CHAPTER VII

CONCLUSION AND FUTURE PERSPECTIVES

7.1. Conclusion

In the present study, for the first time, we provided a comprehensive analysis of the venom toxinome composition of an Indian red scorpion (*M. tamulus*) from India. This scorpion venom is enriched in low molecular mass ion-channel toxins, making it one of the deadliest species that give rise to severe neurotoxic symptoms. Because our proteomic analysis relied on a protein database, several toxins in this scorpion venom may have yet to be identified due to the paucity of protein databases. A transcriptomic analysis coupled with the proteomic analysis may have improved the strategy for more robust identification of scorpion venom toxins. Although the toxins identified in *M. tamulus* venom (MTV) show adverse pharmacological effects, several may become a goldmine of drug prototypes and help find therapeutic applications in treating various diseases, including cancer. The clinical manifestations after scorpion stings in western India correlate well with the cumulative relative abundance of MTV toxins identified by proteomic analysis. The meager recognition of the most abundant low molecular mass toxins of MTV by commercial anti-scorpion antivenom (ASA) is a significant hurdle for effective antivenom treatment against scorpion stings. Antibodies specifically raised against scorpion venom's low molecular mass toxins should be used to supplement the commercial antivenom to improve the clinical treatment of scorpion stings.

Another significant finding of this study was the purity, quality, and safety profile of commercial ASAs determined by simple and affordable laboratory tests. Proteomic analysis revealed that ASAs contained 67.16 to 74.30% IgG/ $F(ab')_2$ and small quantities of serum proteins. Improper pepsin digestion of IgG will reduce the $F(ab')_2$ content and increase the IgG-containing Fc portion responsible for complement activation and early adverse reactions. Besides, during its production, antivenom may be contaminated with endotoxin-producing gram-negative bacteria; therefore, the use of preservatives, such as cresol and phenol, is recommended by WHO to prevent bacterial contamination. ASAs did not show IgE contamination or bacterial endotoxin devoid of aggregate content but demonstrated moderate complement activation properties, which may have adverse effects in treated patients.

This study used the spectrofluorometric method to determine venom-antivenom binding and calculated the percent venom-specific antibody from the spectrofluorometric titration curve. The percent of antibodies raised against the venom toxins in commercial

ASAs was determined at the range of 5.3–6.3%, which is a reason for their poor efficacy. Thus the modern approach to antivenom manufacturing should be geared up to reduce complications and better manage scorpion stings.

Drawbacks of conventional therapies using commercial ASA and α1-adrenoreceptor antagonists (AAA) have prompted us to search for an adequate formulation to improve treatment against MT sting. Thus our study presented the efficacy of a formulated drug comprised of low doses of commercial ASA, AAA, and Ascorbic acid, capable of neutralizing the *in vivo* toxic effect of MTV in *Caenorhabditis elegans* and Wistar strain albino rats. Both models exhibited significantly improved efficiency of the developed drug compared to its component against MTV-induced toxicity.

7.2 Future perspectives

In the present study, the MTV was deciphered from India; however, the geographical variation in MTV toxicity and its pharmacological effects often leads to differences in local and systemic symptoms. In-depth proteomic analyses to correlate the geographical variation in MTV composition with sting severity are yet to be explored.

Further, from the proteomic profiling of the MTV, many proteins or peptides of low abundance may not be identified due to the limited number of sequences in protein reference databases. Therefore, additional –omic analyses, such as genomics and transcriptomics, are encouraged to promote the discovery of novel scorpion venom toxins. This research will increase our understanding of MTV and its toxicity mechanism and identify novel drug prototypes from this venom. In addition, venom proteins (toxins) of low abundance can be challenging to detect without optimizing common mass spectrometric methods or applying alternative techniques, such as western blotting with a particular antibody.

While various treatments are available for the clinical management of scorpion stings, early administration of scorpion antivenom is the preferred choice, even though the poor immunogenicity of scorpion antivenom might present additional clinical challenges. Thus designing of protocol to enhance the antigenicity of low molecular mass toxins of scorpion venom and the development of alternatives for antivenom production, such as the use of an adjuvant, expression of antibodies in *Escherichia coli* and mammalian hybridoma cell lines, can also be investigated.

The approaches described in this study for assessing the quality and safety of Indian antivenoms can greatly assist laboratories involved in developing, manufacturing, and quality control of antivenoms and national regulatory organizations. These techniques can be used to evaluate other antivenoms for treating scorpion stings in different parts of the world.

This study demonstrates for the first time that *C. elegans* can be a model organism for screening the neutralization potency of drug molecules against a neurotoxic venom. Our study also presented the efficacy of a formulated drug comprising low doses of commercial ASA, AAA, and Ascorbic acid, capable of neutralising MTV's *in vivo* toxic effect in *C. elegans* and Wistar strain albino rats. However, mechanism of hyperglycemia in MTV-induced Wistar albino rat and its neutralization by medicinal plants and synthetic components can also be evaluated.