CHAPTER VI

CONCLUSION AND FUTURE PERSPECTIVES

6.1 Conclusion

In the present investigation, Russell's Viper venom proteomes from three different geographical locations of India were deciphered using tandem mass spectrometry analysis. In addition, the immunological cross-reactivity and neutralization of RVV by commercial antivenom was assessed and the poorly immunogenic RVV components were identified using antivenomics approach. Finally, a novel apyrase enzyme (Ruviapyrase) was purified and characterized from Indian RVV in the present study.

The study reports for the first time a comprehensive proteomic analysis of Indian RVV from different geographical locales of India. ESI-LC-MS/MS analysis of the RVV samples against Viperidae protein entries of the NCBI database suggests the presence of 55 to 73 proteins in Indian RVV that are clustered into 14 to 15 enzymatic and non-enzymatic protein classes. Phospholipase A_2 is the most abundant enzymatic component of all the RVV samples, while Kunitz-type serine protease inhibitor and snaclec are abundant non-enzymatic components of western and eastern India RVV, and southern India RVV, respectively. 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 qualitative and quantitative differences in RVV composition. Further, 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.

Further, the RVV proteome compositions of eastern, western and southern India are well correlated with the biochemical and pharmacological properties of the respective RVV samples and clinical manifestation of RV-envenomation from that region. Unique neurotoxic symptoms exhibited by RV bite patients from southern India and to some extent western India is in good agreement with the presence of different quantities of neurotoxic phospholipase A_2 enzymes in these RVV samples.

The second important finding of this study is the poor recognition and neutralization of low and mid-molecular weight RVV proteins such as phospholipase A_2, and snake venom serine proteases by commercial polyantivenom. Further, since the primary source of snake venoms for raising commercial PAV is Irula Snake Catchers

Industrial Cooperative Society, Tamil Nadu, southern India, the neutralization potency and immuno-recognition of southern India RVV by commercial polyvalent antivenoms are relatively better than the western and eastern India RVV samples. Antivenomics studies revealed that phospholipase A_2, Kunitz-type serine protease inhibitor, snake venom serine proteases, and snaclec are the major poorly recognized toxins of Indian RVV. In addition, antivenoms lacked sufficient antibodies against metalloproteases and neurotoxic phospholipase A_2 enzymes of eastern and southern India RVV, respectively. These findings urge a need to design improved immunization protocols to mitigate the toxic effects of low molecular mass RVV components as well as the development of region-specific antivenom for better hospital management of RV bite patients.

The third major finding of the study is the purification and characterization of a novel apyrase (Ruviapyrase) enzyme from Indian RVV. This is the first report of an apyrase enzyme from snake venom. Ruviapyrase demonstrated dose-dependent preferential hydrolysis of ATP over ADP with a ~1.6 fold higher specific activity towards the former substrate. Transient stability of Ruviapyrase under physiological conditions, low *Km* values and high turnover numbers toward its substrates indicate that this class of enzymes may have evolved in snake venoms to rapidly accomplish its task of depletion of their cellular harmony (ATP). Metal ions play an important role in the catalytic activity of Ruviapyrase and the enzyme contains disulfide linkages. Ruviapyrase is highly glycosylated with neutral carbohydrates and N-linked oligosaccharide, and contains α-D-mannosyl and α-D-glucosyl residues. Further, reversal of ADP-induced platelet aggregation by Ruviapyrase may contribute to platelet dysfunction and increased bleeding risk in RV bite patients.

6.2 Future perspectives

The proteomic profiles of RVV from different geographical locations of the Indian sub-continent have been documented; however, studies on RVV samples from Nepal, Bhutan, and Bangladesh are yet to be deciphered. Since the antivenoms manufactured in India are commercially distributed to these neighbouring countries, there is a need to expand the proteomic and antivenomics studies on RVV samples from these geographic locations of the Indian sub-continent in the future. In addition, future studies on lethality neutralization potency (*in vivo*) of commercial polyvalent antivenom are also warranted. Further, the design of strategies for enhancing the immunogenicity of pharmacologically active low molecular mass (<20 kDa) RVV toxins and

development of equine alternatives for antivenom production, such as expression of antibodies in *Escherichia coli* and mammalian hybridoma cell lines, can also be explored.