CHAPTER 2
REVIEW OF LITERATURE

2.1 CRATAEGUS OXYCANtha

It is a deciduous tree of Rosaceae family conventionally well known for its cardioprotective effect from first century A.D by Greek Herbalist, Dioscorides. In late 19th century, the herb became widespread among researchers in America and Europe [76].

2.1.1 Phytoc nstituents of Crataegus oxycantha

Crataegus oxycantha is a fruit-bearing plant with a long history as a traditional active therapeutic substance for different ailments. It has been used traditionally since ancient times as a cardio tonic and currently it gained the popularity in the treatment of hypertension, angina, arrhythmias and congestive heart failure [77]. The leaves, flowers and berries of hawthorn consist a variety of bioflavonoids and antioxidants that appear to be primarily responsible for the cardiac actions of the plant. Hawthorn is rich in triterpenic acids like ursolic acid and oleanolic acid, polyphenols and antioxidants like epicatechin, procyanidins, isoquercitrin, hyperoside, and chlorogenic acid [78].
Table 2.1 Phytoconstituents of *Crataegus oxycantha*

<table>
<thead>
<tr>
<th>Plant Part</th>
<th>Active Compound Name</th>
<th>Research area</th>
<th>Year</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruits, Leaves</td>
<td>Heptahydroxyflavan glycoside</td>
<td>Germany</td>
<td>1995</td>
<td>[79]</td>
</tr>
<tr>
<td>Fruits, Leaves</td>
<td>Flavan polymers</td>
<td>Germany</td>
<td>1967</td>
<td>[80]</td>
</tr>
<tr>
<td>Leaves, Flowers</td>
<td>Monoamines noradrenaline, adrenaline, dopamine and L-DOPA</td>
<td>Italy</td>
<td>2007</td>
<td>[81]</td>
</tr>
<tr>
<td>Leaves, Fruits</td>
<td>Procyanidins</td>
<td>Poland</td>
<td>2007</td>
<td>[82]</td>
</tr>
<tr>
<td>Leaves, Flowers</td>
<td>Flavonoids such as vitexin-2''-O-rhamnoside, hyperoside and oligomeric</td>
<td>Italy</td>
<td>2007</td>
<td>[83]</td>
</tr>
<tr>
<td>Fruits, Flowers, Leaves</td>
<td>Isobutylamine, O-methoxy phenylethylamine, Ursolic acid, Oleanolic acid, Crategolic acid, Adenosine Adenine, Guanine, Caffeic acid, Quercetin, Hyperoside, Rutin, Vitamin C, Vitexin-4'-'rhamnoside, epicatechol, Tyramine</td>
<td>India</td>
<td>2007</td>
<td>[84]</td>
</tr>
</tbody>
</table>

2.1.2 Taxonomic classification of *Crataegus oxycantha*

The name *Crataegus oxycantha* originates from the Greek word kratos meaning strength and refers to the nature of the wood [85] *Crataegus* is a large genus of trees and shrubs in the, Rosaceae family, with about 250 currently documented species native to northern temperate zones [86, 87]. It consists of bright green leaves, white flowers, and bright red berries (as shown in fig no 26). *Crataegus oxycantha* Linn. Is an official plant in homeopathic system of medicine, traditionally used as a cardiotonic herbal medicine used in various cardiac ailments [88, 89].
Table 2.2 Taxonomic classification of *Crataegus oxycantha*

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Division</td>
<td>Angiospermae</td>
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<tr>
<td>Class</td>
<td>Magnoliopsida</td>
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<tr>
<td>Order</td>
<td>Rosales</td>
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<tr>
<td>Family</td>
<td>Rosaceae</td>
</tr>
<tr>
<td>Sub-family</td>
<td>Maloideae</td>
</tr>
<tr>
<td>Tribe</td>
<td>Crataegaeae</td>
</tr>
<tr>
<td>Genus</td>
<td>Crataegus</td>
</tr>
<tr>
<td>Species</td>
<td>Oxyacantha</td>
</tr>
</tbody>
</table>

2.1.3 Vernacular Names of *Crataegus oxycantha*

**English:** Hawthorn, Mayblossom, Maythorn, Maybush

**Hindi:** Vansangli

2.1.4 Geographical Distribution of *Crataegus oxycantha*

These small sized trees are grown as a hedge plant in Europe. The distribution of the plant is mostly seen in temperate areas including countries like North Africa, Western Asia, India, China and North America. In India, it is seen in the temperate Himalayas, Kashmir and Himachal Pradesh, at a high altitude regions of 1800-3000 m [84].

2.2 ETHNOPHARMACOLOGICAL PROPERTIES OF *CRATAEGUS OXYCANtha*

Numerous medicinal uses have been documented in the literature regarding *Crataegus oxycantha* and its effect on MI, hypertension, atherosclerosis, hypercholesterolemia, congestive heart failure, oxidative stress, coronary blood flow, cardiac arrhythmias have been studied in *in vitro* and *in vivo* models of animals.
Clinical trials are going on throughout the world to evaluate the cardioprotective and other effects of *Crataegus oxycantha*.

**2.2.1 Myocardial infarction**

A study was conducted in Chennai to explore the pre-treatment effect of alcoholic extract of *Crataegus oxycantha* in a dosage of 0.5ml/100g body weight administered orally for 1 month to the Isoproterenol induced myocardial infarction in rats. Study reported that, alcoholic extract of *Crataegus oxycantha* protected heart against mitochondrial oxidative stress by enhancing the activities of Kreb’s cycle enzymes and prevented lipid peroxidative damage caused by Isoproterenol validating the cardioprotective role of *Crataegus oxycantha* on rat heart [90].

*Crataegus oxycantha* given in a dose of 25mg/kg body weight and 50mg/kg body weight orally in Isoproterenol induced myocardial necrosis among wistar rats showed that, *Crataegus oxycantha* reduced the levels of cardiac Troponin-I, CK MB and other cardiac marker enzymes. The plant extract had prevented lipid peroxidation, increased endogenous anti-oxidant superoxide dismutase, catalase and reduced glutathione in the heart proving its cardiotonic, cardioprotective and anti-oxidant property of *Crataegus oxycantha* in experimentally induced myocardial infarction rat models. Authors concluded that, cardiotonic effect of *Crataegus oxycantha* may be due to its ursolic acid content binding to digitalis site on Na$^+$ K$^+$ ATPase enzyme [91].

A study conducted on rat cardiomyocytes, to assess the effect of commercially available two alcoholic extract of Hawthorn to measure the calcium transients reported that, initiation of robust calcium transients and calcium overload was seen with extract 1 and while addition of extract 2 caused increased calcium sparking, initiation of calcium transients, and an increased beating rate but no calcium overload. Study concluded that, positive inotropic effect of hawthorn is via the Na$^+$, K$^+$-ATPase and also by influencing intracellular calcium concentrations [92].
A study was aimed to explore the inotropic effect of *Crataegus* special extract WS 1442 and its fractions in human myocardial tissue. Authors reported that, *Crataegus* special extract WS 1442 has a positive inotropic effect independent of cAMP action probably through inhibition of the sarcolemmal Na\(^+\),K\(^+\)-ATPase\[93\].

### 2.2.2 Anti-atherosclerotic effect

A study conducted among 80 stable angina patients aged between 45 and 65 years to compare the effect of 12 weeks of aerobic exercise, *Crataegus oxycantha* extract and their combination on cell adhesion molecules on endothelial cell surface revealed a significant reduction in the serum levels of ICAM-1 and E-selectin evidencing the anti-atherosclerotic property of *Crataegus oxycantha*\[94\].

A study conducted among atherogenic diet fed rats to investigate the anti-atherosclerotic and hypocholesterolemic effect of Tincture of Crataegus showed an augmentation of LDL receptor activity indicating additional binding of 125I-LDL to the liver plasma membranes. Authors summarised that, antitherosclerotic effect is thought to be due to the increased activity of LDL-receptors in the liver leading to more entry of plasma cholesterol into the liver. The test drug also increased breakdown of cholesterol to bile acids, thereby restraining cholesterol synthesis proving the anti-atherosclerotic and hypocholesterolemic effect of Tincture of *Crataegus* \[95\].

A study was designed to evaluate the hypolipidemic activity of hawthorn fruit among New Zealand white rabbits. The animals were divided into 3 Groups. Group 1 rabbits were fed with Cholesterol-free diet. Group 2 were administred with 1g/100g high cholesterol diet and Group 3 received 2g/100g hawthorn fruit powder along with high cholesterol diet. Study reported that, there was reduction in total cholesterol and triglycerides by 23.4% and 22.2% respectively in Group 3 after 12 weeks. There was 50.6% reduction in cholesterol accumulation on aorta. A possible mechanism for hypolipidemic activity of hawthorn is probably due to the inhibition of intestinal acyl CoA: cholesterol acyltransferase enzyme without disturbing hepatic
A study was conducted among mice to examine the hypolipidemic and anti-atherosclerotic effect of hawthorn fruit compound. Animals were divided into 4 Groups of 6 animals each. Group A fed with normal diet and Group B were administered with high cholesterol diet. Group C received high cholesterol diet with simvastatin and Group D received high cholesterol diet along with hawthorn fruit compound for eight weeks. Study noticed a substantial decrease in triglyceride levels, ratio between low-density lipoprotein cholesterol (LDL-C) and serum cholesterol among Group C and D. There was marked decline in LDL cholesterol in Group D compared to Group C. Study proved the hypolipidemic activity of hawthorn fruit compound, suggesting its use in the atherosclerosis [97].

Tincture of *Crataegus* in a dose of 0.5ml/100g body weight/ day for 6 weeks in experimentally induced atherosclerotic rats prevented the decrease in the levels of Glutathione and α tocopherol content of liver, aorta and heart. The drug exhibited potent anti-oxidant property by maintaining the normal levels of SOD, CAT, GPx and GST enzymes and prevented the oxidation of atherogenic lipo proteins LDL & VLDL ascertaining the anti-oxidant and anti-atherosclerotic property of Tincture of *Crataegus* in rats [98].

An invitro study conducted to investigate the antioxidant property of phenolic extract of hawthorn showed that, oxygen scavenging property was found to be high in fresh young leaves, fresh floral buds and pharmaceutical dried flowers. Anti-oxidant property was due to the presence of phenolic proanthocyanidin and flavonoid contents present in the hawthorn [99].

### 2.2.3 Anti-arrhythmic effect

A study that was designed to assess the anti-arrhythmic effect of alcoholic extract of *Crataegus oxycantha* on digoxin induced arrhythmias in anaesthetised wistar rats showed that duration of atrial and ventricular arrhythmias were shorter among drug treated Groups compared to controls. The study suggested the potential
use of alcoholic extract of *Crataegus oxyacantha* as an alternative treatment in digoxin induced arrhythmias in humans [100].

A study was intended to assess the anti-arrhythmic effect of 3-month pre-treatment of *Crataegus oxyacantha* (LI 132) flower and leaves dried extract after global no flow ischemia using Langendorff heart of the rat. Study inferred that, pretreatment with *Crataegus* considerably decreased the ischemic episode and malignant arrhythmias. Although occurrence of ventricular tachycardias were similar in control and drug treated Groups, the duration was strongly reduced in drug treated Group compared to control. Authors concluded that pretreatment with *Crataegus* extract was highly effective in protecting the heart against reperfusion arrhythmias in *Invitro* experiment [101].

An *invitro* study done in Caco-2 cells showed inhibition of acylCoA: cholesterol acyltransferase (ACAT) activity by hawthorn extract proving its hypolipidemic potential. Study was further supported by animal study conducted among Male Lakeview Golden (LVG) Syrian hamsters to examine the lipid lowering potential of Hawthorn and its possible mechanism of action. Animals were administered for 4 weeks with semi-synthetic diet containing 0.08% (w/w) cholesterol (control) (i) hawthorn dichloromethane extract, (ii) Plant sterol extract (iii) hawthorn dichloromethane extract + PSE (iv) oleanolic acid (OA) and ursolic acid (UA) mixture for 4 weeks. Study inferred that, compared to control Group, all other Groups showed considerable reduction in the levels of serum LDL & VLDL and the hepatic cholesterol ester. Possible mechanism of action proposed was the inhibition of ACAT activity by OA and UA content of hawthorn [102].

An *invitro* study using isolated cardiac myocytes of adult rats conducted to determine the anti-arrhythmic effect of *Crataegus* leaf and flower extract LI 132 (*Crataegus*), which contains 2.2% flavonoids was designed. The hawthorn extract presented a positive inotropic effect and reasonable rise in the energy turnover by mechanical and ionic processes. Hawthorn extract showed reasonably good myocytes energetics compared to beta receptor agonist, isoprenaline or cardiac glycoside, or elevation of the extracellular Ca++-concentration. Prolongation of
an apparent refractory period ascertained an antiarrhythmic potential of hawthorn extract [103].

2.2.4 Antioxidant effect

A pre-treatment study with tincture of *Crataegus oxycantha* with an oral dose of 0.5 ml/100g body weight for one month was conducted. Pretreatment prevented lipid peroxidation, pathological changes and maintained anti-oxidant enzymes of heart in Isoproterenol induced myocardial infarction among rats substantiating the cardio protective effect of *Crataegus oxycantha* [104].

2.2.5 In Endothelial dysfunction

A study was conducted to investigate the effect of *Crataegus* special extract WS1442 on aging-related endothelial dysfunction in wistar rats using mesenteric artery rings. Prolonged *Crataegus* treatment enriched aging-related impairment of endothelium-dependent relaxations and the induction of endothelium-dependent contractile responses. This could be due to decrease in the prostanoid-mediated contractile responses, presumably by augmenting the oxidative stress and the overexpression of cyclooxygenase enzymes [105].

An *in vivo* study conducted to examine the effect of WS 1442, a special extract of *Crataegus* leaves and flowers on isolated rings of rat aorta and human mammalian artery showed that WS 1442 caused an NO-liberation from human coronary artery due to endothelial eNOS phosphorylation at serine 1177 leading to vasodilatation [106].

A study conducted to investigate the effect of mixture of flavonoids and procyanidins extracted from hawthorn, *Crataegus oxycantha* L. and *C.monogyna* Jacq. On rat aorta. Study showed that procyanidins in *Crataegus* extract caused endothelium-dependent nitric oxide-mediated relaxation in isolated rat aorta, maybe through the activation of tetraethyl ammonium-sensitive K⁺ channels [107].
2.2.6 Anti-anxiety effect

The clinical efficacy of fixed quantities of two plant extracts (\textit{Crataegus oxycantha} and \textit{Eschscholtzia californica}) and magnesium versus placebo in a double-blind, randomised, placebo-controlled trial involving 264 patients presenting with generalised anxiety was conducted. The outcome was, the preparation containing fixed quantities of \textit{Crataegus oxycantha}, \textit{Eschscholtzia californica}, and magnesium proved to be safe and more effective than placebo in treating mild-to moderate anxiety disorders [108].

2.2.7 Anti-hypertensive effect

A study was done to evaluate the effect of aqueous extract of \textit{Crataegus oxycantha} leaves on blood pressure in normal anaesthetised rats. The authors of the study found that there was a significant decrease in the systolic, diastolic and mean blood pressure evidencing the hypotensive effect of \textit{Crataegus oxycantha} [109].

A randomised controlled trial conducted among 79 type II diabetic patients, were divided into two Groups. One Group of 39 patients took 1200mg of hawthorn extract and second Group took placebo for 16 weeks. The Study showed greater decrease in diastolic BP in hawthorn Group compared to placebo Group but such difference was not seen in systolic BP among two Groups supporting the hypotensive effect of hawthorn among diabetic patients [110].

A study was designed to explore the hypotensive effect of hawthorn among 36 mild hypertensive patients. Patients were divided into 4 Groups. Group 1 received 600 mg of Magnesium, Group 2 received 500 mg hawthorn extract, Group 3 were administered combination of Magnesium and hawthorn and Group 4 were administered placebo, daily for 10 weeks. Systolic and diastolic BP were measured at 5 and 10 weeks intervals and findings showed that resting diastolic BP was significantly decreased among hawthorn Group compared to other 3 Groups and study also added that hawthorn Group showed a substantial anti-anxiety effect as an added advantage in this study [111].
2.2.8 Anti-inflammatory and anti-apoptotic effect

A study was aimed to assess the anti-inflammatory and anti-apoptotic effect of alcoholic extract of the berries of *Crataegus oxycantha* on Isoproterenol-induced myocardial infarction in wistar albino rats. The study showed that plant extract improved catalase and SOD enzymes, prevented lipid peroxidation, reduced the levels of CK and LDH enzymes of heart, reduced the nitrite concentration with decreased expression of both iNOS and COX-2 confirming the anti-oxidant and anti-inflammatory activity of *Crataegus oxycantha* [112].

An *in vivo* study was conducted among rats to assess the anti-apoptotic effect of *Crataegus oxycantha* extract among experimental myocardial ischemia reperfusion model. Study revealed that COC extract at 100mg/kg presented a substantial reduction in the infarct size and creatine kinase activity. Hawthorn extract caused considerable increase in phospho Akt and c-Raf levels in the cardiac tissue with significant decline in caspase-9 and caspase-7 proving the anti-apoptotic potential of COC extract. Study also reported a significant reduction in pro-apoptotic proteins, such as nuclear factor –kB, cytochrome c, apoptosis inducing factor, adenosine diphosphate ribose among hawthorn Group compared to control Group further supporting anti apoptotic efficacy of COC extract in this study [113].

2.2.9 Hypoglycaemic effect

A study was conducted to explore the hypoglycemic activity of aqueous extract of *Crataegus oxycantha* leaves administered as single dose or 9 daily doses by oral route among normal and streptozotocin induced diabetic rats. Study revealed substantial reduction in blood sugar levels in streptozotocin induced diabetic rats without affecting blood glucose in normal rats proving the antihyperglycemic potential of *Crataegus oxycantha* in experimental animals [114].

2.2.10 Immunomodulatory effect

A middle cerebral artery occlusion of 75 minutes and reperfusion of either 3hr or 24hr in male Sprague Dawley rats, pretreated with ethanolic extract of
Hawthorn in an oral dose of 100mg/kg for 15 days revealed a substantial reduction of pro-inflammatory mediators like TNF-a, IL-6, IL-1b and ICAM-1 in the brain. Pretreatment had increased IL-10 and Foxp3-positive Tregs, thereby subduing the triggered inflammatory mediators in the brain. Hawthorn treatment also minimizes apoptotic cell death by inducing phosphorylation of STAT-3 and increasing the expression of Bcl-xL in the brain supporting the immunomodulatory effect of ethanolic extract of hawthorn in MCAO induced stroke model [115].

2.2.11 Congestive heart failure

A placebo controlled, randomised, parallel Group, multicentre clinical trial was conducted among cardiac failure patients of NYHA class II to evaluate the efficacy and safety of Crataegus oxyacantha fresh berry extract. In this trial, 69 patients were administered Crataegus berry extract and 74 patients were on placebo for 8 weeks. Study revealed a substantial progress in the exercise tolerance in the Crataegus Group compared to placebo, supporting the chronic use of test drug in heart failure patients without any serious adverse effect profile [116].

A meta-analysis of thirteen trials were used to evaluate the effectiveness of hawthorn extract as adjuvant in chronic heart failure. The analysis showed an improved physiologic outcome of maximal workload and remarkable reduction in blood pressure - heart rate product without any serious adverse effects proposing the use of hawthorn extract in chronic heart failure [117].

A study was designed to examine the chronic use of Crataegus extract WS 1442 in comparison to use of diuretics in heart failure patients(NYHA Class III). Study included 209 patients who were randomised to receive 1800mg of WS1442 or 900mg of WS1442 or placebo for 16 weeks. Study highlighted a greater improvement in exercise capacity and lesser adverse effect with 1800mg WS1442 extract advising its use in chronic heart failure patients [118].

A randomized crossover trial was carried out to assess the effect of Crataegus special extract WS 1442 on pharmacokinetics of digoxin. Study involved 8 healthy volunteers who were administered digoxin 0.25 mg alone (D) for 10 days and
digoxin 0.25 mg with 450mg Crataegus special extract WS 1442 twice daily (D + H) for 21 days. Authors of this Study noticed no major differences in the pharmacokinetic parameters with the addition of Crataegus special extract WS 1442 to digoxin and substantiating its simultaneous use with digoxin in heart failure patients [119].

2.2.12 Hepato protective effect

A study was aimed to examine the effect of Crataegus extract in comparison to silymarin on liver injury induced by carbon tetrachloride (CCl4) among rats.

Sprague–Dawley rats of both sexes were orally administered with Crataegus extract (10, 20 or 40 mg/kg), silymarin (25 mg/kg) or saline (control) along with 2.8 ml/kg of CCl4 once daily for 7 days. Crataegus extract caused dose dependent decrease in the serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase compared to the CCl4 control Group. Crataegus extract administered at 40 mg/kg dose was found to be highly effective and decreased the serum nitric oxide (nitrite/nitrate) level, the histological liver damage but enhanced the mucopolysaccharide and protein content of hepatocytes further supporting the use of Crataegus extract in CCl4-induced hepatic injury among rats [120].

An in vitro assay of cultured neonatal murine cardiomyocyte were carried out to evaluate negative chronotropic effect of hawthorn extract on atrial and ventricular cardiomyocytes. Study examined the assumption of test drug acting via muscarinic receptors, and was tested with hawthorn extract in the presence of non-selective muscarinic antagonist, atropine or selective M2 receptor antagonist, himbacine. Hawthorn showed significant effect on atrial cardiomyocytes than ventricular cardiomyocytes favouring the assumption of the study. Quantification of hawthorn extract showed inhibition of quinuclidinyl benzilate binding to murine cardiomyocytes, proposing muscarinic receptor agonistic activity of hawthorn extract in cultured cardiomyocytes [121].

A study was carried out to assess the hepato protective effect of methanolic extract of hawthorn on chronic alcoholic damage among rats. In this study,
coadministration of 35% ethanol in a dose of 3g/kg/day BD and methanolic extract of hawthorn in a dose of 50 mg/kg/day for twelve weeks showed a significant reduction in the AST, ALT, γ-GT and ACP levels and presence of normal liver tissue demonstrating the hepato protective effect of hawthorn. *Crataegus oxyacantha* co-administration also decreased the levels of triglycerides, total cholesterol and LDL without affecting HDL levels in ethanol induced hepatic damage among rats proving its hypolipidemic potential. Antioxidant effect of the hawthorn was evidenced by reduction in the liver lipid peroxidation and increased serum TAC levels. Authors proved the antioxidant, hypolipidemic and preventive effect of hawthorn on alcoholic liver damage among rats [122].

### 2.3 ANIMAL MODELS OF MYOCARDIAL INFARCTION

- Acute ischemia by injection of microspheres in dogs [123]
- Occlusion of coronary artery in anesthetized Dogs [124]
- Myocardial infarction after coronary ligation in rats [125]
- Isoproterenol induced myocardial necrosis in rats [126]
- Coronary artery ligation in isolated working rat heart [127]
- Ischemia/reperfusion injury in rats [124]

#### 2.3.1 Isoproterenol – Animal model of MI

Isoproterenol [1(3, 4) dihydroxy phenyl 2-iso-propylaminoethanol] hydrochloride is a synthetic catecholamine, a beta-adrenergic agonist. The basic mechanism of Isoproterenol is causing severe oxidative stress in myocardium which in turn leads to infarct like necrosis of the heart muscle thus serving as the standard model for the study of potentially beneficial properties of several drugs on cardiac function. The chemical structure is similar to epinephrine (adrenaline) and it acts selectively on beta-adrenergic receptors and stimulates it [Fig 2.1] [126].
2.3.2 Isoproterenol as an inducer of MI

![Fig 2.1 The structural similarity of ISO and Epinephrine. [128]](image)

The effects of ISO on heart are produced through $\beta_1$ and $\beta_2$ adrenoceptors. Both $\beta_1$- and $\beta_2$- adrenoceptors facilitate the positive inotropic and chronotropic effects to $\beta$-adreno receptor agonists [129]. ISO induces ischemia or hypoxia due to myocardial hyperactivity and coronary hypotension and produce myocardial ischemia due to cytosolic Ca²⁺ overload [130].

![Fig 2.2 Schematic representation of Isoproterenol mechanism of action. [131]](image)
ISO produces cardiac necrosis by various mechanisms, including increased oxygen consumption, poor oxygen utilization, increased calcium overload and accumulation, altered myocardial cell metabolism, increased myocardial cAMP levels, deranged electrolyte status, intracellular acidosis, altered membrane permeability and increased levels of lipid peroxidation [132]. The several mechanisms proposed to describe ISO-induced cardio toxicity include the generation of highly cytotoxic free radicals through the auto oxidation of catecholamines, which has been implicated as one of the important causative factor for its effects on myocardium [133].

Oxidation of catecholamine generates quinoid compounds which in turn produces superoxide anions and subsequently, hydrogen peroxide, which in the presence of iron, forms highly reactive hydroxyl radicals and damages protein, lipid, and DNA thus increasing the size of infarction [134]. In addition, excessive accumulation of free radicals leads to loss of function and integrity of myocardial membranes in turn weakening the myocardium [135]. These free radicals may interact with polyunsaturated fatty acids (PUFAs) within the membranes and forms peroxyl radicals. These free radicals can then attack neighbouring fatty acids, causing a chain reaction of lipid peroxidation (LPO). The lipid hydroperoxide end products are harmful and may cause further tissue and organ damage in the myocardium [136].