Quantifying Demyelination in NK venom treated nerve using its electric circuit model

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Reduction of myelin in peripheral nerve causes critical demyelinating diseases such as chronic inflammatory demyelinating polyneuropathy, Guillain-Barre syndrome, etc. Clinical monitoring of these diseases requires rapid and non-invasive quantification of demyelination. Here we have developed formulation of nerve conduction velocity (NCV) in terms of demyelination considering electric circuit model of a nerve having bundle of axons for its quantification from NCV measurements. This approach has been validated and demonstrated with toad nerve model treated with crude Naja kaouthia (NK) venom and also shows the effect of Phospholipase A2 and three finger neurotoxin from NK-venom on peripheral nerve. This opens future scope for non-invasive clinical measurement of demyelination.

Speedy and efficient transmission of action potential sequences carrying neuro-signals depends on axon membrane sheath made by myelin¹². Demyelination in nerve axon of peripheral nerve results in reduction of nerve conduction velocity (NCV) occurred in most of neuro diseases such as chronic inflammatory demyelinating polyneuropathy (CIDP)³⁴, Guillain-Barre syndrome (GBS)⁵⁶ etc. In fact, Node of Ranvier (NoR) is distorted by demyelination leading to slow movement of Na⁺/K⁺ ions. Clinical analysis of these diseases requires quantification of demyelination. In this direction, invasive measurement of demyelin/myelin thickness using SEM imaging and nerve biopsy is a destructive and clinically critical and harmful for the patients. Moreover, these techniques have risk for site selection of the damaged nerve and may cause discomfort and infection after surgery for nerve testing⁶⁷. Hodgkin and Huxley first demonstrated quantitative description of axonal membrane currents for giant axon of a squid with the use of electrical circuit model developed by them⁹. Later, experiments were performed on toad model with consideration of node of Ranvier to prove that the membrane currents are dependent on Na⁺, K⁺ ions and other Ca²⁺ ions (contributing leakage current). As seen in previous studies¹⁰–¹², snake venom especially Naja kaouthia venom causes degradation of myelin sheath due to having high percentage of phospholipase A2 (PLA₂), neurotoxins and cytotoxins¹³–¹⁶. In fact, NK-PLA₂ belonging to lypolytic enzyme family catalyzes the hydrolysis of fatty acid esters at position 2 of 1, 2 diacyl-sn-phosphoglycerides producing lysophospholipids and free fatty acids¹⁷. So Naja kaouthia venom is one of the strong candidates for demyelination of myelin sheath due to the presence of NK-PLA₂. Previous studies show that PLA₂ also causes Alzheimer’s disease¹⁸, Multiple Sclerosis (MS)¹⁹, Epilepsy²¹ due to motor dysfunction. In this direction the development of toad nerve model has revolutionized the field of neuroscience especially for human peripheral clinical treatment²²,²³. Moreover, the membrane transport and activity of neurotransmitter are better examined on isolated toad nerve having bundle of axons²⁴,²⁵.

Electric model of a demyelinated nerve. A rigorous theory of electrical circuit model of a demyelinated nerve was made by previous authors²⁶–²⁸. Since frog nerve consists of bundle of axons (Fig. 1(a,b)), H-H electrical circuit model for giant nerve is modified considering bundle of axons as shown in Fig. 1(c). The ephaptic interactions between action potential impulses of parallel axons in a bundle is demonstrated to describe NoR misalignment among axons (Fig. 1(a,d)). The nerve conduction velocity with incorporation of demyelinating factor γ, alignment parameter A and an ephaptic coupling constant α among axon-1 surrounded by six axons derived as

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where, $N$ = number of nerves surrounding a demyelinated nerve (here $N$ is considered to be six), as shown in Fig. 1(b,c). $R = R_0 \frac{(N-1)(1-\gamma)}{(1-\alpha)}$, $\alpha = \frac{R_1}{R_0 + R_2 A (N-1)(1-\gamma)}$, $\gamma$ = demyelination factor which is defined as the ratio of change in myelin thickness due to demyelination to the actual amount of myelin thickness of the nerve. The effect of demyelination factor upon resistance, capacitance and conductance in the circuit has already been discussed in our previous work\textsuperscript{29,30}. The total resistance in

The NCV of toad sciatic nerve decreases with increase of demyelination. The experimental points of black dots are obtained from conduction of action potential (latency between proximal and distal action potential) in sciatic nerve of frog model demyelinated by \textit{Naja kaouthia} crude venom with concentration 0.1, 1.0 and 10 μg/ml. Similarly experimental points of triangle are obtained from sciatic nerves treated with NK-PLA\textsubscript{2} (purified from crude venom). The inset of Fig. 1(e) shows demyelination $\Delta D$ in μm versus concentration of crude venom/NK-PLA\textsubscript{2} ($\Delta D$ is the difference between normal nerve thickness and demyelinated nerve, obtained from SEM images shown in Fig. 2). The solid line and dashed line in inset figure are drawn by using experimental points with minimum deviation. The lines are almost close to each other proving NK-PLA\textsubscript{2} mainly responsible for demyelination of the nerve of toad model.
the circuit is mainly divided into internal resistance ($R_i$) and external resistance ($R_o$). The $I_n$ and $V_n$ are the ionic current and voltage at node $n$. The total ionic current $I_{ion,n}$ at node $n$ consists of current contributed by sodium ion as ($I_{Na,n}$), fast potassium as ($I_{Kf,n}$), slow potassium as ($I_{Ks,n}$) and also leakage current contributed by mainly calcium and chlorine as ($I_{L,n}$), through demyelinated ionic resistances $1/G_{Na}(1 + \gamma)$, $1/G_{Kf}(1 + \gamma)$, $1/G_{Ks}(1 + \gamma)$ and $1/G_{L}(1 + \gamma)$ respectively, where $G_{Na}$, $G_{Kf}$, $G_{Ks}$ and $G_{L}$ are ionic conductance of sodium ion, fast potassium, slow potassium and leakage ions (mainly calcium, chlorine etc.). The voltage $V_n$ at node $n$ depends on $V_{Na}$, $V_{Kf}$, $V_{Ks}$ and $V_{L}$. Nernst (diffusion) potentials of sodium, fast potassium, slow potassium and leakage ions at which corresponding currents return to be zero. $V_{thNa}$, $V_{thKf}$, $V_{thKs}$ and $V_{thL}$ are threshold voltages of sodium, fast potassium, slow potassium and leakage currents start to flow at active node as shown in Fig. 1(d). The values of ionic conductance, Nernst potential and threshold voltage of sodium, slow potassium, fast potassium and leakage ions of frog are obtained from previous works[29,30]. The variation of NCV is determined by using equation (1), decreases with increase of demyelinating factor (Fig. 1(e)). To validate our formulation of neuro-signal conduction, the experiments were performed on toad nerves treated with crude venom of *Naja kaouthia* (NK) for six times and the decrease of myelin sheath (demyelination) was observed every time (Fig. 1(e)).

**Results**

For experimental estimation of demyelination, sciatic nerves were isolated from toad and its storage was made in Ringer’s solution. During experiments on nerve conduction by using AD instruments, the sciatic nerves were kept moist in nerve chamber using Ringer’s solution. The reduction of myelin thickness (i.e., demyelination) with increase of crude venom concentration was confirmed by SEM images in Fig. 2(A–D). We have obtained experimentally propagation of action potential stimuli applied on sciatic nerves treated with different crude venom concentration as shown in Fig. 2(a–d). The nerve conduction signals obtained from proximal and distal action potential nerve treated with 0.1 μg/ml crude venom concentration shows reduction of nerve conduction velocity (NCV) due to demyelination of axons in the nerve (Fig. 2). It also shows the reduction of action potential amplitude with crude venom concentration. The experimental values of NCV in Fig. 1(e)
matches well with theoretical values of NCV which was obtained by using the formulation (equation 1) using MATLAB and these values of NCV may be used to predict the amount of demyelination as shown in the curve of the figure. From the SEM images (Fig. 2(B–D)), the demyelination factor \( \gamma \) of sciatic nerve treated with 0.1 μg/ml, 1.0 μg/ml and 10 μg/ml crude venom concentration are estimated as ~0.21, 0.37 and 0.5 respectively (as shown in supplementary table S1 and supplementary Fig. S1(a)) which are close to those obtained from NCV results of Fig. 1(e). Estimation of demyelination from NCV formulation will avoid the invasive difficulties of SEM imaging and nerve biopsy. The rate of increase of demyelination (\( \Delta D \)) with crude venom concentration is very fast up to 1 μg/ml and it becomes saturated after that (inset of Fig. 1(e)). As seen in Fig. 3, NK-PLA2 present in crude venom is mainly responsible for demyelination as per the following reaction of NK-PLA2 with phospholipids of myelin sheath.

\[
\text{NK-PLA2} + \text{Myelin} \xrightarrow{\text{Hydrolysis}} \text{Lyosphospholipids} + \text{free fatty acids}
\]

The rate of reaction increases with increase of NK-PLA2 molecules (as NK-PLA2 concentration increases with crude venom concentration/dose) and as a result more demyelination takes place with increase in concentration of NK-PLA2 (Fig. 3). The nerve conduction experiment of NK-PLA2 (purified from NK crude venom) treated sciatic nerves shows reduction of NCV due to demyelination in which the variation of demyelination thickness (\( \Delta D \)) with NK-PLA2 concentration for NK-PLA2 treated nerve is almost close to that of crude venom treated nerve proving major contribution of demyelination by NK-PLA2 (Fig. 1(e)). The demyelination factor \( \gamma \) of sciatic nerve (treated with 0.1 μg/ml, 1.0 μg/ml and 10 μg/ml NK-PLA2 concentration) estimated from the SEM images (Fig. 3(A–C)) are also almost close to those obtained from NCV results of Fig. 1(e) as shown in supplementary table S1 and supplementary Fig. S1(b).

The similar results are obtained after repeated nerve conduction experiments of normal sciatic nerve and nerves demyelinated with both crude venom and NK-PLA2 and NCV are estimated with ±SD (standard deviation) statistically to minimize the percentage of error as per standard method. The theoretical curve of NCV versus demyelination factor (Fig. 1(e)) validated with nerve conduction experiments, establish a hypothesis to quantify demyelination in the patients and the demyelination of nerve is obtained from known NCV using...
were determined according to Doley and Mukherjee40 using egg yolk as a substrate. One unit of PLA2 activity was
treated with
Naja kaouthia
sciatic nerves of toads of group A is not treated with crude venom whereas the sciatic nerves of B, C and D groups
demyelination measurements from nerve conduction velocity and divided into four groups (A, B, C and D). The
different concentration of snake venom. We have taken 48 toads of same age (~1–2 months) for validation of
D. melanostictus
toad (Duttaphrynus melanostictus)
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Preparation of Sciatic nerve.

Materials and Methods

Methods

Ethics Statement. Common Asian toads (Duttaphrynus melanostictus) were used for neurotoxicity stud-
ies. All animal experiments were performed in accordance with the guidelines from the Defence Research
Laboratory, Tezpur, Assam (India) under Registration Number 1227/bc/07/CPCSEA and approved by Tezpur
University Animal Ethical Committee (TUAEC) (Approval no: DORD-Pro/TUAEC/10-56/14/Res-06). Efforts
were made to minimize the number as well as sufferings of animals during the experiments.

Animals. For validation of NCV in a demyelinated nerve, we have extracted sciatic nerve from common Asian
toad (D. melanostictus). Further, we have attempted to see the variation of demyelination in the sciatic nerve using
different concentration of snake venom. We have taken 48 toads of same age (~1–2 months) for validation of
demyelination measurements from nerve conduction velocity and divided into four groups (A, B, C and D). The
sciatic nerves of toads of group A is not treated with crude venom whereas the sciatic nerves of B, C and D groups
are treated with Naja kaouthia venomos of 0.1 μg/ml, 1 μg/ml and 10 μg/ml respectively.

Collection of snake venom. Crude venom from Naja kaouthia of North East India was collected from the
wild and stored as explained in Das et al.38. The permission for milking of snakes from Assam was obtained from
Principal Chief Conservator of Forest (Wild Life) and Chief Wild Life Warden of Assam, India (WL/FG.27/tissue
Collection/09 dated 07/10/2011).

Determination of protein content. Protein concentration of the crude venom and purified toxin was
determined according to Lowry's method using BSA as a standard39.

Partial purification of fraction containing Phospholipase A2 (PLA2) and three finger toxin (3FTx).

PLA2 and three finger toxin was purified as described in Das et al.37. Briefly, crude venom was sub-
jected to single step fractionation on RP-HPLC using symmetry C18 column (5 μ, 4.6 × 250 mm, 300 Å) (Waters,
USA). Fractionation was carried out in a linear gradient of buffer B (80% ACN containing 0.1% TFA) in a
pre-equilibrated column with buffer A (0.1% TFA in milli Q water) on a HPLC system (Waters, USA). Eluted
protein peaks were monitored at 215 and 280 nm and collected manually. Considering the retention time of the
protein peaks likely to contain PLA2 and 3FTxs were isolated.

Phospholipase A2 activity assay. PLA2 activities of the crude venom as well as the fractionated peaks
were determined according to Doley and Mukherjee40 using egg yolk as a substrate. One unit of PLA2 activity was
defined as the amount of protein which produces a decrease in 0.01 absorbance in 10 mins at 740 nm.

Preparation of Sciatic nerve. The method as described by Katsuki et al. was followed for sciatic nerve
preparation37. In brief, common Asian toads (Duttaphrynus melanostictus) weighing 30–35 gms of either sex were
decapitated and then pithed. Sciatic nerve of length 4–6 cm and 0.5–1 mm diameter was dissected from lumber
plexus to the knee joint. Throughout the procedure the nerve was continuously flooded with Ringer’s solution.
Finally, the dissected nerve was treated with venom sample and mounted on nerve chamber (AD Instruments,
Powerlabs, Australia) containing Ringer’s solution.

Recording of compound action potential (CAP) and determination of nerve conduction velocity
(NCV). Various concentrations of crude NK venom and partially purified PLA2 from the crude venom (0.1 μg/μl,
1 μg/μl and 10 μg/μl) was treated in-vitro to the dissected nerve for 2 mins. Standard techniques for extracellular recordings were followed. CAP was measured in a nerve chamber (AD Instruments, Powerlabs, Australia) equipped with 15 stainless steel electrodes as shown in Fig. 5. A dual Bio Amp/stimulator was used to obtain and

Figure 4. Mechanism of channel blocking in cell membrane. We have performed channel blocking of sciatic nerve using 3FTx neurotoxin for 5–6 times and the results obtained from repeated experiments are almost same. We have shown the results which are estimated statistically. (a) transportation of Na⁺ into cell through cell membrane and K⁺ transportation leaving from cell through membrane and provides saltatory movement of the nerve impulse (action potential). (b) Shows less blocking of Na⁺ and K⁺ channel by NK crude venom in which 3FT weak neurotoxin binds with acetylcholine receptors (AchR) and then blocks channel. Number of blocking is small if crude venom concentration is less. (c) Represents more blocking if crude venom concentration becomes more because of presence of more 3FT neurotoxin. (d) Represents most of channels are blocked if crude venom concentration is very high (more than 10 μg/ml). (e) Neuro conduction signal (proximal and distal action potential) obtained by using AD instruments. There is a reduction of peak of distal action potential with respect to proximal action potential showing Na⁺ and K⁺ channel blocking. (f) Neuro conduction signal of 1 μg/ml 3FTx-weak neurotoxin treated sciatic nerve shows a sharp reduction of peak of action potential. (g) Neuro conduction signal of 10 μg/ml 3FTx neurotoxin shows almost same sharp reduction of distal action potential peak. As in 1 μg/ml 3FTx treated sciatic nerve, most of channels are blocked (Fig. 4(d)), same reduction of distal action potential was observed even after treatment of 10% 3FTx treated sciatic nerve. (h) SEM image of toad sciatic nerve treated with 10 μg/ml 3FTx shows no reduction of myelin sheath even at high concentration. The myelin thickness of which is found to be 1.74 ± 0.18 μm, which is similar to normal sciatic nerve.
record CAP. In brief, the dissected sciatic nerve was externally stimulated with a frequency of 1 Hz where pulses at 0.1 ms duration were used to determine the CAP31. The setup was comprised of two male BNC (Bayonet Neill–Concelman) connectors to three micro-hooks constructed of gold-plated beryllium copper which was used to stimulate the nerve. The electrodes for proximal and distal stimulus recording were placed at a distance of 3 cm. Nerve end with lumber plexus of spinal cord was connected with proximal recording electrode and electrode at nerve end connecting knee joint acted as distal recording electrode. Each experiment for recording of CAP was completed within 20 s of timeframe to avoid drying of the dissected nerve. CAP was analyzed by SCOPE (Powerlabs, Australia). Nerve only treated with Ringer’s solution was considered as control. The experiments were performed six times and ±SD was estimated statistically to minimize the percentage of error as per standard method31. The NCV was calculated using the latency and distance data from the signal.

SEM imaging of sciatic nerve treated with different concentration of venom. Sciatic nerves were subjected for SEM analysis to check the effect of crude venom on morphology of the isolated nerve. Untreated nerve is served as control for the analysis. Sciatic nerve preparations were incubated for 15 mins with various concentrations of crude venom (0.1 mg/ml–10 mg/ml) and primary fixation was done using 2.5% gluteraldehyde for 4 hr. Further the nerve was subjected for secondary fixation using 1% OsO4 (Osmium tetroxide) for 4 hr for better penetration. Cross section of sciatic nerve was made by slicing at a length of 10 mm using glass cutter in a microtome maintaining uniformity. The sliced nerve segments were further observed under SEM for the structural change. Demyelination or reduction of myelin thickness considering 6–7 nerves in a bundle was quantified with the use of measuring scale installed in the SEM.

References
1. Jessen, K. R. & Mirsky, R. The origin and development of axon ensheathment and myelin growth. Nat. Rev. Neurosci. 6, 671–690 (2005).
2. Rasband, M. N. Composition, assembly, and maintenance of excitable membrane domains in myelinated axons. Semin. Cell Dev. Biol. 22, 178–184 (2011).
3. Stephanova, D. L., Daskalova, M. S. & Alexandrov, A. S. Differences in membrane properties in simulated case of demyelinating neuropathies, intermodal focal demyelination with conduction block. J. Biol. Phys. 32, 129–144 (2006).
4. Toothaker, T. B. & Brannagan, T. H. Chronic inflammatory demyelinating polyneuropathies: current treatment strategies. Curr. Neurol. Neurosci. Rep. 7(1), 63–70 (2007).
5. Berg, B. V. D. et al. Guillain-Barre syndrome: pathogenesis, diagnosis, treatment and prognosis. Nat. Rev. Neurol. 10, 469–482 (2014).
6. Kuwabara, S. Guillain-Barre Syndrome. Curr. Neurol. Neurosci. Rep. 7(1), 57–62 (2007).
7. Whitney, M. A. et al. Fluorescent peptides highlight peripheral nerves during surgery in mice. Nat. Biotechnol. 29(4), 352–356 (2011).
8. Goldman, L. & Aussiello, D. Shy ME. Peripheral neuropathies. Cecil Medicine, 23rd edn. (eds. Goldman, L.) Ch. 446 (Elsevier, Philadelphia, 2007).
9. Hodgkin, A. L. & Huxley, A. F. A quantitative description of membrane and its application to conduction and excitation in nerve. J. Physiol. 117, 500–544 (1952).
10. Banik, N. L., Gohil, K. & Davison, A. N. The action of Snake Venom, Phospholipase A and Trypsin on Purified Myelin in vitro. Biochem. J. 159, 273–277 (1976).
11. Mukherjee, A. K. Phospholipase A2-interacting weak neurotoxins from venom of monocled cobra Naja kaouthia display cell-specific cytotoxicity. Toxicon 51, 1538–1543 (2008).
12. Ogar, A. Y., Rževski, D. L., Murashov, A. N., Tselin, V. I. & Utkin, Y. N. Weak neurotoxin from Naja kaouthia cobra venom affects haemodynamic regulation by acting on acetylcholine receptors. Toxicon 45, 93–99 (2005).
13. Mukherjee, A. K. Correlation between the phospholipids domains of the target cell membrane and the extent of Naja kaouthia PLA2-induced membrane damage: Evidence of distinct catalytic and cytotoxic sites in PLA2 molecules. Biochim. Biophys. Acta 1770(2), 187–195 (2007).
14. Kini, R. M. Excitement ahead: structure, function and mechanism of snake venom phospholipase A2 enzyme. *Toxicon* **42**, 827–840 (2003).
15. Kini, R. M. Evolution of three-finger toxins - A versatile mini protein scaffold. *Acta Chim. Slov.* **58**, 693–701 (2011).
16. Kini R. M. & Doley, R. Structure, function and evolution of three-finger toxins: Mini proteins with multiple targets. *Toxicon* **56**, 855–867 (2010).
17. Doley, R., King, G. F. & Mukherjee, A. K. Differential hydrolysis of erythrocyte and mitochondrial membrane phospholipids by two phospholipase A2 isoenzymes (NK-PLA2-I and NK-PLA2-II) from the venom of the Indian monocled cobra *Naja kaouthia*. *Arch. Biochem. Biophys.* **425**, 1–13 (2004).
18. Yagami, T. Cerebral arachidonate cascade in dementia: Alzheimer’s disease and vascular dementia. *Curr. Neuropharmacol.* **4(1)**, 87–100 (2006).
19. Ransohoff, R. M. Animal models of multiple sclerosis: the good, the bad and the bottom line. *Nat. Neurosci.* **15**(8), 1074–1077 (2012).
20. Nylander, A. & Hafler, D. A. Multiple sclerosis. *J. Clin. Invest.* **112**, 1188–1180 (2008).
21. Yegin, A., Akbas, S. H., Ozben, T. & Korgun, D. K. Secretory phospholipase A2 and phospholipids in neural membranes in an experimental epilepsy model. *Acta Neurol. Scand.* **106**(5), 258–262 (2002).
22. Steinbach, J. H. & Stevens, C. F. Neuromuscular Transmission. *Frog Neurobiology A Handbook*, (eds. Llinas, R. & Precht, W.) Ch. 2, 33–77 (Springer, New York, 1976).
23. Purvaningsih, E. H., Ibrahim, N. & Zain, H. The nerve protection and in vivo therapeutic effect of Acalypha indica extract in frogs, *Med. J. Indones.* **19**(2), 96–102 (2010).
24. Wallingford, J. B., Liu, K. J. & Zheng, Y. Xenopus, *Curr. Biol.* **20**(6), 263–264 (2010).
25. Harland, R. M. & Grainer, R. M. Xenopus research: metamorphosis by genetics and genomics. *Trends Genet.* **27**(12), 507–515 (2011).
26. Jiang, W., Chen, L. & Yang, F. X. Analysis and control of the bifurcation of Hodgkin Huxley model. *Chaos Soliton. Fract.* **31**, 247–256 (2007).
27. Koles, Z. J. & Rasmisky, M. A computer simulation of conduction in demyelinated nerve fibers, *J. Physiol.* **227**, 351–364 (1972).
28. Schnabel, V. & Johannes, J. S. Evaluation of the Cable Model for Electrical Stimulation of unmyelinated Nerve Fibers. *IEEE Trans. Biomed. Eng.* **48**(9), 1027–1033 (2001).
29. Das, H. K. & Sahu, P. P. Effect of Demyelination on conduction velocity in demyelinating polyneuropathic patients. *Int. J. Currit. Sci. Tech.* **1**(4), 101–104 (2013).
30. Das, H. K. & Sahu, P. P. Coupled Nerve: A Technique to increase the Nerve Conduction Velocity in Demyelinating Polyneuropathic Patients. *Proc. Eng.* **64**, 275–282 (2013).
31. Katsuki, R. et al. Phospholipase A2 isozymes (NK-PLA2-I and NK-PLA2-II) from the venom of the Indian monocled cobra *Naja kaouthia* (2015). 32. Tan, K. Y., Tan, C. H., Fung, S. Y. & Tan, N. H. Venomics, lethality and neutralization of *Naja kaouthia* (monocled cobra) venoms from three different geographical regions of Southeast Asia. *J Proteomics* **120**, 105–125 (2015).
33. De Weille, J. R., Schweitz, H., Maes, P., Tartar, A. & Lazdunnii, M. Calcispetine, a peptide isolated from black mamba venom, is a specific blocker of the L-type calcium channel. *Proc. Natl. Acad. Sci. U.S.A.* **88**, 2437–40 (1991).
34. Katoh, E. et al. Structural basis for the biological activity of dendrotoxin-I, a potent potassium channel blocker. *Biopolymers* **54**, 44–57 (2000).
35. Smith, L. A., Lafaye, P. J., LaPenotiere, H. F., Spain, T. & Dolly, J. O. Cloning and functional expression of dendrototoxin K from black mamba, a K+ channel blocker. *Biochem.* **32**, 5692–5697 (1993).
36. Yasuda, O. et al. FS2: a mamba venom toxin, is a specific blocker of the L-type calcium channels. *Artery* **21**, 287–302 (1994).
37. Das, D., Sharma, M., Das, H. K., Sahu, P. P. & Doley, R. Purification and Characterization of Nk-3FTx, A Three Finger Toxin (3FTx) from the venom of North East Indian Monocled Cobra. *J. Biochem. Mol. Toxicol.*, doi:10.1002/jbt.21734 (2015).
38. Das, D., Ura, N., Hiramath, V., Vishwanath, B. S. & Doley, R. Biochemical and biological characterization of *Naja kaouthia* venom from North-East India and its neutralization by polyclonal antivenom. *J. Venom Res.* **4**, 31–38 (2013).
39. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275 (1951).
40. Doley, R. & Mukherjee, A. K. Purification and characterization of an anticoagulant phospholipase A2 from Indian monocled cobra (*Naja kaouthia*) venom. *Toxicon* **41**, 81–91 (2003).

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**Author Contributions**

H.K.D., D.D. and P.P.S. contributed equally in performing the animal experiments. H.K.D. and P.P.S. wrote the main manuscript text and prepared Figures 1–4. D.D. and R.D. thoroughly reviewed the manuscript.

**Additional Information**

**Supplementary information** accompanies this paper at http://www.nature.com/srep

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