**Abstract**

Thrombosis and related cardiovascular disorder are one of the major blood disorders of mankind.

Present day anticoagulant drugs to counter these disease conditions are expensive and give rise to

harmful side effects. Heparin and Warfarin are the two most widely used anticoagulants for the

treatment of thrombotic disorders like deep vein thrombosis, acute myocardial infarction,

thromboembolism and prevention of systemic embolism in patients with atrial fibrillation. One of the major limitations of these anticoagulants are their adverse side effects including hemorrhage, necrosis, osteoporosis and induced thrombocytopenia. Search for the newer class of anticoagulants with lower side effects and capable of digesting existing blood clots gained importance during the past two decades. Snake venom was found to be one of the richest natural sources for anticoagulant factors, mostly metalloproteinases. Some of the SVMPs are undergoing clinical trials worldwide for treating blood disorders. Ancrod / Viprinex is a defibrinogenating agent derived from the venom of the Malayan pit viper. Aggrostat puede from saw-scaled viper venom is presently used in combination with heparin and aspirin for management of unstable angina or myocardial infarction. It is also prescribed to patients who have undergone percutaneous trans-luminal coronary angioplasty to decrease the rate of refractory ischemic condition. But the search for ideal anticoagulant with minimal side effects has not ended.

In this study, we have purified two anticoagulant proteins form Indian monocled cobra venom

using various chromatographic techniques and named them as KT-6.9 and NKV 66.

KT-6.9 is a low molecular weight (6.9 kDa) polypeptide with strong antiplatelet properties. It has

sequence homology with several 3FTx family members. KT-6.9 specifically inhibited ADP,

thrombin and arachidonic acid induced platelet aggregation in a dose dependent manner which

emphasize the possibility of KT-6.9 binding to G protein coupled receptors (GPCRs). SPR analysisusing BIAcore X100 and other protein binding studies revealed the binding of KT-6.9 on theplatelet surface. Neurotoxicity studies on RPND models showed dose dependent and time

dependent inhibition of muscle contraction when treated with KT-6.9. It also inhibited

acetylcholine induced muscle contraction. KT-6.9 probably binding competitively with

acetylcholine on to post synaptic acetylcholine receptors on muscle cells.

NKV 66 is a 66 kDa metalloproteinase anticoagulant protein which showed chain specific

fibrinogenolytic activity where it has digested alpha chain of bovine fibrinogen in a dose and time dependent manner. NKV 66 is not a phospholipase and showed minimal hemolytic activity when tested on human RBC. NKV 66 completely hydrolyzed fibrin clots developed *in vitro* in 18 hours.Anticoagulant activity of NKV 66 was totally inhibited by EDTA treatment suggesting its

metalloproteinase activity whereas phenyl methyl sulfonyl fluoride treatment (PMSF), a serine

protease inhibitor did not affect its anticoagulant activity. NKV 66 was found highly thermostable and even survived exposure to 100º C for 1 min. Cleaving di-sulphide bonds by β-mercaptoethanol caused complete loss of activity and unfolding the protein with 4M urea also caused NKV 66 to loose of activity. NKV 66 inhibited ADP and collagen induced platelet aggregation process in a dose dependent manner possibly by enzymatic degradation of platelet receptors or by digesting fibrinogen and making it unavailable for platelet aggregation. NKV 66 was tested for disintegrin like activity on A549 cell lines. NKV 66 at concentration of (100μg) clearly affected the morphology of A549 cells. Most cells assumed a rounded appearance and detached from the basement membrane. To further assess the disintegrin like activity of NKV 66 cell adhesion assay was performed using collagen treated plates. About forty percent reduction in the cell adhesion was observed in case of NKV 66 treated cells. The results indicate disintegrins like activity of NKV 66 on A549 cells.