8.1 Conclusion

In the present investigation, active fractions/ components from the aqueous leaf extract of *L. indica* and whole fruit of *M. charantia* affecting the process of blood coagulation and fibrinolysis have been isolated, characterized and their mechanism of anticoagulant action have been evaluated. The possible therapeutic applications of the isolated fractions/protein/component have also been explored.

The first major finding of the study is the purification, characterization and mechanism of anticoagulant action of active anticoagulant preparation (AAP) and active anticoagulant fraction (AAF) from *L. indica* and M. charantia, respectively. AAP demonstrated strong *in vitro* as well as *in vivo* anticoagulant properties by virtue of its ability to inhibit coagulation factors, thrombin and factor Xa whereas anticoagulant mechanism of AAF is correlated to its defibrinogenation and partial antiplatelet activity. Both AAP and AAF were found to be non toxic in tested rodent model, suggesting their probable application as an antithrombotic agent. Moreover, AAP is the first report of a natural fraction isolated from medicinal plants, with the dual ability of inhibiting thrombin and factor Xa. This property makes it an interesting herbal drug with broader specificity. This new basic knowledge about AAP permits us to foresee the development of this herbal drug as a therapeutic agent to prevent and manage unwanted blood clot formation (thrombosis) and fight against hyperfibrinogenemia related disorders.

The second important finding of the present study is the purification, characterization and elucidation of anticoagulant mechanism of a 35 kDa (m/z 34747.5230) serine protease (lunathrombase) showing fibrin(ogen)olytic activity from aqueous leaves from *L. indica* Lunathrombase is an αβ-fibrinogenase, demonstrating anticoagulant activity with its dual inhibition of thrombin and FXa by a non-enzymatic mechanism. Further it inhibited collagen/ADP/arachidonic acid-induced mammalian platelet aggregation, and demonstrated antiplatelet activity via COX-1 inhibition and the upregulation of the cAMP level. Lunathrombase showed *in vitro* thrombolytic activity and was not inhibited by endogenous protease inhibitors α_2 macroglobulin and antiplasmin. Lunathrombase was non-cytotoxic to mammalian cells, non-hemolytic. Lunathrombase (10 mg/kg) did not show toxicity or adverse pharmacological effects in treated animals. Besides, increased tail bleeding time and *in vivo* anticoagulant and defibrinogenation in

mice undoubtedly suggested the pharmacological significance of lunathrombase for the prevention and/or treatment of thrombosis associated cardiovascular disorders.

The third important finding is the characterization of plant-derived β-sitosterol with antithrombotic, *in vivo* anticoagulant, and thrombus-preventing activities in a mouse model. The natural soy bean phytosterol, β-sitosterol (BSS) demonstrated anticoagulant activity by dose-dependent inhibition of thrombin in an uncompetitive manner. BSS demonstrated thrombolytic activity by activating plasminogen albeit it is devoid of protease (fibrinogenolytic) activity. BSS was non-cytotoxic to mammalian cells, non-hemolytic, demonstrated its *in vivo* anticoagulant activity when administered orally, and inhibited k-carrageen-induced thrombus formation in the tails of mice. Our results suggest that dietary supplementation of BSS may help prevent thrombosis-associated cardiovascular disorders.

8.2 Future perspectives

Future studies on the structural aspects of lunathrombase and BSS designed to understand the mechanisms involved in its binding to thrombin and factor Xa as well as its therapeutic potential in terms of its inhibitory effects on thrombin and factor Xa is warranted. A more advanced research focusing on the antiplatelet nature of active fractions/lunathrombase/BSS for development as antiplatelet drugs holds promising ventures. Further, long term sub-acute toxicity study of active fractions of *L. indica* and *M. charantia* will provide deeper insight in drug safety for future clinical trial.