CHAPTER 6
DISCUSSION

In the present study, the effect of *Crataegus oxycantha* and its phytopharmacological potential in preventing the changes seen during MI was studied. Due to rich anti-oxidant properties of *Crataegus oxycantha*, its use in the prevention of endothelial dysfunction was elaboratively studied by using Isoproterenol induced oxidative stress in the rat model of myocardial infarction. The following parameters were studied to demonstrate its effects on the biomarkers and histopathological changes in Isoproterenol induced MI rat model.

Phase I

6.1 Acute and sub-acute toxicities of *Crataegus oxycantha*

In the acute toxicity study, rats were treated with different doses of *Crataegus oxycantha* fruit extract from 100 mg/kg B.W to 2000 mg/kg B.W which did not produce any signs of toxicity, behavioural changes, and mortality in the test Groups as compared with controls. In the present study, oral administration of ethanolic extract of COC did not produce any death or signs of acute toxicity up to the dose of 2000 mg/kg B.W during the period of observation, indicating the high margin of safety. There was no significant changes in the body weight, organ weight, haematological parameters and biochemical parameters with different doses of COC showing high safety margin of *Crataegus oxycantha* among rats (Table No.5.1, Table No.5.2, Table No.5.3, Table No.5.4, Table No.5.5) which was found to be statistically significant (P<0.05).

A study designed to evaluate acute and sub-acute toxicological profile of combination of *Crataegeus oxycanthes*, Passiflora incarnata and Valeriana officinalis administered to rats, mice and dogs reported that, combination of 3 plants given at
high doses for a chronic period of 180 days had not caused any serious toxicity indicating the wide margin of safety of combination.

This study where *Crataegus oxycaynthia* was one of the constituent of combination, is strongly supporting the present study, where 2000mg/kg B.W of *Crataegus oxycaynthia* was used and found to be safe among rats. [168]

A double-blind, placebo-controlled clinical trials conducted among 750 patients with NYHA class I – III CHF, revealed that treatment with hawthorn extract in a dose of 240 to 1800 mg/day taken for a period of 21 to 90 days enhanced the quality of life in these patients. This study is in association with the present study where 2000 mg/kg hawthorn extract was found to be safe, [169-171].

A preclinical study conducted in Germany found that hawthorn dose upto 300 mg/kg administered for 26 weeks had not caused any toxicity to rats and dogs and thus found to be safe which is in agreement with present study indicating its safe use. [172,173].

**Phase II**

### 6.2 Dose standardisation of Isoproterenol

There were several studies demonstrated the ISO-induced myocardial infarction. It is manifested by alteration in electrocardiography and increased release of cardiac biomarkers [174]. These manifestations and pathophysiological changes are similar to human MI, thus in several pharmacological interventions this model of MI is well standardized and widely accepted to study the cardioprotective effects [175,176]. However various authors performed the difference in the dosage of ISO in inducing MI. The dosage of ISO was ranging from 5 mg to 300 mg/kg B.W in various studies used as agent to induce MI [177,178]. Hence we standardized the ISO proterenol dose by selecting 3 different doses by observing the serum biomarkers and histopathological changes.
In the present study, 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. CK –MB levels were 186 IU/L, 223 IU/L and 411 IU/L and serum Troponin-I levels were found to be 0.76 ng/ml, 1.06 ng/ml and 1.85 ng/ml at 5 mg/kg B.W, 85 mg/kg B.W and 150 mg/kg B.W of Isoproterenol respectively.

Even though levels of serum cardiac markers were highest at 150 mg/kg body weight Isoproterenol, histopathological findings (Figure no 5.15) showed that complete tissue destruction at this dose 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, 85 mg/kg body weight of Isoproterenol was chosen to induce MI and to carry out the effect of ethanolic extract of *Crataegus oxycantha* in rats (Table No.5.6).

A study conducted among rats to test the effect of alcoholic extract of *Crataegus oxycantha* on anti-oxidant status and lipid peroxidation, we noticed a significant reduction in anti-oxidant enzymes, an increase in mitochondrial lipid peroxidation, fall in manganese SOD and mitochondrial swelling and disruption of mitochondrial cristaein the heart of 85 mg/kg B.W Isoproterenol induced MI rat model.. Pre-treatment with alcoholic extract of *Crataegus oxycantha* improved anti-oxidant status, prevented lipid peroxidation. Thus in the current study, these results justify the usage of 85 mg/kg B.W of Isoproterenol to induce MI among rats [90].
Phase III

6.3  Effect of *Crataegus oxycantha* L. on Isoproterenol Induced myocardial infarction and endothelial dysfunction in rats

6.3.1 Effect of COC on Serum Lipid Profile

In the present study, increase in serum triglyceride level was found in ISO group. Serum TG levels were reduced in COC and COC+ METO groups compared to ISO group.

In the present study, increase in serum total cholesterol were observed in ISO group. Serum total cholesterol levels were reduced in COC and COC+METO groups compared with ISO group indicates the hypolipidimic actions of COC. Serum HDL level was decreased in ISO group. But there was gradual improvement in the percentage of serum HDL levels observed in COC and COC+METO group treated rats.

Serum LDL and VLDL levels were raised in ISO group. With drug treatment, in Group V, VI& IX there was reduction in LDL and VLDL levels were observed (Table No. 5.7) the above findings were highly authenticated the hypolipidimic potential of the *Crataegus oxycantha* and its combination with Metoprolol used in the present study.

A study was aimed to find out the effect of combination of tincture of *Crataegus* (TCR) and *Mangifera indica* L. (MNG) on lipid and antioxidant profile of rats induced with atherosclerosis. Co-administration of these two plant extracts inhibited the elevation of lipid levels in the serum and heart and also produced a substantial fall in lipid accumulation in the liver and aorta confirming the anti hyperlipidaemic effect of these two drugs which support the present study findings[179].

A study designed to evaluate the effect of Tincture of Crataegus (TCR), on hyperlipidemic diet fed rats observed a substantial decline in the levels of
cholesterol, triglycerides, LDL and VLDL. Study also noticed, a marked reduction in glutathione and α tocopherol deposition on liver, heart and aorta among these rats confirming its hypolipidemic effect, which is in association with the present study observations [71].

A study was conducted among male mice to measure the hypolipidemic potential of Shan-Zha (Crataegus pinnatifida) fruit extract, which is generally used in Chinese medicine as hypolipidemic agent. Hyperlipidemia was induced by high fat diet in in C57BL/6 J and Shan-Zha fruit extract was administered at an oral dose of 250 mg/kg for 7 days showed an increase of PPARα expression in liver which regulates fatty acid oxidation. Study also presented increased expressions of the β-oxidation-related genes such as CPT-1 and ACOX-1 which intended for lipid degradation and blood lipid decrement. This study offers a scientific indication that Shan-Zha is appropriate to use as an alternative approach for dyslipidaemia therapy. The above study findings highly supports current findings of COC and its use in the cardiovascular diseases [180].

A study was carried out to estimate the protective effect of *Crataegus oxycantha* in male albino rats, orally fed with palm kernel oil for a month. Study showed an elevation in the levels of total cholesterol, LDL cholesterol, triglycerides and abnormal protein profile due to palm kernel oil administration. *Crataegus oxycantha* given in a daily oral dose of 0.5ml /kg.B.W. To Palm Kernel Oil Group, presented a decrease in total cholesterol, triglycerides, (LDLc & VLDLc) probably due to the upregulation of LDL receptors. Study concludes *Crataegus oxycantha* as a possible hypocholesterolaemic agent in cardiovascular disorders [181].

To explore the Hypolipidemic and antioxidant effect of ethanolic extract of *Crataegus oxycantha*, a study was conducted among male albino wistar rats. Animals were divided into 3Groups. Group 1 had free access to food and water, Group 2 exposed to drinking water containing 1% H2O2 and Group 3 had drinking water containing 1% H2O2 and 300 mg / kg B.W. of crude ethanolic extract of *Crataegus oxycantha* for a period of 30 days. At the end of 1 month, there was a substantial fall in TC, TAG, LDL- C, VLDL-C concentrations with raise in the serum HDL-C and
serum GSH in Group 3 compared to Group 2 demonstrating the hypolipidemic and antioxidant effect of *Crataegus oxycantha* in the animal study which further confirms its phyotherapeutic potential [182].

A study was directed to measure the hypocholesterolemic and antiatherosclerotic effect of 2% hawthorn powder among Sprague-Dawley rats. Animals were divided into 3 Groups, Group 1 received normal rat diet, Group 2 received high cholesterol diet and Group 3 were administered with high cholesterol diet supplemented with 2% hawthorn powder for 4 weeks. In HCD-fed rats, an increased plasma total cholesterol and LDL-cholesterol with a decreased HDL-cholesterol was observed. Consumption of hawthorn markedly suppressed the elevated total cholesterol and LDL-lipoprotein levels plus an increased HDL-cholesterol level. Antioxidant enzyme activities in the liver (SOD & CAT) and Kidney (SOD) were re-established by hawthorn powder supporting its claim of being hypolipidemic and antioxidant potential [183].

A randomized, double blind, placebo controlled trial conducted among newly diagnosed essential hypertensive patients at Baghdad for a period of 12 weeks compared the effect of hawthorn extract capsule administered twice daily in a dose of 450 mg on blood pressure and serum lipid profile. At the end of 12 weeks, there was marked reduction in the serum total cholesterol and low density lipoprotein with substantial increase in HDL levels in hawthorn treated Group. Systolic and diastolic BP were decreased in the hawthorn Group, highlighting the protective effect of hawthorn on cardiovascular diseases like hypertension and atherosclerosis [184].

A study was conducted to compare the effect of simvastatin with hawthorn fruit compound among atherosclerotic ApoE-deficient mice to evaluate their effect on blood lipids. Mice were fed with high cholesterol diet (HCD) for eight weeks, and were divided into 3 Groups. Group A received HCD, Group B received HCD with simvastatin and Group C received HCD along with hawthorn fruit powder for 8 weeks. Study showed in Group C there was a substantial decline in serum TG, and ratio between LDL-C and serum cholesterol compared to Group B. There was
marked decrease in LDL-C in Group C compared to simvastatin Group justifying the use of hawthorn fruit compound in the treatment of atherosclerosis [185].

A study was planned to explore the hypolipidemic and antioxidant effect of hawthorn fruit among ApoE-deficient mice. Male ApoE-deficient mice were allocated to two Groups. Group 1 fed with standard diet and Group 2 was received standard diet + 1% hawthorn fruit for 16 weeks. At the end of experimental period, there was remarkable reduction in the size of atherosclerotic lesions and marked decline in the serum levels of cholesterol, triglycerides, LDL & VLDL in Group 2 confirming hypolipidemic effect of hawthorn. There was obvious increase in the levels of T-AOC, SOD &GSH-PX activities in hawthorn fruit Group affirming its antioxidant potential. Possible molecular mechanisms for hypolipidemic effect was the downregulation of hepatic fatty acid synthase (FAS) due to the decreased level of sterol regulatory element binding protein-1c (SREBP-1c) mRNA expression in Group 2. Authors of the Study speculated that is flavonoids present in the hawthorn are responsible for the downregulation of SREBP-1c gene expression by regulating PPARs [186].

A study was conducted to observe the antihyperlipidemic effect of **Crataegus oxyacantha** berries among albino rabbits. Albino rabbits were fed with atherogenic diet for 30 days. Animals were divided into two Groups. Group 1 were fed with control diet and Group 2 fed with a combination of control diet with 100mg/kg body weight of hawthorn berries for 60 days. At the end of the experiment, Group 2 showed considerable drop in the levels in triglyceride, total cholesterol and LDL (84.03%), as compared to Group 1. But no variation was documented in the HDL profile between the two Groups. Hypolipidemic activity of hawthorn berries were ascribed to the presence of flavonoids and procyanidins as bioactive compounds [187].

A case control interventional study was conducted to compare the antihyperlipidemic effect of **Crataegus** and simvastatin among male albino rats. Animals were allocated to 5 Groups. Group A was normal control, Group B was experimental control, Group C was given **Crataegus** extract, Group D received
simvastatin and E Group was given combination of both *Crataegus* and simvastatin. Except Group A, all other Groups were fed with hyperlipidemic diet for 8 weeks. Study indicated that both Group C and Group D are equally effective in reducing cholesterol, triglyceride, LDL and VLDL levels. While Group E was significantly effective in lowering LDL-c as compared to Crataegus or simvastatin alone thereby further supporting its hypolipidemic tendency in the animal studies [188].

A study conducted in Pakistan to explore the hypolipidemic effect of oral administration of hawthorn berry crude extract in albino rabbits. Animals were divided into 2 Groups, Group 1 received atherogenic diet. Group 2 received atherogenic diet plus hawthorn extract in a dose of dose 100 mg/kg body weight. Blood samples were collected from both the Groups to study biochemical parameters and lipid profile before starting experiment, 30th day of atherogenic diet, 20th, 40th and 60th day of treatment. Authors observed a substantial decline in the triglyceride, total cholesterol, and LDL without any change in the HDL levels in both Groups, thus substantiating the antihyperlipidemic activity of hawthorn extract in rabbits which further confirmed present study findings [189].

**6.3.2 Effect of COC on Tissue Anti-oxidant status**

Super oxide dismutase levels in cardiac tissue homogenate were reduced in ISO group. However in drug treatment groups (COC and COC+METO), the levels were increased in the present study. These findings clearly substantiated the anti-oxidant potential of test drug (COC). In the present study, catalase levels were dropped in Isoproterenol induced MI Groups. There were steady increase in catalase levels of COC and COC+ METO groups compared to ISO group.

During induction of MI by Isoproterenol, GST and glutathione peroxidase levels in the cardiac tissue homogenate declined substantially. After drug treatment (COC and COC+METO), GST and glutathione levels were increased which proved its antioxidant effects.
Glutathione (GSH) and Vitamin E levels were diminished in Isoproterenol induced MI Group. Among drug treated Groups (COC and COC+METO) GSH and Vitamin E levels were markedly increased in comparison with ISO group. Lipid peroxidation was decreased in COC and COC+ METO groups in comparision with ISO group which confirmed the antioxidant property of *Crataegus oxycantha* (Table No 5.8).

A study was conducted to evaluate the alcoholic, hydroalcoholic and aqueous extracts of *Crataegus oxycantha* L. to find out phenolic, flavonoid and anthocyanin content and its anti-oxidant, anti-microbial activity. DPPH radical transformation value of 89.9% was revealed by fruit extract (methanol-water (50/50, v/v %)) confirming its high anti-oxidant potential the study findings are in association with the current study [190].

A study was carried out to measure the anti-microbial and anti-oxidant activities of Illicium verum, *Crataegus oxycantha* species monogyna and Allium cepa red and white varieties. In this study authors reported that flavonols extract presented high antioxidant activity as compared with anthocyanins and standard antioxidants like ascorbic acid and quercetin. These findings also support the antioxidant property of *Crataegus oxycantha* that was observed in the current study [179].

A study was conducted in Chennai to explore the effect of alcoholic extract of *Crataegus oxycantha* administered with oral dose of 0.5ml/100g for 30 days to male albino rats with Isoproterenol induced myocardial infarction. The study revealed significantly reduced levels of anti-oxidant enzymes in Isoproterenol Group, which was improved alone in alcoholic extract of *Crataegus oxycantha* and captopril by itself. Research findings are in association with present study [179].

Akhila M.et.al evaluated the effect of combination of tincture of *Crataegus* (TCR) and *Mangifera indica* L. (MNG) on lipid and antioxidant profile of rats induced with atherosclerosis. Their results showed considerable improvement in the levels of antioxidant enzymes such as superoxide dismutase, catalase, glutathione
peroxidase, and Glutathione, there by re-establishing the antioxidant status to near normal levels. Once again these data confirm the rich antioxidant property of the *Crategus oxycantha* [191].

A study was carried out among rats to assess the protective effect of quercetin and α-tocopherol on mitochondrial damage and myocardial infarct size in Isoproterenol-induced myocardial infarction. Pretreatment of rats for 14 days with quercetin (10 mg/kg) alone, α-tocopherol (10 mg/kg) alone, and combination of quercetin (10 mg/kg) and α-tocopherol (10 mg/kg) carried out. Myocardial infarction was induced by Isoproterenol in a dose of 100 mg/kg for 2 days. After induction of MI, there was a substantial upsurge in the mitochondrial lipid peroxides with major fall in mitochondrial antioxidants. Isocitrate, succinate, malate, and α-ketoglutarate and NADH dehydrogenases and cytochrome-c-oxidase activities were reduced. There appeared a rise in calcium levels, and fall in adenosine triphosphate in the mitochondria. Combination of quercetin and α-tocopherol regulated all the biochemical parameters to normal levels, reduced the infarct size and maintained the integrity of cardiac tissue and re-established mitochondrial function in these rats. These study research findings were in association with the present study and confirmed COC positive effects on lipid peroxidation [192].

**6.3.3 Effect of COC on Cardiac membrane bound enzymes**

Level of Na$^+$/K$^+$-ATPase in ISO group was reduced and in the drug treated rats (COC and COC+METO), there was statistically significant improvement in the Na$^+$/K$^+$-ATPase levels compared to ISO group in the present study (Figure No.5.1).

A study conducted to evaluate cardioprotective effect of *Crataegus oxycantha* on Isoproterenol-induced myocardial necrosis reported that pre-treatment with *Crataegus oxycantha* rich in ursolic acid showed dose dependent inhibition of Na$^+$/K+ -ATPase enzyme present in cardiac tissue homogenate. The study findings were contradicting the present study, in which Na$^+$/K$^+$ -ATPase enzyme levels were improved in post treatment Groups. [55].
In the present study, Ca\textsuperscript{2+} ATPase levels were increased in ISO group predicting its increase in Ca\textsuperscript{2+} overload effect in myocardium. With drug treatment (COC and COC+METO), Ca\textsuperscript{2+} ATPase levels were reduced in Group V, VI & IX compared to Group ISO group (Figure No. 5.2).

Mg\textsuperscript{2+}-ATPase levels were reduced in ISO group. Following the drug treatment (COC and COC+METO), levels of this enzyme were increased among Groups V, VI & IX compared to ISO group. (Table No.5.9) (Figure No. 5.3).

An animal study was carried out in Isoproterenol induced MI to estimate the cardiotonic effect of Lagenaria siceraria fruit juice. The study revealed reduction in Na\textsuperscript+/K\textsuperscript{+} ATPase and Mg\textsuperscript{2+} - ATPase with marked increase in Ca\textsuperscript{2+}-ATPase activity in Isoproterenol Group compared to control. In post-treatment Groups, Ca\textsuperscript{2+} - ATPase was reduced significantly with improvement in Na\textsuperscript+/K\textsuperscript{+} -ATPase and Mg\textsuperscript{2+} - ATPase, which was supporting the findings observed in the present study [94].

6.3.4 Effect of COC on Serum Cardiac MI markers

In the present study, Troponin-I levels were undetected in the control Group. There was marked decline in the levels of Troponin-I in Group V, VI & IX compared to ISO group. The results are highly supporting the therapeutic benefits of COC and COC+ METO combination in the treatment and prevention of MI (Figure No. 5.4).

In this study, lactate dehydrogenase levels in ISO group was increased compared to controls. The LDH levels were markedly decreased in drug treated Groups (V, VI & IX) compared to Isoproterenol Group (Figure No. 5.5).

CK-MB levels were increased in ISO group when compared to Control group rats. Enzyme levels were reduced in post treatment Groups (Group V, VI & IX) compared to ISO group which confirmed the COC use in treatment and prevention of MI (Figure No. 5.5).
A study carried out to demonstrate the cardiotonic effect of *Crataegus oxycantha* on Isoproterenol induced myocardial infarction stated that pre-treatment with *Crataegus oxycantha* considerably reduced the serum levels of cardiac markers such as Troponin-I, creatinine kinase-MB, lactate dehydrogenase, glutamate oxalotransaminase, glutamate pyruvate transaminase and uric acid in Isoproterenol induced MI Group. Study outcomes are in agreement with the present study results [91].

A study was carried out to assess the effect of alcoholic extract of berries of *Crataegus oxycantha* on inflammation and apoptosis in Isoproterenol-induced myocardial infarction (MI) among rats. Authors of the study noticed a considerable increase in the levels of LDH, CK in serum, and decrease in the antioxidant status of the heart along with an increase in lipid peroxidation in Isoproterenol Group. Pre-treatment with the *Crataegus* preserved LDH, CK levels to near normal status. Study findings are in accordance with present study results [112].

AST levels were increased in Isoproterenol Group compared to control Group. There was decline in AST levels in Groups V, VI & IX compared to Isoproterenol Group (Figure No. 5.6).

In the present study, ALT levels were increased in ISO group compared to control Group. On drug treatment (COC and COC+ METO), levels of ALT was reduced in Groups V,VI & IX compared to ISO group further confirmed COC use in prevention of MI (Figure No. 5.6).

Serum uric acid levels were increased in Isoproterenol Group compared to control Group. However, there was a gradual decrease in serum uric acid levels in Groups (COC and COC+METO) compared to ISO group. (Table No.5.10) (Figure No. 5.7).

A study was designed to investigate the cardioprotective effect of *Crataegus oxycantha* in Isoproterenol induced myocardial necrosis among rats. In this study authors reported that pre-treatment with *Crataegus oxycantha* restored the serum levels of SGOT, SGPT and uric acid to near normal level compared with the
Isoproterenol Group in a dose dependent manner confirming its cardio protective potential. This study results are consistent with present study findings [91].

An animal study was directed to assess the effect of quercetin and α-tocopherol in Isoproterenol induced myocardial infarction. For 14 days, rats were pretreated with combination of quercetin (10 mg/kg) and α-tocopherol (10 mg/kg) daily. This was followed by induction of myocardial infarction by 100 mg/kg of Isoproterenol. Study findings showed an augmented serum troponin levels, plasma lipid peroxidation, plasma uric acid, myocardial calcium in Isoproterenol treated rats. In addition, enhanced serum lactate dehydrogenase-1 and -2 isoenzyme band strengths was also reported. However substantial reduction in antioxidant enzymes with raised ST segment was reported in electrocardiogram among Isoproterenol Group. Combined pre-treatment with quercetin and α-tocopherol showed cardioprotective effect by abolishing free radicals, improving antioxidants and conserving Ca$^{2+}$ levels in Isoproterenol induced myocardial infarction [192].

6.3.5 Effect of COC on Serum Endothelial markers

V-CAM levels in the present study were increased in ISO group compared to control Group. In post-treatment (COC and COC+METO) Groups (V, VI& IX) levels were reduced compared to ISO group, the findings demonstrated COC beneficial effects in the prevention of endothelial dysfunction (Figure No. 5.8).

A study was conducted in unstable angina patients to compare the effect of leaves and flower extract of *Crataegus oxycantha*, aerobic exercise and their combination. The study revealed that combination of aerobic exercise and *Crataegus oxycantha* extract significantly reduced the levels of endothelial adhesion molecules such as ICAM-1 and E-selection confirming it’s anti-atherosclerotic and cardioprotective effect. The results from this study were in association with present study [58].

Present study is one of its first to evaluate the effect of *Crataegus oxycantha* on the levels of V-CAM and revealed that there was significant decline in the
V-CAM levels in the post-treatment Groups compared to Isoproterenol Group further adding to its cardioprotective potential.

In the present study, CRP levels were found to be increased in Isoproterenol Group compared control Group. However, upon treatment with standard and test drugs (COC and COC+METO) in Groups V, VI & IX the CRP levels were decreased (Figure No. 5.8).

Present study shows increased TNF-α levels in Isoproterenol Group compared Group I, where as there was a gradual decrease in serum TNF-α levels seen in Groups V, VI& IX compared to ISO group. The findings proven COC use in the prevention of endothelial dysfunction (Table No.5.11) (Figure No. 5.9).

Atherosclerotic pathology is based on inflammatory process [194]. At the site of inflammation, there will be release of mediators like TNFα, IL-1B, IL-6, IL-10, IL-1ra, sTNF- R [195]. For leukocyte adhesion, there will be accumulation of V CAM, I CAM-1[194] and E selectin [196] on endothelial cells and changes in these adhesion molecules will affect inflammatory factors such as CRP, IL-6, TNFα and creatinine kinase.

A study conducted among stable angina patients revealed that combination of aerobic exercise and *Crataegus oxycantha* extract significantly reduced the levels of endothelial adhesion molecules such as I CAM-1 and E-selectin. The study highly supporting the current findings and the results are in association with present study positively [193].

Since the levels of ICAM-1 and E-selectin is reduced by *Crataegus oxycantha*, there should be associated decrease in inflammatory factors like CRP and TNFα. In the present study, alcoholic extract of *Crataegus oxycantha* considerably reduced the levels of CRP and TNFα confirming its anti-inflammatory, anti-atherosclerotic and endothelial protection potential.

A study was conducted to find out the neuroprotective and immunomodulatory mechanism of ethanolic hawthorn extract in middle cerebral
artery occlusion-(MCAO) induced stroke among rats. Male sprague Dawley rats pretreated with 100 mg/kg of hawthorn extract for 15 days undergone middle cerebral artery occlusion for 75 minutes followed by reperfusion showed augmented levels of proinflammatory cytokines (IL-1β, TNF-α, IL-6), ICAM-1, IL-10 and pSTAT-3 expression in the brain. However with hawthorn pre-treatment, levels of proinflammatory cytokines, ICAM-1, IL-10 and pSTAT-3 expression were found to be reduced, which is consistent with our study findings [196].

To study the effect of quercetin in acute myocardial infarction induced by left coronary artery ligation among rats, animals were segregated into 4 Groups. Group 1 as control, Group 2 as model Group and Group 3 received 100 mg/kg of quercetin and Group 4 received 400 mg/kg quercetin. Isometric normal saline once daily for one week was given to Group 1 and Group 2 rats. The mRNA expression and protein levels of TNF-α, IL-1β and MDA levels were considerably improved with substantial fall in SOD and CAT activities in the Groups 2, 3 and 4 compared to Group 1. Study noted an improvement in the cell apoptosis index, which is statistically significant. Nonetheless in comparison to Group 2, a reduction in the mRNA and protein levels of TNF-α and IL-1β and MDA levels in myocardial tissue of rats in the Group 3 and Group 4 with greater improvement in SOD and CAT activities were reported. Research showed reduction in the cell apoptosis index thus proving the anti-inflammatory, antioxidant, and anti-apoptotic effects of quercetin in acute myocardial infarction. The study results are in association with present study as quercetin is one of the important phytoconstituent in Crataegus oxycantha which further supported the current study findings [198].

A study was conducted to assess the effect of quercetin in myocardial ischemia/reperfusion induced myocyte injury in isolated rat hearts. Myocardial ischemia was induced by coronary occlusion followed by reperfusion and was treated with 1.0 mg/kg, i.v of quercetin. It resulted in decreased expression of TNF-alpha (TNF-α) and interleukin-10 (IL-10), reduced infarct size and inflammatory cytokines levels in the serum. However, quercetin increased baseline hemodynamic parameters thus proving its anti-inflammatory potential in the treatment of myocardial infarction the results were in association with present study [199].
A study was carried out to examine the anti-inflammatory effects of *Crataegus* pinnatifida ethanolic extracts (CPEE) in an ovalbumin (OVA)-induced murine asthma model. Eosinophilic mucus secretion and improved cytokine levels were seen in airways during OVA challenge in OVA sensitised mice. Pre-treatment with CPEE was given during OVA challenge. CPEE was given at doses of 100 and 200 mg/kg once daily on days 18-23 to the mice. There appeared noteworthy reduction in the Th2 cytokines including IL-4 and IL-5 levels, decrease in the number of inflammatory cells in BALF and airway hyperresponsiveness upon CPEE treatment. The test drug also inhibited the infiltration of eosinophil-rich inflammatory cells and mucus hypersecretion and the expression of ICAM-1, VCAM-1 and MMP-9 and the activity of MMP-9 in lung tissue were decreased in OVA-challenged mice. Thus proving that hawthorn is having definite actions on the expression of VCAM & ICAM, the results are in association with the present study [200].

**6.3.6 Effect of COC on Morphometric parameters of the rat Heart**

Body weight of the rats was decreased in ISO group compared to controls. On treatment, in Groups V, VI& IX body weight increased slightly. In the present study, weights of the hearts were increased in ISO group. Post-treatment (COC and COC+METO) Groups (V, VI& IX) reported gradual decline in weight of the heart compared to ISO group (Figure no 5.10).

Weight of the left ventricle was increased in the Group II compared to controls. However, in Groups (COC and COC+METO) V, VI& IX, weight had been reduced respectively compared to ISO group (Figure no 5.10).

In the present study, left ventricular wall thickness increased in ISO group. Post-treatment (COC and COC+METO) Groups (V, VI& IX) reported gradual decline in left ventricular wall thickness (Table No.5.12). The above findings from morphometric analysis showed COC cardioprotective benefits and COC+ METO group confirmed its use in the prevention of cardiac remodelling in MI.

A study was conducted to compare the effects of racemic carvedilol and metoprolol on cardiac hypertrophy caused by Isoproterenol among rats showed that
body weight of the animals were decreased in Isoproterenol Group compared to control Group. However body weight gradually increased in the carvedilol and metoprolol Groups compared to Isoproterenol Group. Heart weight, left ventricular weight of the animals were increased in Isoproterenol Group compared to controls and drug treatment reduced the heart weight and left ventricular weight in this study and showed the preventive benefits of metoprolol which was used in the current study in combination with COC. The study results confirmed its anti cardiac remodelling effects [201].

In the present study, effect of different doses of *Crataegus oxycaantha* was compared with control, Isoproterenol and metoprolol Group. Study reported that, effects on body weight decreased in Isoproterenol Group compared to control and was improved in metoprolol and combination of metoprolol with *Crataegus* Group. Heart weights, left ventricular weight of the animals were increased in Isoproterenol Group compared to controls. In combination of metoprolol with *Crataegus* Group reduction in the heart weight and left ventricular weight was observed compared to Group II. Present study findings are in agreement with the research findings of Hanada et al [202].

An animal study carried out to elucidate the effect of *Crataegus oxycaantha* on Isoproterenol induced myocardial necrosis showed decreased body weight and increased heart weight in Isoproterenol Group. Gradual improvement in body weight and considerable decrease in heart weight was seen with *Crataegus oxycaantha* treatment, which is in association with the research findings of the present study [91].

In the present study, left ventricular wall thickness was increased evidencing the cardiac hypertrophy in Isoproterenol Group compared to controls. But in post - treatment Groups (COC and COC+METO), left ventricular wall thickness was decreased (Figure no. 5.11)
6.3.7 Effect of COC on Histopathological changes in the rat Heart and TTC Enzyme mapping

In the present study, in Group II there was necrosis and waviness of myocardial fibers with accumulation of neutrophils seen. However, in post-treatment Groups, normal cardiac muscle fiber with little neutrophils and normal histoarchitecture was seen. These results show cardioprotective effect of *Crataegus oxycantha* and its synergistic effect with metoprolol. Biochemical findings supported the histopathological results of *Crataegus oxycantha*, which further confirmed the cardio protective effect of the ethanolic plant extract during myocardial infarction (Figure No.5.16). Necrotic areas were seen in Isoproterenol treated rats, which was revealed by TTC negative regions as indicated by arrow marks. (Figure no 5.17) Necrotic areas were reduced in the COC treatment Group and exhibited normal cardiac tissue with co-administration of metoprolol. These results established the therapeutic potential of plant extract in the prevention of myocardial infarction as pre-treatment and its synergistic action while co-administering with metoprolol.

Previous studies showed anti left ventricular remodelling and anti myocardial dysfunction effect of Hawthorn in early pressure overload-induced cardiac hypertrophy, which supports the present study findings [100].

The above findings from the present study showed that the COC extract had beneficial effects in the prevention of endothelial dysfunction and myocardial necrosis. In addition, in treatment Group there was reduction in the endothelial and myocardial markers. This may be due to the effect of *Crataegus oxycantha* extract and the synergistic effect on co-administration with metoprolol.

A systematic review and Meta analysis conducted to evaluate the safety of *Crataegus* spp. (hawthorn) monopreparations in the treatment of congestive heart failure included 24 clinical studies and 5,577 patients for adverse drug event monitoring. In the clinical trials were WS 1,442 containing 18.75% oligomeric procyanidins and LI 132 containing 2.25% flavonoids were used in the range of 160
to 1,800 mg dosage for 3 to 24 weeks. Hawthorn extracts were well tolerated and it has been associated with mild to moderate side effects. Eight serious side effects were reported regarding LI 132 use. Author concluded that hawthorn preparations were found to be safe except for some serious adverse effects associated with LI132 which needs proper vigilance when prescribed along with other drugs [204].

A randomised, double-blind, placebo-controlled multi center study was conducted to examine the efficacy and safety of *Crataegus* extract WS® as an add-on-treatment in adults with NYHA class II or III CHF and reduced left ventricular ejection fraction. Trial included 2681 patients who received either 900 mg/day WS® 1442 or placebo for 24 months. Primary end point of the study was advent of first cardiac event which was reported as 620 days for WS® 1442 and 606 days for placebo. Study inferred that, effect of WS 1442 on the primary endpoint was statistically insignificant. WS 1442 can possibly cut down the occurrence of sudden cardiac death in patients with less compromised left ventricular function. The study findings are in association with present study [205,206].