CONCLUSION
Conclusion

In this present investigation, isoenzyme pattern of phospholipase A\textsubscript{2} enzymes of *Daboia russelli* venom was studied. This study reveals the presence of twelve basic, one neutral and six acidic PLA\textsubscript{2}s i.e., a total of 19 PLA\textsubscript{2} enzymes in Russell’s viper venom of east India origin.

Three PLA\textsubscript{2} enzymes of different nature (i.e., one acidic, one neutral and one basic in nature) were isolated and purified to homogeneity by combination of ion exchange, gel filtration and RP-HPLC. RVVA-PLA\textsubscript{2}-I, RVVN-PLA\textsubscript{2}-I and RVVB-PLA\textsubscript{2}-I has a molecular mass of 58.0 kDa (homodimer), 12.8 kDa (monomer) and 6.7 kDa (monomer) respectively and were determined by SDS-PAGE and ESI/MS analysis. The secondary structure of these three PLA\textsubscript{2}s showed strong α-helical structure which is well consistent with secondary structure of other snake venom PLA\textsubscript{2} enzymes. Further, heat inactivation study as well as secondary structure of all these three PLA\textsubscript{2}s from RVV documented that they are thermostable in nature like most of the earlier reported PLA\textsubscript{2}s of snake venom.

The understanding of structure-function relationships in PLA\textsubscript{2} enzymes is complicated, and contradictory results are presented to explain the anticoagulant action of venom PLA\textsubscript{2} enzymes. However, this present study advocates that anticoagulant activity of PLA\textsubscript{2}s is mainly attributed to the enzymatic hydrolysis of pro-coagulant phospholipids and partly by binding to the phospholipids of plasma by a non-enzymatic mechanism. Therefore, it may be concluded that strong anticoagulant effect of the PLA\textsubscript{2} under study is contributed by both enzymatic and non-enzymatic mechanisms.

RVVA-PLA\textsubscript{2}-I and RVVB-PLA\textsubscript{2}-I preferentially hydrolysed phospholipids of erythrocytes membranes compared to liver mitochondrial
membranes whereas in case of RVVN-PLA₂-I this preference was reversed. None of these PLA₂s could hydrolyze HT-29 colon adenocarcinoma cell membrane phospholipids within 4 hours of treatment suggesting a differential mode of attack on membrane phospholipids by these three PLA₂s purified from Russell’s viper venom. These PLA₂s show distinct preference for hydrolyzing those phospholipid domain(s) of intact membranes which are specific towards particular PLA₂s.

The GC analysis of saturated/unsaturated fatty acids release pattern from intact mitochondrial and erythrocytes membranes after the addition of RVVA-PLA₂-I and RVVB-PLA₂-I suggested the existence of a significantly greater number of RVVA-PLA₂-I and RVVB-PLA₂-I sensitive domains in erythrocyte membrane as compared to mitochondrion membrane while RVVN-PLA₂-I sensitive domains exist more in mitochondrial membrane than the erythrocytes. Further studies to identify the nature of these PLA₂ sensitive membrane domains are in progress.

None of the PLA₂s in the present study at tested dose showed lethality in mice even after 48 hours injection suggesting that they are devoid of any lethal effects in mice. Moreover, immunodiffusion test documented very week immunogenic nature of RVVA-PLA₂-I, RVVN-PLA₂-I and RVVB-PLA₂-I.

In this study, four medicinal plants which have been used traditionally by the local people of North-East India were screened for anti snake venom effect. Methanol extract of leaves of A. indica, C. sinensis and X. Strumarium and root of A. marmelos has showed significant inhibitory activity against crude RVV and also against the three purified phospholipase A₂ (PLA₂) enzymes of Daboia russelli venom. AIPLA1 (A. indica PLA₂ inhibitor) purified from A. indica leave extract and also the other three plant extracts are highly promising source for the development of novel anti-snake venom drug in future.