Government of West Bengal
Department of Science & Technology and Biotechnology

Report on the BOOST Programme

(to be submitted by the grantee Department)

1. **Name of Institute:** Uluberia College
2. **BOOST Programme: BOOST II Programme** (Sanction no: 339/WBBDC/IP-2/2013 dt. 03.09.2014)
3. **Name of the grantee department(s):** School of Life Sciences
4. **Name and affiliation of the Programme Coordinator:**

   Dr. Siddhartha Sankar Bhattacharya
   Associate Professor *and* Head,
   Department of Zoology
   Uluberia College
   Uluberia, Howrah - 711 315

5. **Grant received:** Rs. 40,00,000.00/- (Rupees Forty Lakh)
6. **Grant utilized:** Rs. 41,35,187.00/- (Rupees Forty One Lakh Thirty Five Thousand One Hundred Eighty Seven)
7. **Unspent amount: :** Rs. 0.00/-
8. **Unspent amount refunded (if any):** NA
9. **Report submitted:** Yes
10. **UC submitted:** Yes (please attach the scanned UC)
11. **Audited Statement of Expenditure submitted:** Yes (please attach the scanned SoE)
12. **Details of the BOOST funded equipment:** (please attach the scanned Sanction Letter)

Sl.	Instrument sanctioned	Purchased (Y/N)	Installed (Y/N)	Utilized for Research and/or students' practical	AMC status	Remarks (if any)
1	Protein Gel	Y	Y	Both	NA	
2	Trinocular Microscope	Y	Y	Both	NA	
3	UV Spectro	Y	Y	Both	NA	
4	Refrigerated Centrifuge	Y	Y	Both	NA	
5	Thermocycler	Y	Y	Both	NA	
6	Micropipette	Y	Y	Both	NA	
7	Pipetting Aid	Y	Y	Both	NA	
8	pH meter	Y	Y	Both	NA	
9	Magnetic Stirrer	Y	Y	Both	NA	
10	Vortex Mixer	Y	Y	Both	NA	
11	Table Micro centrifuge	Y	Y	Both	NA	
12	DNA Gel Power Pack	Y	Y	Both	NA	

13	Digital Balance	Y	Y	Both	NA	
14	Water Bath	Y	Y	Both	NA	
15	Heating Plate	Y	Y	Both	NA	
16	Gel Rocker	Y	Y	Both	NA	
17	Chest Freezer	Y	Y	Both	NA	
18	Vertical Autoclave	Y	Y	Both	NA	
19	Gel Docu & Image system	Y	Y	Both	NA	
20	Double Distillation	Y	Y	Both	NA	
21	Bio Saf/Laminar Flow	Y	Y	Research	NA	
22	CO_2 incubator	Y	Y	Research	Running	

13. Students (from grantee and/or other departments) who utilized the BOOST sponsored facility to fulfil their academic curriculum:

	Name of the Department(s)	**Total no. of students** (Starting from 1st academic year after installation of the facility)						
		………… **(Year 1)**	………… **(Year 2)**	………… **(Year 3)**	………… **(Year 4)**	………… **(Year 5)**	………… **(Year 6)**	………… **(Year 7)**
Grantee Department	School of Life Sciences, Uluberia College, Uluberia, Howrah - 711 315, India	**70**	**75**	**75**	**80**	**80**	**90**	**90**
Other Departments	Dept. of Chemical Biological & macromolecular Sciences S. N. Bose National Centre for Basic Sciences, J. D. Block, Sector III, SaltLake, Kolkata 700 098, India	**4**	**4**	**5**	**5**	**5**	**5**	**5**
	Department of Microbiology, St. Xavier's College , 30 Mother Teresa Sarani Kolkata 700016, India	**NA**	**NA**	**NA**	**1**	**1**	**1**	**1**
	Department of Biotechnology, Thapar Institute of Engineering and	**NA**	**NA**	**NA**	**NA**	**NA**	**1**	**NA**

	Technology, Bhadson Road, Patiala, Punjab 147004, India							
Total		**74**	**79**	**80**	**86**	**86**	**97**	**96**

14. Details of the research projects (of grantee or other departments) in which the BOOST sponsored facility was utilized:

Sl. no.	Title of the research project	Name & Affiliation of P.I.	Equipment used	No. of research fellow benefitted
1	Effectiveness of novel nano formulation in treatment of ROS mediated neurodegenerative disorders	Dr. Debasish Pal, Principal & Associate Professor, Department of Zoology, Uluberia College, Uluberia Howrah - 711 315 & Prof. Samir Kumar Pal Adjunct Professor, Department of Zoology, Uluberia College, Uluberia Howrah - 711 315	pH meter , UV Spectro, Trinocular Microscope , Micropipette, Pipetting Aid, Table Micro centrifuge, Magnetic Stirrer, Vortex Mixer, Digital Balance, Double Distillation, Chest Freezer	**4**
2	Sub chronic toxicity and biodistribution of nanomaterials	Dr. Siddhartha Sankar Bhattacharya Associate Professor and Head, Department of Zoology, Uluberia College, Uluberia Howrah - 711 315 & Prof. Samir Kumar Pal Adjunct Professor, Department of Zoology, Uluberia College, Uluberia Howrah - 711 315	UV Spectro, Micropipette, Pipetting Aid, Trinocular Microscope , Table Micro centrifuge, Magnetic Stirrer, Vortex Mixer, Digital Balance, Double Distillation, Chest Freezer	**4**
3	Effectiveness of novel nano formulation in treatment of inflammatory disorders	Dr. Debasish Pal, Principal & Associate Professor, Department of Zoology, Uluberia College, Uluberia	UV Spectro, Trinocular Microscope , Micropipette, Pipetting Aid, Table Micro centrifuge,	**4**

		Howrah - 711 315 & Prof. Samir Kumar Pal Adjunct Professor, Department of Zoology, Uluberia College, Uluberia Howrah - 711 315	Magnetic Stirrer, Vortex Mixer, Digital Balance, Double Distillation, Chest Freezer	
4	Effectiveness of new metal hybrid formulae in treatment of diabetes and associated disorders	Dr. Debasish Pal, Principal & Associate Professor, Department of Zoology, Uluberia College, Uluberia Howrah - 711 315 & Prof. Samir Kumar Pal Adjunct Professor, Department of Zoology, Uluberia College, Uluberia Howrah - 711 315	pH meter , UV Spectro, Trinocular Microscope , Micropipette, Pipetting Aid, Table Micro centrifuge, Magnetic Stirrer, Vortex Mixer, Trinocular Microscope, Digital Balance, Double Distillation, Chest Freezer	**5**
5	Role of novel nano formulation in treatment of hematological disorders	Dr. Siddhartha Sankar Bhattacharya Associate Professor and Head, Department of Zoology, Uluberia College, Uluberia Howrah - 711 315 & Prof. Samir Kumar Pal Adjunct Professor, Department of Zoology, Uluberia College, Uluberia Howrah - 711 315	pH meter , UV Spectro, Trinocular Microscope , Micropipette, Pipetting Aid, Table Micro centrifuge, Magnetic Stirrer, Vortex Mixer, Trinocular Microscope, Refrigerated Centrifuge, Digital Balance, Double Distillation, Chest Freezer	**5**
6	Role of nanomaterial in treatment of wound healing	Dr. Siddhartha Sankar Bhattacharya Associate Professor and Head, Department of Zoology, Uluberia College, Uluberia Howrah - 711 315 & Prof. Samir Kumar Pal	Trinocular Microscope , Micropipette, Pipetting Aid, Table Micro centrifuge, Magnetic Stirrer, Vortex Mixe, Digital Balance, Double Distillation	**5**

		Adjunct Professor, Department of Zoology, Uluberia College, Uluberia Howrah - 711 315		
7	Ultrafast Spectroscopy on DNA-Cleavage by Endonuclease in Molecular Crowding	Prof. Samir Kumar Pal Adjunct Professor, Department of Zoology, Uluberia College, Uluberia Howrah - 711 315	UV Spectro, DNA Gel Power Pack, Gel Docu & Image system, Micropipette, Pipetting Aid, Table Micro centrifuge, Magnetic Stirrer, Vortex Mixer, Digital Balance, Double Distillation	**4**
8	Synthesis and Characterization Nanohybrid for Potential Photodynamic Therapeutic Application	Prof. Samir Kumar Pal Adjunct Professor, Department of Zoology, Uluberia College, Uluberia Howrah - 711 315	UV Spectro, pH meter, Bio Saf/Laminar Flow, Vertical Autoclave, CO_2 incubator, Protein Gel, Gel Rocker, Refrigerated Centrifuge, Thermocycler, Digital Balance, Double Distillation	**4**
9	Rationalization of Traditional Liver Medicine Using Systems Biology Approach and Its Evaluation in Preclinical Trial	Dr. Debasish Pal, Principal & Associate Professor, Department of Zoology, Uluberia College, Uluberia Howrah - 711 315 & Prof. Samir Kumar Pal Adjunct Professor, Department of Zoology, Uluberia College, Uluberia Howrah - 711 315	pH meter , UV Spectro, Trinocular Microscope , Micropipette, Pipetting Aid, Table Micro centrifuge, Magnetic Stirrer, Vortex Mixer, Trinocular Microscope, Refrigerated Centrifuge, Protein Gel, Gel Rocker, Digital Balance, Double Distillation, Chest Freezer	**3**
10	Role of Natural Flavonoids in Detoxification of Lead Poisoning	Dr. Debasish Pal, Principal & Associate Professor, Department of	pH meter , UV Spectro, Trinocular Microscope , Micropipette,	**4**

		Zoology, Uluberia College, Uluberia Howrah - 711 315 & Prof. Samir Kumar Pal Adjunct Professor, Department of Zoology, Uluberia College, Uluberia Howrah - 711 315	Pipetting Aid, Table Micro centrifuge, Magnetic Stirrer, Vortex Mixer, Trinocular Microscope, Refrigerated Centrifuge, Digital Balance, Double Distillation, Chest Freezer	

15. Training programmes/workshops organized utilizing the BOOST sponsored facility:

Sl. no.	Title of the programmme	Year	Name and affiliation of the Programme Co-ordinator	Total no. of participants
1	Workshop on Advanced Nanotherapeutics	2017	Dr. Debasish Pal, Principal & Associate Professor, Department of Zoology, Uluberia College, Uluberia Howrah - 711 315 & Dr. Siddhartha Sankar Bhattacharya Associate Professor and Head, Department of Zoology, Uluberia College, Uluberia Howrah - 711 315	30
2	Workshop on "Perfect Pipetting Techniques and Cleaning Procedures"	2017	Dr. Debasish Pal, Principal & Associate Professor, Department of Zoology, Uluberia College, Uluberia Howrah - 711 315 & Dr. Siddhartha Sankar Bhattacharya Associate Professor and Head, Department of Zoology, Uluberia College, Uluberia Howrah - 711 315	35

Government of West Bengal
Department of Science & Technology and Biotechnology

16. Participation in National/International/Provincial Seminar/Conference/Workshop arising from work done using the BOOST sponsored facility:

Sl. no.	Title of the Seminar/Conference/Workshop	National/ International/ Provincial	Year	Participant Department	No. of participants
1	Gordon Research Seminar- Oxygen Radicals 2020, Ventura, USA	International	2020	Department of Zoology, Uluberia College, Uluberia Howrah - 711 315	1
2	International Conference on Nanoscience and Technology (ICONSAT 2020) held at SN Bose National Centre for Basic Sciences, India	International	2020	Do	3
3	Gordon Research Seminar- Clusters and Nanostructures 2019, Switzerland	International	2019	Do	1
4	APDW-2019 organized by Asian Pacific Digestive Week Federation held at Kolkata, India	International	2019	Do	1
5	Paris Redox-2019 held at University Pierre and Marie Curie, Paris, France	International	2019	Do	1
6	International Conference on Nanoscience and Technology (ICONSAT 2018) held at Indian Institute of Science, Bangalore	International	2018	Do	1
7	Nanochallenge 2017 organized by Suny Polytechnic Institute, New York, USA and PSG Institute of Advanced Studies, Coimbatore, India	International	2017	Do	1
8	International Conference on Nanoscience and Technology (ICONSAT 2016) held at Indian Institute of Science Education and Research, Pune, India	International	2016	Do	1
9	NanoBioCon 2016 organized by Institute of Electrical and Electronics Engineers (IEEE) and MaulanaAbulKalam Azad University of Technology (MAKAUT; formerly WBUT), India	International	2016	Do	1

10	International Conference on Biomedical Science and Instrumentation 2016 (ICBSI - 2016)' organized by University of Calcutta, India	International	2016	Do	1
11	Ultrafast Science- 2015 held at SN Bose National Centre for Basic Sciences, India	National	2015	Do	1
12	BESHCON 2020 5th Annual Conference of Bengal Society of Hematology,India	National	2020	Do	2
13	Bose Fest-2019, SN Bose National Centre for Basic Sciences, India	National	2019	Dept. of Chemical Biological & macromolecular Sciences S. N. Bose National Centre for Basic Sciences, J. D. Block, Sector III, SaltLake, Kolkata 700 098	3
14	Bose Fest-2016, SN Bose National Centre for Basic Sciences, India	National	2019	Do	1
15	Bose Fest-2017, SN Bose National Centre for Basic Sciences, India	National	2019	Do	1
16	Workshop on Advanced Nanotherapeutics 2017 (WANT 2017) held at Uluberia Colleg, India	National	2017	Department of Zoology, Uluberia College, Uluberia Howrah - 711 315	2
17	National Conference on Nanotechnology in Medicine-2017' organized by Chettinad Academy of Research and Education, India	National	2017	Do	1
18	7th Masterclass in Liver Diseases (MCLD) 2017 organized by Institute of liver Disease and Transplantation (ILDT), Gleneagles Global Hospitals, Chennai, India	National	2017	Do	1
19	Workshop on Advanced	National	2017	Do	09

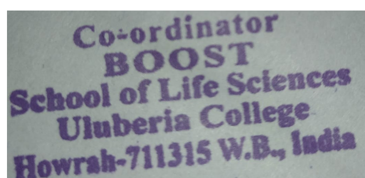
	Nanotherapeutics 2017 organized by Uluberia College, India				
20	Workshop on “Advances in Biological Techniques” 2017 organized by Raja Pyari Mohan College, India	National	2017	Do	20
21	Workshop on “Perfect Pipetting Techniques and Cleaning Procedures” 2017 organized by Uluberia College, India	National	2017	Do	10

17. Post-curricular achievements by the students having been benefitted from the BOOST sponsored facility:

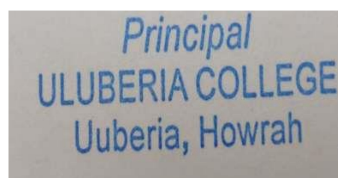
Achievements by the students	**Total no. of students** (Starting from 1st academic year after installation of the facility)						
	………… **(Year 1)**	………… **(Year 2)**	………… **(Year 3)**	………… **(Year 4)**	………… **(Year 5)**	………… **(Year 6)**	………… **(Year 7)**
Joined Ph.D	**NA**	**NA**	**1**	**NA**	**NA**	**2**	**NA**
Secured job	**NA**	**NA**	**NA**	**2**	**2**	**NA**	**NA**

Declaration: We do hereby declare that the above information are correct and based on the documentation by the Grantee Department(s). I also understand that the above information are subject to physical verification by the Department of Science & Technology and Biotechnology, Govt. of West Bengal at any point of time.

……………………………………………………………
Signature of the
Programme Coordinator(s) (With seal)
Date: 12/01/2021



……………………………………………………………
Signature of the
Head of the Department (With seal)
Date: 12/01/2021



Banerjee Abhijit & Associates
Chartered Accountants
Mobile : 9830177234
Office : Room No. - 28, Sree Hari Shopping Complex, P.O. - Chanditala, Dist. - Hooghly, Pin-712702 (W.B.)
Ph. : (03212)- 237043(O) Mobile : 9531672353 (O), E-mail : caabhijitbanerjee@gmail.com

UTILISATION CERTIFICATE

This is to certify that the grant of Rs. 40,00,000/= (Rupees Fourty lakh only) was sanctioned by West Bengal Biotech Development Corporation Ltd. Department of Biotechnology vide Sanction No:339/WBBDC/1P-2/2013 dated 03.09.2014, towards Biotechnology Instruments –BOOST-II (Instruments Grants to College) in favour of ULUBERIA COLLEGE , Uluberia, Howrah. Against the said grant, the bill or proforma in voice of Rs. 30,92,095/= (Rupees Thirty lakh ninety two thousand ninety five only) has been paid or debited and remaining amount Rs. 10,43,092/= for Reagents & Consumables under Processing as per details given in the attached statement and accordance with the terms and conditions laid down by Government Of West Bengal Office of The West Bengal Biotech Development Corporation Ltd. Department of Biotechnology for the purpose for which it was sanctioned.

If as a result of check or audit objection, some irregularity is noticed at a later stage, action will be taken to refund or regularize the objected amount. It is further certified that inventories of permanent or semi – permanent assets created/acquired wholly or mainly out of the grants given by the Government Of West Bengal Office of The West Bengal Biotech Development Corporation Ltd. Department of Biotechnology as indicated above are being maintained in the prescribed form and are being kept up-to-date and these assets have not been disposed of , encumbered or utilized for any other purpose.

Place: Chanditala, Hooghly
Date : 07.04.2016

For: **BANERJEE ABHIJIT & ASSOCIATES**
Chartered Accountants

(ABHIJIT BANERJEE)
M - 056016
B.COM (Hons), FCA, DISA (ICAI)

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ULUBERIA COLLEGE

Uluberia : Howrah
West Bengal : Pin -711 315

RECEIPT & PAYMENT ACCOUNT

For : Biotechnology Instruments - Grant Received from West Bengal Biotech Development Corporation Ltd.
Department of Biotechnology

Vide : Sanction No:339/WBBDC/1P-2/2013 dated 03.09.2014

Receipts	Amount (Rs.)	Payments	Amount (Rs.)
To Grant Received From Boost -II: Vide: Sanction No: 339/WBBDC/1P-2/2013 dt. 03.09.2014	4000000.00	By Purchase of Biotechnology Equipment (As per Annexure)	2933919.00
(Ch No-792751 Dated 04.09.2014		By Bank Charges	9042.00
To Interest On SB	135187.00	By Commission for Airport & Dock	149134.00
		By Reagents & Consumables [Processing]	1043092.00
	4135187.00		4135187.00

Place: Chanditala, Hooghly

Date :07-04-2016

Signed in Terms of our Separate Report of even date.

For: **BANERJEE ABHIJIT & ASSOCIATES**
Chartered Accountants

(ABHIJIT BANERJEE)
M - 056016
B.COM (Hons), FCA, DISA (ICAI)

Principal
ULUBERIA COLLEGE
Uuberia, Howrah

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ULUBERIA COLLEGE

ULUBERIA, HOWRAH, PIN-711 316

DETAILS STATEMENT OF BIOTECHNOLOGY EQUIPMENTS (ANNEXURE)

SL NO	Item Description	Supp. Name	Address	Invoice No & Date	Amount	Cheque No & Date	Nature Of Transtion
1	Protein Gel	ASE Instruments	5A/1, Abinash Chandra Banerjee Lane,Beliaghata, Kolkata - 10	I/071-14/13,DT-07.05.2015	113400.00	Ch No-79895,DT-21.05.15	INDIA
2	Trinocular Microscope	Digitech Systems	12/1/9, Monoharpukur Road,1st Floor, Kolkata- 700 026	DS/15-16/020,DT-14.05.2015	237938.00	Ch No-234111,DT-10.07.15	INDIA
3	UV Spectro	TECHCOMP LIMITED	Macao Commercial offshore Em Macau, Ruade Pequim, Ns 202A-246	M115/001942.DT-07.05.2015	429166.00	ChNo.NEFT ,DT-31.03.2015	Out Side India
4	Refrigerated Centrifuge	Hermle Labortechnik	Siemensstr 25,78564 Welingen, Germany	205610-AU 20889,DT-08-04.15	364337.00	Ch No.NEFT ,DT-31.03.15	Out Side India
5	Thermocycler	BIBBY SCIENTIFIC LTD	Beacon Road,Staffordshire ST-15 OSA,United Kingdom	177113,DT-07-04-2015	227016.00	Ch No.NEFT ,DT-31.03.15	Out Side India
6	Micropipette	Digitech Systems	12/1/9, Monoharpukur Road,1st Floor, Kolkata- 700 026	DS/15-16/021,DT-14.05.2015	21620.00	Ch No-234111,DT-10.07.15	INDIA
7	Pipetting Aid	Digitech Systems	12/1/9, Monoharpukur Road,1st Floor, Kolkata- 700 026	DS/15-16/034,DT-25.06.2015	9380.00	Ch No-234111,DT-10.07.15	INDIA
8	PH Meter	Labchem & Labortenik Instruments	48/C,Co-operative Colony, Kolkata - 700 118	I/L46-14/05,DT-16.02.2015	17175.00	Ch No-79896,DT-20.05.15	INDIA
9	Magentic Stirrer	Labchem & Labortenik Instruments	48/C,Co-operative Colony, Kolkata - 700 118	I/L48-14/09,DT-16.02.2015	14198.00	Ch No-79899,DT-12.06.15	INDIA
10	Vortex Mixer	Labchem & Labortenik Instruments	48/C,Co-operative Colony, Kolkata - 700 118	I/L47-14/08,DT-16.02.2015	8015.00	Ch No-79899,DT-12.06.15	INDIA
11	Table Micro Centrifuge	ASE Instruments	5A/1, Abinash Chandra Banerjee Lane,Beliaghata, Kolkata - 10	I/076-14/14,DT-12.02.2015	22050.00	Ch No-79895,DT-21.05.15	INDIA

Principal
ULUBERIA COLLEGE
Uuberia, Howrah

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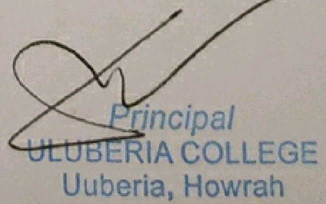
ULUBERIA COLLEGE

ULUBERIA, HOWRAH, PIN-711 316

DETAILS STATEMENT OF BIOTECHNOLOGY EQUIPMENTS (ANNEXURE)

SL NO	Item Description	Supp. Name	Address	Invoice No & Date	Amount	Cheque No & Date	Nature Of Transtion
12	DNA Gel Power Pack	ASE Instruments	5A/1, Abinash Chandra Banerjee Lane,Beliaghata, Kolkata - 10	I/077-14/15,DT-12.02.2015	14490.00	Ch No-79895,DT-21.05.15	INDIA
13	Digital Balance	ASE Instruments	5A/1, Abinash Chandra Banerjee Lane,Beliaghata, Kolkata - 10	I/075-14/24,DT-12.02.2015	36750.00	Ch No-79898,DT-25.06.15	INDIA
14	Water Bath	Digitech Systems	12/1/9, Monoharpukur Road,1st Floor, Kolkata- 700 026	DS/15-16/022,DT-14.05.2015	21470.00	Ch No-234111,DT-10.07.15	INDIA
15	Heating Plate	Labchem & Labortenik Instruments	48/C,Co-operative Colony, Kolkata - 700 118	I/L49-14/10,DT-16.02.2015	5610.00	Ch No-79899,DT-12.06.15	INDIA
16	Gel Rocker	ASE Instruments	5A/1, Abinash Chandra Banerjee Lane,Beliaghata, Kolkata - 10	I/078-14/25,DT-12.02.2015	13740.00	Ch No-79898,DT-25.06.15	INDIA
17	Chest Freezer	ASE Instruments	5A/1, Abinash Chandra Banerjee Lane,Beliaghata, Kolkata - 10	I/073-14/16,DT-12.02.2015	57750.00	Ch No-79895,DT-21.05.15	INDIA
18	Vertical Autoclave	ASE Instruments	5A/1, Abinash Chandra Banerjee Lane,Beliaghata, Kolkata - 10	I/074-14/23,DT-12.02.2015	45800.00	Ch No-79898,DT-25.06.15	INDIA
19	Gel Docu & Image System	Synoptics Limited	Nuffield Road, Cambridge,CB4	0000005457A,DT-13.05.2015	542242.00	Ch No. NEFT DT-31.03.15	Out Side India
20	Double Distillation	ASE Instruments	5A/1, Abinash Chandra Banerjee Lane,Beliaghata, Kolkata - 10	I/072-14/12,DT-12.02.2015	89934.00	Ch No-79895,DT-21.05.15	INDIA
21	Bio Saf/Lamninar Flow	ESCo Micro Pte Ltd	21 Changi South Street-1 Singapore	SO-192231,DT-13.04.2015	302688.00	Ch No.NEFT DT-31.03.15	Out Side India
22	CO2 Incubator	Eppendorf India Limited (Made In Germany)	1C & 2C, Kaberi Abasan,Salimpur Road, Kolkata - 700 031	2612, DT-14.03.2016	339150.00	Ch No.234115, DT-02.04.16	INDIA
TOTAL					2933919.00		



Principal
ULUBERIA COLLEGE
Uuberia, Howrah

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West Bengal Biotech Development Corporation Lt
(A Govt. of WB Undertaking)

No. 339 / WBBDC /1P-2/2013 Dated: 03.09.2014

From: **Managing Director**
West Bengal Biotech Development Corporation Ltd.

To : The Principal
Uluberia College
Sijberia, Sizberia
Howrah, West Bengal 711315

Sub : Disbursement of fund in connection with BOOST SCHEME.

Sir / Madam,

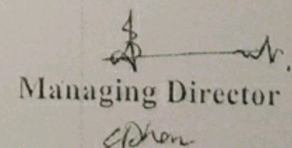
Enclosed herewith kindly find a Cheque bearing No. 792751 (Rs 406 Gv, dated 06.09.2014, issued by the West Bengal Biotech Development Corporation Ltd. in respect of academic programme by the Department of Biotechnology for improvement of Biotechnology based Infrastructure in Undergraduate/Postgraduate Colleges (BOOST-I and BOOST-II) for the following equipments and reagents & consumable.

Equipments: Protein gel apparatus including blotting apparatus (2.0 lakhs); Inverted Microscope (2.6 lakhs); Uv-Vis spectrophotometer (4.0 lakhs); Refrigerated centrifuge (6.0 lakhs); Water purification system (7.0 lakhs); Laminar Flow Hood (1.5 lakhs); Thermocycler (3.5 lakhs); Miscellaneous (3.4 lakhs) comprising Pipetteman, pH meter, Magnetic stirrer, Vortex, Table top centrifuge, DNA gel apparatus, Semi micro balance, Waterbath, Microwave, Heating plate, Gel rocker, -20°C freezer and Autoclave

Reagents & Consumables: 10.0 lakhs.

You are requested to kindly follow the terms and conditions for the grant released under 'BOOST" scheme.

Kindly acknowledge receipt of the cheque.



Managing Director

EN 24 Salt Lake Sector V, Kolkata, Biotech Park, Kolkata 700 091
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Incorporation of a Biocompatible Nanozyme in Cellular Antioxidant Enzyme Cascade Reverses Huntington's Like Disorder in Preclinical Model

*Aniruddha Adhikari, Susmita Mondal, Monojit Das, Pritam Biswas, Uttam Pal, Soumendra Darbar, Siddhartha Sankar Bhattacharya, Debasish Pal, Tanusri Saha-Dasgupta, Anjan Kumar Das, Asim Kumar Mallick, and Samir Kumar Pal**

The potentiality of nano-enzymes in therapeutic use has directed contemporary research to develop a substitute for natural enzymes, which are suffering from several disadvantages including low stability, high cost, and difficulty in storage. However, inherent toxicity, inefficiency in the physiological milieu, and incompatibility to function in cellular enzyme networks limit the therapeutic use of nanozymes in living systems. Here, it is shown that citrate functionalized manganese-based biocompatible nanoscale material (C-Mn_3O_4 NP) efficiently mimics glutathione peroxidase (GPx) enzyme in the physiological milieu and easily incorporates into the cellular multienzyme cascade for H_2O_2 scavenging. A detailed computational study reveals the mechanism of the nanozyme action. The in vivo therapeutic efficacy of C-Mn_3O_4 nanozyme is further established in a preclinical animal model of Huntington's disease (HD), a prevalent progressive neurodegenerative disorder, which has no effective medication to date. Management of HD in preclinical animal trial using a biocompatible (non-toxic) nanozyme as a part of the metabolic network may uncover a new paradigm in nanozyme based therapeutic strategy.

1. Introduction

Over the past decade, nanozymes, nanomaterials with intrinsic enzyme-like properties have attracted significant interest for application in multiple fields owing to their advantages (i.e., high and tunable catalytic activity, low cost, easy large scale production, and high stability) over the drawbacks of natural enzymes (i.e., low stability, high cost, laborious preparation, and low recyclability).[1] Since the discovery of the first iron-containing nanozyme in the last decade,[2] numerous nanomaterials have been elucidated to have oxidase,[3] catalase,[4] SOD,[5] peroxidase,[6] monooxygenase,[7] hydrolase,[8] and laccase[9] mimicking activities and therefore been used in diverse applications like the destruction of biofilm, removal of algal bloom, immunoassay, tissue staining, cancer treatment, and glucose biosensing. Most of these nanozymes

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Animals were randomly divided into five groups ($N = 10$ per group). The treatments were as follows,

Group 1: Control (Normal saline 16 days).
Group 2: 3-NPA treated (10 mg kg^{-1} body weight (BW) for alternative 4 days).
Group 3: 3-NPA (10 mg kg^{-1} BW for alternatively first 4 days days) + C-Mn_3O_4 NPs (0.5 mg kg $^{-1}$ (BW) for 16 days).
Group 4: 3-NPA (10 mg kg^{-1} BW for alternatively first 4 days) + Citrate (0.5 mg kg $^{-1}$ (BW) for 16 days).
Group 5: C-Mn_3O_4 NPs (0.5 mg kg $^{-1}$ (BW) for 16 days).

All treatments were performed intraperitoneally. Necessary ethical permissions were taken from Institutional Animal Ethics Committee, Uluberial College (Reg. No.: 2057/GO/ReRcBi/S/19/CPCSEA). All tests were performed following the standard guideline of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India.

Behavioral Assays: All mice were subjected to sequential tests started with OFT then followed by tail-flick, rotarod, beam walking, EPM, light, and dark box, FST, SPT, pole test, novel object recognition, and MWM. Some tests like OFT, hind-limb clasping test, beam walking, EPM were performed in an interval of 2 days. Other Tests were done before starting treatment and at the end of 16th day. Between two consecutive tests, there was a period of 1 h when the animals were left undisturbed. Details of the behavioral studies are described in Supporting Information.

Serum Isolation and Blood Biochemistry: At the end of the experimental period, the animals were euthanized and decapitated after being fasted. Blood was collected from retro-orbital plexus just before sacrifice, kept in sterile non-heparinized tubes in slanting position for 45 min and centrifuged at 3500 × g for 20 min. The clear serum was obtained and used in subsequent biochemical analysis.

Histopathological Examination: For microscopic evaluation, a conventional technique of paraffin wax sectioning and differential staining was used.[45] 2 h, dehydrated in graduated ethanol (50–100%), cleared in xylene, and embedded in paraffin. Microtome was used to prepare ultrathin sections (4–5 µm), followed by staining with hematoxylin and eosin and silver stain. Histopathological changes were examined under the microscope (Olympus BX51) equipped with a CCD based camera.

Antioxidant Enzyme Activity and Mitochondrial Studies: Details of the experimental methods are described in Supporting Information.

Statistical Analysis: All quantitative data are expressed as Mean ± Standard Deviation (SD) unless otherwise stated. One-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test was performed for comparison between multiple groups. Beforehand, the normality of each parameter was checked by normal quantile–quantile plots. No data were discarded or preprocessed during analysis. Sample size in our animal studies was determined following the standard sample sizes previously been used in similar experiments as per relevant literature. Designated sample size (in figure legends) always refers to biological replicates (independent animals). GraphPad Prism v8.0 (GraphPad Software), and Sigmaplot v14.0 (Systat Software, Inc.) were used for statistical analysis. For all comparisons, $p < 0.05$ was considered significant.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

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Manganese neurotoxicity: nano-oxide compensates for ion-damage in mammals†

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Here, we have compared the behavioral neurotoxicity of a manganese nanoformulation (citrate functionalized Mn_3O_4 nanoparticles; C-Mn_3O_4 NPs) with that of the well-known neurotoxicant, ionic Mn, in an animal model. We found that mice administered with C-Mn_3O_4 NPs showed no signs of a neurobehavioral disorder, but the NPs instead ameliorated Mn-induced neurotoxicity (Parkinson's-like syndrome) through the chelation of excess Mn ions and subsequent reduction of oxidative damage.

Manganese (Mn) is an essential trace element ubiquitously present in the Earth's crust.[1] It is required as an activator or cofactor of various enzymes (*e.g.*, galactosyltransferase,[2] agmatinase,[3] arginase,[4] glutamine synthetase,[5] pyruvate carboxylase[6,7] and Mn-superoxide dismutase,[8,9] as well as several kinases, hydrolases, transferases, and decarboxylases[10]) that govern several important physiological processes, including bone development, connective tissue growth, metabolism, mitochondrial antioxidant systems, and cell death.[11] However, in the *in vivo* cellular environment, Mn behaves like a double-edged sword. At physiological concentration, this redox-active transition metal acts as an interface between the genome of the organism and exposome (*i.e.*, the environment)[11,12] to facilitate the proper functioning of the aforementioned processes. On the other hand, excessive occupational, iatrogenic, medical, and environmental exposure causes its accumulation in the liver, kidney and brain, resulting in various symptoms, including hepatic cirrhosis, dystonia, polycythemia, and hypermanganesemia.[13–15] Excessive Mn in its ionic form (both 2+ and 3+) accumulates in the brain, particularly in the basal ganglia, cerebellum and hippocampus, and causes clinical signs and morphological lesions analogous to idiopathic Parkinson's disease (PD).[16–18] The etiology of Mn-induced neurotoxicity is multifactorial and numerous studies have illustrated the complex mechanism that starts from mitochondrial inactivation and subsequent enhancement of reactive oxygen species (ROS) in the affected brain areas, resulting in dysregulation of the nigrostriatal dopaminergic pathway.[19–21] Further induction of classical activation (M1 activation) of microglial cells and astrocytes in the central nervous system (CNS) leads to the production of proinflammatory cytokines that cause neuronal damage.[20,22] This 'Dr Jekyll and Mr Hyde' nature of Mn in physiological milieu is well documented.[1,21,23,24] However, little is known about its *in vivo* behavior and toxicity in the nano form. Over the past decade, the use of Mn-containing nanomaterials in a wide range of applications, from catalysis,[25–27] gas sensing,[28–30] energy storage,[31–33] molecular adsorption[34,35] and magnetic data storage,[31,36] to biomedical imaging[37–40] and therapeutics (*e.g.*, in treatment of neonatal hyperbilirubinemia,[41] hepatic fibrosis,[42,43] *etc.*), has grown in leaps and bounds, as has the potential risk of human exposure. Recently, we explored organ-specific toxic effects of surface-coated Mn_3O_4 nanoparticles (NPs) in BALB/c mice upon repeated dose (90 days) oral exposure. Mice given an NP dose of 0.5 mg kg^{-1} body weight (BW) showed impaired liver function and male-specific reproductive toxicity, owing to the inherent ability of NPs to generate ROS and modulate cellular redox equilibrium. However, due to the limited scope of the study, we were unable to explore the neurologic consequences of chronic Mn_3O_4 NP exposure, and as a result, significant ignorance still exists about its interference in neurobehavioral function. The work presented here extends these observations and focuses on the effect of chronic repeated dose exposure of manganese oxide nanoparticles at a similar dose to that leading to hepatic and reproductive toxicity shown in earlier studies (0.5 mg per kg BW) on some selected behavioral,

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capacity. This *in vitro* antioxidant activity was exactly reflected in the brain antioxidant enzyme system. As a result, the $MnCl_2$-induced decrease in the activities of SOD, CAT and GPx were recovered upon C-Mn_3O_4 NP treatment.

To understand the molecular mechanism of action, we examined the effect of C-Mn_3O_4 NPs on mitochondria, as the critical role of mitochondrial injury in the pathogenesis of Mn toxicity has been well documented.[77] It has been found that Mn is accumulated in the mitochondrial matrix *via* calcium transporters.[78,79] After its accumulation, Mn ions can cause mitochondrial damage by disrupting the respiratory electron transport chain (ETC), resulting in ATP depletion, which may cause apoptosis.[77,78] Mn ions can further induce cell death *via* the opening of a non-specific pore in the inner mitochondrial membrane, *i.e.*, the mitochondrial permeability transition (mPT), which alters the mitochondrial membrane, potentially leading to necrosis.[43,77] In addition, manganese ions also facilitate Fenton-like reactions (the conversion of $O_2^{\bullet-}$ radicals to OH radicals), which in turn induce apoptosis *via* DNA damage and lipid peroxidation. In the current *in vivo* model of manganism, we found that brain mitochondrial function was impaired in Mn-treated animals (Fig. 5). Increased mitochondrial permeabilization (mitochondrial swelling or mPTP formation) (Fig. 5a), decreased mitochondrial dehydrogenase activity (Fig. 5b), dysregulated mitochondrial membrane potential (Fig. 5c), decreased ATP level (Fig. 5d), decreased complex IV activity (Fig. 5e) and increased mitochondrial lipid peroxidation (Fig. 5f) were evident in the brain tissue of Mn-exposed animals. These deteriorating changes in mitochondrial parameters resulted in neuronal degeneration observed in the histological findings. C-Mn_3O_4 NPs were able to protect mitochondria from the aforementioned damage (Fig. 5). The ability of the NPs to chelate Mn ions and their subsequent antioxidant activity are both responsible for the observed ameliorative activity. Chelation of Mn ions protects the mitochondrial membrane from pore formation, whereas the antioxidant activity helps in scavenging the free radicals and reducing the associated oxidative damage. The aforementioned possible molecular biology mechanism of the Mn-induced brain injury at convalescence is schematically represented in Fig. 5g.

In summary, from a neurobehavioral point of view, C-Mn_3O_4 NPs are completely biocompatible and do not exhibit the characteristic PD-like symptoms of manganese toxicity. Furthermore, they are capable of ameliorating Mn-induced neurotoxicity owing to their unique property of chelating harmful excess Mn^{2+} ions *in vivo* and their subsequent antioxidant activity to heal the oxidative damage caused by mitochondrial dysfunction. To the best of our understanding, engineering such biocompatible nanoparticles, capable of chelating harmful metal ions and possessing substantial antioxidant activity, may pave the way in designing therapeutic strategies against other neurobehavioral disorders, such as Parkinson's, Alzheimer's and Huntington's, which are pathophysiologically modulated by mitochondrial oxidative damage.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article and/or its supplementary materials. Any other related data that support the findings of this study are available from the corresponding author, S. K. P., upon reasonable request.

Conflicts of interest

The authors disclose no conflict of interest.

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Novel one pot synthesis and spectroscopic characterization of a folate-Mn_3O_4 nanohybrid for potential photodynamic therapeutic application†

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Treatment of cancer using nanoparticles made of inorganic and metallic compounds has been increasingly used, owing to their novel intrinsic physical properties and their potential to interact with specific cellular sites, thereby significantly reducing severe secondary effects. In this study, we report a facile strategy for synthesis of folate capped Mn_3O_4 nanoparticles (FA-Mn_3O_4 NPs) with high colloidal stability in aqueous media using a hydrothermal method for potential application in photodynamic therapy (PDT) of cancer. The capping of FA to Mn_3O_4 NPs was confirmed using various spectroscopic techniques. In adenocarcinomic human alveolar basal epithelial cells (A549), the nanohybrid synthesised with a combination of FA and Mn_3O_4 shows remarkable PDT activity *via* intracellular ROS generation (singlet oxygen). As established by a DNA fragmentation assay and fluorescence studies, the nanohybrid can cause significant nuclear DNA damage by light induced enhanced ROS generation. In the assessment of Bax, Bcl2 provides strong evidence of apoptotic cellular death. Cumulatively, the outcomes of this study suggest that these newly synthesized FA-Mn_3O_4 NPs can specifically destroy cells with overexpressed folate receptors, thereby providing a solution in the journey of cancer eradication.

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Introduction

Cancer has become one of the major threats to life expectancy of the global population during the last few decades. According to the Global Cancer Observatory (GLOBOCAN) database and World Health Organization (WHO), approximately 9.6 million deaths occurred from cancer in 2017,[1,2] among which lung cancer caused the highest rate of mortality compared to other cancer types. The available curatives for cancer *e.g.*, chemotherapy, immunotherapies and surgeries are painful, troublesome and extremely costly. Besides the high cost and side effects, the available curatives have failed to reduce the mortality rate due to cancer, hence, new alternative treatment strategies are extremely necessary.

From its discovery (by Dougherty *et al.* in 1960) the photodynamic therapy (PDT) is gradually becoming an efficacious alternative.[3] PDT requires minimal invasion for complete destruction of malignant cells, and has less side effects. In most of the cases PDT activates the ROS mediated apoptosis pathway in cancer cells that results in the annihilation of carcinoma.[4] PDT involves administration of photosensitizing agent, which may require some modulation after internalisation in specific cells followed by activation of the agent by radiation of a specific wavelength. As a result, some irreversible photo induced damage takes place in targeted cell.[3] Although, proved effective, several limitations (*i.e.*, poor water solubility, aggregate formation, high dermal toxicity and low clearance)[5,6] of the conventional organic PDT agents hinder widespread application of it in the clinical settings. In recent studies, inorganic nanoparticles emerged as an effective alternative because of its biocompatibility, high retention time in circulation, target specificity (by attaching ligand) and low toxicity.[7–9] Another problem that confines the use of PDT is the absence of a mechanism that can ensure target specific delivery (sometimes the normal cells become as vulnerable as cancer cells). In this regard, folic acid (FA) could be used as an inexpensive and stable ligand for targeting folate receptors (FR), a tumour-associated protein over-expressed in cancer cells having high binding affinity towards folic acid ($K_d \approx 10^{-10}$ M).[10] The strategy of targeting cancer cells through FRs is a well-recognized strategy due to overexpression of FR in a variety of cancer cells including those in breast, kidney, colon, ovaries, cervix and renal cell carcinomas.[10]

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light irradiation. After irradiation with blue light for 20 min, the intracellular ROS level reached saturation, which is consistent with both extracellular ROS generation and photo-induced cytotoxicity assay. The considerable light induced enhanced intracellular ROS activity and optimum dark toxicity of 0.31 μg mL^{-1} dose of FA-Mn_3O_4 NPs indicates its potential in PDT.

Next, to understand the mode of cell death we performed microscopic studies. In bright field microscopic analysis apoptotic changes like reduction in cell size, clear cytoplasm *etc.* (morphometric data are shown in ESI Fig. S2†) become evident in case of light irradiated cells treated with FA-Mn_3O_4 NPs, whereas, the cells without light illumination showed normal morphology.[38,39] The extent of apoptosis was further evaluated using nuclear staining dye DAPI (Fig. 5a). DAPI can bind specifically to nuclear DNA and emit a blue light visible with a fluorescence microscope.[40,41] When the nuclei of A549 cells were studied by DAPI staining, we found that normal nuclei had a homogeneous pattern of staining (Fig. 5a). In contrast, FA-Mn_3O_4 NP treated (with photo irradiation) nuclei were smaller and brighter than normal cells, and nuclear fragmentation and condensation (apoptotic bodies) were evident (Fig. 5a). These findings suggest that the photo-induced FA-Mn_3O_4 NPs demonstrated changes consistent with apoptosis.[42]

The damage of nuclear DNA as observed in DAPI staining was further accessed by DNA fragmentation assay using gel electrophoresis. DNA was extracted from the nanoparticle treated cells (both in presence and in absence of blue light) and run on 1.5% agarose gel. Fig. 5c shows that in presence of blue light there was a significant damage of nuclear DNA in terms of ladder formation, a biochemical hallmark of apoptosis,[43] compared to both control and without light FA-Mn_3O_4 NP treated ones. Various studies suggest that the balance between proapoptotic (Bax/Bad) and antiapoptotic (Bcl-2/Bcl-xL) members of the Bcl-2 protein family determine the fate of a cell in physiological milieu.[40,43] Fig. 5d displays the expression of Bax which was increased in the cells that were incubated with FA-Mn_3O_4 in presence of light whereas, the expression of Bcl2 protein was drastically decreased in these group of cell. It is well know that Bcl2 protein family acts as an anti-apoptotic actor by inhibiting Bax. Thus in summary, FA-Mn_3O_4 NPs in presence of blue light increases the cellular ROS level, which triggers the Bax expression in cells. On the other hand, FA-Mn_3O_4 NPs down regulate the Bcl2 expression, in turn reduces the inhibition on Bax. As a result, increased Bax can induce the nuclear DNA fragmentation thereby dictating the cells to undergo apoptosis.

Conclusion

From this study we can conclude that we have developed a facile and cost effective method for synthesis of folic acid capped Mn_3O_4 nanoparticles without using any additional organic linker (confirmed by spectroscopic studies). This folic acid capped nanoparticle can generate ROS in presence of blue light (as depicted in both intracellular and extracellular DCFH assay) and can be used as an efficacious PDT agent against adenocarcinomic human alveolar basal epithelial cells (lung cancer cells). Nuclear DNA fragmentation, apoptotic body formation, overexpression of Bax and down regulation of Bcl2 proteins suggest the underlying mechanism to be apoptosis. Although, we have described the photo induced cytotoxicity of FA-Mn_3O_4 NPs in lung cancer cells as a model, the similar effects can be observed in other carcinomas too. Hence, blue light induced PDT can be effectively used to treat carcinomic tissues (*e.g.* lung cancer, colorectal cancer, stomach cancer, throat cancer *etc.*) by introduction of blue light using optical fibres. Finally, this work elicits a new approach towards the synthesis of effective, low cost nanohybrids for diagnosis and therapy of alveolar adenocarcinoma.

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article and/or its ESI.† Any other related data that support the findings of this study are available from the corresponding author, SKP, upon reasonable request.

Conflicts of interest

The authors confirm no conflict of interest to disclose.

Acknowledgements

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A Smart Nanotherapeutic Agent for in vitro and in vivo Reversal of Heavy-Metal-Induced Causality: Key Information from Optical Spectroscopy

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Human exposure to heavy metals can cause a variety of life-threatening disorders, affecting almost every organ of the body, including the nervous, circulatory, cardiac, excretory, and hepatic systems. The presence of heavy metal (cause) and induced oxidative stress (effect) are both responsible for the observed toxic effects. The conventional and effective way to combat heavy metal overload diseases is through use of metal chelators. However, they possess several side effects and most importantly they fail to manage the entire causality. In this study, we introduce citrate-functionalized Mn_3O_4 nanoparticles (C–Mn_3O_4 NPs) as an efficient chelating agent for treatment of heavy metal overload diseases. By means of UV/Vis absorbance and steady-state fluorescence spectroscopic techniques we investigated the efficacy of the NPs in chelation of a model heavy metal, lead (Pb). We also explored the retention of antioxidant properties of the Pb-chelated C–Mn_3O_4 NPs using a UV/Vis-assisted DPPH assay. Through CD spectroscopic studies we established that the NPs can reverse the Pb-induced structural modifications of biological macromolecules. We also studied the *in vivo* efficacy of NPs in Pb-intoxicated C57BL/6j mice. The NPs were not only able to mobilize the Pb from various organs through chelation, but also saved the organs from oxidative damage. Thus, the C–Mn_3O_4 NPs could be an effective nanotherapeutic agent for complete reversal of heavy-metal-induced toxicity through chelation of the heavy metal and healing of the associated oxidative stress.

Introduction

Chelation therapy, introduced by Alfred Werner and Paul Ehrlich in early 1900 s,[1] is still the most effective and widely used method for treatment of heavy metal overload and associated spectrum of disorders that includes heavy metal toxicity,[2] anemia,[3] Alzheimer's,[4] Parkinson's,[5] Friedreich's ataxia,[6] and Wilson's disease.[7] All of the aforementioned conditions involve the accumulation of metal ions in certain tissues or cellular compartments of the body and the inability of the physiological systems to mobilize them.[7a] Being non-redox in nature, most of the heavy metals lack pro-oxidant catalytic activity, rather they covert to some indirect mechanisms (through destruction of sulfhydryl (–SH) containing enzymes essential for in vivo antioxidant defense) to exert pathogenesis through free radical mediated damage.[8] Thus, regulating the free radical-induced intracellular damage is equally essential in combating heavy metal overload diseases besides the elimination of the same from various organ systems.[8b] The conventionally used chelating agents for treatment of heavy metal overload disorders were introduced more than four decades ago (i.e., $CaNa_2EDTA$-1985;[9] BAL-1949;[9] DMSA-1978;[10] DMPS-1958[11]), and they suffer from inherent toxic effects owing to non-specificity and unwanted distribution throughout the body including several essential organs.[12] Most dangerously, some of the chelators redistribute the heavy metals to the brain instead of excretory organs resulting in severe cognitive disorders, particularly in children.[2c] The aforementioned causal relationship between heavy metal and oxidative stress and inherent toxicity of the

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Treatments

Animals were randomly divided into 3 groups. First group (Control) of animals ($n=10$) orally received 200 μL physiological saline (0.9% NaCl) for 49 days (the entire study period). Remaining two groups ($n=12$/group) received $Pb(NO_3)_2$ solution (45 mg kg^{-1} body weight (BW)) intraperitoneally (i.p.) on alternative days for a period of 28 days in order to induce Pb(II) mediated damage. The second group (Pb(II)) was left untreated for the rest of the experimental period (negative control) while, the third group (Pb(II)+C–Mn_3O_4 NP) was orally administered with C–Mn_3O_4 NPs (0.25 mg kg^{-1} BW) for the last 21 days. All agents were administered postprandial.

Blood collection and serum isolation

Mice were fasted for 12 h on the 50th day, and blood samples were collected by orbital exsanguination from the retro-orbital sinus plexus of the mice in micro centrifuge tubes with and without EDTA (2.0%). Serum was separated following standard procedure[14b] and kept at −20 °C until further use.

Assessment of hematological and liver function parameters

All serum biochemical parameters (ALT, AST, ALP, GGT, total protein, direct and total bilirubin) were analyzed by using commercially available assay kits (Span Diagnostic, Surat, India) following the protocol recommended by manufacturer. δ-amino-levulinic acid dehydratase (δ-ALAD) activity was measured using the European standardized method.[30] Complete blood count (CBC) was obtained using an automated cell counter (Medonic CA 620, Boule Diagnostics, Sweden).

In vivo distribution of Pb

ICP-AES (ARCOS, SPECTRO Analytical Instruments GmbH, Germany) was carried out to determine the amount of Pb in blood and other organs like liver, kidney, spleen and, brain. The open acid digestion method was used for sample preparation. In brief, tissues were dried using liquid nitrogen and weighted. The freeze-dried samples were dissolved in an acid mixture that contained HNO_3 (3 mL), H_2SO_4 (2 mL), and H_2O_2 (1 mL), heated at 120 °C until only a residue remained, and then diluted with deionized water to 10 mL.

Measurement of intracellular antioxidant enzymes

The degree of lipid peroxidation was measured in terms of thiobarbituric acid reactive substances (TBARS) formation following a standard procedure.[31] SOD, CAT, GPx and GSH activity were determined using commercially available test kit (Sigma-Aldrich, MO, USA) following the protocol suggested by the manufacturer.

Histopathology

Liver was collected from animals of each group and washed separately with ice-cold phosphate buffer (pH 7.0). After drying with tissue paper, the organs were fixed in neutral buffered formalin solution (10%), dehydrated in graduated ethanol (50–100%), cleared in xylene and, embedded in paraffin. 4–5 μm sections were cut using microtome, deparaffinized and hydrated. Sections were stained with hematoxylin-eosin (H&E), and serius red.

Statistical analysis

All experimental results are expressed as Mean ± standard deviation (SD) unless otherwise stated. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post-hoc test was implemented for comparing different parameters across the groups using GraphPad Prism (v5.00 for Windows, GraphPad Software, CA, USA).[32].

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: Heavy metal toxicity · Nanomedicine · Surface functionalized Mn_3O_4 nanoparticles · Chelation · Lead toxicity

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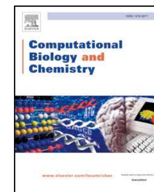
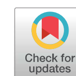


Research Article

Rationalization of a traditional liver medicine using systems biology approach and its evaluation in preclinical trial



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ABSTRACT

'Bottom-up', i.e., molecule to medicine strategy for the discovery of new drugs takes enormous time and cost. In most of the cases, inherent toxicity and undesired side effects of the developed drug hinder its way beyond the early stages of development. In this regard, the systems pharmacology can play an excellent role by reducing the cost and time of drug development through rationalization and/or repurposing of traditional drugs with known side effects. In the present study, our aim was to develop an integrated systems biology method for the prediction of active ingredients of a traditional medicine and their potential targets inside the body. Further, we evaluated the predictive capacity of the developed method in a preclinical animal model.

Here, we have prepared a formulation (SKP17LIV01) from an extract of eight medicinal plants traditionally used as liver medicine and identified the constituents using UHPLC-MS technique. Using systems biology approach, we have rationalized the components of the formulation for potential use in the treatment of heavy metal-induced hepatotoxicity. The active ingredients and potential therapeutic targets were also predicted. A detailed biochemical, histopathological and molecular study on the mice model of lead toxicity confirms the efficacy of the formulation as per prediction by the systems pharmacology approach. The study may open a new frontier for re-discovery of drugs that are already used in traditional medicine.

1. Introduction

Over the past decade, there has been a severe decline in the rate at which new drug candidates are being translated into effective therapies in the clinics (Kola and J, 2004). In particular, the lack of efficacy and clinical safety of the synthetic drugs resulted into a worrying rise in late-stage attrition in phase-2 and phase-3 clinical trials (Sams-Dodd, 2005). These late-stage attrition rates are at the heart of the relative decrease in productivity of the pharmaceutical industry creating a major financial shock (Hopkins, 2008). In this regard, natural products with diverse chemical scaffolds can be an invaluable alternative source of compounds in drug discovery and development (Fang et al., 2017). Several of these natural products have been in use as ancient traditional remedies (e.g., Traditional Chinese Medicine, Ayurveda, etc.) since time immemorial (Jiang, 2005). They are considered less toxic as they have undergone the mill of coevolution with human protein (Dobson, 2004). However, limited knowledge about the active ingredients and molecular mechanism of action restricts their use in modern medicine (Atanasov et al., 2015; Pan et al., 2013). The current paradigm of systematic identification of drug targets of the natural products at the human proteome level via various experimental assays is highly expensive and time-consuming (Fang et al., 2017). So, an alternative approach is required, which will preferably include *in silico, in vitro,* and *in vivo* methods in a synchronous manner to reduce the cost and time of drug discovery. Network pharmacology encompassing systems biology, network analysis, connectivity, redundancy, and pleiotropy can be an alternative strategy for the invention of new drugs (Hopkins, 2008). Although several studies have used computational approaches to identify target molecules (Das et al., 2017; Li et al., 2015; Sun et al., 2016; Wang and J, 2016; Zheng et al., 2013), a systematic modus operandi for rationalization of the medicinal activities of natural products and their mechanism of action is lacking. In recent years, Wu et al. (2019), and Gao et al. (2018) have successfully used a combined computational and experimental approach to predict hepatotoxicity

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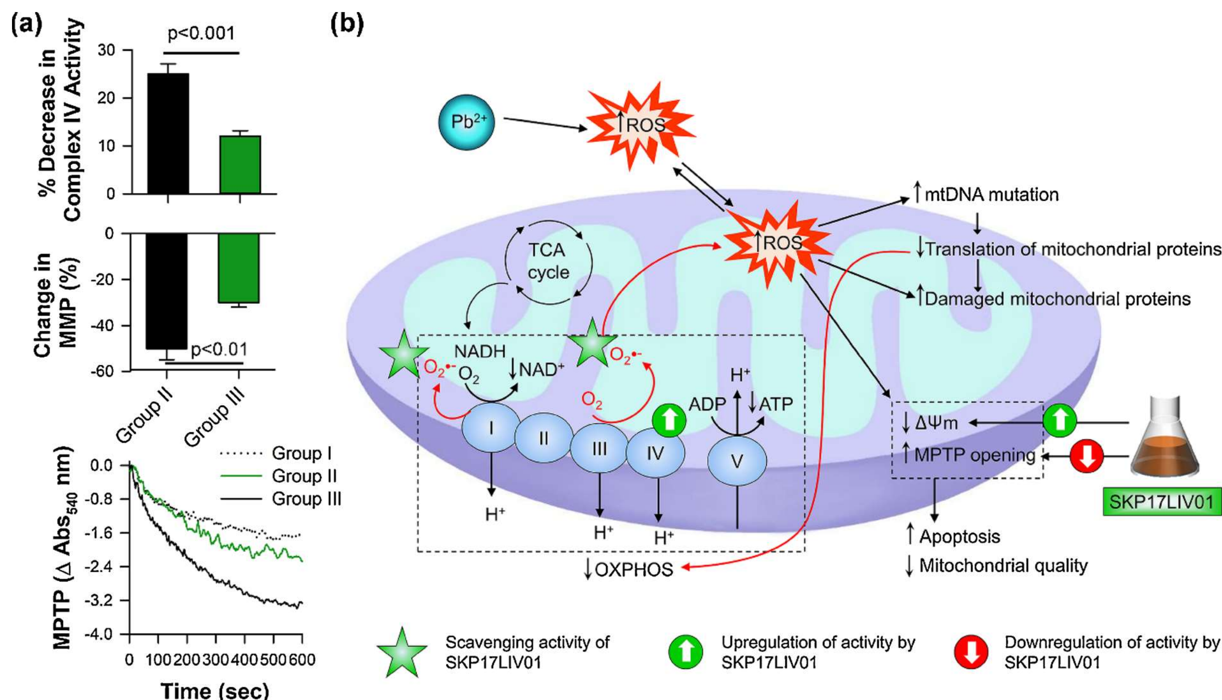

Fig. 11. The molecular mechanism behind the therapeutic action of SKP17LIV01. (a) Effect on mitochondria. Change in complex-IV activity, change in mitochondrial membrane potential (MMP or $\Delta\Psi_m$) and effect in Ca^{2+} induced opening of mitochondrial permeability transition pore (MPTP). (b) Schematic representation of the protective action of the extract (SKP17LIV01) on mitochondria. Group I: Control; Group II: $Pb(NO_3)_2$; Group III: $Pb(NO_3)_2$ + SKP17LIV01. All data represented as Mean ± Standard Deviation (SD). N = 6 for each measurement.

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Declaration of Competing Interest

None.

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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.compbiolchem.2019.107196.

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Spectroscopic Studies on Dual Role of Natural Flavonoids in Detoxification of Lead Poisoning: Bench-to-Bedside Preclinical Trial

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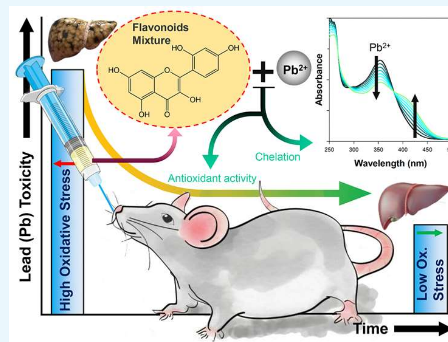
S *Supporting Information*

ABSTRACT: Ubiquitousness in the target organs and associated oxidative stress are the most common manifestations of heavy-metal poisoning in living bodies. While chelation of toxic heavy metals is important as therapeutic strategy, scavenging of increased reactive oxygen species, reactive nitrogen species and free radicals are equally important. Here, we have studied the lead (Pb) chelating efficacy of a model flavonoid morin using steady-state and picosecond-resolved optical spectroscopy. The efficacy of morin in presence of other flavonoid (naringin) and polyphenol (ellagic acid) leading to synergistic combination has also been confirmed from the spectroscopic studies. Our studies further reveal that antioxidant activity (2,2-diphenyl-1-picrylhydrazyl assay) of the Pb–morin complex is sustainable compared to that of Pb-free morin. The metal–morin chelate is also found to be significantly soluble compared to that of morin in aqueous media. Heavy-metal chelation and sustainable antioxidant activity of the soluble chelate complex are found to accelerate the Pb-detoxification in the chemical bench (in vitro). Considering the synergistic effect of flavonoids in Pb-detoxification and their omnipresence in medicinal plants, we have prepared a mixture (SKP17LIV01) of flavonoids and polyphenols of plant origin. The mixture has been characterized using high-resolution liquid chromatography assisted mass spectrometry. The mixture (SKP17LIV01) containing 34 flavonoids and 76 other polyphenols have been used to investigate the Pb detoxification in mouse model. The biochemical and histopathological studies on the mouse model confirm the dual action in preclinical studies.



■ INTRODUCTION

Lead (Pb), one of the most widely used metals in the industry and simultaneously a versatile, subtle, and persistent poison present in Earth's crust, can induce a wide array of physiological, biochemical, and behavioral dysfunctions affecting almost every organ system (central and peripheral nervous, hematopoietic, respiratory, cardiovascular, renal, hepatic and reproductive system) cumulating in death.[1−4] Although Pb toxicity has been documented as early as 2000 BC and found to be preventable, victims of such toxicity in human civilization are evident in recent times.[5] Even in 2015, 494 550 deaths occurred due to Pb exposure, which is 0.6% of the global burden of diseases and 9.3 million disability-adjusted life years.[4,6,7] Of particular concern is the role of Pb exposure in the development of intellectual disability in children.[7] Though there is wide recognition of this problem and many countries have acted to stop its use, exposure to Pb, particularly in childhood, remains a key concern for health care providers and public health officials worldwide. Though Pb itself is a nonredox metal and does not possess any prooxidant catalytic activity, it rather converts to some indirect mechanisms [e.g., auto-oxidation of hemoglobin (Hb), accumulation, and subsequent auto-oxidation of δ-aminolevulinic acid induced by Pb] for imparting pathogenesis via oxidative disturbances.[4,8−10] Thus, controlling the free-radical-induced intracellular damage is equally important in combating Pb poisoning alongside the removal of the same from organs. On the other hand, the currently approved treatment for Pb toxicity (use of chelating agents such as 2,3-dimercaptosuccinic acid and $CaNa_2EDTA$) can neither be used at a therapeutically adequate dose for a prolonged period of time owing to a number of shortcomings and inherent toxicity (e.g., nephrotoxicity, cardiotoxicity, zinc dieresis, nausea, fever, and breathing trouble) nor reduce the significant oxidative stress

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Thiol-based antioxidant system plays second line of cellular protection against reactive free-radical-mediated oxidative damage in pathophysiological situation.[54] Cellular metabolite such as GSH has been presented in Figure 8a. The level of GSH has been considerably decreased because of intoxication along with the increased level of oxidized glutathione. Post-treatment with SKP17LIV01 after the Pb(II) exposure restored the levels of cellular metabolites close to normal, demonstrating its protective nature. Figure 8b provides a schematic overview of the proposed mechanism of action of SKP17LIV01 in the protection of oxidative stress induced by Pb. The model clearly indicates that SKP17LIV01, despite direct sustainable free-radical scavenging activity, boosts the in vivo enzymatic antioxidant defense system resulting in protection from increased levels of ROS. The model is further supported by the observations of lipid peroxidation and protein carbonylation, the two important markers of oxidative stress. The increase in the MDA level (Figure 8c) in the serum and liver (~3.8 fold compared to control in both cases, $p < 0.01$) upon $Pb(NO_3)_2$ treatment suggests enhanced peroxidation, leading to tissue damage and failure of antioxidant mechanism to prevent the formation of excessive free radicals. Post-treatment with SKP17LIV01 ($Pb(NO_3)_2$ + SKP17LIV01) significantly attenuated these changes (decrease in MDA level by ~1.8-folds (liver) and ~1.5-folds (serum) compared to $PbNO_3$-treated group, $p < 0.05$ in all cases). The flavonoids and polyphenols exert their antioxidant effects in the body by preventing the generation of ROS, direct scavenging of it, or through enhancement of cellular antioxidant system. Thus, the observed recovery of antioxidant enzyme pool in the animal model of Pb intoxication after the administration of SKP17LIV01 is the consequence of synergistic activity between its various phytoconstituents as evident from our spectroscopic studies. The flavonoids may have played a crucial role in the reduction of the treatment period by controlling the accumulation and bioavailability of Pb(II) in the tissue through complexation, subsequent sustainable antioxidant activity, and removal.

CONCLUSIONS

The disaster due to Pb poisoning is evident from the ancient human civilization till date, although it is concluded to be preventable. Understanding the mechanism of Pb detoxification by low-cost medicinally important molecules and cost-effective herbal mixture is very important for the promotion of lots of folk medicines in developing countries. Here, we have explored the detailed mechanism of Pb detoxification by morin, a model flavonoid, which is omnipresent in medicinal herbs using steady-state and picosecond-resolved optical spectroscopy. We have shown that Pb−morin chelate becomes a sustainable antioxidant revealing dual role of the flavonoid in the heavy-metal detoxification process. Our spectroscopic studies also reveal that the dual nature in the detoxification remains intact in the controlled mixture of few flavonoid and polyphenol molecules (MNEA), and even in a mixture, many such molecules were extracted from medicinal plants (SKP17LIV01). We have studied the efficacy of the complex mixture in the mouse model for the detoxification of Pb poisoning in detail. Subsequent, biochemical, histopathological, and biodistribution studies confirm that the molecular mixture (SKP17LIV01) facilitates excretion of Pb from the mammal (mice) body after heavy-metal chelation and ROS scavenging. The study is expected to have an impact in the discovery of new drugs of ethnobotanical origin.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b02046.

> Composition of ingredient(s) present in SKP17LIV01, AST and ALT activity, summary of the hematological parameters, determination of binding constant of the Pb(II)−Morin complex, interaction of Pb(II) with narigin, interaction of Pb(II) with EA, interaction of EA with morin, effect of Pb2+ chelation on aqueous solubility of morin, compounds and metabolites identified in positive mode, compounds and metabolites identified in negative mode of UHPLC-MS (PDF)

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Notes

The authors declare no competing financial interest.

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Ultrafast spectroscopy on DNA-cleavage by endonuclease in molecular crowding

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ABSTRACT

The jam-packed intracellular environments differ the activity of a biological macromolecule from that in laboratory environments (*in vitro*) through a number of mechanisms called molecular crowding related to structure, function and dynamics of the macromolecule. Here, we have explored the structure, function and dynamics of a model enzyme protein DNase I in molecular crowing of polyethylene glycol (PEG; MW 3350). We have used steady state and picosecond resolved dynamics of a well-known intercalator ethidium bromide (EB) in a 20-mer double-stranded DNA (dsDNA) to monitor the DNA-cleavage by the enzyme in absence and presence PEG. We have also labelled the enzyme by a well-known fluorescent probe 8-anilino-1-naphthalenesulfonic acid ammonium salt (ANS) to study the molecular mechanism of the protein-DNA association through exited state relaxation of the probe in absence (dictated by polarity) and presence of EB in the DNA (dictated by Förster resonance energy transfer (FRET)). The overall and local structures of the protein in presence of PEG have been followed by circular dichroism and time resolved polarization gated spectroscopy respectively. The enhanced dynamical flexibility of protein in presence of PEG as revealed from excited state lifetime and polarization gated anisotropy of ANS has been correlated with the stronger DNA-binding for the higher nuclease activity. We have also used conventional experimental strategy of agarose gel electrophoresis to monitor DNA-cleavage and found consistent results of enhanced nuclease activities both on synthetic 20-mer oligonucleotide and long genomic DNA from calf thymus.

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1. Introduction

Many *in vivo* enzymatic processes including repression or activation of transcription in gene regulatory network are triggered by binding of proteins to their respective target sites on DNA. However the study of enzyme-DNA interactions have been usually designed as dilute solution experiments, which differ substantially from *in vivo* conditions as 40% volume of cytosol is occupied by a wide variety of macromolecules and solutes [1–3]. Due to this reason, the diffusion of any solutes in intracellular environment is get affected either being reduced or presenting anomalous diffusion at short times [4–8]. Contemporary studies have revealed that macromolecular crowding inside the cell does not only affect diffusion processes but also biochemical reaction processes by inducing the enzyme to undergo protein folding, self-association, or protein-binding processes, which in turn may alter the activity of the enzyme [9–12]. Thus, in order to obtain more accurate rates for enzymatic reactions, it is important to perform the studies of biochemical processes in nature-like microenvironments that try to mimic the effect of macromolecular crowding.

As the quantitative studies of enzyme-DNA interactions within a living cell are challenging, subsequently to mimic the intracellular like environment the high concentration of crowding agents are often used in the *in vitro* studies. For this purpose polyethylene glycol and polysaccharides are often considered as a convenient macromolecular crowder as it is highly soluble in water, it does not precipitate the biological macromolecules used for the study and also it does not bind with the biological macromolecules before and after the reaction [13]. Polymer cosolutes (PEG) usually generate an area inaccessible to other biological macromolecules known as excluded volume and these excluded volume per cosolutes increases as size of cosolutes increases. Apart from this, inclusion of cosolutes causes the decrease of water activity of solution and hence generates an osmotic pressure [14,15]. The dependence of

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Table 3
Rotational time constants of ANS at the enzyme (DNase I) surface at various systems.

system	τ_1 ns [%]	τ_2 ns [%]	τ_{avg} ns
ANS-DNase I	1.32 (11.3)	45 (88.7)	39.6
ANS-DNase I-DNA	0.9 (14.7)	45 (85.3)	38.4
ANS-DNase I-PEG	0.20 (66.4)	40 (33.6)	13.5
ANS-DNase I-DNA-PEG	0.13 (70.8)	40 (29.2)	11.7
Buffer	0.07 (100)	–	0.07
PEG	0.19 (100)	–	0.19

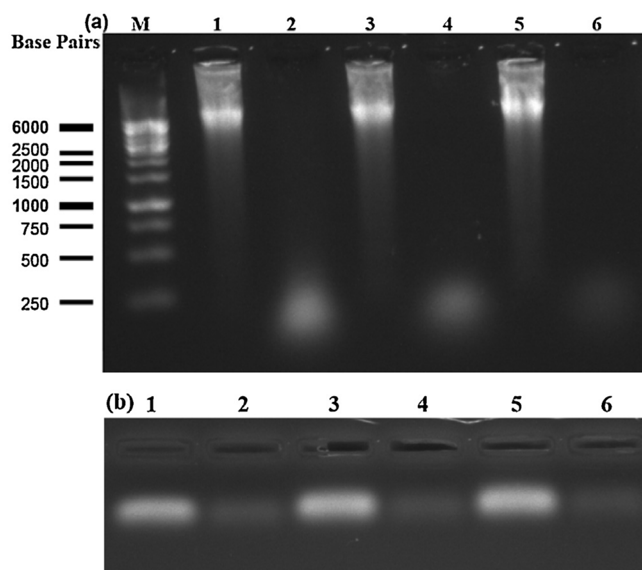

Fig. 7. a) Hydrolysis of calf thymus DNA by DNase I at 25 °C. Lane M shows the DNA size marker. Lanes 1, 3 and 5 show DNA in presence of 0, 5 and 15 wt% PEG. Lanes 2, 4 and 6 shows hydrolysis by DNase I in the presence of 0, 5 and 15 wt% PEG. b) Hydrolysis of a short oligonucleotide DNA (20-mer dsDNA) by DNase I at 25 °C. Lanes 1, 3 and 5 shows DNA in presence of 0, 5 and 15 wt% PEG. Lanes 2, 4 and 6 shows hydrolysis by DNase I in presence of 0, 5 and 15 wt% PEG.

The effect of molecular crowding on the catalytic activity of DNase I was also analysed using agarose gel electrophoresis. Initially we examined the hydrolysis of larger genomic DNA (calf thymus) by DNase I at 25 °C in absence and presence of different PEG concentration. Before hydrolysis by DNase I the migration of substrate DNA in absence and presence of 5 and 15 wt% PEG were found to be same (Fig. 7(a)), validate PEG does not significantly affect the stability of DNA (lane 1, 3 and 5). Migration of bands were found to be faster (lane 2, 4 and 6) upon hydrolysis with DNase I with respect to substrate corroborate the formation of degraded product from the substrate. The diffused band of degraded product in absence of PEG were found to be smeared and of higher intensity, however, the smearing and intensity of bands were found to be decreased as the PEG wt% was increased corroborate the formation of higher degraded product from the substrate in presence of molecular crowding. To evaluate the effect of molecular crowding on hydrolysis of short DNA using agarose gel electrophoresis, 20-mer dsDNA was used as a substrate shown in Fig. 7(b). Like genomic DNA, the migration of 20-mer dsDNA was also found to be same in absence and presence of 5 and 15 wt% PEG (lane 1, 3 and 5). Upon hydrolysis by DNase I the intensity of substrate DNA was decreased as concentration of molecular crowding was increasing (lane 2, 4 and 6). The result shows that dsDNA hydrolysis by DNase I was greatly enhanced by the addition of 5 and 15 wt% PEG. Overall, molecular crowding increases the cleavage yield of DNase I not only for the large DNA (calf thymus) but also for the short (20-mer) DNA oligonucleotide.

4. Conclusions

Our results reveal that the enzymatic activity of DNase I has been increased in presence of a molecular crowding agent, PEG 3350. While, steady state and ultrafast time resolved spectroscopy on a florescence probe EB intercalated to substrate DNA reveal the hydrolysis of the substrate by DNase I, the spectroscopic information including picosecond resolved fluorescence polarization gated studies on the enzyme-bound fluorescence probe ANS shows enhanced surface flexibility of DNase I in the presence of PEG. The reduced water activity at the enzyme surface due to osmotic stress of the molecular crowding agent enhancing the dynamical flexibility of the enzyme is concluded to increase the DNA binding eventually accelerate the hydrolysis reaction of DNase I. In summary the study attempts to unravel the molecular picture of DNA hydrolysis by an endonuclease in presence of an osmotic stress generating molecular crowding agent. A clear correlation of the flexibility of the endonuclease with the rate of DNA hydrolysis within the overall structural integrity of the enzyme has also been established.

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