Development of Analytical Methods for Identification of Indian Snake Venoms and Indian Red Scorpion Venom

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CHAPTER VI

CONCLUSION AND FUTURE PERSPECTIVES

6.1 Conclusion

Distinguishing between wet and dry snakebite circumstances is crucial for effective antivenom treatment. This paper proposes an analytical approach for detecting envenomation in snakebite patients, particularly in the Indian subcontinent. Due to the labour-intensive and expensive nature of isolating toxins from snake venom, we used an alternate strategy, including mapping antigenic epitopes of toxins, synthesizing bespoke peptides, and generating antibodies against these peptides to detect snake venom. The high-titre polyclonal antibodies generated against custom peptides synthesized from the predominant low-molecular-mass toxins present in the proteomes of the Indian 'Big Four' snakes (*Naja naja, Bungarus caeruleus, Daboia russelii*, and *Echis carinatus*) exhibited in vitro and in vivo detection of these snakes and *Naja kaouthia* venoms. The formulation obtained from polyclonal antibodies (FPAb) demonstrated the ability to detect venoms at a low concentration of 1 pg/µL under *in vitro* conditions and immunologically recognized snake venoms in envenomed rat plasma for up to 240 minutes' post-venom injection. The immunological recognition of FPAb with antibodies targeting antigenic custom peptides derived from the low-molecular-mass poisons of the 'Big Four' snakes was determined to be superior to that of commercially available polyvalent snake antivenom.

We employed the colourimetric sensing capability of AuNPs, wherein the AuNP-FPAb conjugates transitioned in colour from red to blue/purple upon contact with the examined snake venoms, attributable to the aggregation of the AuNPs. This study employed high-resolution photos obtained from smartphone cameras to identify colour variations. This work employed digital image colourimetry using smartphone photos to identify snake venoms in envenomed rat plasma, utilizing the colour shift resulting from the agglomeration of gold nanoparticles. Consequently, our portable smartphone-based colourimetric approach will assist in identifying envenomation in patients at hospitals, clinics, or health centres in distant areas without advanced instrumental equipment.

A diagnosis method to detect and assess the toxins in the patient's body fluids is essential for efficient therapy against scorpion stings. In this study, we have proposed a novel, simple, and rapid method for detecting *Mesobuthus tamulus* venom (MTV) using polyclonal antibodies raised against the custom peptides representing the antigenic epitopes of K^+ (Tamapin) and Na^+ (α-neurotoxin) channel toxins, the two major MTV toxins identified by proteomic analysis. The optimum polyclonal antibody formulation, PAbF, acted synergistically, demonstrating significantly higher *in vitro* immunological

recognition of MTV than anti-scorpion antivenom (developed against native toxins) and individual antibodies against peptide immunogens. The PAbF could optimally detect MTV in envenomed rat plasma (by intravenous and subcutaneous administration) at 30-60 minutes post-injection. This study's acetonitrile precipitation approach enhanced MTV detection sensitivity by concentrating low molecular mass peptide toxins in envenomed rat plasma.

Colourimetric assays provide a simple visual detection method that does not require sophisticated equipment. This work employed absorbance spectra and localized surface plasmon resonance (LSPR) detection by monitoring the colour change in the gold nanoparticle (AuNP) colloidal solution resulting from the interaction of MTV toxins and AuNP-PAbF conjugate. This is the inaugural study identifying Indian red scorpion venom in the plasma of envenomed animals, and it shows significant potential for detecting venom in human bodily fluids.

6.2 Future perspectives

This study presents a proof-of-concept for a portable smartphone-based colourimetric technique for identifying and quantifying venom from the Indian 'Big Four' snakes and *Naja kaouthia* in the plasma of envenomated rats. The suggested detection method requires testing using clinical samples derived from the body fluids of snakebite patients. Research utilizing clinical samples must include a statistically sufficient sample size to substantiate the usability of the FPAb for detection in clinical environments. Clinical testing is also necessary for the alternative proof-of-concept strategy presented in this work for the sensitive detection of MTV utilizing a species-specific antibody formulation, PAbF.

This work uses polyclonal antibodies generated in rabbits to identify snake and scorpion venoms in animal models. Nevertheless, other methods may be investigated to enhance the antibody manufacturing process, hence minimizing the discomfort experienced by the animals involved. Additionally, to improve the specificity and selectivity of venom toxins, monoclonal antibodies can be produced from the suggested antigenic-tailored peptides to provide a more sensitive venom detection method.

Furthermore, the smartphone-based detection method introduced in this study necessitates enhancements in real-time result processing, interpretation, and

communication to relevant personnel before it can be used in essential rural health centres. The MTV detection approach outlined in this work requires a visible range spectrophotometer. However, it may be possible to develop devices or kits utilizing image analysis, microfluidics, or lateral flow assays to quickly detect MTV at the point of care.

While we have employed gold nanoparticle-based colourimetric sensing for expedited analysis, alternative methodologies, such as quantum dot-based or electrochemical sensors, might be developed for toxin or species-specific venom detection in real-time biological fluid samples.

Further research into the pharmacokinetics and pharmacodynamics of the venom toxins utilized in developing the detection system may prove beneficial. Pharmacokinetics may provide insights into the distribution, metabolism, and elimination of venom from the body. This understanding aids in determining the optimal timing for testing and the appropriate sample collection to maximize the likelihood of toxin identification. In our study, we picked toxins based on their prolonged presence in circulation; however, comprehensive future investigations may enhance the development of a more sensitive detection method for trace venom identification. Pharmacodynamics may elucidate how venom toxins induce symptoms and identify which venom proteins are responsible for specific types of damage. These investigations enhance the correlation between detection test findings and the patient's clinical status.