PUBLICATIONS

A. Publications in peer-reviewed international journals from thesis work

1. **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.
2. **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.
3. **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.
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.**, 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.

B. Mass spectrometry data submitted to public depository

1. The mass spectrometry proteomic data of Eastern India Russell's Viper venom samples have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with project name "Proteomic analysis of *Daboia russelli* venom samples from Burdwan and Nadia districts of West Bengal, eastern India" and accession id PXD008607.

C. Other publications in peer-reviewed international journals

1. Mukherjee, A. K., **Kalita, B.**, & Thakur, R. (2014). Two acidic, anticoagulant PLA_2 isoenzymes purified from the venom of monocled cobra *Naja kaouthia* exhibit different potency to inhibit thrombin and factor Xa via phospholipids independent, non-enzymatic mechanism. ***PloS One 9*(8)**, e101334.

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

3. 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***, 138-147.

4. 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.

5. Mukherjee, A. K., Bhagowati, P., Biswa, B. B., Chanda, A., & **Kalita, B.** (2017). A comparative intracellular proteomic profiling of *Pseudomonas aeruginosa* strain ASP-53 grown on pyrene or glucose as sole source of carbon and identification of some key enzymes of pyrene biodegradation pathway. ***Journal of Proteomics 167***, 25-35.

6. 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.

7. Gogoi, D., Arora, N., **Kalita, B.**, Sarma, R., Islam, T., Ghosh, S. S., Devi, R., & Mukherjee, A. K. (2018). Anticoagulant mechanism, pharmacological activity, and assessment of preclinical safety of a novel fibrin (ogen) olytic serine protease from leaves of *Leucas indica*. ***Scientific Reports 8*(1)**, 6210.

8. 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.

9. 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.
10. 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***, 16 (2), 171-184.

D. Publications in National and International conferences

1. **Kalita, B.**, Mukherjee, A.K. Characterization and mechanism of two anticoagulant PLA_2 isoenzymes purified from the venom of monocled cobra *Naja kaouthia*. National Seminar on Recent Advances in Biotechnological Research in North East India: Challenges and Prospects, Tezpur University, 27-29 November, 2014.

2. **Kalita, B.**, Thakur, R., Mukherjee, A.K. Characterization and mechanism of two anticoagulant PLA_2 isoenzymes purified from the venom of Indian monocled cobra *Naja kaouthia*. 83rd Annual Conference of Society of Biological Chemists of India, KIIT University, Bhubaneswar, 17-21 December, 2014.

3. **Kalita, B.**, Patra, A., Mukherjee, A.K. Exploring the venom proteome of *Daboia russelii* from western India: Correlation between RVV composition and its clinical manifestations. Recent Advances in Snakebite Research and Snakebite Therapy: National and International Perspective: SnakSymp2016, Tezpur University, 22-24 November, 2016.

4. **Kalita, B.**, Patra, A., Mukherjee. A.K., Proteomic investigation of western and eastern India *Daboia russelii* venom: Evidence of variation in clinical manifestation of RV bite owing to different geographical location. 3rd International Conference on Translational Research: Application in Human Health and Agriculture, Amity University, Kolkata, 23-25 September, 2017.

E. Awards and achievements

1. Received the best poster award in the 3rd International Conference on Translational Research: Application in Human Health and Agriculture, Amity University, Kolkata, 23-25 September, 2017.

Journal of proteome research

Article

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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

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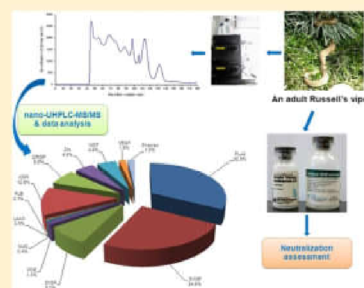
Supporting Information

ABSTRACT: The proteome composition of western India (WI) Russell's viper venom (RVV) was correlated with pharmacological properties and pathological manifestations of RV envenomation. Proteins in the 5−19 and 100−110 kDa mass ranges were the most predominate (~35.1%) and least abundant (~3.4%) components, respectively, of WI RVV. Non-reduced SDS-PAGE indicated the occurrence of multiple subunits, non-covalent oligomers, self-aggregation, and/or interactions among the RVV proteins. A total of 55 proteins belonging to 13 distinct snake venom families were unambiguously identified by ESI-LC-MS/MS analysis. Phospholipase A_2 (32.5%) and Kunitz-type serine protease inhibitors (12.5%) represented the most abundant enzymatic and non-enzymatic proteins, respectively. However, ATPase, ADPase, and hyaluronidase, detected by enzyme assays, were not identified by proteomic analysis owing to limitations in protein database deposition. Several biochemical and pharmacological properties of WI RVV were also investigated. Neurological symptoms exhibited by some RV-bite patients in WI may be correlated to the presence of neurotoxic phospholipase A_2 enzymes and Kunitz-type serine protease inhibitor complex in this venom. Monovalent antivenom was found to be better than polyvalent antivenom in immuno-recognition and neutralization of the tested pharmacological properties and enzyme activities of WI RVV; nevertheless, both antivenoms demonstrated poor cross-reactivity and neutralization of pharmacological activities shown by low-molecular-mass proteins (<18 kDa) of this venom.



KEYWORDS: *venom proteome, ESI-LC-MS/MS, pro-coagulant, anti-coagulant, venom−antivenom cross-reactivity, neurotoxicity*

■ INTRODUCTION

Russell's viper (*Daboia russelii*) is a medically important snake in many South Asian countries, including India (Figure 1). RV bites account for a large amount of snakebite morbidity and mortality and show complex clinico-pathological manifestations in human victims.[1−4] Russell's viper venom (RVV) primarily targets blood coagulation in victims and promotes tissue damage, edema, necrosis, hypotension, hemostatic imbalance, hemorrhage, and acute renal failure.[2,4,5] In addition, a few RV bite patients in western India (WI) showed neurological symptoms like flaccid paralysis,[3,4] although such clinical symptoms are uncommon in eastern India.[2] The RVV toxin or toxins accountable for neurotoxic symptoms post RV bite in WI remain to be identified. Further, marked differences in clinical manifestations of RV bites in different parts of the Indian subcontinent[2,4,6−8] may not be well explained without analyzing in detail the proteome composition of venom of RVs from particular geographical locations.

About 35 000−50 000 snakebite deaths have been reported in India, of which the "Big Four" species (*Naja naja*, *D. russelii*, *Echis carinatus*, and *Bungarus caeruleus*) account for the maximum share.[9] Therefore, Indian polyvalent antivenom is raised in equines against venoms from the "Big Four". Nonetheless, efficacy, potency, and safety of antivenoms are of immense concern[10−12] because geographical, seasonal, and ontogenic variations in snake venom composition may affect the antivenom treatment.[12−15] Consequently, knowledge on the composition of the venom of a snake from a particular geographical location, as well as assessment of efficacy of commercial antivenom to neutralize the enzymatic activities and pharmacological properties of RVV, is necessary for efficient hospital management of snakebites and appraisal of antivenom efficacy.

Recent proteomics studies have documented significant qualitative as well as quantitative variations in venom composition of RV from southern India, Sri Lanka, and Pakistan.[16−18] This suggests further characterization of RVV from different regions of the Indian peninsula would be valuable for a better understanding of the pharmacological

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Proteomic Analysis and Immuno-Profiling of Eastern India Russell's Viper (*Daboia russelii*) Venom: Correlation between RVV Composition and Clinical Manifestations Post RV Bite

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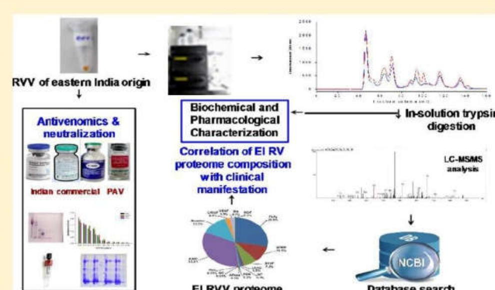
Supporting Information

ABSTRACT: The proteomes of Russell's viper venom (RVV) from Burdwan (RVV B) and Nadia (RVV N), the two districts of West Bengal, eastern India (EI), were investigated by gel-filtration chromatography (GFC) followed by tandem mass spectrometry of tryptic fragments of the fractions. A total of 73 and 69 proteins belonging to 15 snake venom protein families were identified in RVV B and RVV N, respectively, by MS/MS search against Viperidae (taxid 8689) protein entries of the nonredundant NCBI database. The minor differences in venom composition of both the EI RV were established unequivocally by their biochemical and pharmacological properties and by SDS-PAGE, gel filtration chromatography, and LC-MS/MS analyses. The composition of EI RVVs was well correlated with published reports on the pathophysiology of RV-envenomed patients from this part of the country. Venom−antivenom cross-reactivity determined by ELISA, Western blotting, and antivenomics approaches demonstrated poor recognition of low molecular mass (<20 kDa) RVV proteins by commercial polyvalent antivenoms, which was substantiated by neutralization of RVV enzymes by antivenom.



KEYWORDS: *ESI-LC-MS/MS, pro-coagulant, thrombocytopenia, venom enzyme neutralization, venom-antivenom cross-reactivity*

1. INTRODUCTION

Russell's viper (*Daboia russelii*; RV), a category I medically important snake and member of the "Big Four," is responsible for a heavy toll of snake bite mortality and morbidity in the Indian subcontinent.[1−3] Epidemiological studies suggest that the highest incidence of RV envenomation is in eastern India (EI).[3,4] RV venom (RVV) is abundant in proteases and phospholipase A_2 (PLA_2) that primarily inflict hemostatic disturbances in victims upon envenomation.[1−3,5−7] Nevertheless, compositional variation of RVV according to the geographical locale of this snake[5−8] is indicative of the severity and variability of clinical manifestations observed post RV envenomation.[1,3,9,10] Therefore, the variation in clinical symptoms of RV envenomation in different locales of the Indian subcontinent and their possible correlation to the proteome composition of RVV in the given region needs to be explored to develop suitable and potent antivenoms.

The Indian polyvalent antivenom (PAV) is raised in horses against a pool of the "Big Four" snake venoms (*D. russelii, Naja naja, Bungarus caeruleus,* and *Echis carinatus*) by conventional immunization schedules. Administration of this equine antivenom consisting of IgG or F(ab')2 fragments still remains the inevitable choice for treating snake envenomation. Nevertheless, cost, efficacy, safety, and the potency of commercial antivenoms are important for the efficient clinical management of envenomed patients, which, in turn, depends on the efficient neutralization of lethality induced by venom toxins.

Keeping in mind the frequency and severity of RV envenomations in eastern India, the present study investigated the venom proteome composition of EI RVV using gel filtration chromatography and LC-MS/MS analysis of each fraction. The proteomic data were correlated to hospital-based published reports on clinical manifestations of RV bites in this region.[3] In addition, some of the biochemical and pharmacological properties of the venom and its immunological cross-reactivity toward commercial PAVs were studied. Further, potency of commercial PAVs to neutralize the enzymatic activities and pharmacological properties of EI RVV was also assessed.

2. MATERIALS AND METHODS

Russell's viper venoms (pooled from four adult snakes) from Burdwan (RVV B) and Nadia (RVV N), the two districts of West Bengal, eastern India (EI) were procured from Calcutta Snake Park, Kolkata. Pooled RVV of western India origin (WI RVV) was procured from the Haffkine Institute, Mumbai. RVV

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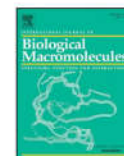

Quantitative proteomic analysis and antivenom study revealing that neurotoxic phospholipase A_2 enzymes, the major toxin class of Russell's viper venom from southern India, shows the least immuno-recognition and neutralization by commercial polyvalent antivenom

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ABSTRACT

The proteome composition of Russell's viper venom (RVV) from southern India (SI) was investigated by 1D-SDS-PAGE of venom followed by tandem mass spectrometry analysis of protein bands. A total of 66 proteins belonging to 14 snake venom protein families were identified by LC-MS/MS analysis against Viperidae (taxid 8689) protein entries from the non-redundant NCBI database. Phospholipase A_2 (43.25%) and snaclec (14.57%) represented the most abundant enzymatic and non-enzymatic proteins, respectively. SI RVV was characterized as containing a higher quantity of PLA_2 and a lower amount of Kunitz-type serine protease inhibitors, in comparison to RVV from other regions of the Indian subcontinent. The enzymatic activities, pharmacological properties, and clinical manifestations of RV envenomation in SI were well correlated with its proteome composition; however, ATPase, ADPase, and hyaluronidase enzymes were not identified by LC-MS/MS analysis, owing to paucity of the existing database. Neurological symptoms exhibited by RV-bite patients in SI were correlated to the presence of abundant neurotoxic phospholipase A_2 enzymes (15.66%) in SI RVV. Neutralization studies, immunological cross-reactivity, and antivenomics studies unequivocally demonstrated the poor recognition and lowest neutralization of PLA_2 enzymes by commercial polyvalent antivenom, which is a major concern for the treatment of RV-envenomed patients in SI.

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1. Introduction

Russell's viper (*Daboia russelii*) (RV), a member of the "Big Four" is a medically important snake that accounts for a large number of morbidity and mortality cases across the Indian sub-continent [1, 2]. Variation in snake venom composition owing to geographical location is a ubiquitous phenomenon and striking differences have been reported in the clinical manifestation of RV envenomation from different parts of the Indian sub-continent [1–4]. Russell's viper venom (RVV) primarily causes interference with the blood hemostasis system that finally leads to consumption coagulopathy by the concerted action of the proteases and phospholipase A_2 (PLA_2) enzymes [1, 2, 5–7]. Interestingly, RV envenomation from southern India (SI) and Sri Lanka (SL) also manifests neurotoxic symptoms [8, 9]. The venom proteome of SL RV is reported to contain significant amounts of neurotoxic PLA_2s (>30%) that are responsible for this observed clinical manifestation [5].

In a previous study that provided a tandem mass spectrometric analysis of gel filtration fractions of SI RVV [10], the proteome of SI RVV was expressed only in terms of the number of proteins identified by LC-MS/MS analysis (a qualitative analysis), rather than the quantitative distribution of different proteins/toxins in this venom [11]. Further, detailed information on the percent abundance of individual proteins in each family of venom toxins was not described, so the above study did not report on the detailed relative composition of SI RVV [10, 11]. Moreover, since several of the venom components act synergistically by forming protein complexes [12, 13], the relative composition of individual toxins in a venom must be understood, in terms of the geographical differences

Abbreviations: ADP, adenosine diphosphate; AMP, adenosine monophosphate; APTT, activated partial thromboplastin time; ARF, acute renal failure; ATP, adenosine triphosphate; BAEE, Nα-benzoyl-L-arginine ethyl ester hydrochloride; Bio-E, Biological E Limited; BSVL, Bharat serum and Vaccines Ltd.; CP, carboxypeptidase; CRISP, cysteine-rich secretory protein; EI, eastern India; GC, glutaminyl cyclase; ISCICS, Irula Snake Catchers' Industrial Cooperative Society; KSPI, Kunitz-type serine protease inhibitor; LAAO, L-amino acid oxidase; NGF, nerve growth factor; NT, 5′-nucleotidase; PAV, polyvalent antivenom; PDE, phosphodiesterase; PLA_2, phospholipase A_2; PLB, phospholipase B; PSVPL, Premium Serum and Vaccines Pvt. Ltd.; PT, prothrombin time; RV, Russell's viper; SI, southern India; SVMP, snake venom metalloprotease; SL, Sri Lanka; SVSP, snake venom serine protease; TAME, Nα-p-tosyl-L-arginine methyl ester hydrochloride; VBPL, Virchow Biotech Pvt. Ltd.; VEGF, vascular endothelial growth factor; WI, western India.

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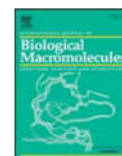

First report of the characterization of a snake venom apyrase (Ruviapyrase) from Indian Russell's viper (*Daboia russelii*) venom

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ABSTRACT

A novel apyrase from Russell's viper venom (RVV) was purified and characterized, and it was named Ruviapyrase (Russell's viper apyrase). It is a high molecular weight (79.4 kDa) monomeric glycoprotein that contains 2.4% neutral sugars and 58.4% N-linked oligosaccharides and strongly binds to Concanavalin A. The LC-MS/MS analysis did not identify any protein in NCBI protein database, nevertheless some *de novo* sequences of Ruviapyrase showed putative conserved domain of apyrase superfamily. Ruviapyrase hydrolysed adenosine triphosphate (ATP) to a significantly greater extent ($p < .05$) as compared to adenosine diphosphate (ADP); however, it was devoid of 5′-nucleotidase and phosphodiesterase activities. The *Km* and *Vmax* values for Ruviapyrase towards ATP were 2.54 μM and 615 μM of Pi released min^{-1}, respectively with a turnover number (*Kcat*) of 24,600 min^{-1}. Spectrofluorometric analysis demonstrated interaction of Ruviapyrase with ATP and ADP at *Kd* values of 0.92 nM and 1.25 nM, respectively. Ruviapyrase did not show cytotoxicity against breast cancer (MCF-7) cells and haemolytic activity, it exhibited marginal anticoagulant and strong antiplatelet activity, and dose-dependently reversed the ADP-induced platelet aggregation. The catalytic activity and platelet deaggregation property of Ruviapyrase was significantly inhibited by EDTA, DTT and IAA, and neutralized by commercial monovalent and polyvalent antivenom.

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1. Introduction

Envenomation by Russell's viper (*Daboia russelii*), a member of Viperidae family is a major health hazard in many parts of South East Asia [1, 2]. Russell's viper venom (RVV) comprises a myriad of different enzymatic and non-enzymatic proteins or polypeptides [3, 4] which primarily targets the blood vascular system of victim/prey [1, 4, 5]. However, presence of enzymes in snake venoms which are acclaimed to cause distinct adverse effects other than the haemostatic imbalance is also reported [3, 4]. Examples are nucleotidases responsible for catalysing the hydrolysis of adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP), termed as ATPase, ADPase and AMPase, respectively, to release inorganic phosphates [6, 7]. Some nucleotidases of other than snake venom origin, termed apyrase, were shown to cleave both ATP as well as ADP in a decreasing order of preference [8]. The apyrase, a Ca^{2+}-activated enzyme, sequentially catalyses ATP to ADP and then ADP is further catalysed to AMP [8]. Both ATPase and ADPase play important roles in many biological processes including the modulation of neural cell activities [9], prevention of intravascular thrombosis [8, 10], and regulation of immune responses [11].

The first report describing that incubation of ATP with snake venom liberates pyrophosphate dates back to 1950; an enzyme present in snake venom is known to hydrolyse ATP into either ADP and phosphate or AMP and pyrophosphate [12]. Although biochemical analysis has unambiguously indicated the occurrence of ATPase and/or ADPase enzyme(s) in numerous snake venoms [1, 3, 4, 12, 13], to date there is no report on purification and characterization of ATPase, ADPase or apyrase enzyme from snake venoms. Their low abundance in venom, transient stability, and sensitivity to denaturing agents are the major bottlenecks that have been encountered in the isolation and detailed biochemical and pharmacological characterization of apyrases or nucleotidases [8].

Zeller [12] demonstrated that ATPase is a toxic component of snake venom, as it was thought to produce shock symptoms by the depletion of ATP and therefore can contribute to prey immobilization [12]. Purines, a class of multi-toxin plays a pivotal role in prey immobilization by inducing hypotension and vascular permeability *via* activation of A1 (neuronal) receptor, A2 (vascular smooth muscle) and A3 (mast cell) receptors [14, 15]. These multi-toxins are integral components of

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REVIEW

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

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ABSTRACT

Introduction: The Russell's Viper (RV) (*Daboia russelii*), a category I medically important snake, is responsible for a significant level of morbidity and mortality in the Indian sub-continent.

Areas covered: The current review highlights the variation in RV venom (RVV) composition from different geographical locales on the Indian sub-continent, as revealed by biochemical and proteomic analyses. A comparison of these RVV proteomes revealed significant differences in the number of toxin isoforms and relative toxin abundances, highlighting the impact of geographic location on RVV composition. Antivenom efficacy studies have shown differential neutralization of toxicity and enzymatic activity of different RVV samples from the Indian sub-continent by commercial polyvalent antivenom (PAV). The proteome analysis has provided deeper insights into the variation of RVV composition leading to differences in antivenom efficacy and severity of clinical manifestations post RV-envenomation across the Indian sub-continent.

Expert commentary: Variation in RVV antigenicity due to geographical differences and poor recognition of low molecular mass (<20 kDa) RVV toxins by PAV are serious concerns for effective antivenom treatment against RV envenomation. Improvements in immunization protocols that take into account the poorly immunogenic components and geographic variation in RVV composition, can lead to better hospital management of RV bite patients.

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Russell's Viper; antivenomics; tandem mass spectrometry analysis

1. Epidemiology of snakebite, with special reference to Russell's Viper bites in tropical countries

Snake venoms contain a myriad of proteins and polypeptides that have diversified via accelerated evolution facilitated by positive Darwinian selection [1–3]. This often diverse suite of toxins aid in immobilizing, killing and digesting prey [4]. Bestowed with high affinity and selectivity, these biological toxins primarily affect the cardiovascular and neuromuscular systems, and blood coagulation of prey or victims [5–7]. Nevertheless, some of these medically important toxins of snake venom, following purification and extensive characterization, also hold promise as excellent candidates for producing lifesaving drugs [8–10].

Snake envenoming is an occupational health hazard that primarily affects rural agricultural workers in developing countries of Africa, Asia, Latin America, and Oceania [11]. Snakebite victims in these regions are subjected to high morbidity and mortality rates due to poor access to health services, and in many instances, a scarcity of antivenom, the only effective choice of treatment for snakebite [12]. In spite of the severe physical, psychological and socio-economic impacts of snakebite, this abundant health problem has received scant attention from regional and national health authorities, as well as research funding agencies throughout the world. Recently (2017), the World Health Organization (WHO) has again recognized snake envenoming as a neglected tropical disease (www.who.int/neglected_diseases/diseases/en). Subsequently, interest in understanding and addressing this grave problem is gradually emerging among health authorities, governmental and non-governmental organizations, antivenom manufacturers, and researchers across the globe.

Conservative estimates suggest globally at least 421,000 snakebites occur annually and approximately 20,000 of these result in death. The estimations of snakebite in India are even more poignant, with approximately 81,000 envenoming per year resulting in 11,000 deaths; these numbers are the highest for any country [13]. Mortality data from the Million Death Study (2001–03) conducted by the Registrar General of India and the Centre for Global Health Research provided the first-ever direct estimate of 45,900 annual snakebite deaths in India [14]. Nevertheless, accurate numbers of snake envenomings and fatalities are difficult to estimate due to inadequate reporting and record-keeping [13,15]. Further, occurrence and mortality is only part of the picture: a significant (but unknown) number of envenomings result in permanent morbidities, such as loss of limbs, digits and/or function, and these effects can debilitate a victim for life.

India is inhabited by more than 60 species of venomous snakes, and among them, the 'Big Four' snakes – Russell's Viper (*Daboia russelii*), Spectacled Cobra (*Naja naja*), Common Krait (*Bungarus caeruleus*), and Saw-scaled Viper (*Echis carinatus*) – are distributed throughout the country and are responsible for most cases of envenoming, morbidity and mortality [16,17]. In Asia, Russell's

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Addendum

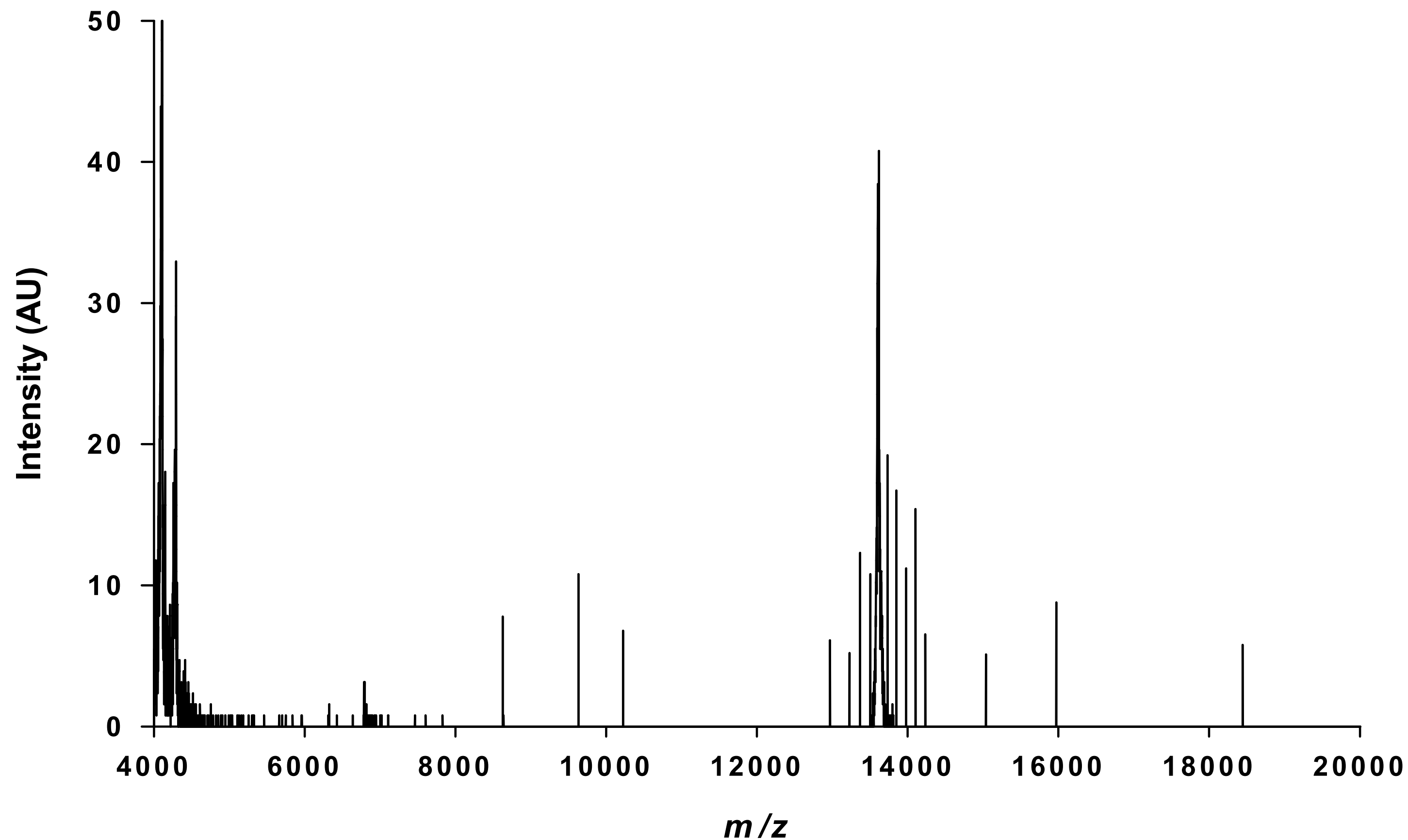

Figure A1: MALDI-TOF-MS spectrum of SI RVV (5 µg) recorded in the range of 4 to 20 kDa. The spectrum was analyzed on a Bruker Daltonics UltrafleXtreme mass spectrometer at 40000 resolutions full width at half maximum.

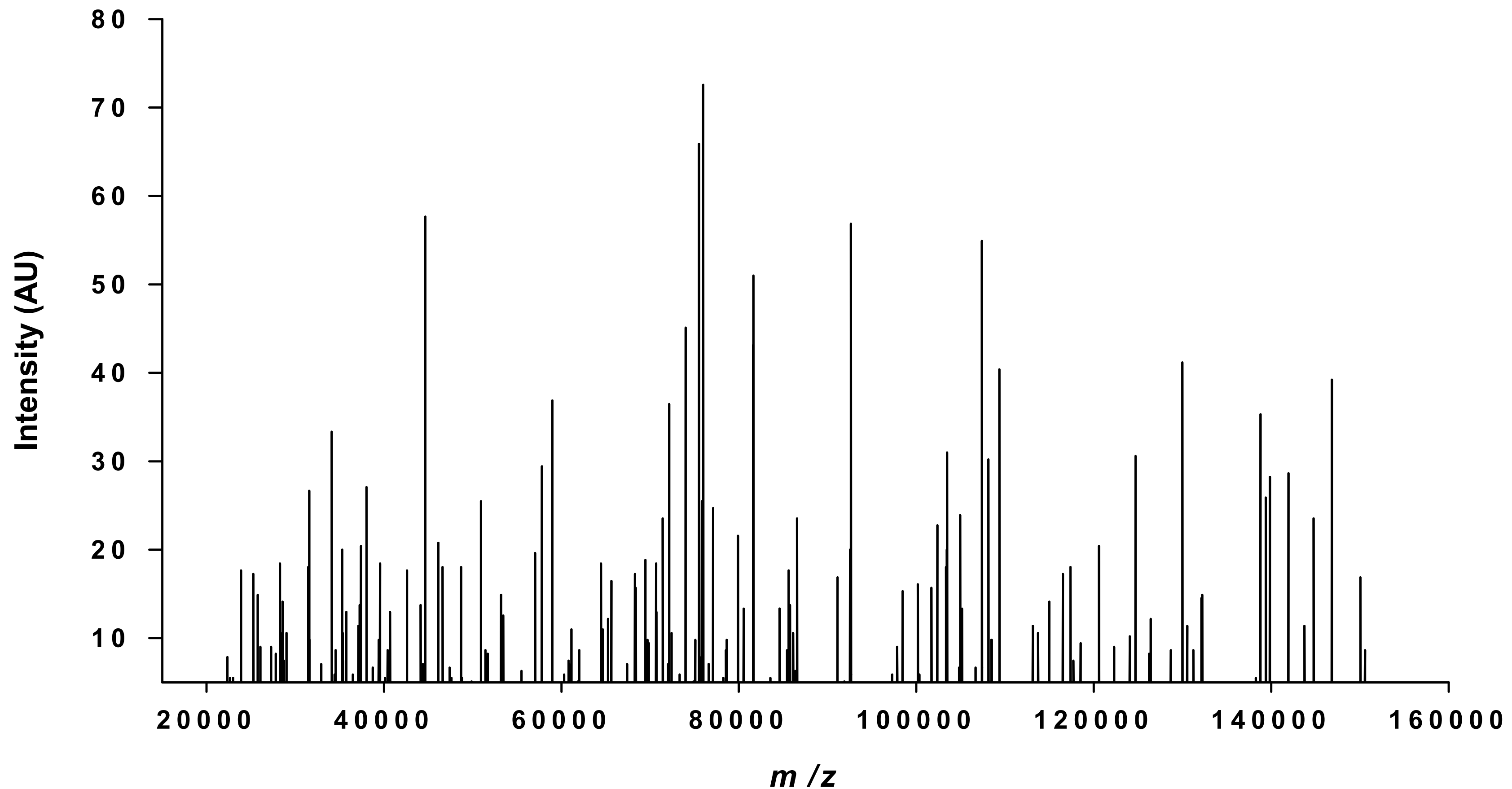

Figure A2: MALDI-TOF-MS spectrum of SI RVV (5 µg) recorded in the range of 20 to 160 kDa. The spectrum was analyzed on a Bruker Daltonics UltrafleXtreme mass spectrometer at 40000 resolutions full width at half maximum.

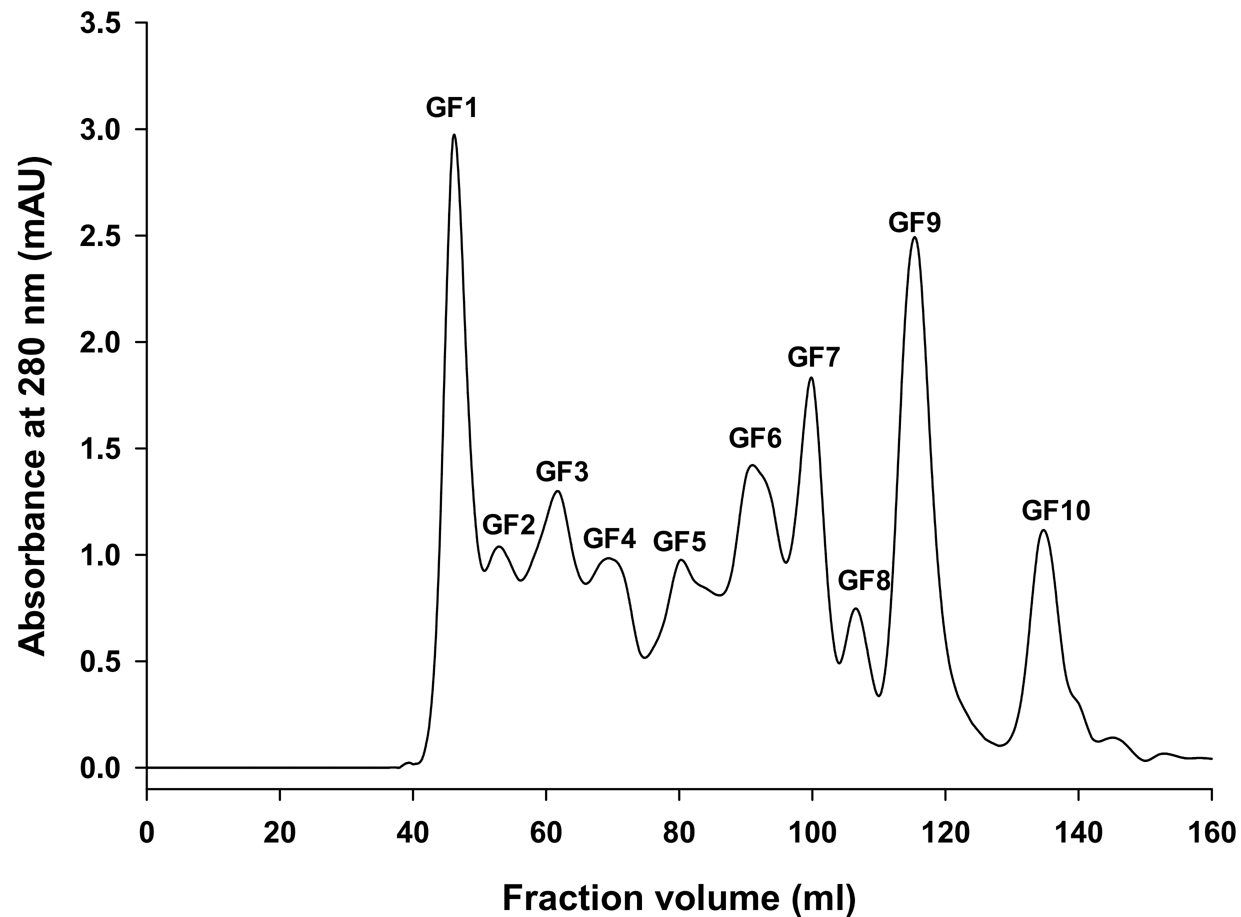

Figure A3: Fractionation of WI RVV (75 mg dry weight) on a HiLoad 16/600 Superdex 75 pg GF column. The flow rate of buffer (25 mM HEPES containing 50 mM NaCl, pH 7.0) was maintained at 10 ml/h and fractions of 2.0 ml were collected. The fractionation was carried out at 4 °C.