DEPARTMENT OF BIOTECHNOLOGY
MINISTRY OF SCIENCE AND TECHNOLOGY, GOVT. OF INDIA
FUNDED RESEARCH PROJECT

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Venomics and antivenomics of Indian Cobra and Russell's viper: implications in drug discovery and quality control of antivenoms

FINAL PROJECT REPORT

(11th December, 2014 to 10th December, 2018)

Submitted By

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Progress Report for R&D Projects [Final report]

Section A: Project Details

A1. Project Title	Venomics and antivenomics of Indian Cobra and Russell's viper: implications in drug discovery and quality control of antivenoms
A2. DBT sanction no. and date	TI412/NE/U-Excel/2013; December 11, 2014
A3. Name of the PI	Prof. A. K. Mukherjee,
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A6. Total cost	Rupees 132.20 lakhs
A7. Duration of report	11th December, 2014 to 10th December, 2018
A8. Approved objectives of the project	**1.** Proteomics strategy to analyse cobra (*N. naja* and *N. kaouthia*) and Russell's viper (*D. russelii*) venom proteomes by using Multidimensional chromatographic techniques, toxin mass fingerprinting and N-terminal peptide sequencing. **2.** The *de novo* sequencing of tryptic digested peptides and BLAST search of homologous proteins in current snake venom databases to identify the different families of venom proteins, post translations modifications and pharmaceutically important proteins present in cobra and Russell's viper venom with special emphasis on eastern/NE India samples. **3.** Using proteomics tool to determine the degree of immunological cross-reactivity between cobra and Russell's viper venom of different geographical location of India with commercial polyvalent antivenom.
A9. Specific recommendation by task force, if any	No

Dated: 17th January, 2019

(A. K. Mukherjee)

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Section-B: Scientific and Technical Progress

B1. Progress made against the Approved Objectives, Targets & Timelines during the Reporting Period

B1.1. Venomics and antivenomics of western (WI), eastern (EI), and southern India (SI) Russell's viper (*Daboia russelii*) venom (RVV)

B1.1.1. Venom de-complexation of WI RVV by gel filtration (GF) and anion exchange (AEX) chromatography, ESI-LC-MS/MS analysis of venom fractions

WI RVV (200 mg dry weight) by fractionation on GF chromatography on a HiLoad 16/600 Superdex 75 pg column (1.6 x 60 cm) resolved into 10 major peaks. Subsequent fractionation of each GF peak by anion-exchange chromatography resulted in separation of venom proteins in 137 peaks.

Thereafter, each protein peak was subjected to in-solution trypsin digestion for subsequent LC-MS/MS analysis on an Agilent 1200 nHPLC interfaced to an LTQ Orbitrap Discovery hybrid mass spectrometer (Thermo Fisher Scientific) via a Nanomate Triversa (Advion BioSciences), equipped with an LC coupler and an ESI nano-spray chip. Fragmentation was collision-induced dissociation and the LC-MS/MS data was acquired in a data-dependent acquisition (DDA) mode by Xcalibur software (Thermo Fisher Scientific). The LC-MS/MS data was searched with taxonomy set to Viperidae against the entries in the non-redundant NCBI database (taxid: 8689 with 53,236 protein entries) using Proteome Discoverer 1.3 software. The relative abundance of identified WI RVV proteins was determined by MS1-based label-free proteomics.

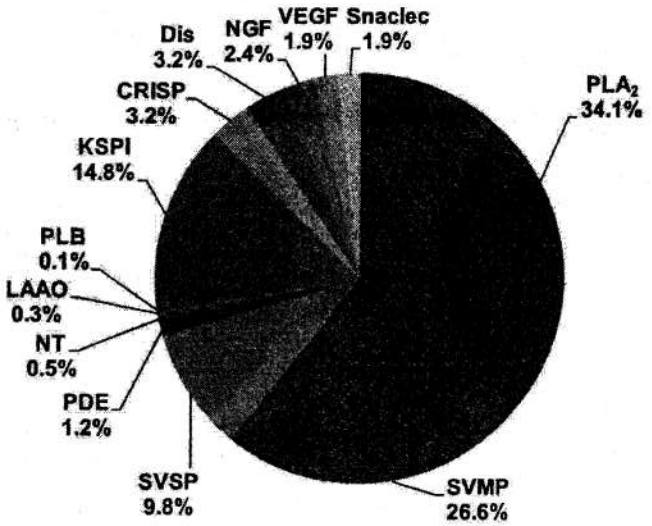

Figure 1: Protein family composition of WI RVV proteome. The pie chart represents the relative occurrence of different enzymatic and non-enzymatic proteins families of RVV, determined by MS1 based

label free proteomics. Abbreviations: PLA_2 (phospholipase A_2); SVMP (snake venom metalloprotease); SVSP (snake venom serine-protease); PDE (phosphodiesterase); NT (nucleotidase); LAAO (L-amino acid oxidase); PLB (phospholipase B); KSPI (Kunitz type protease inhibitor); CRISP (cysteine rich secretory protein); Dis (disintegrin); NGF (nerve growth factor); VEGF (vascular endothelial factor); Snaclec (C-type lectin)

A total of 55 proteins belonging to 13 distinct snake venom families were unambiguously identified by ESI-LC-MS/MS analysis of IEX fractions of WI RVV. Phospholipase A_2 (32.5%) and Kunitz type serine protease inhibitors (12.5%) represented the most abundant enzymatic and non-enzymatic proteins, respectively (Fig. 1).

B1.1.2. Venom de-complexation of EI RVV by gel filtration (GF) and ESI-LC-MS/MS analysis of venom fractions

EI RVVs (45 mg dry weight) of Burdwan and Nadia districts of West Bengal by fractionation on GF chromatography on a HiLoad 16/600 Superdex 75 pg column (1.6 x 60 cm) resolved into 10 major peaks each. Thereafter, each protein peak was subjected to in-solution trypsin digestion for subsequent LC-MS/MS analysis (see section B1.1.1).

Mass spectrometry analysis of the gel filtration fractions and the subsequent MS/MS search against the Viperidae protein entries of the NCBI database identified 73 and 69 proteins distributed among 15 snake venom protein families in EI RVV B and EI RVV N, respectively (Figures 2B, C). A comparison of the EI RVV proteomes (based on occurrence of homologous distinct peptides) from the two neighboring districts of West Bengal suggested that both venoms share 45 proteins in common, whereas 28 and 24 protein entries were uniquely identified in EI RVV B and EI RVV N, respectively.

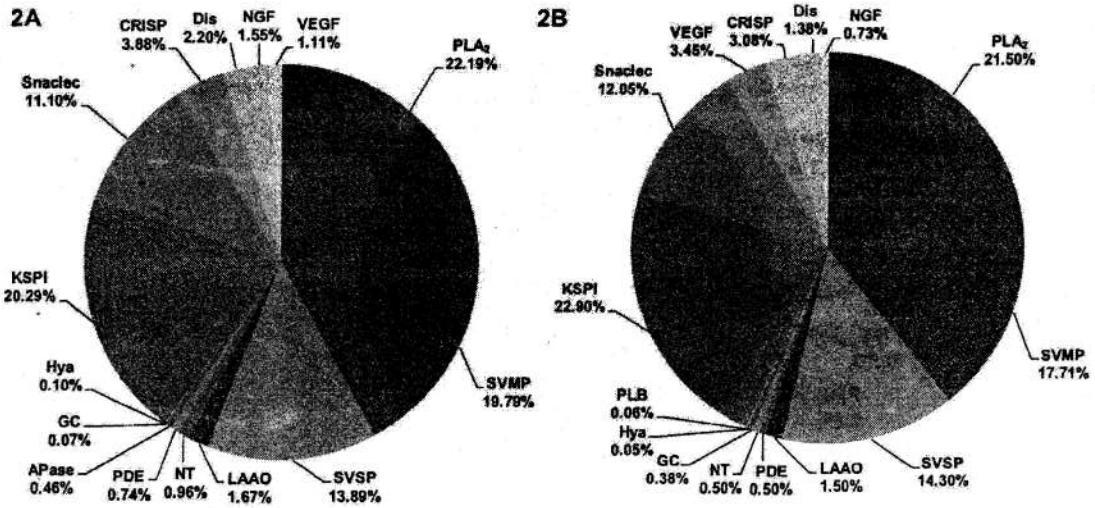

Figure 2: Protein family composition of **A.** EI RVV B and **B.** EI RVV N. The pie chart represents the relative abundance of different enzymatic and non-enzymatic proteins families of RVV as determined by MS2 based Label free quantitative proteomics.

B1.1.3. Venom de-complexation of SI RVV by 1D-SDS-PAGE and ESI-LC-MS/MS analysis of the protein bands

The in-gel trypsin digestion and subsequent tandem mass spectrometric analysis of 10 gel sections of SI RVV resulted in the identification of 66 distinct proteins in this venom, distributed in 14 snake venom protein families (Fig. 3).

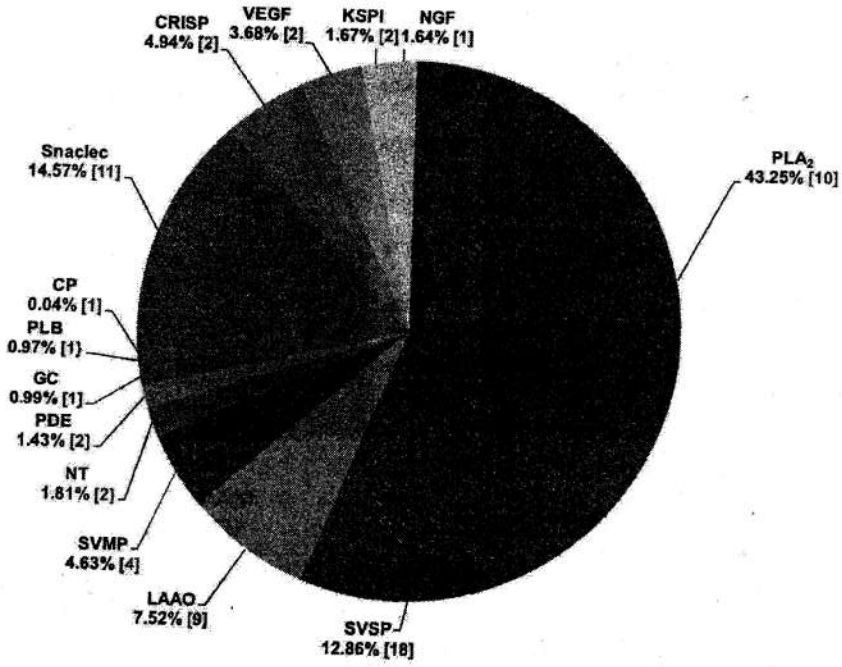

Figure 3: Protein family composition of SI RVV. The relative occurrences of different enzymatic and non-enzymatic protein families in SI RVV are expressed as an average of relative abundances calculated using MS1 (Summed Peptide-Spectrum Match Precursor Intensity) and MS2 (NSAF) based label-free quantitation techniques.

B1.2. Assessment of immunological cross-reactivity between WI, EI, and SI RVV and commercial antivenoms by second generation antivenomics approach

Antivenomics study also revealed that WI, EI, and SI RVV proteins in molecular weight range of 10 to 20 kDa were not efficiently captured by PAV coupled immunoaffinity columns (Fig. 4A-D). Subsequently, the SDS-PAGE bands of PAV unbound WI, EI, and SI RVV proteins were excised for in-gel trypsin digestion and subjected to ESI-LC-MS/MS analysis for protein identification and quantification.

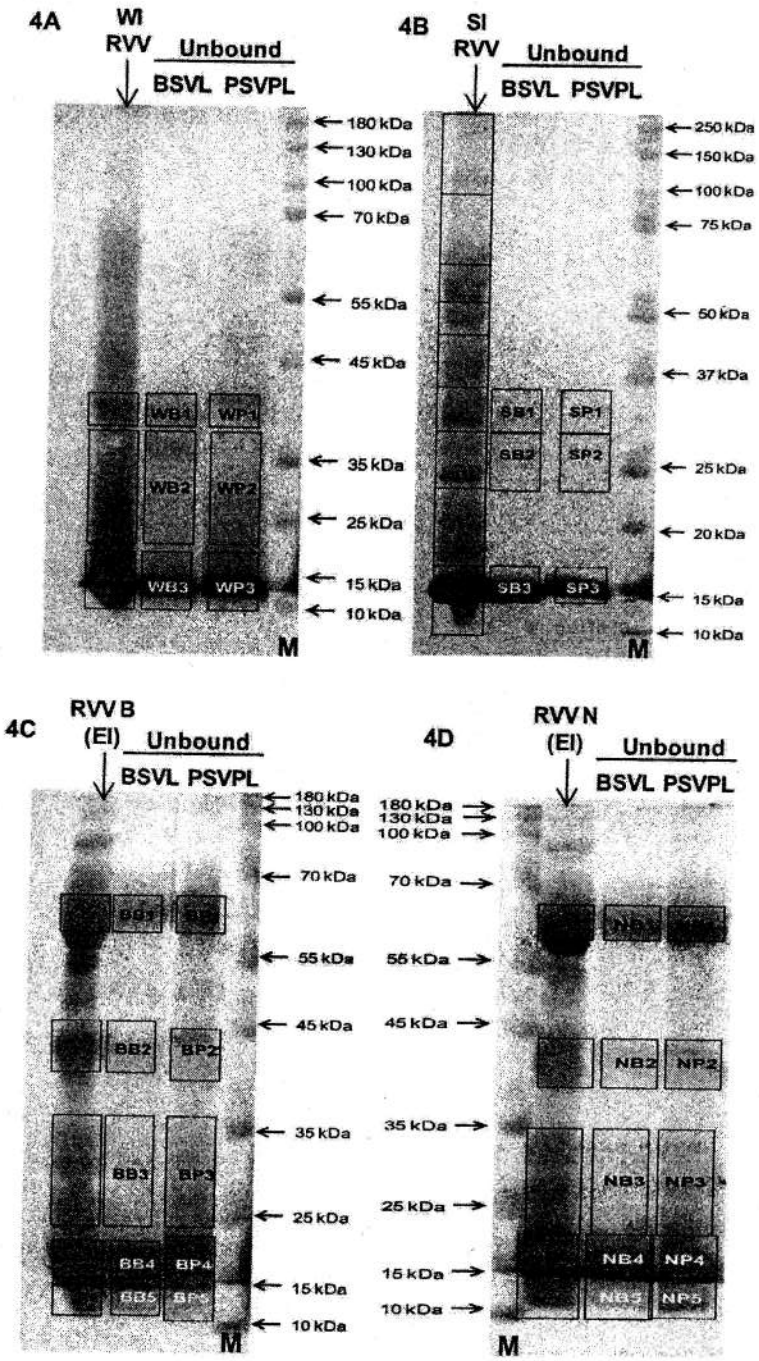

Figure 4: Second generation antivenomics of WI, EI, and SI RVV. 12.5% SDS-PAGE analysis of PAV-unbound fractions of **A.** WI RVV, **B.** SI RVV, **C.** EI RVV (B), and **D.** EI RVV (N). The PAVs used for the study were manufactured by Bharat Serums and Vaccines Ltd. (BSVL) and Premium Serums and

Vaccines Pvt. Ltd. (PSVPL). The protein bands of the unbound fractions were excised, in-gel digested and subjected to ESI-LC-MS/MS analysis for protein identification.

ESI-LC-MS/MS analysis of the PAV unbound SDS-PAGE protein bands suggested that the major PAV-unbound toxins in WI, EI, and SI RVV belonged to PLA_2, KSPI, snaclec, CRISP, VEGF, and NGF protein families. In addition, the tested PAVs also lacked sufficient antibodies against proteases (SVMP and SVSP) of EI RVV. Nevertheless, MS2 based quantitative analysis indicated that PLA_2 and KSPI, the two major protein families in RVV, were least recognized by commercial PAVs (Fig. 4E). These proteins play a significant role in RVV-induced toxicity and exhibit diverse pharmacological effects in bite victims; therefore, poor recognition of these components by commercial PAVs is a serious concern for effective antivenom therapy. Interestingly, the extent of immuno-recognition of SI RVV was relatively better compared to WI and EI RVV samples. Most of the commercial antivenom manufacturing companies procure snake venom from Irula Snake Catchers' Industrial Cooperative Society, Tamil Nadu for raising equine antivenom, which likely explains the observed differential cross-reactivity of commercial PAVs against RVV samples from different locations. Therefore, there is urgency in developing improved immunization schemes that include venoms collected from wide geographic locations in order to render PAV effective throughout the country. Alternatively, efforts can also be made to develop region-specific and species-specific antivenom for better treatment of RV-bite patients.

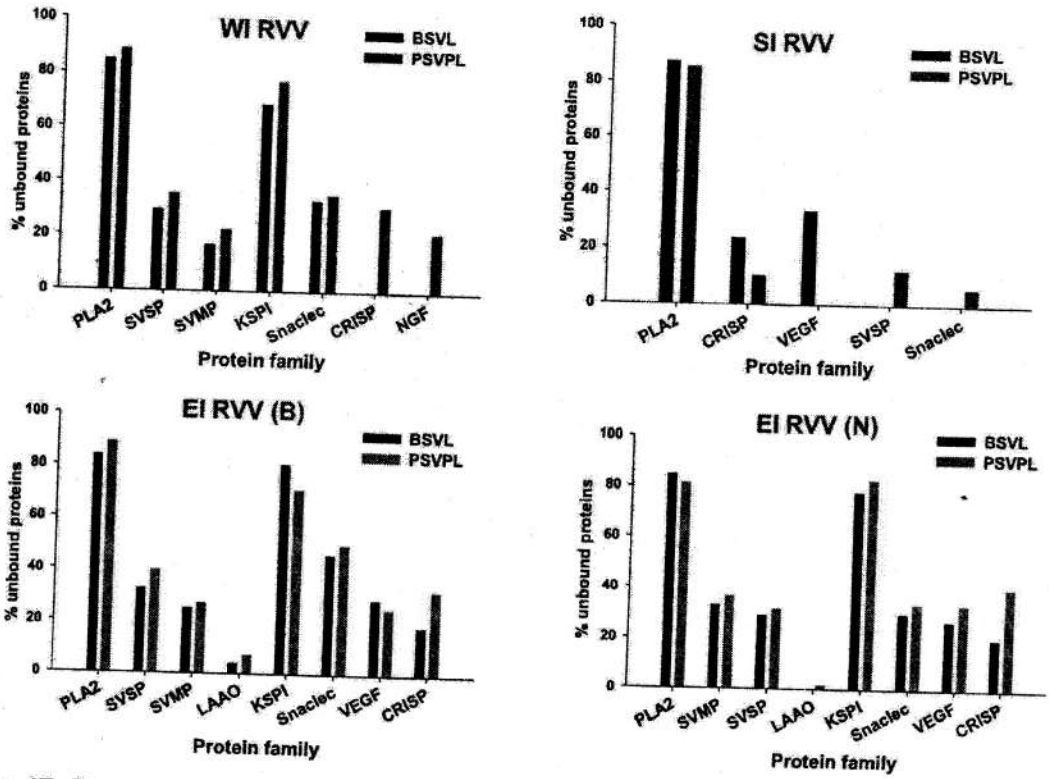

Figure 4E: Percentage of unbound proteins of WI, SI, and EI RVV eluted from immunoaffinity columns coupled with PAV (BSVL and PSVPL).

B1.3. Clinical manifestations of envenomations by RV and their correlation with RVV proteome composition

The common clinical features of RV bites, such as rapid swelling and extreme pain of the bitten body part, local ecchymosis and hemorrhage, and intense blebs over the affected extremities are primarily caused by the abundant SVMPs in RVV. Therefore, the variation in SVMPs observed among the RVV samples from different localities is expected to result in different levels of severity of SVMP-induced toxicity, and clinical manifestations in RV bite patients across the Indian sub-continent are likewise expected to be variable. Largely due to the activities of SVSPs, RVV initially affects the vascular system by provoking hemostatic disturbances, including rapid thrombosis and hypofibrinogenemia that ultimately results in consumptive coagulopathy and incoagulable blood. This results from the concerted action of the serine proteases and some metalloproteases (FX activator) that activate prothrombin, Factor X and V, and fibrin(ogen)olytic enzymes that catalyze hydrolysis of fibrinogen and/or fibrin. Subsequently, abundant anti-coagulant RVV proteins such as PLA2s, KSPIs, and snaclecs exert anti-coagulant action by inhibiting various blood factors such as thrombin, and/or Factor Xa, further promoting incoagulable blood.

Intravascular hemolysis and related complications observed in RV-envenomed patients primarily result from the action of PLA_2 isoenzymes that can cause lysis of phospholipids of erythrocyte membranes, leaving the cells vulnerable to dissolution. Acute kidney injury (previously termed as acute renal failure or ARF) is a persistent clinical manifestation observed in RV-envenomed patients throughout the Indian sub-continent. RVV factor X activators, LAAO, and PLA2 isoforms are the likely components responsible for RVV-induced acute kidney injury, and significant variation in the amounts of these components in RVV, as observed by proteomic analyses, may lead to differences in the severity of RVV-induced acute kidney injury in different regions. However, due to a lack of clinical data on snakebite from different regions of Indian subcontinent, this presumption cannot yet be verified. Another component, VEGF, may also exacerbate kidney injury; it exhibits potent hypotension and enhancement of vascular permeability activities, thereby resulting in overall bleeding complications.

In addition to the common clinical symptoms mentioned above, RV bite patients from SI, SL, and occasionally from WI are also reported to show neuroparalytic symptoms such as ptosis, bulbar palsy, inter-nuclear ophthalmoplegia, and respiratory paralysis due to pre-synaptic neuromuscular block. While the neurological symptoms are quite severe in SL RV-envenomed patients, they are moderate in SI and very rarely reported in WI. These differences in severity of neurological symptoms can be explained on the basis of variation in the relative abundances of neurotoxic PLA_2 isoforms in RVV samples from SL (>30%), SI (15.7%) and WI (3.2%).

The proteomic analyses of RVV from different regions of the Indian sub-continent have enabled us to document significant levels of variation in venom composition of this medically relevant snake. Further, as discussed above, the proteomic findings were consistent with the clinical manifestations of RV envenomation reported across the country.

B1.4. Venomics and antivenomics of western and eastern India *Naja naja* venom (NnV) and eastern India *N. kaouthia* venom (NkV)

B1.4.1. Venom de-complexation of WINnV by GFC and RP-HPLC followed by SDS-PAGE analysis, tandem mass spectrometry analysis of venom fractions, and data mining to unveil the complex venom proteome

WINnV (75 mg dry weight) upon GF chromatography on a HiLoad 16/600 Superdex 30 pg column resolved into 4 major peaks. Thereafter, each protein peak was subjected to SDS-PAGE under reduced condition. Peaks GF1 and GF2 were resolved into 11 and 5 bands, respectively, where GF3 and GF4 fractions showed only one broad protein band in the mass range of 5-15 kDa. All the protein bands were subjected to in-gel trypsin digestion for subsequent LC-MS/MS analysis (see section B1.1.1).

Tandem mass spectrometry analysis of the SDS-PAGE proteins against Elapidae database of NCBI resulted in identification of 54 distinct proteins from 16 different venom protein families in WINnV. SVMP (7.32%) and 3FTx (66.51%) represented the most abundant enzymatic and non-enzymatic protein families identified in WINnV, respectively (Fig. 5A). A high proportion 3FTx in WINnV is responsible for blurring of vision, loss of consciousness, early neuroparalysis, and rapid onset of respiratory failure observed in cobra envenomed patients in WI. Other symptoms of cobra bite, such as severe pain at the bite site; rapid progression of swelling, local tissue damage due to the large, non-healing ulcer formation may be attributed to the presence of Ohanin-like protein (OLP), cobra venom factor (CVF), PLA_2 and SVMP.

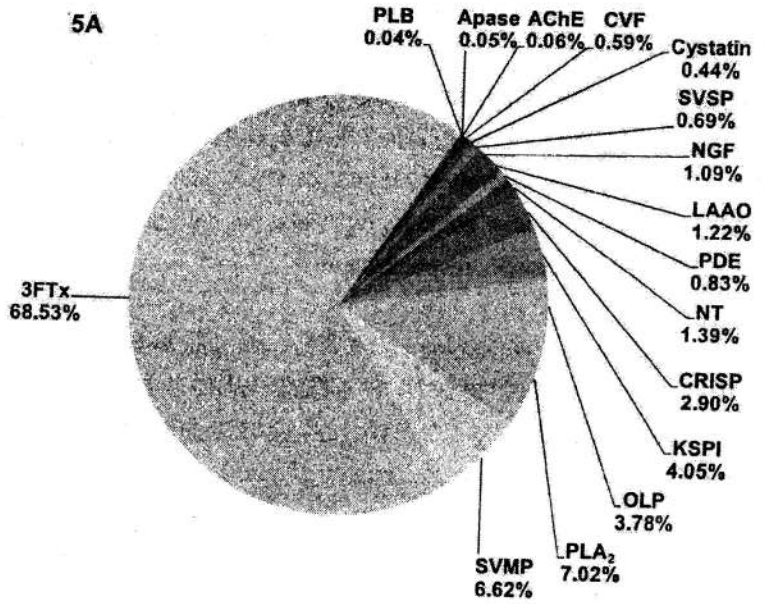

Figure 5A: Protein family composition of WINnV proteome deciphered from the LC-MS/MS analysis of the SDS-PAGE bands.

RP-HPLC fractionation of WINnV (2 mg protein) resolved the proteins into 30 protein peaks (Fig. 5B). The SDS-PAGE of these peaks revealed 71 heterogeneous protein bands (Fig. 5C).

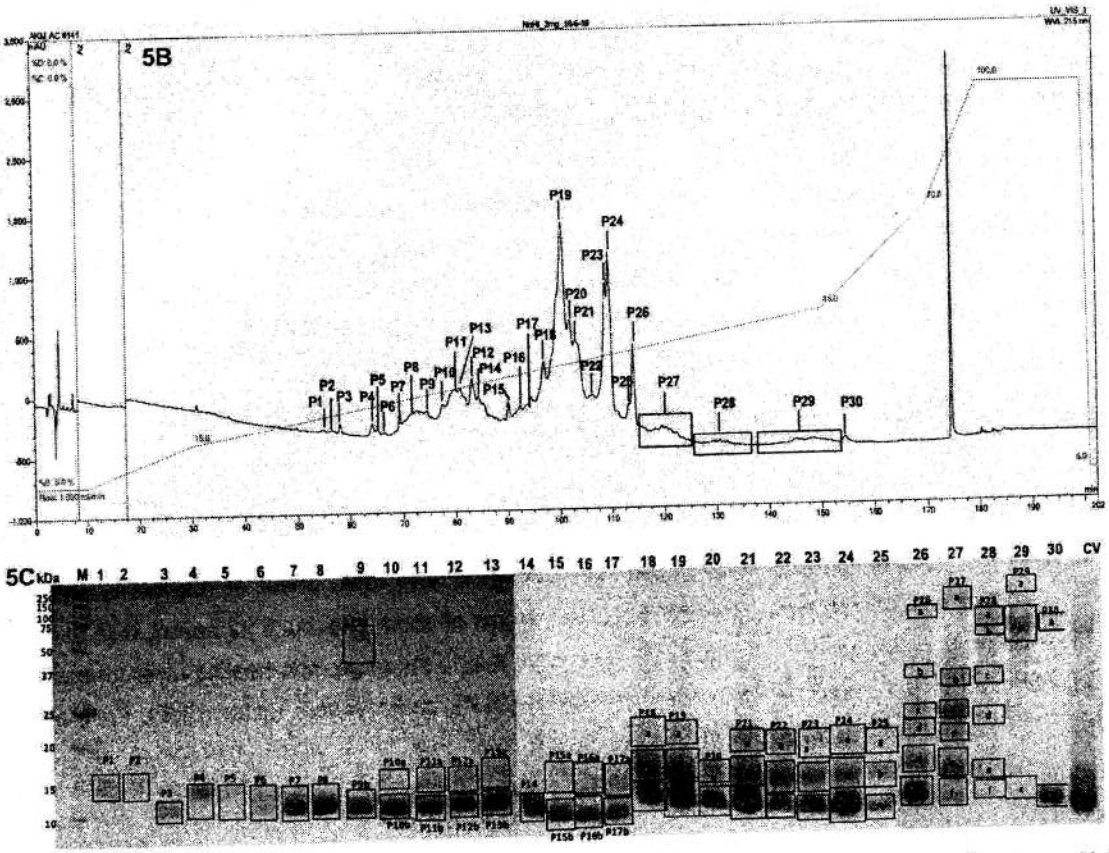

Figure 5B: RP-UHPLC analysis of WINnV (1.5 mg) fractionated on a Waters Sunfire® C18 column (4.6 × 250 mm, 5 µm particle size) coupled to Dionex Ultimate 3000 system. The flow rate was maintained at 1.0 mL/min and elution of protein was monitored at 215 nm. **C.** 12.5% SDS-PAGE of the RP-HPLC peak fractions under reduced conditions. The lane numbers represent the corresponding peaks; Lane M and CV represent protein ladder and crude venom, respectively.

The in-gel trypsin digestion and subsequent LC-MS/MS analysis (see section B1.1.1) of the 71 protein bands of RP-HPLC peaks of WINnV led to the identification of 40 distinct proteins distributed over 12 different snake venom protein families (Fig. 5D). The PLA_2 (27.1%) and 3FTx (50.9%) were found to be the most abundant enzymatic and non-enzymatic proteins, respectively (Fig. 5D).

5D

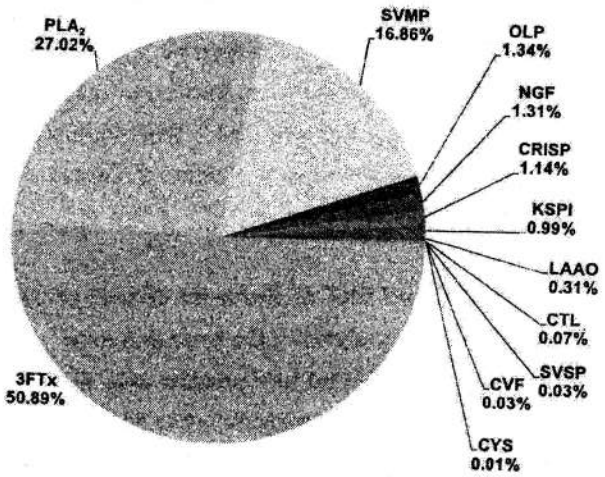

Figure 5D: Pie chart representing the protein family composition and their relative occurrence in WINnV proteome deciphered from the LC-MS/MS analysis of the SDS-PAGE bands of RPHPLC peaks, when the MS/MS data were searched against NCBI protein entries with taxonomy set to Elapidae (taxid 8602).

B1.4.2. Venom de-complexation of ElNnV by cation-exchange chromatography followed by ESI-LC-MS/MS analysis of the protein fractions

El NnV (25 mg dry weight) by fractionation on cation-exchange chromatography on a HiPrep CM FF 16/60 FPLC column resolved into 6 major peaks. The tryptic digested proteins of each fraction were subjected to LC-MS/MS analysis (see section B1.1.1).

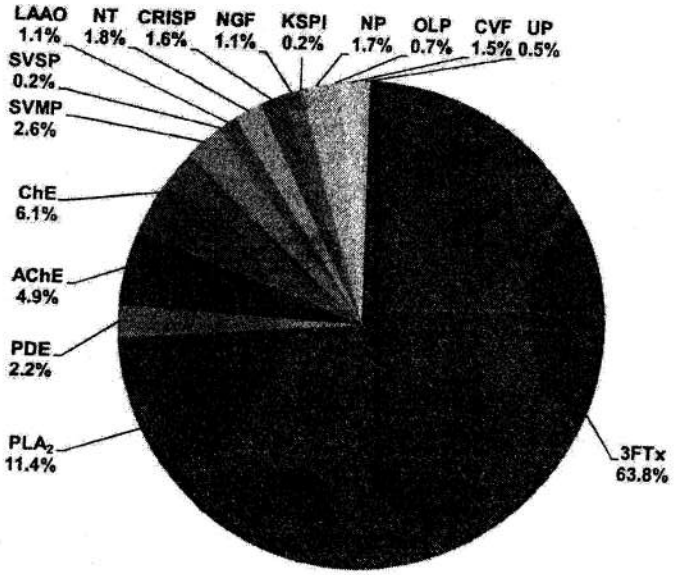

Figure 6: Protein family composition of ElNnV proteome deciphered from the LC-MS/MS analysis of the cation-exchange fractions.

Proteomic analysis revealed the presence of 43 enzymatic and non-enzymatic proteins in EINnV which were distributed over 15 snake venom protein families (Fig. 6). The three finger toxins (63.8%) and phospholipase A_2s (11.4%) were the most abundant families of non-enzymatic and enzymatic proteins, respectively. ESI-MS/MS analysis demonstrated the occurrence of acetylcholinesterase, phosphodiesterase, cholinesterase and snake venom serine proteases in *N. naja* venom which were not detected previously by proteomic analysis.

B1.4.3. De-complexation of NnV and NkV of Burdwan district of West Bengal, EI by SDS-PAGE analysis followed by ESI-LC-MS/MS analysis of the protein bands

The SDS-PAGE (reduced) of NnV and NkV proteins were resolved at the molecular mass range of ~6–130 kDa. NnV was excised into 11 gel sections, while NkV into 13 gel sections. The gel sections were then subjected to in-gel trypsin digestion followed by ESI-LC-MS/MS analysis for protein identification (see section B1.1.1).

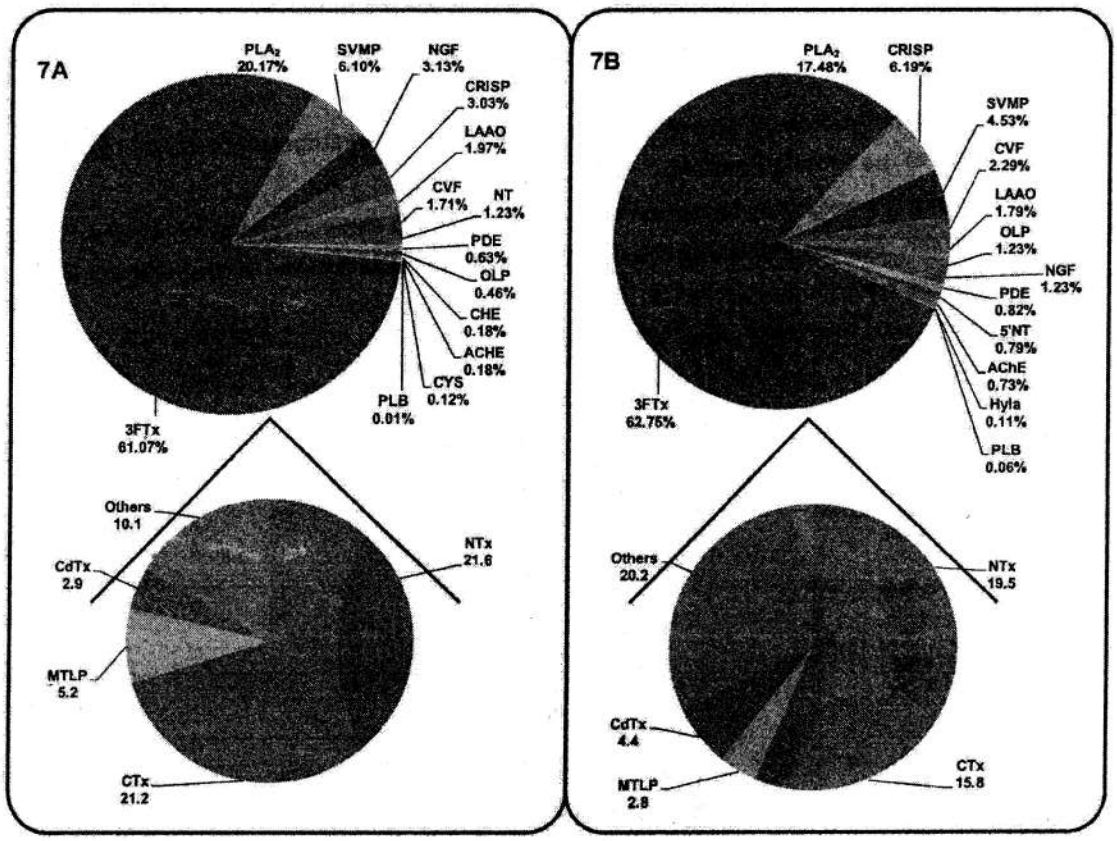

Figure 7: Protein family composition of **A.** NnV and **B.** NkV proteomes by searching against the Elapidae database. The figures below represent the relative abundances of 3FTx sub-classes in NnV and NkV proteomes. The relative abundances of toxins were calculated using the MS2 spectral count method.

By searching the elapid database, 52 proteins belonging to 14 different snake venom protein families were identified in NnV (Figure 7A), whereas 55 proteins from 13 snake venom protein families were identified in NkV (Figure 7B). Interestingly, by proteomic analysis, ChE and cystatin were identified only in NnV, while Hya was exclusively found in NkV (Figures 7A, B). A comparison shows that 29 proteins are common (based on homologous peptides) to both the venom proteomes; however, 23 and 26 proteins (toxins) are uniquely expressed in NnV and NkV, respectively. These unique proteins account for ~50% of the total Nn and Nk venom proteomes. Further, 11 and 16 3FTxs and PLA_2, the two major protein classes of cobra venom were found to be uniquely expressed in NnV and NkV, respectively, showing qualitative differences in venom composition between the two species of cobra under study. In addition, the relative abundances of PLA_2, SVMP, 5'NT, and NGF are slightly higher in NnV, compared to NkV (Figures 7A, B).

B1.5. Assessment of immunological cross-reactivity between WI and EI NnV, and EI NkV by second generation antivenomics study

Antivenomics study suggested that the low molecular mass (<20 kDa) cobra venom proteins were evidently not immuno-captured by PAVs (Figure 8A-C).

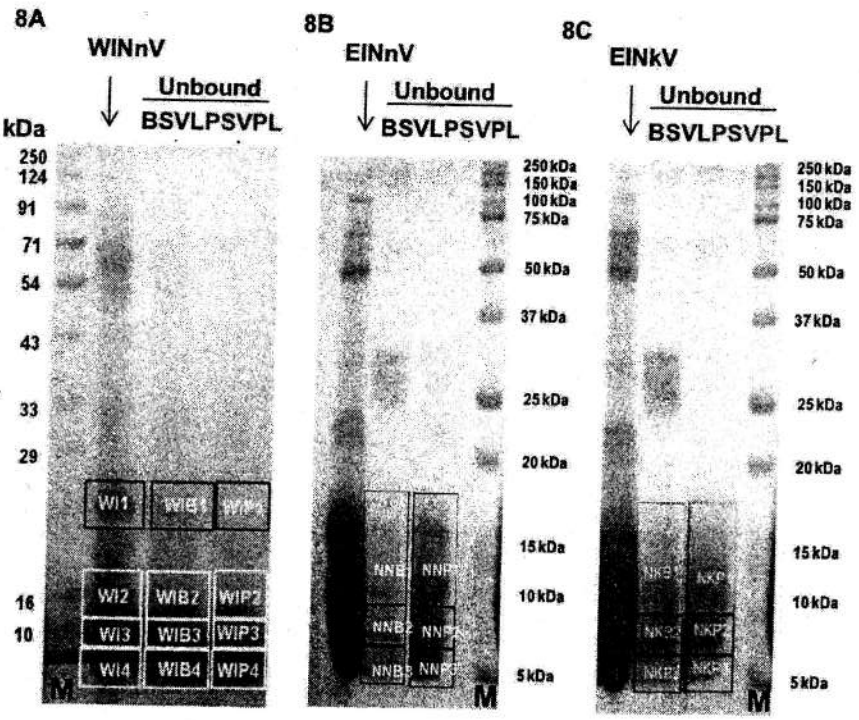

Figure 8: Second generation antivenomics of WI and EI NnV, and EI NkV. 15% SDS-PAGE analysis of PAV-unbound fractions of **A.** WINnV, **B.** EINnV, and **C.** EINkV. The PAVs used for the study were manufactured by Bharat Serums and Vaccines Ltd. (BSVL) and Premium Serums and Vaccines Pvt. Ltd.

(PSVPL). The protein bands of the unbound fractions were excised, in-gel digested and subjected to ESI-LC-MS/MS analysis for protein identification and quantification.

The proteomic analysis of PAV immunoaffinity unbound cobra venom proteins demonstrated that PLA_2, 3FTx, NGF, CRISP, and OLP are the least immunologically recognized low molecular mass toxins of cobra venom (Figures 8D-F). These proteins which play a significant role in cobra venom-induced toxicity and lethality; therefore, the poor recognition of these components by commercial PAVs should be of utmost concern for the development of effective antivenom therapy against cobra bite. In addition, the percentage of PAV unbound proteins in NkV is higher than that of NnV, indicating a lower immunological recognition of the former venom by PAV (Figures 8B, C). This finding reinforces the idea that antivenom against NkV should be added to commercial PAV to improve the treatment *against N. kaouthia* bite.

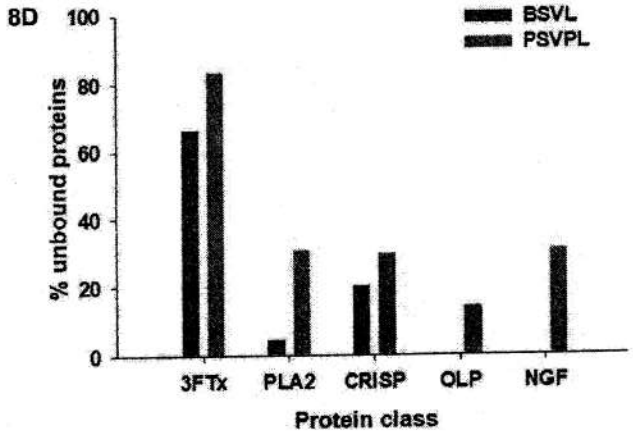

Figure 8D: Percentage of unbound proteins of WINnV eluted from immunoaffinity columns coupled with PAV (BSVL and PSVPL).

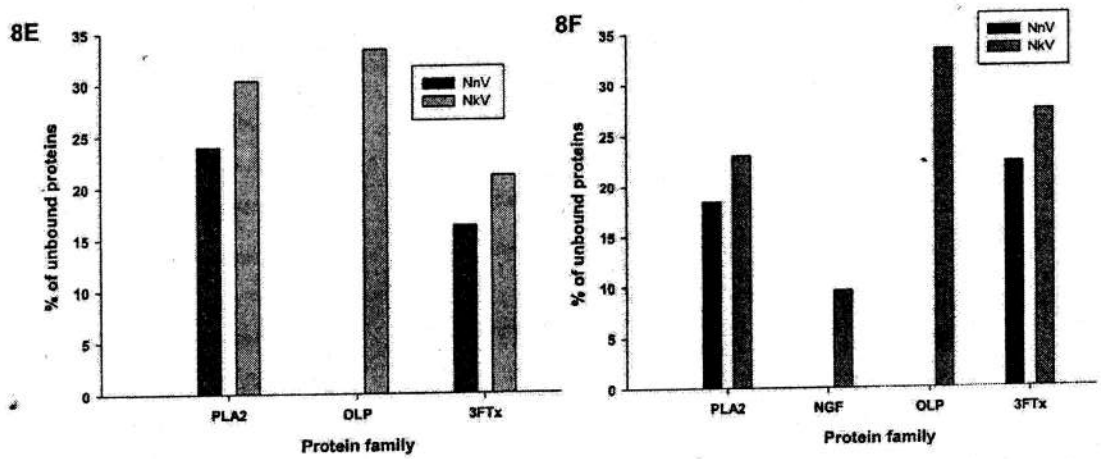

Figure 8: Percentage of unbound proteins of EINnV and EINkV eluted from immunoaffinity columns coupled with **E.** BSVL PAV and **F.** PSVPL PAV.

B1.6. Correlation of NnV and NkV proteome compositions with lethality, pathophysiology, and clinical manifestations of these venoms

Several of the clinical manifestations exhibited by cobra bite patients are corroborated well with the proteome composition of WI and EINnV, and EINkV determined in this study. For example, the major pathological symptoms exhibited by cobra envenomed victims are blurring of vision, loss of consciousness, early neuroparalysis, and rapid onset of respiratory failure. These symptoms may be attributed to the occurrence of a high proportion of 3FTxs, more specifically the neurotoxins in these venoms. Further, other toxins like OLP, with an abundance of 3FTxs could also account for the neuroparalysis seen in cobra-envenomed victims. Other symptoms of cobra bite, such as severe pain at the bite site, rapid progression of swelling, local tissue damage due to the large, non-healing ulcer formation may be attributed to the presence of OLP, CVFs, PLA_2s, and metalloproteases in the venoms under study. PDEs and NTs, identified in the three venom sources, are known to be involved in the release of cellular nucleic acids that can cause smooth muscle relaxation, vasodilation, and other cardiovascular effects of cobra envenomation.

In addition, the higher lethality of NnV compared to NkV may be explained by the occurrence of slightly higher cumulative proportions of neurotoxins and cytotoxins, the two subclasses of 3FTx in EINnV (42.8%) compared to EINkV (35.3%). Similarly, the occurrence of slightly higher quantity of PLA_2 and SVMP in NnV (26.27%) compared to NkV (22.01%) explains the higher edema-inducing activity and myotoxicity inflicted by NnV in mice. Further, the interaction of PLA_2 with low molecular mass neurotoxins from cobra venom shows a marked synergistic ability to potentiate their cytotoxicity and haemolytic activities. Therefore, the occurrence of slightly higher quantities of PLA_2 and neurotoxins in NnV (41.8%), compared to NkV (36.9%), may shed light on higher lethality and haemolytic activity of NnV compared to NkV.

B2. Summary and conclusions of the progress made so far:

The venom proteomes of Indian Russell's Viper (*Daboia russelii*) from western, eastern and southern India, Indian Spectacled Cobra (*Naja naja*) from eastern and western India, and Indian Monocled Cobra (*Naja kaouthia*) from eastern India have been analyzed by proteomic analysis and the proteome composition of these venom samples was well correlated with the pathophysiological symptoms in envenomed patients in that particular region. Proteomic analyses suggested that PLA_2, proteases (SVMP and SVSP), KSPI, and snaclec are the abundant protein families in RVV, while 3FTx and PLA_2 are the predominant protein classes in NnV and NkV. Second generation antivenomics studies unequivocally indicated the poor recognition of low molecular mass (<20 kDa) proteins of RVV, NnV, and NkV. Therefore, these findings suggest inclusion of antibodies raised specifically against low molecular mass components in commercial PAV for better hospital management of snake bite patients.

B3. Details of new leads obtained, if any:

(A) This is the first report on proteomic analyses of RVV and NnV from different geographical locations of India.

(B) The proteomic analyses have provided a sound correlation between WI, EI, and SI RVV / WI and EI NnV / EI NkV composition and pathophysiological manifestations post RV or cobra bite envenomation in different parts of the country.

(C) A major breakthrough was identification of WI and SI RVV components responsible for showing unique neurotoxic symptom in RV bite patients in western India.

(D) By LC-MS/MS analysis, significant variation in venom composition of RVV and NnV from different geographical locale of India was observed. These differences will have great impact in designing region specific antivenom for efficient treatment of snakebite patients.

(E) Identification of poorly recognized RVV and cobra venom proteins by commercial antivenom. This finding will play an importance role in improving the quality of antivenom for better treatment of snakebite patients.

B4. Future plan of work:

1. Glycoproteomic analysis of Indian Russell's Viper and Cobra venoms from different geographical locations of India.
2. Designing of strategies for development of region-specific antivenoms.
3. Exploration of candidate drug molecules from the catalogue of proteins generated by proteomic analysis.

B5. Details of Publications & Patents, if any:

B5.1. Publications in peer-reviewed international journal:

1. Chanda, A., Kalita, B., Patra, A., Senevirathne, W.D.S.T., & **Mukherjee, A.K.** (2018). Proteomic analysis and antivenomics study of Western India *Naja naja* venom: Correlation between venom composition and clinical manifestations of cobra bite in this region. ***Expert Review of Proteomics***, (Just accepted manuscript). DOI: 10.1080/14789450.2019.1559735.
2. Chanda, A., Patra, A., Kalita, B., & **Mukherjee, A. K.** (2018). Proteomics analysis to compare the venom composition between *Naja naja* and *Naja kaouthia* from the same geographical location of eastern India: Correlation with pathophysiology of envenomation and immunological cross-reactivity towards commercial polyantivenom. ***Expert Review of Proteomics***, 15 (11), 949-961.
3. Kalita, B., Mackessy, S.P., & **Mukherjee, A. K.** (2018). Proteomic analysis reveals geographic variation in venom composition of Russell's Viper in the Indian subcontinent: Implications for clinical manifestations post-envenomation and antivenom treatment. ***Expert Review of Proteomics***, 15 (10), 837-849.

4. Kalita, B., Patra, A., Das, A., & **Mukherjee, A. K.** (2018). Proteomic analysis and immuno-profiling of eastern India Russell's viper (*Daboia russelii*) venom: Correlation between RVV composition and clinical manifestations post RV bite. ***Journal of Proteome Research***, 17 (8), 2819-2833.
5. Kalita, B., Singh, S., Patra, A., & **Mukherjee, A. K.** (2018). Quantitative proteomic analysis and antivenom study revealing that neurotoxic phospholipase A_2 enzymes, the major toxin class of Russell's viper venom from southern India, shows the least immuno-recognition and neutralization by commercial polyvalent antivenom. ***International Journal of Biological Macromolecules,*** 118 (A), 375-385.
6. Kalita, B., Patra, A., Jahan, S., & **Mukherjee, A. K.** (2018). First report of the characterization of a snake venom apyrase (Ruviapyrase) from Indian Russell's viper (*Daboia russelii*) venom. ***International Journal of Biological Macromolecules***, 111, 639-648.
7. Patra, A., Kalita, B., & **Mukherjee, A. K.** (2018). Assessment of quality, safety, and pre-clinical toxicity of an equine polyvalent anti-snake venom (Pan Africa): Determination of immunological cross-reactivity of antivenom against venom samples of Elapidae and Viperidae snakes of Africa. ***Toxicon***, 153, 120-127.
8. Patra, A., Kalita, B., Chanda, A., & **Mukherjee, A. K.** (2017). Proteomics and antivenomics of *Echis carinatus carinatus* venom: Correlation with pharmacological properties and pathophysiology of envenomation. ***Scientific Reports***, *7*(1), 17119.
9. Dutta, S., Chanda, A., Kalita, B., Islam, T., Patra, A., & **Mukherjee**, A. K. (2017). Proteomic analysis to unravel the complex venom proteome of eastern India *Naja naja*: Correlation of venom composition with its biochemical and pharmacological properties. ***Journal of Proteomics***, *156*, 29-39.
10. Kalita, B., Patra, A., & **Mukherjee, A. K.** (2017). Unraveling the proteome composition and immuno-profiling of western India Russell's viper venom for in-depth understanding of its pharmacological properties, clinical manifestations, and effective antivenom treatment. ***Journal of Proteome Research***, *16*(2), 583-598.
11. **Mukherjee, A.K.,** Kalita, B., Mackessy, S. P. (2016). A proteomics analysis of Pakistan *Daboia russelii russelii* venom and assessment of potency of Indian polyvalent and monovalent antivenom. ***Journal of Proteomics,*** 144, 73-86.
12. **Mukherjee, A.K.,** Dutta, S., Kalita, B., Jha, D.K., Deb, P., Mackessy, S. P. (2016) Structural and functional characterization of complex formation between two Kunitz-type serine protease inhibitors from Russell's Viper venom. ***Biochimie,*** 128-12, 138-147.

B5.2. Conference presentation:

i. **Mukherjee, A. K.,** and Dutta, S., (2019) Protein and peptide-based Antithrombotic Cardiovascular Drug Development from Snake Venoms: Prospects and Challenges at the 106th Indian Science Congress, New Biology section, Lovely Professional University, Phagwara, Punjab, 3-7 January, 2019.

ii. **Mukherjee, A. K.,** (2018) Assessment of Efficacy and Quality Control of Commercial Antivenom for Efficient Hospital Management of Snakebite at the International Toxicology Conference, Bach Mai Hospital, Hanoi, Vietnam, 5th November, 2018.

iii. **Mukherjee, A. K.,** and Kalita, B., (2018) Proteomic analyses show geographical variation in venom composition of Indian Russell's Viper (*Daboia russelii*): Requirement of well-designed antivenom production at the 10th Annual Meeting of the Proteomic Society of India and International Conference on Proteomics for Cell Biology and Molecular Medicine, NCCS, Pune, India, 12 - 14 December, 2018.

iv. **Mukherjee, A. K.** (2018) Venomics and antivenomics of Indian cobra *Naja naja* venom: Failure to immuno-recognition of low molecular mass venom proteins by commercial polyvalent antivenom at Trends in Biochemical and Biomedical Research: Advances and Challenges, Department of Biochemistry, Banaras Hindu University, Varanasi, February 13-15, 2018.

v. **Mukherjee, A. K.** (2018) Green medicine for snakebite: From therapeutic applications to conservation strategies at the National Conference on Drug Discovery from Natural Products and their Traditional Uses, University of Science and Technology, Meghalaya, March 23-24, 2018.

vi. **Mukherjee, A. K.** (2018) Assessment of Quality of Antivenom for Efficient Hospital Management of Snakebite at the National Conference on Recent Advances on Applied Biological Sciences, Department of Biotechnology and Bioinformatics, North Eastern Hill University, Shillong, 4-5 May, 2018.

vii. **Mukherjee, A. K.,** Patra, A., Kalita, B., Chanda, A. (2017) Studies on venom proteome composition of Indian Saw-scaled Viper (*Echis carinatus carinatus*) and cross-reactivity of venom proteins with commercial polyvalent antivenom at the SNAKSYMP 2017: Conference on Recent Advances in Research on Snake Venom and Snakebite Therapy: National and International Perspectives, Centre for Cellular and Molecular Biology, India, 30 November - 3 December, 2017.

viii. Dutta, S., Chanda, A., Kalita, B., Islam, T., Patra, A., **Mukherjee, A. K.** (2017) Proteomic characterization of India spectacled cobra *Naja naja* venom: Correlation with biological activity and assessment of immuno-recognition of venom proteins by commercial polyvalent antivenom at the 3rd International Conference on Translational Research: Application in Human Health and Agriculture, Amity University, India, 23 - 25 September, 2017.

ix. Kalita, B., Patra, A., Das, A., **Mukherjee, A. K.** (2017) Assessment of Immunological cross-reactivity and neutralization potency of Indian antivenom against biochemical and pharmacological properties of Indian Russell's viper venom at the 3rd International Conference on Translational Research: Application in Human Health and Agriculture, Amity University, India, 23 - 25 September, 2017.

x. Kalita, B., Patra, A., **Mukherjee, A.K.** (2017) Geographical variation in venom proteome composition of Indian Russell's Viper (*Daboia russelii*) is associated with differences in clinical manifestations of RV bite in Indian subcontinent at the 3rd International Conference on Translational

Research: Application in Human Health and Agriculture, Amity University, India, 23 - 25 September, 2017.

xi. **Mukherjee, A.K.**, Dutta, S., Kalita, B., Jha, D.K., Deb, P., Mackessy, S.P. (2016) Characterization of Rusvikunin complex isolated from *Daboia russelii russelii* venom to comprehend its potent biological activity at the National seminar on Snake Venom Research and Snake-bite Therapy: National and International Perspective, Tezpur University, India, 22 - 24 November, 2016.

xii. Kalita, B., Patra, A., **Mukherjee, A. K.** (2016) Exploring the venom proteome of *Daboia russelii* from western India: Correlation between RVV composition and its clinical manifestations at the National seminar on Snake Venom Research and Snake-bite Therapy: National and International Perspective, Tezpur University, India, 22 - 24 November, 2016.

xiii. Dutta, S., Chanda, A., Kalita, B., Islam, T., Patra, A., **Mukherjee, A.K.** (2016) Proteomic, biochemical and pharmacological characterization of eastern India *Naja naja* venom and correlation of these properties with clinical manifestation of cobra bite at the National seminar on Snake Venom Research and Snake-bite Therapy: National and International Perspective, Tezpur University, India, 22 - 24 November, 2016.

xiv. Chanda, A., Islam, T., Patra, A., Kalita, B., Dutta, S., **Mukherjee A.K.** (2016) Assessment of efficacy of three Indian polyvalent antivenoms against Eastern and Western India *Naja naja* venom at the National seminar on Snake Venom Research and Snake-bite Therapy: National and International Perspective, Tezpur University, India, 22 - 24 November, 2016.

xv. Patra, A., Kalita, B., **Mukherjee, A.K.** (2016) Studies on neutralization of biochemical and pharmacological properties and antivenomics of Indian polyvalent and monovalent antivenom against Western India Russell's viper (*Daboia russelii*) venom at the National seminar on Snake Venom Research and Snake-bite Therapy: National and International Perspective, Tezpur University, India, 22 - 24 November, 2016.

B5.3. Awards and achievements of the PI:

1. Received the "Sreenivasaya Memorial Award-2014" for his outstanding contribution in Biochemistry and allied sciences by the Society of Biological Chemists (India), held at KIIT University, Bhubaneswar, December, 2014
2. Awarded Doctor of Science (D. Sc.) in Biotechnology for snake venom work by Calcutta University (2018)
3. Received "Visitor's Award for Research in Basic and Applied Sceinces-2018" for his research on snake venom from honorable President of India at Rashtrapati Bhawan on 2 May, 2018
4. Awarded "Tata Innovation Fellowship (2017-18)" by Department of Biotechnology, Ministry of Science and Technology, Govt. of India (2018)
5. Elected as Fellow of the Royal Society of Biology (FRSB), United Kingdom (2018)

6. Received "Indian Council of Medical Research Prize for Biomedical Research Conducted in Underdeveloped Areas" for his work on snake venom (Prize for the year 2017)
7. Received "Prof. Sohail Ahmad Award-2018" from Indian Academy of Biomedical Sciences, Lucknow for outstanding research contribution in the field of Biomedical Sciences and snake venom (basic research)

Section-C: Details of Grant Utilization

C1. Equipment Acquired or Placed Order with Actual Cost:

N/A

(Signature of Principal Investigator)

Date: 18/11/2019

C2. Manpower Staffing and Expenditure Details: UC/SE under preparation.

(Signature of Principal Investigator)
Date:

C3. Details of Recurring Expenditure (April 2017 - March 2018): UC/SE under preparation.

C4. Financial Requirements for the Next Year with Justifications: NA

(Signature of Principal Investigator)
Date:

FORMAT FOR ASSESSMENT OF COMPLETION REPORTS
(This brief report should not exceed 2 pages including pictures)

1. **Title of the project:** Venomics and antivenomics of Indian Cobra and Russell's Viper; implications in drug discovery and quality control of antivenoms

2. **DBT Sanction Order No., date, duration, total budget of the project:** BT/412/NE/U-Excel/2013, dated December 11, 2014; the project was initially sanctioned for 3 years, 1 year no-cost extension is provided till 10 December, 2018; sanctioned budget - Rs. 132.20 lakh.

3. **Name & contact details (tel., mob. & email) of the Principal Investigator:** Prof. A.K. Mukherjee, Department of Molecular Biology and Biotechnology, Tezpur University, Napaam, Sonitpur, Assam - 784028, India. Telephone: +91-3712-275405, mobile: +91-7896003886, email: akm@tezu.ernet.in

4. **Aims & objectives of the project/study (maximum 150 words):**

The major objective of the project was to study the venom composition of Russell's viper (*Daboia russelii*) (RV) and Spectacled Cobra (*Naja naja*) (Nn) / monocle cobra *N. kaouthia* (Nk) from different regions of India using proteomic analysis. Further, we aimed to correlate the proteome composition of these snake species with the clinical manifestation of RV and Nn/Nk envenomation. Another aim of the study was to evaluate the extent of immunological cross-reactivity between RV and Nn venoms of different geographical locations of India with commercial polyvalent antivenom (PAV) and subsequently, to identify the poorly immunogenic components of RVV and NnV using mass spectrometry analysis.

5. **Outcome and salient achievements (maximum 250 words) under the project:**

5.1 Outcome of the project: The venom proteome composition of RV, Nn, Nk and Saw-scaled Viper (EC) were analyzed by tandem mass spectrometry and geographical differences in venom composition was identified. The variation in snake venom composition was well correlated with the pathophysiological symptoms in envenomed patients in different regions of the country. The neurotoxic symptom exhibited by RV bite patients in southern India was well correlated with presence of neurotoxic PLA_2 enzyme in this venom. Proteomic analyses resulted in identification of 55, 66, and 69 to 73 proteins belonging to 13 to 15 protein families in RVV samples from western (WI), southern (SI), and eastern (EI) India, respectively. Enzymatic proteins predominated in RVV and ECV whereas reverse is true for Nn and Nk venoms. By LC-MS/MS analysis, 43 and 54 proteins belonging to 15 to 16 protein families were identified in EI and WI NnV, respectively. Further, proteomic analysis identified 90 proteins distributed over 15 protein families in ECV.

Immunological cross-reactivity and antivenomics studies against commercial PAV unequivocally indicated the poor recognition of low molecular mass (<20 kDa) proteins of RVV, NnV, NkV and ECV. Further, the enzymatic activities and pharmacological properties exhibited by these proteins were also poorly neutralized by PAV. Therefore, these findings suggest inclusion of antibodies raised specifically against these low molecular mass components in commercial PAV for better hospital management of snake bite patients.

5.2. Achievements of the PI:

a. Received the "Sreenivasaya Memorial Award-2014" for his outstanding contribution in Biochemistry and allied sciences by the Society of Biological Chemists (India), held at KIIT University, Bhubaneswar, December, 2014.

b. Awarded Doctor of Science (D. Sc.) in Biotechnology for snake venom work by Calcutta University (2018).

c. Received "Visitor's Award for Research in Basic and Applied Sceinces-2018" for his research on snake venom from honorable President of India at Rashtrapati Bhawan on 2 May, 2018.

d. Awarded "Tata Innovation Fellowship (2017-18)" by Department of Biotechnology, Ministry of Science and Technology, Govt. of India (2018).

e. Elected as Fellow of the Royal Society of Biology (FRSB), United Kingdom (2018).

f. Received "Indian Council of Medical Research Prize for Biomedical Research Conducted in Underdeveloped Areas" for his work on snake venom (Prize for the year 2017).

g. Received "Prof. Sohail Ahmad Award-2018" from Indian Academy of Biomedical Sciences, Lucknow for outstanding research contribution in the field of Biomedical Sciences and snake venom (basic research).

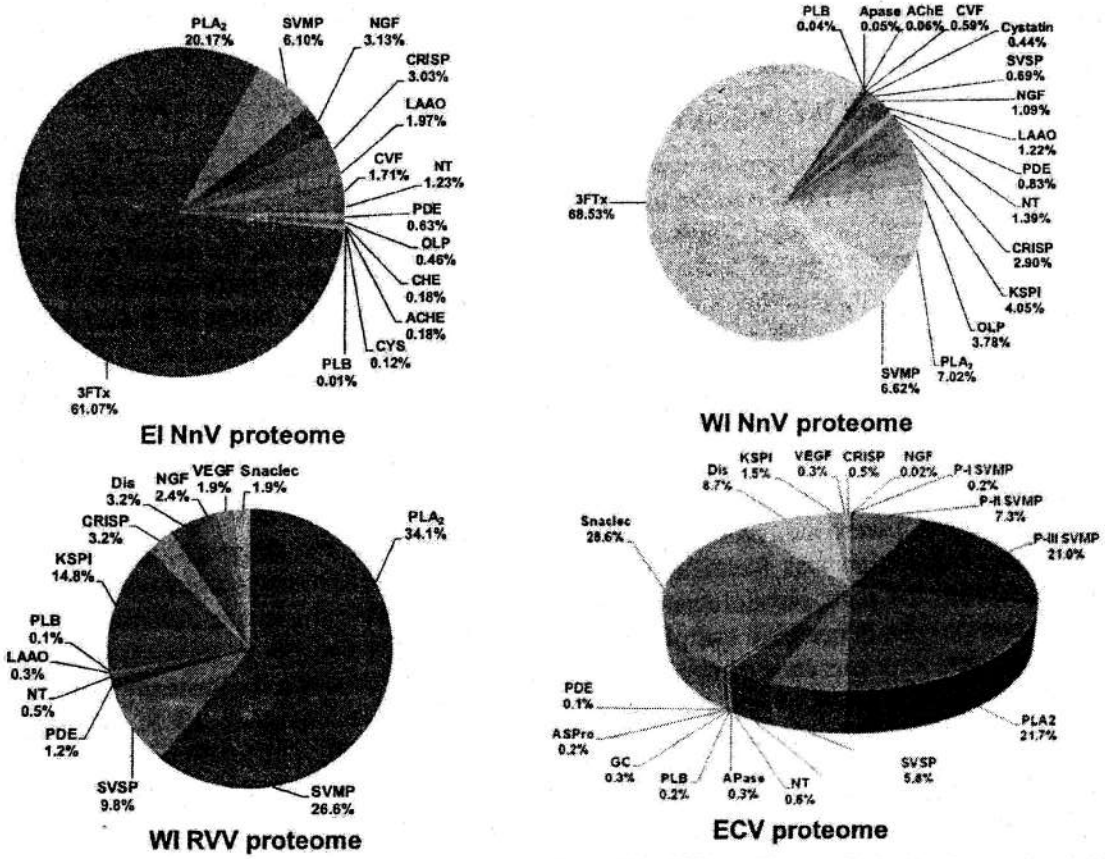

Fig. 1: Proteome compositions of EI NnV, WI NnV, WI RVV, and ECV determined by proteomic analysis.

6. Whether the outcome has lead to early translation research?

The findings of project provides the basis for the following early translation research -

- Development of region and species-specific antivenom.
- Design of novel strategies for development of antivenom that will be effective against the poorly immunogenic low molecular weight venom toxins.
- Detection of species-specific snake envenomation.

7. Research publications emanated from the project (a list of publications may be provided along with the impact factor and citation index of the journal).

a. Chanda, A., Kalita, B., Patra, A., Senevirathne, W.D.S.T., & **Mukherjee, A.K.** (2018). Proteomic analysis and antivenomics study of Western India *Naja naja* venom: Correlation between venom composition and clinical manifestations of cobra bite in this region. ***Expert***

Review of Proteomics **(IF: 3.489),** (Just accepted manuscript). DOI: 10.1080/14789450.2019.1559735.

b. Chanda, A., Patra, A., Kalita, B., & **Mukherjee, A. K.** (2018). Proteomics analysis to compare the venom composition between *Naja naja* and *Naja kaouthia* from the same geographical location of eastern India: Correlation with pathophysiology of envenomation and immunological cross-reactivity towards commercial polyantivenom. ***Expert Review of Proteomics*** **(IF: 3.489)**, 15 (11), 949-961.

c. Kalita, B., Mackessy, S.P., & **Mukherjee, A. K.** (2018). Proteomic analysis reveals geographic variation in venom composition of Russell 's viper in the Indian subcontinent: Implications for clinical manifestations post-envenomation and antivenom treatment. ***Expert Review of Proteomics*** **(IF: 3.489)**, 15 (10), 837-849.

d. Kalita, B., Patra, A., Das, A., & **Mukherjee, A. K.** (2018). Proteomic analysis and immuno-profiling of eastern India Russell's viper (*Daboia russelii*) venom: Correlation between RVV composition and clinical manifestations post RV bite. ***Journal of Proteome Research*** **(IF: 3.950)**, 17 (8), 2819-2833.

e. Kalita, B., Singh, S., Patra, A., & **Mukherjee, A. K.** (2018). Quantitative proteomic analysis and antivenom study revealing that neurotoxic phospholipase A_2 enzymes, the major toxin class of Russell's viper venom from southern India, shows the least immuno-recognition and neutralization by commercial polyvalent antivenom. ***International Journal of Biological Macromolecules*** **(IF: 3.909),** 118 (A), 375-385.

f. Kalita, B., Patra, A., Jahan, S., & **Mukherjee, A. K.** (2018). First report of the characterization of a snake venom apyrase (Ruviapyrase) from Indian Russell's viper (*Daboia russelii*) venom. ***International Journal of Biological Macromolecules*** **(IF: 3.909)**, 111, 639-648.

g. Patra, A., Kalita, B., Chanda, A., & **Mukherjee, A. K.** (2017). Proteomics and antivenomics of *Echis carinatus carinatus* venom: Correlation with pharmacological properties and pathophysiology of envenomation. ***Scientific Reports*** **(IF: 4.122)**, *7*(1), 17119.

h. Dutta, S., Chanda, A., Kalita, B., Islam, T., Patra, A., & **Mukherjee**, A. K. (2017). Proteomic analysis to unravel the complex venom proteome of eastern India *Naja naja*: Correlation of venom composition with its biochemical and pharmacological properties. ***Journal of Proteomics*** **(IF: 3.722)**, *156*, 29-39.

i. Kalita, B., Patra, A., & **Mukherjee, A. K.** (2017). Unraveling the proteome composition and immuno-profiling of western India Russell's viper venom for in-depth understanding of its pharmacological properties, clinical manifestations, and effective antivenom treatment. ***Journal of Proteome Research*** **(IF: 3.950)**, *16*(2), 583-598.

j. **Mukherjee, A.K.,** Kalita, B., Mackessy, S. P. (2016). A proteomics analysis of Pakistan *Daboia russelii russelii* venom and assessment of potency of Indian polyvalent and monovalent antivenom. ***Journal of Proteomics*** **(IF: 3.722).** 144, 73-86.

k. **Mukherjee, A.K.,** Dutta, S., Kalita, B., Jha, D.K., Deb, P., Mackessy, S. P. (2016) Structural and functional characterization of complex formation between two Kunitz-type serine protease inhibitors from Russell's viper venom. ***Biochimie*** **(IF: 3.188),** 128-12, 138-147.

8. Patents filed or granted, if any (a list may be provided).

Indian patents filed:

(i) Indian Patent on "TOXIN EPITOPE-BASED DETECTION OF SPECIES-SPECIFIC SNAKE ENVENOMATION" PATENT APPLICATION NO. 201831010002; Filling date: 19 March, 2018.

(ii) Indian Patent on "TOXINS-TARGETED SPECIFIC NOVEL ANTI SNAKE VENOM" to Kolkata Patent Office (patent number is yet to be received).

9. Benefits gained through twinning:

- Scientific & Technical expertise gained through twinning in NER: NA
- No. of NER manpower (including PI & staffs) trained in the Non-NER Institute: 02
- No. of visits by Non-NER Researchers to NER Institutes and vise-versa: NA
- Training in any new techniques, if any: LC-MS/MS analysis and antivenomics

10. Number of Ph.Ds. / JRF/RA/ Students trained/ benefited from the project indicating the genders of the manpower.

JRF/SRF (3-M); Technical person (----); RA/PDF (----); M. Sc. Students (02-F)

11. Whether the project has helped in creating a Technology Platform?

A comprehensive knowledge on venom composition and poorly immunogenic sub-proteomes of RV, Nn, Nk, and EC determined ' y proteomic analysis in the present study is of prime relevance for understanding the pathophysiology of snake envenomation and development of effective antivenom, the only adequate choice for treating snakebite. The poorly immunogenic venom components can be specifically targeted for antibody production, and supplementation with these antibodies can significantly enhance the efficacy of existing PAVs. Further, the proteome analyses have also provided deeper insights into the variation of venom composition that leads to differences in antivenom efficacy across the Indian sub-continent. Therefore, this information can be employed for development of region-specific antivenom that will ensure better hospital management of snakebite bite patients. Also the outcome of the project will be helpful in designing species-specific snake venom detection kit.

12. Technology transferred to any industrial partner, if any. No

13. Future potential and prospects of the outcome in the present project (maximum 150 words).

a. Glycoproteomic analysis of Indian Russell's Viper and Cobra venoms from different geographical locations of India and to understand their role in pathophysiology of envenomation.
b. Designing of strategies for development of region-specific antivenoms (initiated under BIRAC-PACE project in collaboration with a commercial company).
c. Exploration of candidate drug molecules from the catalogue of proteins generated by proteomic analysis.
d. Designing of species-specific diagnostic reagent for detection of snake envenomation.

Date: 17/01/2019

(A.K. Mukherjee)

Prof. A.K. Mukherjee, Ph.D., D.Sc.
Department of Molecular
Biology & Biotechnology
Tezpur University (Central)
Tezpur- 784028, Assam

Progress Report for R&D Projects [Year 2018-2019]

Section-A: Project Details

A1. **Project Title:** Venomics and antivenomics of Indian Cobra and Russell's viper: implications in drug discovery and quality control of antivenoms.

A2. **DBT Sanction Order No. & Date:** Sanction order no. BT/412/NE/U-Excel/2013, Dated- 11-12-2014

A3. **Name of Principal Investigator:** Prof. A. K. Mukherjee, Tezpur University
Name of Co-PI/Co-Investigator: NA.

A4. **Institute:** Tezpur University

A5. **Address with Contact Nos. (Landline & Mobile) & Email:** Department of Molecular Biology & Biotechnology, Tezpur University, School of Science, Tezpur-784028, Contact No- 03712-275405 (O), 7896003886 (M), Email-akm@tezu.ernet.in

A6. **Total Cost:** Rs. 132.20 Lakhs (Rupees one crore thirty two lakhs and twenty thousand only)

A7. **Duration:** Three Years (2014-2017)

Section-B: Scientific and Technical Progress: Shown as Annex-I

B1. **Progress made against the Approved Objectives, Targets & Timelines during the Reporting Period** (1000-1500 words for interim reports; 2500-3500 words for final report; data must be included in the form of up to 3 figures and/or tables for interim reports; up to 7 figures and/or tables for final reports): Submitted

B2. **Summary and Conclusions of the Progress made so far** (minimum 100 words, maximum 200 words): Submitted

B3. **Connectivity of the partnering institutes (Institute wise achievements to be given separately for each objective):** NA

B4. **Details of New Leads Obtained, if any:** Submitted with progress report

B5. **Details of Publications & Patents, if any:** Submitted with progress report

B6. **The details of visits of the Collaborating institutes PI and personnel's** (Purpose and duration of visits): NA

Section-C: Details of Grant Utilization#

C1. **Equipment Acquired or Placed Order with Actual Cost:**

C2. **Manpower Staffing and Expenditure Details:**

C3. **Details of Recurring Expenditure:**

C4. **Financial Requirements for the Next Year (3rd year) with Justifications:**

[Signature of the Principal Investigator]

Prof. A.K. Mukherjee, Ph.D., D.Sc.
Department of Molecular Biology & Biotechnology
Tezpur University (Central)
Tezpur- 784028, Assam

Instructions:

(i) *All the information needs to be provided; otherwise the Progress Report will be treated as incomplete. In case of 'Nil' / 'Not Applicable' information, the same may be indicated.*

(ii) *In case of multicentre project, a combined Progress Report should be submitted incorporating the progress of all components. The Project Co-coordinator/ PI will be responsible for this.*

(iii) **Please indicate the reporting period [i.e. Year 1/2/3/4/5].*

(iv) *Submission of Progress Report by the end of the 11th month of grant sanction is linked with further continuation of the project and timely release of funds for the next year.*

Appendix-B[i]

Utilization Certificate

(For the financial year 1st April, 2017 - 31st March, 2018)

1	Title of the Project/Scheme:	Venomics and antivenomics of Indian Cobra and Russell's viper: implications in drug discovery and quality control of antivenoms.
2	Name of the Organization:	Department of Molecular Biology & Biotechnology, Tezpur University.
3	Principal Investigator:	Prof. Ashis K. Mukherjee
4	Dept. of Biotechnology sanction order No. & date of sanctioning the project:	Sanction order no. BT/412/NE/U-Excel/2013, Dated-11-12-2014
5	Amount brought forward from the previous financial year quoting DBT letter No. & date in which the authority to carry forward the said amount was given:	Rs. 2,190.00 (BC|L/NER-BPMC/2017/618, dated August 9, 2017)
6	Amount received from DBT during the financial year (2017-2018):	Rs. 28,06,000.00
7	Other receipts/interest earned, if any, on the DBT grants:	Rs. 35,605.00
8	Total amount that was available for Expenditure during the financial year (5+6+7):	Rs. 28,43,795.00
9	Actual expenditure (excluding commitments) incurred during the financial year 1st April 2017 to 31st Mar 2018 (statement of expenditure is enclosed):	Rs. 16,09,131.00
10	Unspent balance refunded, if any *(Please give details of cheque No. etc.):*	NA
11	Balance amount available at the end of the financial year 2016-2017 (8-9):	Rs. 12,34,664.00

12 Amount allowed to be carried forward to the next financial year vide letter No. & date: Rs. 12,34,664.00

1. Certified that the amount of Rs. 16,09,131.00 (Rupees sixteen lakhs nine thousand one hundred and thirty one only) mentioned against col. 9 has been utilized on the project/scheme for the purpose for which it was sanctioned and that the balance of Rs. 12,34,664.00 (Rupees twelve lakhs thirty four thousand six hundred sixty four only) remaining unutilized at the end of the year has been surrendered to Govt. (vide No. ___ ___ dated ___)/will be adjusted towards the grants-in-aid payable during the next year 2018-2019.
2. Certified that I have satisfied myself that the conditions on which the grants-in-aid was sanctioned have been duly fulfilled/are being fulfilled and that I have exercised the following checks to see that the money was actually utilized for the purpose for which it was sanctioned.

Kinds of checks exercised:

1. Orders for chemicals were placed after T&PC approval of price.
2. Stock entry of chemical, consumable & contingency items etc.
3. Appointment of JRF following the DBT guidelines after floating the advertisement.
4. Tender for equipment were floated
5.

Prof. A.K. Mukherjee, Ph.D, D.Sc.
Department of Molecular Biology & Biotechnology
Tezpur University (Central)
Tezpur- 784028, Assam

(Signature of Principal Investigator)

(Signature of Finance officer)
Finance Officer
Tezpur University

(Signature of Head of the Institute)
Registrar
Tezpur University

(To be countersigned by the DBT Officer-in-charge)

Appendix-B[ii]

Utilization Certificate

(For the financial year 1st April, 2018 - 10th December, 2018)

1	Title of the Project/Scheme:	Venomics and antivenomics of Indian Cobra and Russell's viper: implications in drug discovery and quality control of antivenoms.
2	Name of the Organization:	Department of Molecular Biology & Biotechnology, Tezpur University.
3	Principal Investigator:	Prof. Ashis K. Mukherjee
4	Dept. of Biotechnology sanction order No. & date of sanctioning the project:	Sanction order no. BT/412/NE/U-Excel/2013, Dated-11-12-2014
5	Amount brought forward from the previous financial year quoting DBT letter No. & date in which the authority to carry forward the said amount was given:	Rs. 12,34,664.00 (BC|L/NER-BPMC/2017/618, dated August 9, 2017)
6	Amount received from DBT during the financial year (2017-2018):	Rs. 0.00
7	Other receipts/interest earned, if any, on the DBT grants:	Rs. 1,339.00
8	Total amount that was available for Expenditure during the financial year (5+6+7):	Rs. 12,36,003.00
9	Actual expenditure (excluding commitments) incurred during the financial year 1st April 2018 to 10th Dec 2018 (statement of expenditure is enclosed):	Rs. 11,78,285.00
10	Unspent balance refunded, if any *(Please give details of cheque No. etc.):*	NA

11	Balance amount available at the end of the financial year 2016-2017 (8-9):	Rs. 56,379.00
12	Amount allowed to be carried forward to the next financial year vide letter No. & date:	Not applicable

1. Certified that the amount of Rs. 11,78,285.00 (Rupees eleven lakhs seventy eight thousand two hundred and eighty five only) mentioned against col. 9 has been utilized on the project/scheme for the purpose for which it was sanctioned and that the balance of Rs. 56,379.00 (Rupees fifty six thousand three hundred seventy nine only) remaining unutilized at the end of the year has been surrendered to Govt. (vide No. 534326 dated 21-02-2020)/will be adjusted towards the grants-in-aid payable during the next year 2018-2019.
2. Certified that I have satisfied myself that the conditions on which the grants-in-aid was sanctioned have been duly fulfilled/are being fulfilled and that I have exercised the following checks to see that the money was actually utilized for the purpose for which it was sanctioned.

Kinds of checks exercised:

1. Orders for chemicals were placed after T&PC approval of price.
2. Stock entry of chemical, consumable & contingency items etc.
3. Appointment of JRF following the DBT guidelines after floating the advertisement.
4. Tender for equipment were floated
5.

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Department of Molecular Biology & Biotechnology
Tezpur University (Central)
Tezpur- 784028, Assam

(Signature of Principal Investigator)

(Signature of Finance officer)
Finance Officer
Tezpur University

(Signature of Head of the Institute)
Registrar
Tezpur University

(To be countersigned by the DBT Officer-in-charge)

Appendix-C[i]

Statement of Expenditure referred to in para 9 of the Utilisation Certificate

Showing grants received the Department of Biotechnology and the expenditure incurred during the period of 1st April, 2017 - 31st March 2018

(Amount in Rupees)

Sl no.	Head	Unspent balance (2016-2017) (Rs.)	Grant received (2017-2018) (Rs.)	Interest earned (1st April 2017 - 31st March 2018) (Rs.)	Total (Rs.) [2+3+4]	Expenditure 2017-2018 (Rs.)	Balance (Rs.) [5-7]	Remarks
	1	2	3	4	5	7	8	
	A. Non-recurring							
1	**Equipment**		Nil		Nil	Nil	Nil	
	B. Recurring							
2	**Consumable**	24,654.00	9,75,000.00		9,99,654.00	4,26,127.00	5,73,527.00	
3	**Contingency**	-196.00	1,00,000.00	35,605.00	99,804.00	59,085.00	40,719.00	
4	**Sample analysis**	-74,611.00	10,00,000.00		9,25,389.00	5,01,966.00	4,23,423.00	
5	**Travel**	-24,997.00	1,00,000.00		75,003.00	53,043.00	21,960.00	
6	**Manpower**	76,001.00	5,32,000.00		6,08,001.00	6,08,001.00	0.00	
7	**Overhead**	0.00	99,000.00		1,00339.00*	62,500.00	37,839.00	
Total		**851.00**	**28,06,000.00**		**28,08,190.00**	**17,10,722.00**	**10,97,468.00**	
Interest earned		**1,339.00**	-	**35,605.00**	**35,605.00**	-	**35,605.00**	
Net total		**2,190.00**	**28,06,000.00**	**35,605.00**	**28,43,795.00**	**17,10,722.00**	**11,33,073.00**	

*interest earned of Rs. 1,339.00 in financial year 2016-2017 was re-app to overhead in financial year 2017-2018

(Signature of Principal Investigator)

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(Signature of Head of the Institute)

Registrar
Tezpur University

(Signature of Finance officer)

Finance Officer
Tezpur University

Appendix-C[ii]

Statement of Expenditure referred to in para 9 of the Utilisation Certificate

Showing grants received the Department of Biotechnology and the expenditure incurred during the period of 1st April, 2018 - 10th December, 2018

(Amount in Rupees)

Sl no.	Head	Unspent balance (2017-2018) (Rs.)	Grant received (2018-2019) (Rs.)	Interest earned (1st April 2018 - 10th December 2018) (Rs.)	Total (Rs.) [2+3+4]	Expenditure 2018-2019 (Rs.)	Balance (Rs.) [5-7]	Remarks
	1	2	3	4	5	7	8	
	A. Non-recurring							
1	**Equipment**	-	-		-	-	-	
	B. Recurring							
2	**Consumable**	5,73,527.00	-		5,73,527.00	5,65,155.00	8,372.00	
3	**Contingency**	40,719.00	-	-	40,719.00	60,124.00	-19405	
4	**Sample analysis**	4,23,423.00	-		4,23,423.00	4,10,400.00	13,023.00	
5	**Travel**	21,960.00	-		21,960.00	3515	18445.00	
6	**Manpower**	0.00	-		0.00	0.00	0.00	
7	**Overhead**	37,839.00	-		37,839.00	37,500.00	339.00	
Total		**10,97,468.00**	-		**10,97,468.00**	**10,76,694.00**	**20,774.00**	
Interest earned		**35,605.00**	-	-	**35,605.00**	-	**35,605.00**	
Net total		**11,33,073.00**	-	-	**11,33,073.00**	**10,76,694.00**	**56,379 .00**	

(Signature of Principal Investigator)

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24.2.20

(Signature of Finance officer)

Finance Officer
Te-pur University

(Signature of Head of the Institute)

FINALCONSOLIDATED STATEMENT OF EXPENDITURE
(FOR FINAL SETTLEMENT OF ACCOUNTS)

1. Title of the Project : Venomics and antivenomics of Indian Cobra and Russell's viper: implications in drug discovery and quality control of antivenoms.

2. Sanctioned Project Cost : Rs. 132.30 Lakh (Rupees One Core thirty two lakh and thirty thousand only)

3. Revised cost, if any : Nil

4. Duration of the project : Three Years (2014-2017)

5. Sanction Order No. & Date : Sanction order no. BT/412/NE/U-Excel/2013, Dated- 11-12-2014

6. Date of commencement of Project : 11-12-2014

7. Extension, if any : 12 months (11th December, 2017 to 10th December, 2018)

8. Date of completion of project : 10th December, 2018

Details of grant, expenditure and balance

Sl. No.	Heads	Sanctioned cost	Year wise releases made					Year wise expenditure incurred					
			1st yr (Rs.)	2nd yr (Rs.)	3rd yr (Rs.)	4th yr (Rs.)	Total (Rs.)	1st yr (Rs.)	2nd yr (Rs.)	3rd yr (Rs.)	4th yr (Rs.)	Total (Rs.)	Balance (Rs.)
A. Non -recurring													
(i)	Equipments	50,00,000.00	50,00,000.00	0.00	0.00	0.00	50,00,000.00	49,92,452.00	0.00	0.00	0.00	49,92,452.00	7,548.00
B. Recurring													
(i)	Human resource	13,20,000.00	4,22,000.00	5,60,000.00	5,32,000.00	0.00	15,14,000.00	2,92,999.00	6,13,000.00	6,08,001.00	0.00	15,14,000.00	0.00
(ii)	Sample analysis	30,00,000.00	10,00,000.00	9,95,000.00	10,00,000.00	0.00	29,95,000.00	9,95,028.00	10,74,583.00	5,01,966.00	4,10,400.00	29,81,977.00	13,023.00
(iii)	Consumable	30,00,000.00	10,00,000.00	10,00,000.00	9,75,000.00	0.00	29,75,000.00	9,99,990.00	9,75,356.00	4,26,127.00	5,65,155.00	29,66,628.00	8,372.00
(iv)	Travel	3,00,000.00	1,00,000.00	1,00,000.00	1,00,000.00	0.00	3,00,000.00	1,22,557.00	1,02,440.00	59,085.00	3,515.00	3,44,206.00	18445.00
(v)	Contingency	3,00,000.00	1,00,000.00	1,00,000.00	1,00,000.00	0.00	3,00,000.00	99,981.00	1,00,215.00	53,043.00	60,124.00	2,56,754.00	-19405.00
(vi)	Overheads	3,00,000.00	1,00,000.00	8,000.00	99,000.00	0.00	2,07,000.00	1,00,000.00	99,899.00	62,500.00	37,500.00	2,99,899.00	-92899
Total interest earned in project							1,21,295.00						
Totals		**1,32,20,000.00**	**77,22,000.00**	**27,63,000.00**	**28,06,000.00**	**0.00**	**1,34,13,634.00**	**76,03,007.00**	**29,65,493.00**	**17,10,722.00**	**10,76,694.00**	**1,33,55,916.00**	**56,379.00**

(PROJECT INVETIGATOR)
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(FINANCE OFFICER)
Finance Officer
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(HEAD OF THE INSTITUTE)
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