Chapter 7

Conclusion and future prospects

7.1 Conclusion

Biochemical and biological properties of *N. kaouthia* venom from northeast Indian origin has not been reported so far. However, considering the venom composition, biochemical and biological properties, it was found to be a homolog when compared with *Naja kaouthia* from other geographical locations. Moreover, this is the first report describing the in vitro biochemical and biological properties *N. kaouthia* venom as well as isolation and characterization of a previously uncharacterized 3FTx (Nk-3FTx) from its venom from Indian origin. The crude venom is highly neurotoxic and has significant anticoagulant effect. The LD_{50} was found to be 0.148mg/kg as tested on experimental mice and was found to be more toxic when compared with lethal doses of this snake reported from other parts of India and Thailand. The crude venom exhibited PLA_2activity, indirect hemolytic activity, edema inducing activity and anticoagulant effect. However, the venom was found to be devoid of hemorrhagic activity, direct hemolytic activity and antibacterial activity even when tested in higher concentrations. Proteolysis by crude venom was found to be weak, however at 100µg, degradation of casein was observed. The venom was found to be cytotoxic towards HEK293 and L6 rat skeletal muscle cell lines at molar concentrations. Most importantly, the crude *N. kaouthia* venom was found to be highly neurotoxic. Neurotoxicity was tested by observing behavioral changes of experimental animals post venom injection and studying its effect on isolated toad sciatic nerve. The crude venom reduced the amplitude of stimulus conduction by nerve axon which suggests decrease in ion flow. This decrease in ion flow is due to effect of crude venom on various ion channels and also might be in other factors associated in propagation of stimulus which finally will lead to neurotoxicity.

We tested the efficiency of commercially available polyvalent antivenom from Bharat Serums. India in neutralizing some of the toxic effect of crude *N. kaouthia* venom from Northeast India. The polyvalent antivenom was found to neutralize

some of the tested biochemical and biological activities at 1:100 (w/w) ratios. In a study; neutralization of Thailand *Naja kaouthia* venom by polyvalent antivenom; the antidote was found to be effective in neutralizing the major proteins such as PLA_2s, but were ineffective against α neurotoxins (which could be due to low immunogenicity)" [67].As discussed in section 3.2, the polyvalent antivenom is raised from "Big four" snakes of India, which are not prevalent in Northeast India. However, species similarity resulted into neutralization of the crude venom but at a higher dose. Administration of polyvalent antivenom has several complexities and neutralization of the crude venom with higher dose can lead to serious patho-physiological complications. This study shows the importance of species-specific antidote with reference to specific geographical location to counter snakebite.

Further, to correlate and establish a strong evidence of biological and biochemical properties and various proteins responsible within the crude *N. kaouthia* venom, the task for identifying various proteins in crude venom was undertaken. We identified different proteins by a combination of fractionating the crude venom using reverse phase HPLC and identifying molecular masses of various proteins by ESI/MS. Correlating the venom composition of *N. kaouthia* of Northeast India with *N. kaouthia* of Thailand, Malaysia and Vietnam, it was found to contain approximately similar percentages of 3FTxs and PLA_2 enzymes which are also the major fraction of the whole venom in all of the compared cases. Some proteins with higher molecular masses could not be detected as explained in chapter 4. However, various biological and biochemical properties of crude *N. kaouthia* venom correlate with the partial compositional analysis where presence of PLA_2 and 3FTxs in major percentage supported our hypothesis of its toxic actions.

This thesis reports the isolation and characterization of a previously uncharacterized non-conventional 3FTx, Nk-3FTx. This the first report of purification and characterization of 3FTx from *N. kaouthia* of Northeast Indian origin. The protein was found to have a molecular mass of 7579.5 ±0.591Da. The identity of Nk-3FTx was determined by a combination of N-terminal sequencing and ESI-LC MS/MS . Various trypsin digested amino acid fragments were assembled and a partial primary structure of Nk-3FTx was built along with the N-terminal sequence. The assembled partial amino acid sequence of Nk-3FTx was of 55 amino acid residues which

showed homology towards CM-9a reported from *N. kaouthia* of Thailand. The primary structure obtained was aligned with other 3FTxs and was found to consist of 8 conserved cysteine residues (four disulfide bonds) and additional two cysteines (fifth disulfide bond) at its first loop. Based on its primary structure it was concluded to be a non-conventional 3FTx. This is the first report of isolation and biophysical characterization of 3FTx from Northeast Indian origin.

Further, some of the biological and biochemical property of Nk-3FTx was determined. However, Nk-3FTx was found to be devoid of most of the tested *in-vitro* and *in-vivo* experiments. The purified protein showed mild anticoagulant activity in citrated human plasma which was insignificant when compared with the crude venom anticoagulant activity. However, Nk-3FTx was observed to exhibit a significant effect on nerve conduction in isolated toad sciatic nerve *in-vitro*. A dose dependent effect in reducing the amplitude of action potential was recorded. Action potential is anion gradient process where K^+ and Na^+ ion plays a vital role. Nk-3FTx might be targeting any of these ions channels for its effect on action potential. With the help of specific sodium and potassium channel blockers we determined that Nk-3FTx has an effect on K^+ channel in a dose dependent manner. However, the receptor-ligand interaction of Nk-3FTx with K^+ channel is essential in determining the mechanism of action of Nk-3FTx.

7.2 Future prospective

The work described in this thesis has addressed some fundamental issues related to the composition of *N. kaouthia* of Northeast India. Some of the futures prospective from the present work are listed below:

1. Neutralization of crude *N. kaouthia* venom using polyvalent antivenom at higher concentration needs attention and antivenomic research, with respect to Indian context and for the Asian *Naja kaouthia*.

2. Detailed proteomic study of venom from this snake can be done which would lead to understanding of the venom composition as well as identification of unique and novel toxins.

3. Venoms are unique source of molecules as despite being from a non-mammalian origin they can specifically target mammalian physiology in a well characterized manner. They are useful in drug discovery processes due high potency and selectivity. Especially, the small peptides which with their three dimensional scaffolds can exhibit specific pharmacological effects.

In case of Nk-3FTx, a previously uncharacterized 3FTx from the venom of *Naja kaouthia* of northeast India can be a newer candidate for various neurological disorders. Hence, detail understanding of the structure and its mechanism of action would be relevant for exploration of its therapeutic potential.