ABSTRACT

Kraits belong to the genus *Bungarus* which form an important group of snakes in India (around 12 to 14 known species) and belong to the Elapidae family. One of these kraits, *Bungarus fasciatus*, is commonly found in the Eastern and North-East regions of India, although they have been reported from other parts of the country as well. Their general distribution ranges from the Indian subcontinent to the South-East Asian countries and Southern China and they have been recorded at an altitude as high as 5000 m above sea level. *B. fasciatus* can be easily identified from its alternating yellow and black bands of equal width encircling throughout its body and it is mostly active at the night. It feeds mainly on the small fish, frog, skinks, eggs of snake and is also known to feed on other snakes as well.

Snakebite envenomation is considered as a neglected tropical disease which is responsible for thousands of death and permanent disability every year in victims worldwide. The venom of snakes is a mixture of several toxins which are pharmacologically active and has evolved over millions of years for the purpose of offence and defense. *B. fasciatus* is of WHO 'Category 2' medical importance consisting of neurotoxic venom and is responsible for occasional snakebite incidents in this region, some even leading to death of the victim. Bite from *B. fasciatus* may cause little to no pain in humans and a minimum local damage to tissue, however, since the venom contains both pre-synaptic and post-synaptic neurotoxins their symptoms become progressively evident with time. Systematic symptoms may include a rapid or delayed progressive respiratory paralysis.

Antivenom serves as the only medically viable option for treating snake envenomation effectively. In India, polyvalent antivenom, which are raised against the venom pool of the "Big-Four" snakes, are mainly used to treat snake envenomation cases including bites from *B. fasciatus*. Although polyvalent antivenom provides some degree of paraspecific protection from *B. fasciatus* envenomation, however, due to inter-species venom variation between *B. caeruleus* and *B. fasciatus*, these antivenom are not fully effective due to which a high dose is often administered by healthcare professionals. Intra-species venom variation among *B. fasciatus* venom from different geographical locations may affect the antivenom efficacy. A high antivenom dosage may lead to adverse side-effects such as serum sickness and anaphylactic reactions in the victims. Hence, studying the

venom variation in *B. fasciatus* venom from different locations of Eastern and North-East India and their cross-reactivity to different Indian polyvalent antivenom becomes necessary to understand the efficacy of these antivenom, and also to identify non-immunodepleted and poorly-immunodepleted venom proteins.

The different aspects of the study are documented in this thesis and organized into six chapters which are briefly summarized as below:

Chapter 1: In the first chapter, the venomous snake *Bungarus fasciatus* is introduced and literature pertaining to its biology, behaviour and venom lethality is described. The use of different antivenom types to treat snakebite envenomation is discussed and previous studies describing the cross-reactivity of *B. fasciatus* venom with different antivenom are summarized. Further, the need of the study and research hypothesis is defined and the objectives of the study are set out.

Chapter 2: In this chapter, *B. fasciatus* venoms collected from three states of Eastern and North-East India (West Bengal, Assam and Mizoram) are assessed to understand variation in their venom profile using gel electrophoresis and Reverse-phase High-performance liquid chromatography. Moreover, biochemical activities of the crude *B. fasciatus* venoms such as Phospholipase A_2 (PLA_2) activity, Proteolytic activity, Direct and Indirect hemolytic activity, and Anti-coagulant activity, are compared to understand the functional difference in the venom.

Chapter 3: In this chapter, the Indian polyvalent antivenom are compared for their immunoglobulin content, and their cross-reactivity against *B. fasciatus* venom from different locations of Eastern and North-East India are studied. Immunochemical analysis using western blotting reveals differences among the antivenom to identify the low molecular weight venom proteins. Moreover, immunodepletion study using second-generation antivenomics highlight the poor immunodepletion capability of polyvalent antivenom mostly for the samples from North-East India.

Chapter 4: In this chapter, the *B. fasciatus* venom samples from North-East India are studied to understand their cross-reactivity to Indian polyvalent antivenom using third-generation antivenomics at different venom-antivenom ratios. The maximum binding capability of the antivenom is determined and the number of antivenom vials, required to immunocapture all venom proteins, are theoretically estimated along with its associated

cost. The poorly-immunodepleted venom proteins from three peaks P5, P6 and P7 are identified using liquid chromatography-tandem mass spectrometry and seven proteins are identified as belonging to the PLA_2 superfamily.

Chapter 5: In this chapter, the in vitro enzymatic activities of poorly-immunodepleted PLA_2 proteins (P5, P6 and P7), such as PLA_2 activity and anti-coagulant activity, are determined. This is followed by the study of in vitro neutralization of their activity by Indian polyvalent antivenom. The seven identified PLA_2 enzymes are compared with known PLA_2 enzymes from the "Big-Four" snakes *in silico*, using multiple sequence alignment, followed by Phylogenetic tree analysis which reveals six of the seven PLA_2s formed distinct clades near the PLA_2s from elapids of "Big-Four". Moreover, secondary and tertiary structure prediction, followed by B-cell epitope and surface cavity prediction are also performed.

Chapter 6: In the final chapter, the steps needed to be taken for manufacturing high quality antivenom at affordable price, and some of the newer and emerging antivenom approaches are critically discussed. Moreover, some of the future prospects of this overall study are also highlighted which may be further explored to gain newer insights in this field.