Abstract

Snake venom has been under investigation for its toxicity, variability in composition as well as for the unique property of the various toxins. The components present in the venom varies greatly among the species as well as within the species [1]. Based on structure and function of the venom components it has been grouped into various families, however functional variations among the isoforms of the families have been also documented [2]. These toxins target various physiological processes in victims/prey with high specificity. Due to their high target specificity venom toxins have been interrogated for therapeutic application or as prototype for development of drugs [3–5].

Naja kaouthia (N. kaouthia) belongs to elapid family and commonly found in South East Asia [6]. This is one of the medically important snakes of northeast India as it is responsible for most of the mortalities and morbidities in this region. Interestingly the biochemical and the pharmacological profile of this snake have not been explored.

The present study describes the biochemical and biological properties of crude *N. kaouthia* venom of northeast India and purification of a three finger toxin from its venom. Biochemical and biological properties of the crude *N. kaouthia* venom of northeast India has been carried out to understand the composition, variation and as well as to document the major toxin family present in this venom. The lethal dose of crude *N. kaouthia* venom was found to be 0.148 mg/kg on experimental mice. The crude venom was found to exhibit phospholipase A_2 activity, indirect hemolytic activity and anticoagulant activity. However, proteolysis by crude *N. kaouthia* venom was found to be negligible which is due to low percentage of proteases in the crude venom. The direct hemolytic, antibacterial and hemorrhagic activity was not observed in this venom when tested *in-vitro* and *in-vivo*. This is in agreement with previous reports of elapid venoms which are rich in PLA_2 family which exhibits these activities [7–9]. The PLA_2 enzymes present in this venom might be also be responsible for the edema inducing activity and myotoxicity as observed on experimental animals. Further the crude venom was found to be toxic to HEK293 and L6 rat skeletal muscle cell lines. Neurotoxicsymptoms were significant,

behavioural changes of the experimental animals. Treatment of toad sciatic nerve preparations with crude venom decreased the CAP (Compound Action Potential) dose dependently and confirms the neurotoxic nature of crude *N. kaouthia* venom. Commercially available polyvalent antivenom was effective in neutralizing some of the tested biochemical and biological activities at 1:100 (w/w) ratios. This cross-neutralization by polyvalent antivenom might be due to the conserved structure of the venom toxin families between *N. kaouthia* and *Naja naja* venom; the latter is used to raise the antivenom.

Venom composition varies among snake families. The elapids are reported to have a major percentage of PLA_2s and lower molecular weight proteins such as three finger toxins, whereas viperids are reported to contain PLA_2 and a majority of high molecular weight proteins such as metalloproteinases, proteinases etc.[10,11]. Hence, we determined the partial composition of crude *N. kaouthia* venom from northeast India based on the molecular mass of various toxins present. Crude *N. kaouthia* venom was analysed by SDS-PAGE, RP-HPLC and ESI/MS. The protein families identified by ESI/MS based on their molecular mass belongs to the families of 3FTxs (69.7%), PLA_2s (15.15%), C type lectins (3.03%) and Ohanin/vespryn/thaicobrin (12.1%). These findings correlate well with the tested biochemical and biological properties of the crude venom. This is this first report on the composition (partial) of the crude venom of *N. kaouthia* from northeast India.

Further, a low molecular protein belonging to the three finger family was purified from crude *N. kaouthia* venom by successive steps of RP-HPLC. The molecular weight was determined using ESI/MS and was found to be 7579.5 ± 0.6 Da. Primary sequence of the purified protein was determined by a combination of N-terminal sequencing and ESI LC-MS/MS. CM-9a (P25679.2) from the venom of *Naja naja kaouthia* of Thailand origin was used as a reference sequence to obtain the partial sequence of the purified protein [12]. This confirms that the purified toxin belong to 3FTx family and is a non-conventional 3FTxas the fifth disulfide bridge is present at its first loop. This purified protein was named as Nk-3FTx *(Naja kaouthia).* This is the first report of 3FTx from northeast Indian origin.

Biochemical and biological activity tests of Nk-3FTx showed it to be devoid of PLA_2 activity, direct hemolytic activity, indirect hemolytic activity, hemorrhagic and edema inducing activities. It was also found to be non-toxic to HEK293 and L6 rat skeletal muscle cell lines. Behavioral studies with experimental animals suggest Nk-3FTx to be a neurotoxin. To confirm the neurotoxic effect of Nk-3FTx, isolated toad sciatic nerve was treated with Nk-3FTx, and a dose dependent decrease in CAP was recorded. Sequence analysis of Nk-3FTx shows it is unlikely to affect neuronal receptors, acetylcholine receptors and calcium channels. However, the observed decrease in amplitude of action potential might be due to effects on sodium or potassium ion channels. Using standard ion channel blockers it was found that Nk-3FTx might be binding to potassium channels, affecting the potassium ion current.

The present work is the first description of biochemical and biological activities of *Naja kaouthia* venom of northeast India and efficacy of commercially available polyvalent antivenom. *Naja kaouthia* venom from this region has not been explored nor have any proteins been isolated and characterized so far. This is one of the medically important species prevalent in this region and responsible for most of the fatal bites. Therefore, the present work will open further avenues to understand the venom and characterization of the protein present in the venom might lead to discovery of novel therapeutic molecules for biomedical application