRAM ON WOMEN ENTREPRENEURS

Impact of PJVB on WE	Frequency	Percent
Decision Making	7	23.3
Children Education	8	26.7
Owning Microenterprise	5	16.7
Supporting Family	7	23.3
Strengthening Entrepreneurial Network	3	10.0
Total	30	100.0



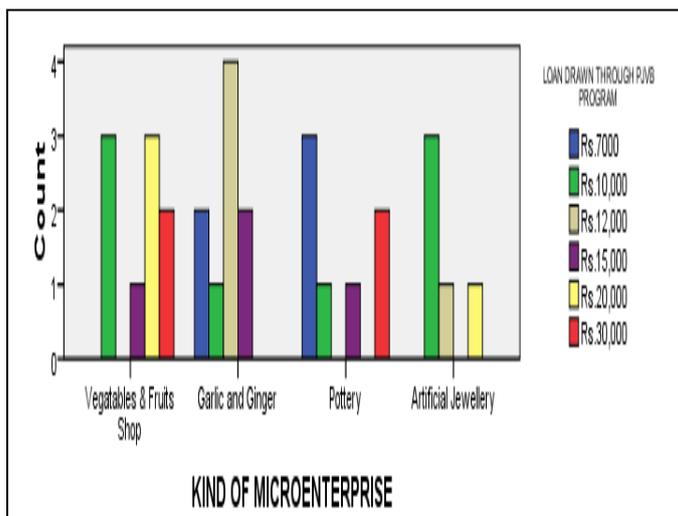
*Source:-FieldSurvey

Majority of 26.7% of the women entrepreneurs have benefitted with the PJVB microfinance program and spent their money to their children’s education; whereas equally 23.3% of the respondents have support their family needs and acquired the decision making capability at household level. Around 16.7% had responded that they started owning their business despite working under someone else. Lastly, around 10% had opined that they strengthen the entrepreneurial network from their localities to district level.

Table: 3

KIND OF MICROENTERPRISE * LOAN DRAWN THROUGH PJVB PROGRAM

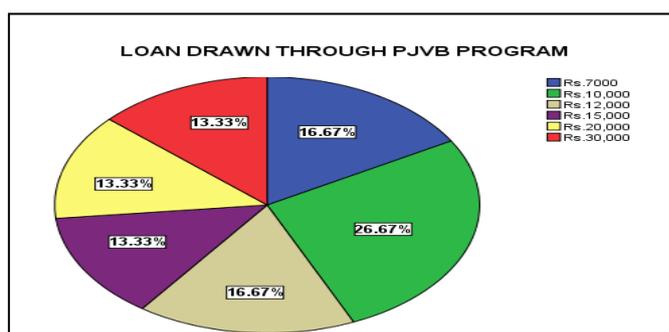
Kind of Microenterprise	LOAN DRAWN THROUGH PJVB PROGRAM						Total
	Rs.7000	Rs.10,000	Rs.12,000	Rs.15,000	Rs.20,000	Rs.30,000	
Vegetables & Fruits Shop	0 .0%	3 37.5%	0 .0%	1 25.0%	3 75.0%	2 50.0%	9 30.0%
Garlic and Ginger	2 40.0%	1 12.5%	4 80.0%	2 50.0%	0 .0%	0 .0%	9 30.0%
Pottery	3 60.0%	1 12.5%	0 .0%	1 25.0%	0 .0%	2 50.0%	7 23.3%
Artificial Jewellery	0 .0%	3 37.5%	1 20.0%	0 .0%	1 25.0%	0 .0%	5 16.7%
Total	5 100.0%	8 100.0%	5 100.0%	4 100.0%	4 100.0%	4 100.0%	30 100.0%



The table: 3 explain about the relationship between the kind of microenterprise started with the support of PJVB program and the loan drawn from the microfinance wing. Majority of equally 30% respondents have started their vegetables and fruits, garlic and ginger business by getting first loan of Rs.7000 and at second loan with Rs10, 000 to Rs.15, 000 and third loan with an amount of Rs.20, 000 to Rs.30, 000. Followed by 23% have started pottery toys and diyaas. Lastly, 17% had started doing business with artificial jewels for their livelihood.

Table: 4
Loan Drawn through PJVB Program

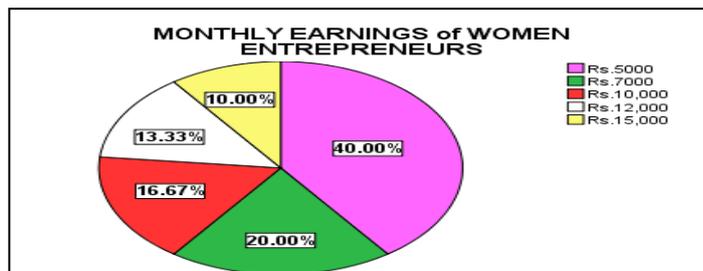
Loan in Rs.	Frequency	Percent
Rs.7000	5	16.7
Rs.10,000	8	26.7
Rs.12,000	5	16.7
Rs.15,000	4	13.3
Rs.20,000	4	13.3
Rs.30,000	4	13.3
Total	30	100.0



The above table 4 (A) explains the loans drawn by the women entrepreneurs from Prayas Microfinance program. The respondent's loan drawing is ranging from Rs.7000 to Rs.30, 000. But the majority of 26.7% are borrowed the loan amount of Rs.10,000 followed by 16.7% of women entrepreneurs borrowed from the first loan cycle with Rs.7000 to second loan cycle of Rs.12,000. Lastly, equally 13.3% of the respondents borrowed the third cycle of loan amount with an increasing range of loan from Rs.15, 000 to Rs.30, 000.

Table: 4(B)
Monthly Earnings of Women Entrepreneurs

Monthly Earnings	Frequency	Percent
Rs.5000	12	40.0
Rs.7000	6	20.0
Rs.10,000	5	16.7
Rs.12,000	4	13.3
Rs.15,000	3	10.0
Total	30	100.0



The above table: 4 (B) explains about the women entrepreneurs monthly income and earnings out of their microenterprises. Majority of 40% have Rs.5000 after all the expenses and loan pay. Around 20% have their earnings of Rs.7000 and 16.7% had their earnings with Rs.10, 000 and 13.3% had with Rs.12, 000 and very of 10% had a good earning amount of Rs.15, 000.

Case Studies of PJVB Women Entrepreneurs

Sumitra Ben is a 35 years old working women and a member of Shree mahamati mahila samuh. She has husband and two sons go on migration to big cities of Gujarat for livelihood. The family decided to stay in village for better upbringing and continuity of education for their children, but they were finding very difficult in fulfilling their family expenses. She was supported by the PJVB program by providing loan. She has brought a cart from a loan amount to carry vegetables and fruits for selling in the market. The positive impact of PJVB microfinance program and her hard work enable her to save Rs.4000 every month after deducting the loan installment and other expenses. Sumitra is looking forward to borrow more loans in next cycles and planning to expand her business from cart to establish her own shop.

Sumali Ben is a 42 year old woman attended a meeting of PRAYAS at her village and decided to start her own microenterprise despite doing labour work for building construction for Rs.%0 per day. She was supported by a small loan with Rs.7000 in first cycle to buy garlic in bulk and sell it in the market. She borrowed Rs.12000 and Rs.15000 in second and third cycle and now expanded her business to sell garlic in wholesale to other shopkeepers at weekly market. After completing the third cycle loan, she has borrowed loan of Rs.50, 000 for constructing her house. The impact of this microfinance program led to avail proper education of her grand children.

Zareena Ben is a member of Acharya Peer Dada Mahila Mandal. Earlier she was involved in household work and was able to earn only Rs.3000 to Rs.3500 per month and is really difficult to meet the basic family needs. Sit he came to know about Prayas microfinance in 2008 and decided to take loan to start a small business of pottery work. After the setting up of small unit to make pottery and expanded their business and successfully settled and able to earn Rs.15000 per month after deducting the installments for loan amount and other expenses. The impact of PJVB program brought her new hope and a sustainable income source despite labour work.

Conclusion

The study concludes with the positive impact of microfinance program on women entrepreneurship and entrepreneurial networks. The Prayas Jan Vikas Bandhol (PJVB) is popularly known as Prayas Microfinance Wing which provides economic support to the marginalized families through the microfinance services in collaboration with banking sector. It has proved that micro-credit is significantly related to self-employment, education of respondent's children, training, skills acquisition, and economic empowerment. Women entrepreneurs need micro-credit to become or remain self-employed either by starting their own businesses or by expanding their existing businesses. To increase their capability and level of empowerment and entrepreneurial skills in their chosen firms would produce and generate a sustainable income. The PRAYAS plays a pivotal role in creating awareness among rural women to start their business by providing Prayas financial support to empower them as women entrepreneurs.

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