CHAPTER 4

SUMMARY AND CONCLUSION

4.1 SUMMARY AND CONCLUSION

Myocardial infarction (MI) results from lack of oxygen supply to the working myocardium. It remains the major cause of death throughout the world. The alarming mortality rate due to MI gives rise to the search for new pharmacological agents. The present investigation was designed with respect to preliminary pharmacology screening and extensive biochemical studies to evaluate the protective effects of an Ashwagandha based herbal formulation, Ambrex thereby, provide preclinical data to support its clinical efficacy.

Research over the last decade has shown that Ambrex has strong therapeutic potential against a variety of tissue damage related disorders and it was also found to be nontoxic to the vital organs such as a liver, kidney, heart and nervous system under experimental conditions in rats. Experimental induction of MI was done by administration of Isoproterenol which is a well established model to study the protective role of various cardioprotective agents. The ischemic changes were confirmed by the increase in the level of cardiac marker enzymes in serum and histological studies of tissues. During ischemia pronounced enhancement in lipid peroxidation products and concomitant decrease in antioxidant enzymes in heart were also observed. The antioxidant status was improved in the rats pretreated with Ambrex. Histological studies revealed marked degenerative changes in heart section from rats treated with ISPH alone. The degenerative changes were less pronounced in ISPH administered rats which received Ambrex pretreatment. Isoproterenol resulted
in a significant increase in gene expression of p53, bax and caspase 3 and decrease in bcl2. Administration of Ambrex to the isoproterenol treated rats prevented the increase in p53, caspase 3 and bax mRNA expression and the decrease in bcl-2 mRNA expression, to the control values. A damage to mitochondria, the energy reservoir of the cell, leads to cell death. The evidence demonstrating mitochondrial dysfunction due to the oxidative stress induced during myocardial infarction is overwhelming. Preservation of mitochondrial integrity is of utmost importance in the design of cardio-protective therapies.

In view of the above claims, an attempt was made to further elucidate the relative contribution of the various ingredients present in the Ambrex formulation by exploring the phytochemistry. It showed the presence of many phytochemicals. The SEM image of Ambrex showed individual, non-aggregated nanoparticles with diameter ranging from 65-260 nm. Nano particles size also increased the therapeutic efficacy of Ambrex. The FT-IR study predicted the presence of groups such as -OH, -CH₂ and-CO. The presence of characteristic functional groups like carboxylic acids, amines, amides, sulfur derivatives and polysaccharides necessary for various medicinal properties of herbal formulation were also present. The aqueous extract of Ambrex was further studied by GC-MS. GC-MS analysis of Ambrex extract revealed the presence of six different compounds and among the identified compounds Methyl Commate A was present in appreciable amount.

In the present study, we also focussed on the role of active principle Withaferrin A present in Withaniasomnifera which is the major ingredient present in Ambrex. Pharmacophore modeling was done using PharmaGist to understand the pharmacophoric potential of withaferin A. Clustering of Withaferin with different existing drug molecules for
cardiovascular disease was performed with ChemMine based on structural similarity and physicochemical properties. The ability of natural active component, Withaferin A to interact with different receptors associated with cardiovascular disease was elucidated with various modeling techniques. These studies conclusively revealed Withaferin A as a potent lead compound against multiple targets associated with cardiovascular disease. Interaction energy calculation with different targets associated with cardiovascular disease shows that Withaferin A has more binding affinity for $\beta_1$-adrenergic receptor than the other five targets. It was also revealed that the active component “Withaferin A” of Ashwagandha may not be a good ligand for estrogen receptor and xanthine dehydrogenase. It has beneficial effect for cardiovascular disease acting in different pathways which involves HMG-CoA reductase, Angiotensinogen-converting enzyme and $\beta$-adrenergic receptors. Pharmacophoric modeling and Clustering analysis supported its activity in inhibition of Angiotensinogen-converting enzyme and HMG-CoA reductase respectively.

It is concluded that Ambrex formulation at the dose of 40 mg/kg body weight can mitigate the cardiotoxic effect of isoproterenol in rat heart by acting on multiple targets. The present study provides experimental evidence that Ambrex formulation has a potent free radical scavenging activity because it augmented the myocardial antioxidant enzyme level. The therapeutic efficacy of Ambrex may be due to its cytoprotective and hypolipidemic property. An effort was also made in this study to standardize the Indian medicine Ambrex to meet modern commercial needs for manufacturing. It is hoped that in today’s stressful life people can take the powerful Amber based supplement used centuries ago to prevent myocardial infarction. This strategy is likely to be cost effective and can extend beyond restoring cardiac functionality to extended survival and improved quality of life.
4.2 SCOPE FOR FUTURE WORK

The outcome of the study will rationalize the traditional use of the herbal formulation Ambrex as a potential supplement and a natural antioxidant for cardiovascular related disorders in future.

- Molecular docking provides only a static view of the protein-ligand interaction, hence in future Molecular dynamics simulation studies need to be done on Withaferin A and the target proteins associated with cardiovascular diseases.

- In future the role of active constituents present in the other four herbs in Ambrex which play a pivotal role in the management of myocardial necrosis and their exact mechanism of action needs to be elucidated.

- In view of the increasing incidence of cardiovascular diseases in the human population, clinical studies need to be carried out to explore the beneficial aspects of the formulation which would help to serve the society at large.