Defence Life Science Journal, Vol. 4, No. 4, October 2019, pp. 231-235, DOI : 10.14429/dlsj.4.14967

One-step Purification and Characterisation of Abrin Toxin from *Abrus Precatorius* Seeds

Swati Banger, Rita Singh, N.K. Tripathi, V. Pal, and A.K. Goel*

DRDO-Defence Research and Development Establishment, Gwalior - 474 002, India
**E-mail: akgoel73@yahoo.co.uk*

ABSTRACT

Abrin is a plant toxin obtained from *Abrus precatorius* seeds. It belongs to the type II ribosomal inactivating proteins consisting of two chains namely, catalytically active A chain and sugar binding B chain linked by a single disulphide bond. Due to high toxicity of abrin, its exposure or consumption can lead to serious public health problems. In the present work, we have extracted and purified the abrin toxin from *Abrus precatorius* seeds. The toxin was purified using a single step anion exchange chromatography. The purified protein was characterised by SDS-PAGE and MALDI- TOF to confirm its purity. The toxicity of purified abrin toxin was also confirmed by injecting the toxin in mice. The purified protein was further used to raise antibodies in mice and characterised by indirect Enzyme Linked Immunosorbent Assay. The results of present study established the use of single step ion exchange chromatography to purify abrin toxin for further development of its detection system.

Keywords: Abrin; Chromatography; ELISA; Extraction; Purification

1. INTRODUCTION

Abrin, a plant toxin is obtained from *Abrus precatorius* seeds, commonly known as jequirity beans or rosary peas. A single seed weighs about 100 mg and contains about 5 per cent abrin toxin of its weight[1]. Abrin is water soluble and highly stable at high temperature (up to 74 °C)[2]. Abrin is highly toxic with 0.1 – 1 µg/kg of lethal dose in human[3, 4]. Clinical signs and symptoms of abrin poisoning include nausea, vomiting, diarrhea and abdominal pain[5]. Contact of abrin with eyes may cause conjunctivitis and even blindness[6]. It is a heterodimeric glycoprotein that contains two polypeptide chains viz. A and B. These chains are linked by a single disulphide bond. Thus, it is a type-II ribosome inactivating protein (RIP), in which A chain and B chain possess N-glycosidase activity and galactose-binding activity, respectively. Both chains together inactivate the eukaryotic ribosomes and inhibit protein synthesis[7]. Abrin seeds also contain agglutinin (a less toxic protein) and other proteins that need to be removed to get purified toxin. Abrin is structurally and functionally similar to ricin toxin obtained from *Ricinus communis* seeds. Both, abrin and ricin can also be used in cancer therapy at sub-lethal doses as they show higher toxicity for cancerous cell as compared to normal cells, probably due to the presence of more glycosylated binding sites on malignant cell surface than normal cells[8]. These toxins in plants deter herbivory and also have antifungal, antiviral and anti-insecticidal characteristics[9].

In vitro studies established that abrin is more potent toxin than ricin[10]. Autoradiography studies confirmed that even a single molecule of abrin is enough to destroy a cell[11]. Thus, it harnesses the potential to be used as a chemical/biological warfare agent. Due to its high toxicity, abrin is listed as a Category B agent by the Centers for Disease Control and Prevention, USA. Besides, abrin toxin is considered as 'Biological Select Agents or Toxins' by United States Department of Health and Human Services[12].

Isolelectric pH of different variants of abrin varies from 5.4 – 8.0 [13]. Different isoforms of abrin differing in toxicity, amino acid composition and molecular weight have been reported using various chromatography techniques including ion exchange, gel filtration, sepharose 4B and lactamyl-sepharose chromatography[14]. Most of these chromatography techniques require more than one step to purify toxic abrin from the seeds. Based on affinity, two isoforms of abrin were recovered using sepharose 4B column, sepharose binding (abrin C) and sepharose non-binding (abrin A), which were further purified using chromatography on Diethylaminoethyl (DEAE) sephadex A 50, Carboxymethyl (CM) cellulose or Diethylaminoethyl cellulose[15]. It has been reported that four isoforms of abrin i.e. abrin-a, abrin-b, abrin-c, and abrin-d can be purified using sepharose 4B, DEAE cellulose and gel filtration column[16]. In another study, three toxin and two agglutinin were purified using lactamyl sepharose, ion exchange and gel filtration chromatography[13].

In the present study, the abrin protein was purified using a single step anion exchange chromatography. The purified toxin was characterised by SDS-PAGE under native and denaturing conditions. Further, the presence of abrin toxin was confirmed by MALDI- TOF/ MS. The purified toxin was used to check the toxicity in mice. Antibodies were also raised in mice using heat inactivated toxin and characterised by indirect ELISA.

Received : 10 April 2019, Revised : 19 June 2019
Accepted : 20 June 2019, Online published : 21 October 2019

2. MATERIAL AND METHODS

2.1 Seeds, Chemicals and Biologicals

Abrin seeds were collected from rural areas of district of Rewari, Haryana, India. Chemicals used in this study were purchased from Himedia, India; SD fine chemicals, India and Sigma, USA. The secondary antibody was procured from Sigma, USA. Chromatography columns were procured from GE Healthcare, Sweden.

2.2 Extraction of Abrin Toxin from *Abrus precatorius* Seeds

Seeds (10 g) of *Abrus precatorius* were decoated and soaked in 50 ml of 5 per cent acetic acid overnight. Further, seeds were homogenised and centrifuged at 9000 rpm. Supernatant was collected and subjected to fractional ammonium sulphate precipitation in two stages, 0 % - 30 % and 30 % - 90 % ammonium sulphate saturation. The 30 % - 90 % ammonium sulphate precipitated pellet was resuspended in 10 ml water. The solution was dialysed against water for two days, and then against buffer (10 mM Tris HCl, 30 mM NaCl, pH 7.8) for three day with three changes at an interval of 24 h. The dialysed sample was named as crude abrin.

2.3 Purification of Abrin Toxin using Ion Exchange Chromatography

Abrin toxin was purified by a simple one step anion exchange chromatography. A 5 ml pre-packed Q sepharose fast flow chromatography column was equilibrated with 4 column volume (CV) of equilibration buffer (10 mM Tris HCl, 30 mM NaCl, pH 7.8) at a flow rate of 1 ml/ min. Dialysed crude abrin extract was filtered using 0.45 µm membrane filter, and the clear filtrate was loaded on the column with 0.5 ml/ min flow rate. Washing of the column was carried out using equilibration buffer. After washing, bound proteins were eluted with step gradient of NaCl. Abrin was eluted with buffer containing 10 mM Tris HCl (pH 7.8) and 70 mM NaCl, while the other bound proteins were eluted with buffer containing 10 mM Tris HCl (pH 7.8) and 150 mM NaCl. The concentration of abrin toxin was determined by BCA kit (Thermo, USA).

2.4 Characterisation of Abrin Toxin

The purified protein was characterised by SDS-PAGE analysis. Gels (3% stacking and 10% separating) were made by using a 29.2% acrylamide and 0.8 % *N,N'*- methylene bisacrylamide, stock solution. The electrode buffer comprised of Tris HCl (0.025 M), pH 8.3, glycine (0.192 M) and SDS (0.1%). Equal volumes of purified abrin and sample buffer (50 mM Tris HCl, pH 6.8, 2% SDS, 20 % glycerol, 2 % 2-mercaptoethanol and 0.04 % bromophenol blue) were mixed and boiled for 10 min. Boiled samples were subjected to SDS-PAGE (both reducing and non-reducing) to analyze the protein size and its purity according to the standard method.

The purified abrin was also characterised by MALDI-TOF. For this purpose, the desired protein band from SDS-Polyacrylamide gel was excised and subjected to MALDI-TOF. The MALDI-TOF spectra were analysed by MASCOT search engine (Bio-tools version 3.1) using National Center for Biotechnology information data.

2.5 Toxicity Studies

Toxicity of purified abrin was evaluated by injecting purified abrin (in PBS) intraperitoneally at a dose of 2 µg/ kg body weight in four BALB/c mice weighing 25 g - 30 g. Two mice, injected with same volume of PBS were treated as control. The injected mice were observed for survival up to 96 h.

2.6 Production and Evaluation of Antibody Titre by Indirect ELISA

Purified abrin was used to raise antibodies in mice. For production of antibodies, six female BALB/c mice (8 week old) were immunised with heat inactivated abrin toxin (25 µg/ mouse/ dose) with Freund complete adjuvant for priming, followed by two booster doses with incomplete adjuvant subcutaneously on days 21 and 42. Blood collected before immunisation was used as control sera in ELISA. Final bleeding was carried out on day 60. The serum was separated from blood and used in ELISA.

The animal experiments were performed according to the Institutional Animal Ethics Committee (IAEC) of Defence Research and Development Establishment (registration number 37/1999/CPCSEA). The animals were maintained as per the approved guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India.

Indirect ELISA was carried out for the determination of anti-abrin antibody titer. ELISA plates (96 well) were used for coating of 200 ng purified abrin in carbonate-bicarbonate (coating) buffer (0.1 M Na_2CO_3; 0.2 M $NaHCO_3$; pH-9.6) in each well. The coated plates were incubated at 4 °C for overnight. After washing the plates with wash buffer [PBS + Tween 20 (0.1 %)] using microplate washer (BioTek, USA), blocking was done by adding 300 µL of blocking buffer [PBS + SMP (5 %)] in each well. The plates were further incubated at 37 °C for 1 h. After three washes, the test sera (anti- abrin mice sera) were two-fold serially diluted in dilution buffer (1% SMP in PBS) and 100 µl of diluted serum samples was dispensed to each well. After 1 h incubation at 37 °C followed by three times washing, 100 µl of anti-mice IgG HRP-conjugate (1:4000 in PBS+1% SMP) was added to each well. The plates were incubated at 37 °C for 1 h. The plates were again washed and 100 µl of 3,3',5,5'-tetramethylbenzidine (TMB) was dispensed per well. The plates were kept at 37 °C for 10 min and then the reaction was stopped by adding 50 µl of 1 N H_2SO_4. The plates were analysed by an ELISA plate reader (BioTek, USA) at 450 nm. For control sample, pre-immunised sera were used in this assay. Each sample was evaluated in duplicate. The mean OD of pre-immune serum plus three standard deviations (SD) was used for the calculation of cut-off values. The results were expressed as reciprocal of the end point dilution.

3. RESULTS AND DISCUSSION

3.1 Extraction, Purification and Characterisation of Abrin Toxin

Abrin is highly toxic and can be used as a biowarfare toxin. Thus, for development of an effective detection system, a simple method of purification to obtain highly purified

protein is necessary. We have developed a simple one step ion-exchange chromatography method for purification of abrin. This purified protein was used for production of hyper immune sera in mice and characterised by ELISA.

After extraction of crude abrin from jequirity seeds, it was analysed by SDS-PAGE and multiple bands were observed as shown in Fig. 1. Various chromatographic techniques are used for purification of the abrin toxin. Most of the reported procedures involve more than one step to achieve purified abrin toxin. These chromatography processes include Sepharose 4B affinity chromatography, ion exchange chromatography; gel filtration chromatography and lactamyl sepharose chromatography[14]. In Sepharose 4B and lactamyl chromatography abrin toxin and agglutinin both bind on column containing these resins. Thus, a second chromatography step for separating agglutinin is required. Isoforms of abrin purified by different methodologies have variations in properties. However, based on the subunit composition and their binding property with sepharose 4B, a gross relationship may be established. In the present method, abrin toxin was purified by a single step anion exchange chromatography. The purified protein was also analysed for binding with sepharose 4B column and resulted in flow-through mode (data not shown).

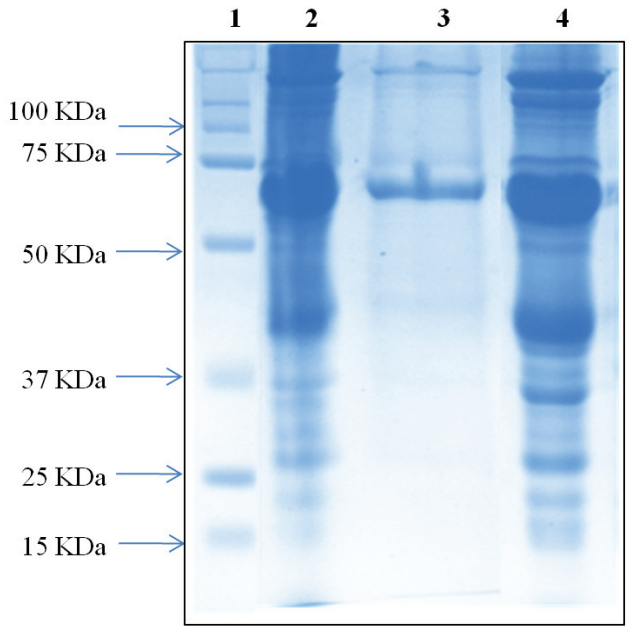

Figure 1. SDS-PAGE analysis of extracted crude abrin toxin: Lane1, Molecular weight marker; Lane 2, 90 per cent precipitate pellet fraction; Lane 3, 90 per cent supernatant fraction; Lane 4, Dialysed sample fraction containing abrin.

The purity of the purified toxin was evaluated by SDS-PAGE analysis as shown in Fig. 2. The size of purified abrin was found to be ~ 65 kDa molecular weight under non-reduced condition. Under reduced conditions, two peptides of ~ 32 kDa and ~28 kDa molecular weight were observed as shown in Fig. 3. The SDS-PAGE confirmed the purity of abrin toxin using anion exchange chromatography. MALDI-TOF analysis confirmed that the excised protein bands were of abrin.

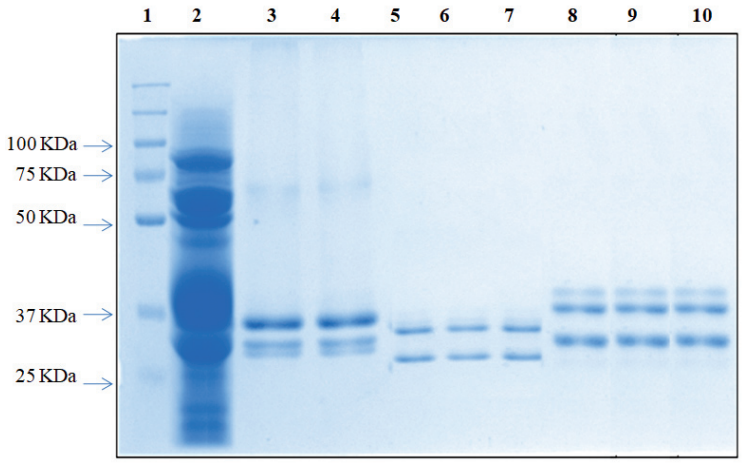

Figure 2. SDS-PAGE analysis for purification of abrin toxin. Lane 1, Molecular weight marker; lane 2, Dialysed sample fraction containing abrin; lane 3 and 4, Flow through fractions; lane 5-7, Eluted fractions with 70 mM NaCl; lane 8-10, Eluted fractions with 150 mM NaCl.

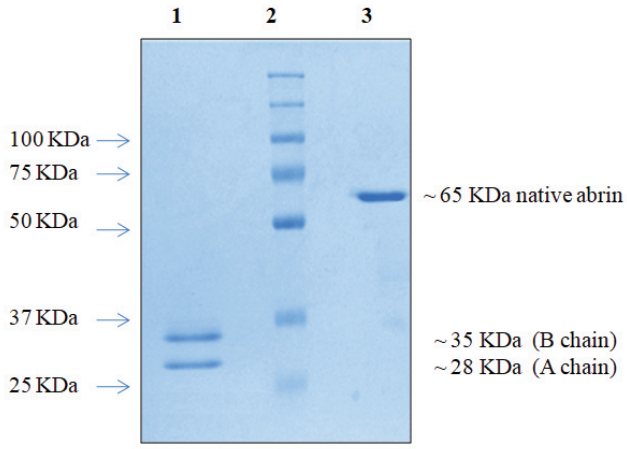

Figure 3. SDS-PAGE profile of purified abrin under native and denatured conditions. Lane 1, Reduced purified abrin toxin; lane 2, Molecular weight marker; Lane 3, Non-reduced purified abrin toxin.

3.2 Toxicity Confirmation

In the present work, mice injected with 2 µg/kg of purified abrin showed the high toxicity of the toxin. Mice were monitored for 96 h after injection. Mice injected with PBS only were normal and healthy, while mice injected with abrin toxin died after 48 h. These mice also showed characteristic symptoms of abrin poisoning i.e. drowsiness, and diminished and then completely absent reflexes[17]. There are several previous reports related to toxicity of chromatography purified abrin toxin. In a study, abrin purified by DEAE chromatography followed by sepharose chromatography showed LD_{50} of 2.8 µg/kg in mice[18]. Four isoforms of abrin purified by sepharose 4B followed by DEAE chromatography had LD_{50} of 10 µg/kg, 25 µg/kg, 16 µg/kg and 31 µg/kg in mice[16]. In another study, LD_{50} of three isoforms purified by combining three methods viz. lactamyl sepharose chromatography, gel filtration and DEAE chromatography was about 18.3 µg/kg, 2 µg/kg and 8.3 µg/kg for abrin I, II and III, respectively[13]. Two toxic isoform purified

by combination of DEAE sephadex A-50, CM- cellulose and DEAE cellulose also showed LD_{50} of 18 µg/kg and 4.8 µg/kg[15]. In another study, LD_{50} of purified abrin toxin was found to be about 20 µg/kg in mice[19].

3.3 Antibody Titre

Various techniques have been used for detection of abrin from various sources including food matrices[19]. Anti-abrin polyclonal antibodies were generated in mice by immunising with heat inactivated abrin toxin The antibody raised in mice sera exhibited a titre of 1: 256000 with abrin toxin in indirect ELISA as shown in Fig. 4.

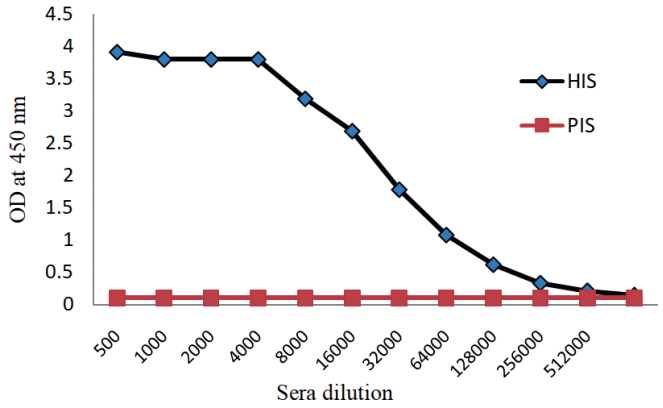

Figure 4. End point titer of anti- abrin polyclonal antibody generated in mice.

4. CONCLUSION

The Abrin toxin was extracted and purified from jequirity beans. Different extraction methods and multi-step chromatography techniques are generally used for obtaining pure abrin toxin. In the present work, a single step anion exchange chromatography was used to obtain purified abrin protein. Purified toxin was highly toxic as determined by injecting native toxin intraperitoneally in mice. The toxin was also characterised by MALDI-TOF analysis for its confirmation. The antibody generated against abrin toxin resulted in high titer of antibodies. Thus, the developed one step purification method can be a useful tool for the purification of abrin toxin.

REFERENCES

1. Lord, J.M. & Spooner, R.A. Ricin trafficking in plant and mammalian cells. *Toxins (Basel),* 2011, **3**(7), 787-801. doi:10.3390/toxins3070787
2. Tam, C.C.; Henderson, T.D.; Stanker, L.H.; He, X. & Cheng, L.W. Abrin Toxicity and Bioavailability after Temperature and pH Treatment. *Toxins (Basel),* 2017, **9**(10). doi:10.3390/toxins9100320
3. Dickers, K.J.; Bradberry, S.M.; Rice, P.; Griffiths, G.D. & Vale, J.A. Abrin poisoning. *Toxicol. Rev.,* 2003, **22**(3), 137-142.
4. Olsnes, S. The history of ricin, abrin and related toxins. *Toxicon,* 2004, **44**(4), 361-370. doi:10.1016/j.toxicon.2004.05.003
5. Chaturvedi, K.; Jadhav, S.E.; Bhutia, Y.D.; Kumar, O.; Kaul R.K. & Shrivastava, N. Purification and dose-dependent toxicity study of abrin in swiss albino male mice. *Cell. Mol. Biol. (Noisy-Le-Grand),* 2015, **61**(5), 36-44.
6. Hart, M. Jequirity-Bean Poisoning. *N. Engl. J. Med.,* 1963, **268**(16), 885-886. doi:10.1056/nejm196304182681608
7. Bagaria S. & Karande A. Abrin and immunoneutralisation: A review. In Toxinology, *edited by* Gopalakrishnakone P. Dordrecht, The Netherlands: Springer, 2014;1-21.
8. Bhutia, S.K.; Mallick, S.K.; Maiti, S.; Mishra, D. & Maiti, T.K. Abrus abrin derived peptides induce apoptosis by targeting mitochondria in HeLa cells. *Cell Biol. Int.,* 2009, **33**(7), 720-727. doi:10.1016/j.cellbi.2009.04.012
9. Stirpe, F. Ribosome-inactivating proteins. *Toxicon,* 2004, **44**(4), 371-383. doi:10.1016/j.toxicon.2004.05.004
10. Griffiths, G.D.; Leek, M.D. & Gee, D.J. The toxic plant proteins ricin and abrin induce apoptotic changes in mammalian lymphoid tissues and intestine. *J. Pathol.,* 1987, **151**(3), 221-229. doi:10.1002/path.1711510310
11. Eiklid, K.; Olsnes, S. & Pihl, A. Entry of lethal doses of abrin, ricin and modeccin into the cytosol of HeLa cells. *Exp. Cell Res.,* 1980, **126**(2), 321-326.
12. Chen, H.Y.; Foo, L.Y. & Loke, W.K. Abrin and Ricin: Understanding Their Toxicity, Diagnosis, and Treatment. *In* Biological Toxins and Bioterrorism, edited by Gopalakrishnakone P, Balali-Mood M, Llewellyn L*, et al.*, eds. Dordrecht: Springer Netherlands, 2015;79-102.
13. Hegde, R. & Podder, S.K. Studies on the variants of the protein toxins ricin and abrin. *Eur. J. Biochem.,* 1992, **204**(1), 155-164.
14. Hegde, R.; Maiti, T.K. & Podder, S.K. Purification and characterization of three toxins and two agglutinins from Abrus precatorius seed by using lactamyl-Sepharose affinity chromatography. *Anal. Biochem.,* 1991, **194**(1), 101-109.
15. Wei C.H.; Hartman, F.C.; Pfuderer P. & Yang W.K. Purification and characterization of two major toxic proteins from seeds of Abrus precatorius. *J. Biol. Chem.,* 1974, **249**(10), 3061-3067.
16. Lin J.Y.; Lee T.C.; Hu S.T. & Tung T.C. Isolation of four isotoxic proteins and one agglutinin from jequiriti bean (Abrus precatorius). *Toxicon,* 1981, **19**(1), 41-51.
17. ElShabrawy, O.A.; ElGengaihi, S.; Ibrahim, N.A. & AliIbrahim, N. Toxicity and teratogenicity of abrin. *Egyptian J.. Vet. Sci.,* 1987, **24**(2), 135-142.
18. Olsnes, S. & Pihl, A. Isolation and properties of abrin: a toxic protein inhibiting protein synthesis. Evidence for different biological functions of its two constituent-peptide chains. *Eur. J. Biochem.,* 1973, **35**(1), 179-185.
19. Garber, E.A. Toxicity and detection of ricin and abrin in beverages. *J. Food Prot.,* 2008, **71**(9), 1875-1883.

ACKNOWLEDGEMENT

The authors are thankful to Director, DRDE, Gwalior for his keen interest, constant support and providing necessary facilities for this study (Manuscript Acc No DRDE/BPT/26/2019).

CONTRIBUTORS

Ms Swati Banger, received the MSc from Jiwaji University, Gwalior, M.P., India, in 2015. Currently working as DBT-SRF at DRDO-Defence Research & Development Establishment and pursuing PhD from Bharathiar University, Coimbatore. She is working on development of detection systems for BW agents.
She performed the experiments and wrote the manuscript in this study.

Ms Rita Singh received Master from Jiwaji University, Gwalior, M.P. India in 2014. Currently working as SRF at DRDO-Defence Research & Development Establishment and pursuing PhD from Bharathiar University, Coimbatore. She is working on development of detection systems for BW agents.
She contributed in purification of toxin.

Dr Nagesh Tripathi received his PhD (Chemical Engineering) from National Institute of Technology, Rourkela. Presently, he is Scientist 'D' at the DRDO-Defence Research and Development Establishment, Gwalior. His research interest includes scale up of biomolecules including recombinant proteins, activated carbon spheres and development of chemical protective suit.
In the current study, he was involved in purification and analysis of toxin, and manuscript writing.

Dr Vijai Pal did his MSc (Biotechnology) from CCS Haryana Agricultural University, Hisar, in 2000 and PhD from Jiwaji University, Gwalior, in 2016. Presently, he is working as Scientist 'E' at DRDO-Defence Research and Development Establishment, Gwalior on development of diagnostic/detection systems for biothreat agents. He has published more than 30 research paper in Journals, besides one book and has filed one Indian Patent.
He designed the experiment for this manuscript.

Dr Ajay Kumar Goel received his PhD (Microbiology) from CCS Haryana Agricultural University, Hisar, in 1999. Currently working as a Scientist 'F' and Head, Bioprocess Technology Division, DRDO-Defence Research and Development Establishment, Gwalior. He has more than 100 research paper, 6 patent, radio talks, books and several overseas presentations to his credit. His current research interest includes development of detection and protection systems for potential biothreat agents.
He conceptualised the experiments for this manuscript.