SUMMARY AND CONCLUSIONS
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Literature concerning to snake venoms and their toxins in general and manifestations (both local and systemic effects) of snake envenomation has been reviewed. Further, the role of extracellular matrix (ECM) degrading enzymes including Snake venom metalloproteinases (SVMPs) and Snake venom hyaluronidases (SVHYs) in snakebite pathology in particular is reviewed. In addition, limitations of antivenom therapy and importance of inhibitors of ECM degrading enzymes in snakebite management is discussed and a literature survey is provided for inhibitors of SVMPs and SVHYs. Furthermore, the significance of clinically approved compounds as an inhibitors of venom hydrolytic enzymes is conferred and literature pertaining to N-acetylcysteine (NAC) and citalopram derivative (DFD, 1-(3-Dimethylaminopropyl)-1-(4-fluorophenyl)-3-oxo-1,3-dihydroisobenzofuran-5-carbonitrile), clinically approved compounds and their derivatives is reviewed.

Initial evaluation of inhibitory efficacy of NAC against various hydrolytic enzymes of viper venoms (Echis carinatus and Vipera russelli) revealed specificity of NAC towards proteases and hyaluronidases whereas NAC did not inhibit PLA$_2$ and 5’ nucleotidase activity. The study also assessed the inhibitory potency of NAC against hemorrhagic activity of both venoms. NAC showed complete inhibition of hemorrhagic activity when incubated with venom prior to testing whereas, little inhibition was observed when venom and NAC were injected independently. Further, the study explored the involvement of possible functional group of NAC (acetyl and thiol) in exerting inhibition of hemorrhage. The observed inhibition of hemorrhage was likely due to zinc chelation as addressed by UV-VIS spectral studies. Further, docking predictions suggested the role of thiol and acetyl groups of NAC in the
inhibition of SVMPs and SVHYs. Along the lines of NAC, N-acetylcysteine amide (NACA) inhibited the hemorrhagic activity of viper venoms efficiently and inhibitory potency of both NAC and NACA was found to be similar.

Further, efficacy of NAC in inhibiting hyaluronidases from different source (venom, testis and serum) was studied. NAC inhibited the enzyme activities dose dependently. Double reciprocal plot analysis was performed to know the type of inhibition and NAC was found to be noncompetitive inhibitor. The study also emphasized the role of thiol group of NAC and glutathione in exerting inhibition. In addition, mechanism of inhibition was established by docking and molecular dynamics (MD) simulation studies for venom and human hyaluronidase. NAC was found to interact with the residues of active site amino acids through hydrogen bond and hydrophobic interactions and also induced changes in dihedral angle of active site amino acids of both the enzymes thereby exerting inhibition. Docking and MD simulation studies also addressed the similar mechanism of inhibition by glutathione.

Further, in the study the inhibitory efficacy of DFD against five medically important viper venoms (Echis carinatus, Echis ocelatus, Echis carinatus sochureki, Echis carinatus leakeyi and Crotalus atrox) has been established. The histological examinations revealed that the compound DFD effectively neutralize the basement membrane degradation and accumulation of inflammatory leukocytes at the site venom injection. Moreover, DFD inhibited PLA2 activities of Crotalus atrox and Echis carinatus leakeyi venoms. Further, docking predictions revealed the binding of DFD to hydrophobic pocket of SVMPs without chelating Zn^{2+} in the active site.
MAJOR FINDINGS OF THE STUDY

- Inhibitory property of NAC against hemorrhage induced by viper venoms and role of functional group of NAC (acetyl and thiol group) in exerting inhibition has been established.

- Inhibitory efficacy of NACA against viper venom induced hemorrhage has been established.

- Inhibitory efficacy of NAC against hyaluronidases from different source (venom, testis and serum), kinetics of inhibition and role of functional group of NAC in exerting inhibition has been addressed.

- Docking and molecular dynamic simulation studies were performed for ligand (NAC and glutathione) and protein (venom and mammalian hyaluronidase) complexes and mechanism of inhibition has been discussed.

- Inhibitory potency of DFD against hemorrhagic activity of five different viper venoms has been established.

In conclusion, the study provides an insight into the role of ECM degrading enzymes (SVMPs and SVHYs) in local pathology of snake envenomation and importance of their inhibition in snakebite management. Inhibition of these spreading factors not only reduces the local tissue damage but also extends the survival time of the victim. In light of the above, present study underlines the need for supportive therapy including topical applications of inhibitors of locally acting enzymes along with regular antiserum therapy. A low molecular weight, easily diffusible compounds which are already in clinical use with low toxicity and good pharmacokinetic profile would be of great standpoint in snakebite management and could facilitate a transit from preclinical to clinical scenario. NAC, NACA and DFD, efficiently inhibited the hemorrhage induced by various medically important viper venoms. Thus it appears
that these compounds are of great value as first aid agents and could complement the antivenom therapy. Moreover, as venom hydrolytic enzymes including SVMPs, SVHYs and PLA$_2$s are homologues to mammalian enzymes including MMPs, hyaluronidases and PLA$_2$s, these inhibitors may find significant importance in developing therapeutic prototypes and lead compounds for various human diseases and ailments.