UGC Major Research Project
Summary of Final Report
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Identification and characterization of novel peptides of clinical importance from salivary glands of *Hyalomma* ticks

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1. Title of the Project: **"Identification and characterization of novel peptides of clinical importance from salivary glands of *Hyalomma* ticks".**

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SUMMARY

Ticks are of vast importance due to their ability to transmit an impressive variety of infectious agents and to cause direct injury by piercing host skin. Despite the hosts' armoury of rejection mechanisms, the tick manages to remain attached and achieve engorgement. Successful feeding of ticks relies on a pharmacy of chemicals located in their complex salivary glands and secreted tick saliva. These chemicals in the salivary glands of the ticks could inhibit host thrombotic, fibrinogenolytic, platelet aggregating and inflammatory responses through variety of mechanisms. So the present study was planned to obtain the functional information on status of antithrombotic, fibrinogenolytic and anti-platelet aggregating peptides in the salivary glands of engorging *H. a. anatolicum* and *H. dromedarii* ticks with a view that these information's could be utilized in raising vaccines, designing synthetic peptides or peptidomimetics which could be further developed as novel therapeutics.

To fulfil the objectives, partially fed adult female *H. a. anatolicum* and *H. dromedarii* ticks were collected from cattle, buffaloes and camels from the villages around Hisar. The female ticks were glued to the bottom of a Petri dish with their dorsal surface upward and placed on ice for 20 min. Using fine scalpel blade and fine tip forceps,

ticks were incised along the dorsal-lateral margin, and the dorsal integuments were removed under a stereoscopic dissection microscope. Then the non-infected salivary glands were removed and transferred into phosphate buffer solution (PBS) containing cocktail of protease inhibitors and were kept at -40°C before the extract was made. The salivary glands from ticks were homogenized under ice using tissue homogenizer in PBS containing cocktail of protease inhibitors. The homogenate was centrifuged at 10000 rpm for 15 min at 4°C. Then the supernatant was either used a fresh or stored at -40°C and used for molecular polymorphism and identification and characterization of anti-thrombotic, fibrinigenolytic peptides in salivary gland extract fractions. Sephadex G-50 column was used for fractionation and 15% SDS-PAGE was used for molecular characterization. 2D-gel electrophoresis and mass spectrometric analysis was done for identification of proteins. Salivary gland antioxidant status was also estimated. Salivary gland tissues were digested to estimate trace elements viz. copper, zinc, manganese and iron concentrations.

Under this project comparative protein profiles of salivary gland extracts of *Hyalomma anatolicum* and *Hyalomma dromedarii* ticks have been done. The two species under investigation showed differences in the number of protein bands, their relative flows, quantitative expressions and their molecular weights. The protein bands numbering 20 and 22 in range of 10kDa to 116kDa and 1 and 3 in range of greater than 116 kDa in *H. anatolicum* and *H. dromedraii* respectively have been reported. The electrophoretic finger printing of peptides reported could be used to identify the *H. a. anatolicum* and *H. dromedarii* ticks if their morphological characters and adjacent structures were damaged due to any reason. So the peptide finger print patterns could serve as an anglestone in identifying species and strains of ticks by using the image analysis system. It has also been reported that an extract of salivary gland of *H. anatolicum* prolonged both PT and APTT, suggesting the presence of an inhibitor of thrombin in salivary gland. These activities were found in fractions 6, 7 and 8. 2D-gel electrophoresis and mass spectrometric analysis revealed a protein band of 27 kDa having anti-thrombotic, fibrinogenolytic and antiplatelet aggregating activities.

When anti-inflammatory activity in these fractions were analysed and compared with the crude extract, the highest value was found in fraction-1 and the lowest in

fraction-6. This suggests that the three peptides of molecular weight 27.8 kDa, 32.2 kDa and 48.2 kDa might be responsible for anti-inflammatory activity. These peptides could have implications in abrogating certain inflammatory diseases of human and animal like rheumatoid arthritis, allergic asthma etc. *H. dromedarii* ticks were found to have more anti-oxidant status to withstand hosts' anti-oxidant stresses for a longer time than *H. a. anatolicum* to suck ample quantity of blood. If anti-oxidant defences of these ticks are disturbed and free radical formation in hosts' body are increased, the damage to the ticks could be more pronounced for controlling tick-borne diseases.

The differences in molecular bases of salivary gland proteins could also have implications for pathogen-vector-host interactions and for analytical strategies in molecular biology research. Such molecular diversities between these two species would also be relevant to the mechanism (s) that might have provided *Hyalomma dromedarii* to suck large amount of blood from the host very successfully and with the ability to adapt rapidly to changed environmental conditions. The results clearly show the variability in biochemical mechanisms of these two species.

The functional information so obtained in the present in the salivary glands of engorging *H. a. anatolicum* and *H. dromedarii* ticks could be utilized in raising vaccines, designing synthetic peptides or peptidomimetics which could be further developed as novel therapeutics for prevention and treatment of blood vascular diseases.