CHAPTER 9
OUTCOME AND FUTURE PERSPECTIVE

- In the present study, by carrying out acute and sub-acute toxicity studies of ethanolic extract of *Crataegus oxycantha*, 200mg/kg B.W. of the dose was found to be safe to use as a cardioprotective dose among rats.

- In the second objective, Isoproterenol was used in 3 different doses such as 5 mg/kg B.W, 85 mg/kg B.W and 150 mg/kg B.W Even though levels of serum cardiac markers were highest at 150 mg/kg body weight Isoproterenol, histopathological findings showed that complete tissue destruction was seen at this dose which was fatal for the animal and it is impossible to carry out the further research with above said dose of Isoproterenol. So, in the present study, we took 85 mg/kg body weight of Isoproterenol to induce MI and to carry out the effect of ethanolic extract of *Crataegus oxycantha* in rats.

- In the present study, myocardial markers such as Troponin-I levels were undetected but lactate dehydrogenase, CK-MB, AST and ALT levels increased in the ISO group compared to control. But on treatment, with metoprolol alone or combination of metoprolol with *Crataegus oxycantha*, there was marked decline in the levels of above markers was observed.

- Endothelial markers like V-CAM, CRP and TNF –α levels increased in ISO group compared to control. However, on treatment with metoprolol alone or combination of metoprolol with *Crataegus oxycantha*, there was marked decline observed in above endothelial markers.

- In the present study, histopathological investigations revealed COC positive effects when compared to ISO group.
In the present study, body weight decreased in Isoproterenol Group compared to COC treated group and was improved in metoprolol and combination of metoprolol with *Crataegus* Group. Heart weight, left ventricular weight of the animals were increased in Isoproterenol and combination of metoprolol with *Crataegus* Group reduced the heart weight and left ventricular weight.

This concludes that, phytophenolic content of COC extract is accountable for anti-cardiac remodelling effect in the present study. It could be due to preventing the oxidative damage and scavenging free radicals produced by Isoproterenol.

The TTC staining of the heart revealed that area of infarction was reduced in COC 200 mg/kg BW and metoprolol Group confirming the cardio protective effect of *Crataegus oxycantha* against tissue necrosis induced by Isoproterenol.

In the future studies, the precise molecular mechanism of action of *Crataegus oxycantha* should be evaluated in higher order of animals to affirm the cardioprotective effect of the plant. It is essential to know its exact role in preventing the progression of endothelial dysfunction. Further studies are warranted to determine its intracellular signalling pathways which protect the cardiomyocytes during myocardial infarction and endothelial dysfunction.

1. A nanosensor coated gold sun particles which is able to detect extremely low troponin levels about 8 times lower than the recommended value for detecting AMI need to be studied in the future.

2. In detailed investigations on COC will be performed using other animal models of MI and histopathological changes in the coronary arteries will be assessed in future studies.

3. Matrix remodelling markers like procollagen, laminin, tenascin, and metalloproteinases which appear during cardiac remodelling in the injured areas after reversible or irreversible ischemia will be next goal of research.
4. Endocan, an endothelium-derived circulating PGs and a prognostic marker of cancers, sepsis, inflammation, and acute lung disorders. Effect of COC on endocan would be studied in association with MI and endothelial dysfunction.
APPENDIX

1. Induction of MI by Isoproterenol
   Isoproterenol Hydrochloride - 5g

2. Plant Extraction
   Ethanol (absolute) (5:1 ratio) - 2500 ml

3. CK-MB Kit
   Biotin antibody - 120 µl
   HRP-avidin - 120 µl
   Biotin antibody diluent - 10 ml
   HRP avidin diluent - 10 ml
   Sample diluent - 20 ml
   Wash buffer - 20 ml
   TMB substrate - 10 ml
   Stop solution - 10 ml

4. C-Reactive protein ELISA kit
   HRP conjugate - 120 µl
   Standard (10x) - 250 µl
   Wash buffer - 1 L
   TMB substrate - 12 ml
   Stop solution - 12 ml
5. **Serum troponin –I ELISA kit**

- cTnI stock lyophilised : 0.40 ml
- cTnI Diluent : 12 ml
- cTnI HRP conjugate : 11 ml
- Wash solution (20X) : 50 ml
- TMB reagent : 11 ml
- Stop solution (1N HCL) : 11 ml

6. **TNF-α ELISA kit**

- Rat TNF-α HRP conjugate : 23 ml
- Assay diluent : 12 ml
- Calibrator diluent : 21 ml
- Wash buffer solution : 21 ml
- Color reagent A : 12 ml
- Color reagent B : 12 ml
- Stop solution : 23 ml

7. **V-CAM ELISA Kit**

- Dilution buffer : 20 ml
- Biotin detection antibody : 60 μl
- Antibody dilution buffer : 5 ml
- HRP- Streptavidin conjugate : 60 μl
- SABC dilution buffer : 5 ml
- TMB substrate : 5 ml
- Stop solution : 5 ml
- Wash buffer (25X) : 15 ml

8. **Macroscopic Enzyme mapping**

- TTC stain (2, 3, 5-triphenyltetrazolium chloride) : 1g