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## Summary and conclusion

*O. hannah* venom interfered in plasma clot formation, clot dissolution and platelet aggregation functions. It exhibited pro-coagulant activity by decreasing the recalcification time of citrated human plasma dose dependently. It also actively interfered in all the tested blood/plasma coagulation experiments such as APTT, PT, TCT, bleeding time and plasmin like activity. *O. hannah* venom moderately decreased aPTT as well as PT when compared with the other three venoms (*N. naja*, *D. russellii* and *E. carinatus*). It decreased TCT and the activity was dose dependent. It showed significant fibrinolytic activity in which it specifically cleaved the B $\beta$ -chain both dose and time dependently and fibrinolytic activity in which it cleaved the  $\alpha$ -polymer partially but completely degraded the  $\alpha$ -chain. Further, the fibrinolytic activities deciphered by *O. hannah* venom though varied in terms of its specificity in cleaving the protein bands of the fibrin, the intensity of degradation was on par with the activities shown by the other three venoms of the sub-continent mentioned above.

Further, in defibrinogenation assay involving fibrinogen estimation in the blood of experimental mice, revealed the MDD of 10  $\mu$ g. The fibrinogen content was not detected in blood for 24 hours when administered MDD of *O. hannah* venom as compared with the PBS injected control mice which showed fibrinogen content of 242  $\pm$  15 mg/dl. The *O. hannah* venom exhibited inhibitory activities on the platelet aggregation process induced by agonists such as collagen, ADP, epinephrine and thrombin similar to the inhibitory activities exhibited by *N. naja*, *D. russellii*, and *E. carinatus* venoms but with varied percentage. *O. hannah* venom caused > 90% inhibition in thrombin induced platelet aggregation in PRP, 36 % inhibition in ADP induced aggregation and 85% in case of epinephrine induced aggregation.

King cobra venom was tested for its cross-reactivity with the two locally available commercial antivenoms named as BSV and VB (produced and supplied by two firms namely Bharat Serum and Vaccines, Maharashtra, and Vins Bioproducts, Andhra Pradesh respectively) in Ouchterlony double immune-diffusion method, ELISA and western blot experiments. BSV and VB antivenoms showed no precipitin bands of

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cross-reactivity with the *O. hannah* venom in ODD, while in both ELISA and Western blot experiments BSV and VB showed very feeble cross-reactivity which accounted to about 10 folds lesser sensitivity seen for other three venoms.

The commercial polyvalent BSV, VB and rabbit raised OH-IgG monovalent antivenoms were tested independently for the neutralization of enzymatic activities of *O. hannah* venom. Both BSV and VB antivenoms did not neutralize while OH-IgG effectively neutralized the protease, hyaluronidase and phospholipase activities of the venom at venom to OH-IgG ratio (w/w) of 1:12, 1:12 and 1:14 ratios respectively. BSV and VB both antivenoms marginally inhibited the pro-coagulant activity of *O. hannah* venom however, with VB exhibiting little more inhibition over BSV. In contrast, OH-IgG antivenom completely inhibited the pro-coagulant activity of the venom at venom: OH-IgG ratios (w/w) of 1:9. Similarly, BSV and VB antivenoms marginally reduced the *O. hannah* venom induced elevated levels of creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) enzymes whereas OH-IgG antivenom completely inhibited *O. hannah* venom induced elevated levels of CPK and LDH enzymes at venom to OH-IgG ratios (w/w) of 1: 13 and 1:15. Further, OH-IgG monovalent antivenom effectively neutralized the edema inducing activity of *O. hannah* venom at venom: OH-IgG ratio (w/w) of 1:9 in contrast to BSV and VB polyvalent antivenoms that showed very little or no inhibition of edema formation by the venom.

Therefore, it can be emphasized that any likely use of the therapeutic commercial polyvalent snake antivenoms (BSV and VB) available in the region to treat the possible life threatening accidental king cobra bite is ruled out since the locally available commercial antivenoms were found to be ineffective towards the king cobra venom. Hence, this study offers scope for making efficient therapeutic antivenom that can neutralize king cobra venom and that could be used to treat the accidental king cobra bite.