CHAPTER 5

SUMMARY AND CONCLUSION

5.1 SUMMARY AND CONCLUSION

This study is aimed at exploring the effect of experimental hyperlipidemia in vital organs and also the hypolipidemic potential of Ambrex, an amber based herbal formulation (Care & Cure Herbs Ltd.). In the traditional medicinal system, many plants have been identified to have hypolipidemic activity but there is an urge to prove the efficacy of therapeutic drugs scientifically so that it could be beneficial to the society and use of such plant medicines are gaining importance. Hence, the present work is focused on determination of the hypolipidemic activity of Ambrex.

Among the cell lines, the preadipose 3T3-L1 is the widely used and convenient cell culture model; hence in vitro studies were done using 3T3-L1 adipocytes. The study revealed that Ambrex did not affect cell viability at the studied concentrations and therefore did not exert toxicity. The results with Oil red O staining demonstrated that Ambrex treatment inhibited accumulation of lipid droplets during differentiation of 3T3-L1 preadipocytes into adipocytes.

PPAR\(\gamma\), SREBP and leptin are key regulators of adipogenesis and are well known to modulate several target genes of lipid metabolism and thus reduce the adipogenesis in 3T3-L1 adipocytes. Interestingly, Ambrex reduced the mRNA expression of PPAR\(\gamma\), SREBP and leptin. Ambrex treatment
decreased TNFα and iNOS expression thereby plays a dual role in alleviating both adipogenesis and inflammation. Additionally, the results from *in vitro* studies show the influence of Ambrex at the molecular level which provide a basis for better treatment strategy in the management of hyperlipidemia and related disorders.

Hyperlipidemia was successfully induced in male wistar rats and during the regression period of 15 days, the Ambrex (amber based herbal formulation) and Atorvastatin (standard drug) treatments were effective in reducing the increased lipid levels such as serum total cholesterol, triglycerides, LDL cholesterol and VLDL cholesterol. Further there was significant increase in HDL-cholesterol levels. In our study, Ambrex and Atorvastatin protected the animals by reducing the atherogenic index by 62.15% and 61.59% respectively.

In the present study Ambrex significantly reduced the activity of HMG-CoA reductase suggesting its potential as an anti-hypercholesterolemic drug like Atorvastatin (a classic HMG-CoA reductase inhibitor). Ambrex has elevated the lipid metabolizing enzymes viz., lipoprotein lipase and lecithin-cholesterol acyl transferase which play a critical role in maintenance of lipid homeostasis.

Ambrex and Atorvastatin treatment restored the marker enzymes to normal levels establishing their hepatoprotective and renoprotective nature.

In our investigation, it was found that Ambrex and Atorvastatin significantly reduced hepatic and cardiac peroxidation products. The observed beneficial effects of Ambrex may be due to active constituents from which its primary medicinal properties emanate which include withanolides, polyphenols, alkaloids, flavonoids, and succinic acid in Ambrex.
Oxidative stress is an outcome of increased generation of free radicals and deficiency of antioxidants. The present study indicates that Ambrex and Atorvastatin treatments had significantly elevated the levels of antioxidant enzymes in serum, liver and heart tissues (superoxide dismutase, catalase and reduced glutathione).

Histopathological study of liver, heart, aorta and kidney tissues were done using haematoxylin-eosin staining. High fat diet fed animals showed accumulation of fat and degeneration whereas Ambrex and Atorvastatin treated animals have restored normal cellular integrity in different organs, affected by hyperlipidemia.

Overall, the results from the studies presented in this thesis provide evidence that Ambrex possess hypolipidemic potential. The medicinal properties of Ambrex may be due to the holistic effect of catechins, flavonoids, withanolides and alkaloids in *Withania somnifera* (Alam et al 2011), succinic acid in Amber (Norbert 2009), triterpenes and tannic acid in *Shorea robusta* (Poornima 2009), phytosterols in *Orchis mascula* and flavanoids in *Cycas circinalis* (Gurav et al 2013). These compounds are known to exhibit antioxidant properties, prevent free radical generation, increase HDL-cholesterol and reduce LDL-cholesterol thus preventing the risk of cardiovascular diseases in humans.

From the *in vitro* and *in vivo* studies, a conclusion may be drawn on the potential of Ambrex as an effective hypolipidemic drug with minimal side effects, which can be extrapolated to human beings to serve a large population of society suffering from cardiovascular disorders worldwide.
5.2 SCOPE FOR FUTURE WORK

The present study highlights the hypolipidemic potential and other beneficial effects of Ambrex as demonstrated by in vivo and in vitro studies. It also provides information regarding possible mechanism of action of Ambrex.

- The elucidation of active principles in the herbal formulation would be helpful for pharmacological intervention of Ambrex.

- The genes coding for signaling molecules would be further studied to explore the exact molecular mechanism and pathways involved in inhibition of lipid metabolism in organs like liver. This will provide a basis for identifying target molecules for enhancing the therapeutic value of Ambrex.

- Clinical studies need to be carried out to explore the beneficial aspects of Ambrex which would help to serve the society at large.