CHAPTER 7

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7.1. Conclusion

The present study has attempted to unveil the venom composition and interspecific venom variability among four species of Indian green pit vipers viz. *Trimeresurus erythrurus*, *T. septentrionalis*, *Viridovipera medoensis* and *Popeia popeiorum*. The results demonstrated substantial differences in electrophoretic and chromatographic profiles indicating differential expression of venom proteins. Such differences in protein expression can be correlated with functional variations in biochemical activities such as procoagulant activity, PLA_2 activity, thrombin-like activity, protease activity and haemolytic activity shown by green pit vipers. The interspecific venom variation among closely related species might contribute to clinical variability of envenomation posing challenges of para-specific inefficacy of antivenom therapy.

Immuno-reactivity studies of Indian green pit vipers with para-specific green pit viper antivenom (GPVAV) revealed good neutralization potential of antivenom to mitigate the toxic effects of venoms. However, Indian polyvalent antivenom (PAV), the only available antivenom in India, exhibited poor cross-reactivity with venom toxins of Indian green pit viper venoms. This finding is reinforced by regional envenomation reports which depict a severe pathophysiological disorder, which is incoagulable blood persisting for many days post bite unresponsive to polyvalent antivenom treatment. The study emphasizes on the need for a suitable antivenom for the treatment of Indian green pit viper envenomated patients. Therefore, studies aimed towards assessing the feasibility and practical implications of the production and optimization of a region-specific polyvalent antivenom for north-eastern India should be urgently considered.

The analysis of crude venom of *T. erythrurus* of Indian origin using ESI-LC-MS/MS exhibits an amalgam of different proteins/peptides belonging to various enzymatic and non-enzymatic families. The major enzymatic toxins include phospholipase A_2 and snake venom serine proteases, and non-enzymatic toxins snaclecs.

Along with these, proteins present in trace amounts include LAAO, CRISPs, c-type lectins, disintegrins, bradykinin potentiating c-type natriuretic peptides and glutaminyl-peptide cyclotransferases. The study reports the first elaborate proteome profile of *T. erythrurus* venom which is well correlated with clinical manifestations of envenomation. Moreover, the venom composition of *T. erythrurus* show a very distinctive pattern in comparison with other green pit vipers of Southeast Asian countries. The study adds additional information on inter-species venom variability along with shedding light on major toxins of *T. erythrurus* which might be exploited for the establishment of "region-specific" antivenoms or designing of APTMERs based alternatives to mitigate the challenges of para-specific inefficacy of present-day antivenom therapy.

The study was further directed towards purification of a haemostatically active protein from the venom of *T. erythrurus*. The purified protein, erythrofibrase is a serine protease with molecular weight of 29 kDa. The protein showed high sequence similarity with snake venom thrombin-like enzymes (SVTLEs). Erythrofibrase showed direct fibrinogenase activity by degrading Aα band of bovine fibrinogen rendering it unsuitable for polymerization. Degradation of fibrinogen causes hypofibrinogenemia and defibrination syndrome in patients, as a result of which clot formation does not occur. The functional attributes of erythrofibrase can be correlated with the major clinical manifestation observed in green pit viper envenomated patients. This study reports for the first time the characterization of an α-fibrinogenase enzyme, erythrofibrase from *T. erythrurus* venom which is crucial for the pathophysiological manifestations observed in envenomated victims.

7.2. Future prospects

1. Proteomics studies of other Indian green pit vipers such as *Popeia popeiorum*, *Trimeresurus septentrionalis*, *Viridovipera medoensis* etc. can be to performed to understand the inter-specific variation in terms of major and unique toxins. Such studies might unveil compositional variation of venom at proteome level.
2. Formulation of a region-specific polyvalent antivenom consisting major toxins of snakes prevalent in north-eastern India such as monocled cobra, green pit viper, banded krait etc. can be solicited to mitigate the challenges of para-specific inefficacy of Indian polyvalent antivenom.

3. The purified protein erythrofibrase can be explored for its therapeutic potential as defibrinogenating agent for the dissolution of unwanted clot in thromboembolic patients as well as molecular marker for diagnostic tools.