CHAPTER I

INTRODUCTION

1.1 Background

Cerebrovascular disease, stroke and transient ischaemic attacks (TIAs) are some of the most common causes of morbidity and mortality (Gatenby, 2004). Stroke happens when blood flow to the brain stops. There are two different types of stroke, the most common one is an ischemic stroke, caused by blood clot that blocks a blood vessel in brain. The other, less common, is hemorrhagic stroke, caused when blood vessel in the brain ruptures and spills blood into the surrounding tissue. Brain cells in the area begin to die, either because they stop getting the oxygen and nutrients needed for functions, or they are killed by the rupture of the vessel and sudden spill of blood. Two commercially drugs available for thrombolytic therapy, streptokinase and tissue plasminogen activator (TPA).

Streptokinase is a first thrombolytic agent. This potent agent is derived from the beta hemolytic streptococci and is consequently associated with the risk of anaphylaxis. Streptokinase treatment or streptococcal infections induce rapid, strong and persistent immune response.

The other thrombolytic agent is tissue plasminogen activator (TPA). This agent produced by recombinant techniques in sufficient quantities for its clinical usefulness in the treatment of pulmonary embolism, deep vein thrombosis and heart attacks. TPA was produced by recombinant DNA technique. Bacterial clones containing human tissue-

type plasminogen activator (t-PA) cDNA sequences were identified in a cDNA library prepared using gel-fractionated mRNA from human melanoma cells (Pennica *et al.*, 1983).

Tissue plasminogen activator (TPA) has been shown to be effective in treating ischemic stroke. In 1996 the U.S. Food and Drug Administration (FDA) approved the use of TPA to treat ischemic stroke in the first three hours after the onset of symptoms. This makes it very important for people who think they are having a stroke to seek help immediately. If given promptly, TPA can significantly reduce the effects of stroke and reduce permanent disability.

Although, tissue plasminogen activator (TPA) can reduce disability from a heart attack or stroke, but there is also a higher risk of bleeding. Additionally, TCA acts to dissolve blood clots rely on the activation of plasminogen to plasmin. Plasmin may lead to platelet activation that promotes reocculation of thrombosis at the original site of the occlusion.

The venom of *Viperideae* and *Crotalideae* snakes contains a large variety of proteins and peptides affecting the haemostatic system. The protein maybe classified as coagulant, anticoagulant or fibrinolytic factor (Braud *et al.*, 2000). The advancetage, clot dissolve agent found in snake venom do not rely on conversion of plasminogen to plasmin or any other blood bone component for activity (Sanchez *et al.*, 2001). Recently, fibrinolytic enzyme from snake venoms was used as alternative drug for thrombosis (Ouyang *et al.*, 1983). Fibrinolytic enzymes have been purified from many types of

snake venoms biochemical prosperities were support the advantage of enzyme. Example, Fibrolase an enzyme isolated from southern copper head (*Agkistrodon controtrix controtrix*) venom induce rapid clot lyses in vitro by directing on fibrin clot. Fibrolase does not rely on the activation of plasminogen to plasmin which increases thrombin activity and platelet activation.

The problem of fibrinolytic enzyme from snake venom is high production cost and not sufficient quantities for clinical usefulness.

1.2 Objective

To study the toxicity of Malayan pit viper venom including antivenom against toxin, purification and characterization of fibrinolytic protein.

1.3 Scope

Determination of LD_{50}, fibrinolytic, hemorrhagic and gelatinase activites. Virginia opossum serum is used to tests against proteolytic toxin. Finally, purification fibrinolytic protein from the venom by HPLC as well as amino acid sequence, three dimension model calculation on active site and post translation modification will be carried out.