11.1. Introduction
Ischemic heart diseases, especially acute myocardial infarction/myocardial necrosis, remain the leading cause of death in both developed and developing countries as seen over the past quarter century (Zhu et al., 1998). Reduction of mortality rate and prevention of myocardial infarction and myocardial necrosis are utmost important. Reactive oxygen species, which possess highly reactive and toxic properties can be generated as a result of ischemia, myocardial infarction, myocardial necrosis and exacerbate the degree of myocardial damage sustained by the ischemic myocardium (Ferrari et al., 1998; Wattanapitayakul and Bauer, 2001). Isoproterenol [L- β- (3, 4-dihydroxy phenyl)- α- isopropyl amino ethanol hydrochloride], a β-adrenergic agonist has been reported to show many metabolic and morphologic aberrations in the heart tissue of the experimental animals similar to those observed in human myocardial infarction (Nirmala and puvanakrishnan, 1996a). It induces myocardial necrosis by a multiple step mechanism (Ravichandran et al., 1990). A growing body of evidence is emerging which suggests that reactive oxygen derived free radicals play a crucial role in the pathogenesis of isoproterenol-induced myocardial necrosis (Nirmala and puvanakrishnan, 1996b). So animals should have to develop an effective defense system to cope with unwanted and toxic oxygen species. In the heart, defense mechanisms include enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) in addition to other endogenous antioxidants. In pathological or diseased conditions, such as myocardial necrosis, diabetes, stroke and others, the production of free radicals may override the scavenging effects of antioxidants leading to oxidative stress (Ji et al., 2004; Zhu et al., 2004). Several medicinal plants have been suggested to be useful in treating myocardial infarction/necrosis due to their antioxidant activity (Fugh bermann, 2000).

In ethnomedical information, *Cytisus scoparius* used for hypnotic & sedative (Siegel, 1976), diabetes (Rebelo and Castro, 1998) and liver disease (Rivera and Obon, 1995). Pharmacological studies have confirmed its hypotensive activity and anti spasmodic activity (Bhakuni et al., 1969). *Cytisus scoparius* contains flavonals namely rutin, quercetin, quercitrin, isorhamnetin and kaempferol (Brum-Bousquet and Paris, 1974) some isoflavones like genistein and sarothenoside (Viscardi et al., 1984) and flavone such as 6'' O acetyl scoparin (Brum-Bousquet et al., 1977). The ethanolic fraction of *Asclepias curassavica* exhibited strong cardiotonic action (Bhagirath and Rastogi, 1969). The fresh (or) dried and pulverized leaf of *Asclepias curassavica* is reported to be used against cancer (Duke and Ayensu, 1985). The latex part of *Asclepias curassavica* is applied to corns, also as an emetic and bactericidal property (Zargari, 1992; Verpoorte and Dihal, 1987). The *Asclepias curassavica* extract stimulated central nervous system as reflected by increased level of serotonin and noradrenaline (Rastogi and Mehrotra, 1993). An inhibitory activity against carcinomatous cells of human nasopharynx has been shown by the plant extract (Neto et al., 2002). Several cardenolides (Abe et al., 1991; Seiber et al., 1982) have been isolated from different parts of plant. Six flavonoid glycosides namely, quercetin 3-O-α-L-rhamnopyranosyl-(1→6) beta -D-glucopyranoside, quercetin 3-O-(2''-O-α-L-rhamnopyranosyl)- β-D-galactopyranoside, quercetin 3-O-beta-D- glucopyranosyl-(1→6)- beta -D-galactopyranoside, quercetin 3-O-(2'',6''-alpha -L- dirhamnopyranosyl)-beta -D-
galactopyranoside, quercetin 3-O-beta -D-galactopyranoside and an unidentified flavonoid mixture were isolated and characterized from this plant (Haribal et al., 1996). Plants containing flavonoids have been reported to possess antioxidant properties (Raj and Shalin, 1999).

The present study was undertaken to evaluate the protective effect of hydroalcoholic extracts of *Cytisus scoparius* and *Asclepias curassavica* against isoproterenol induced oxidative stress and myocardial necrosis in rat upon chronic oral administration.

11.2. Materials and Methods
11.2.1. Plant materials and Extraction
The hydroalcoholic extracts of aerial part of *Cytisus scoparius* and *Asclepias curassavica* (described in Chapter 4) were used in this experiment.

11.2.2. Experimental protocol
Wistar albino rats (200 –250 gm) of either sex were maintained under standard environmental conditions and had free access to feed and water. Seven days after acclimatization, the rats were divided into six groups with six rats each. Group-1 animals served as control and were fed normal saline daily for 30 days. In group- 2, normal animals were administered with isoproterenol (subcutaneous route) in saline at the end of the 30 days. The hydroalcoholic extract of the *Cytisus scoparius* was administered to group-3 and group-4, by oral gavage everyday at a fixed time for 30 days in two different doses of 250 mg/kg and 500 mg/kg body weight, respectively. The hydroalcoholic extract of the *Asclepias curassavica* was administered to group-5 and group 6 animals orally everyday at a fixed time for 30 days in two different doses of 250 mg/kg and 500 mg/kg body weight, respectively. Changes in body weight and intake patterns of water and food in all groups were noted throughout the experimental period at regular intervals. At the end of the 30-days treatment period, rats were administered two doses of isoproterenol (85 mg/kg body weight) (dissolved in distilled water) by subcutaneously, 24 h apart and euthanised 24 h after the last injection for groups- 2, 3, 4, 5 and 6, respectively (Rona, 1985).

11.2.3. Biochemical estimation of markers of oxidative stress
11.2.3.1 Preparation of plasma from blood
After the experimental period the animals were sacrificed under mild chloroform anesthetic condition by cervical decapitation. Blood (2 ml) was aspirated from the left ventricle, collected in a heparinised vial, centrifuged at 3000× g for 30 min and the plasma stored at –20°C for estimation of plasma lactate dehydrogenase (LDH) and thio barbituric acid reactive substance (TBARS). Hearts were removed and stored in liquid nitrogen for biochemical estimation.

11.2.3.2. Preparation of heart homogenate
Heart tissues were carefully exercised and homogenized in cold 1.15% Kcl- 10 mM phosphate buffer with ethylene diamine tetra acetic acid (EDTA, pH 7.4) and centrifuged at 12,000 g for 60 minutes. The supernatant was used for the assay of
marker enzymes (glutathione peroxidase, superoxide dismutase and catalase), thiobarbituric acid reactive substances (TBARS) content and protein estimation.

11.2.3.3. Myocardial TBARS
Lipid peroxidation was measured by the method of Liu et al (1990). The method has been described in chapter 9.

11.2.3.4. Myocardial SOD
SOD activity was analyzed by the method described by Kakkar et al (1984). The method has been described in chapter 9.

11.2.3.5. Myocardial catalase
Catalase activity was measured by the method of Aebi (1974). The method has been described in chapter 9.

11.2.3.6. Myocardial GSH
Reduced glutathione was measured according to the method of Ellman (1959). The method has been described in chapter 9.

11.2.3.7. Myocardial GPx
GPx activity was determined by the method described by Paglia and Valentine (1967) and modified by Wendel (1981). The method has been described in chapter 10.

11.2.3.8. Plasma LDH
LDH was estimated by the method of Moldeus et al (1978) in 1-ml cuvettes, in KH buffer (pH 7.0) using 20 μl sample. Reduction of NADH was monitored at 340 nm against the appropriate controls every 15 s for 60 s.

11.2.3.9. Plasma TBARS
Plasma TBARS were estimated by the same procedure used for measuring myocardial TBARS (Liu et al., 1990), using 0.2 ml plasma. The method has been described in chapter 9.

11.2.4. Histopathology
Myocardial tissue was fixed in 10% formalin, routinely processed and embedded in paraffin. Paraffin sections (3 μM) were cut on glass slides and stained with hematoxylin and eosin (H&E), and periodic acid schiff reagent and examined under a light microscope by a pathologist, blinded to the groups studied.

11.2.5. Statistical analysis
The data were expressed as mean ± standard error of the mean (S.E.M). The biochemical and physiological parameters were analyzed statistically using one way analysis of variance (ANOVA) followed by Dunnet-t-test using the SPSS statistical software for comparison with control group. P<0.05 was considered as significant.

11.3. Results
There was no mortality and no changes in body mass or food and water intake patterns of rats in any group.
11.3.1. Myocardial TBARS
As shown in Figure 11.1, there was significant \( p < 0.001 \) increase in myocardial TBARS in the group-2 (isoproterenol induced rat). Significant decrease in the level of myocardium TBARS was observed in groups-3 \( p < 0.05 \), 4 \( p < 0.01 \), 5 \( p < 0.05 \) and 6 \( p < 0.01 \) in comparison to the control induced rat (group-2).

11.3.2. Myocardial SOD
Significant \( p < 0.001 \) reduction of myocardial SOD activity was observed in group-2 (isoproterenol induced rat), when compared to control group (Figure 11.2). Myocardial SOD increased significantly in rats treated with 500 mg/kg extracts of *Cytisus scoparius* (group 4; \( p < 0.001 \)) and *Asclepias curassavica* (group-6; \( p < 0.05 \)). In groups-3 and 5, there was no significant increase in the level of myocardial SOD activity compared to isoproterenol induced rat

11.3.3. Myocardial catalase
In isoproterenol induced rat group, there was a significant \( p < 0.001 \) decrease in myocardial catalase activity compared to vehicle control group. In groups-3 and 5, there was no significant increase in the level of myocardial catalase activity compared to isoproterenol induced rat (group 2). However, significant increase in myocardial catalase activity was seen in groups 4 \( p < 0.05 \) and 6 \( p < 0.05 \) (Figure 11.1).

11.3.4. Myocardial GSH
In group- 2, significantly \( p < 0.001 \) increased myocardium GSH was observed in comparison to control rats (group 1). There was no significant change in GSH levels in groups- 3 and 5 following isoproterenol administration, when compared to group-2 (isoproterenol induced). However, significant decrease in myocardial GSH activity was seen only in groups- 4 \( p < 0.001 \) and 6 \( p < 0.01 \), respectively (Figure 11.3).

11.3.5. Myocardial GPX
Significant \( p < 0.001 \) reduction in GPX activity was observed in isoproterenol-induced rats without treatment of plant extracts (group-2). Animals of groups-4 \( p < 0.05 \) and 6 \( p < 0.05 \) exhibited significant increase in myocardial GPX activity when compared to control induced rat group (group-2) but no significant change of GPX activity was observed in groups- 3 and 5 in comparison to isoproterenol induced rat group (group 2) (Figure 11.3).

11.3.6. Plasma TBARS
In the groups 3 \( p < 0.05 \), 4 \( p < 0.001 \), 5 \( p < 0.05 \) and 6 \( p < 0.001 \) there was significant decrease in the levels of plasma TBARS as compared to group-2. Isoproterenol induced (group-2) animals showed significantly \( p < 0.001 \) increased plasma TBARS level than the control and plant extracts treated groups (Figure 11.2).

11.3.7. Plasma LDH
Plasma LDH was increased significantly \( p < 0.001 \) in isoproterenol induced group in comparison to control (group-1). Significant reduction of LDH was observed only in group-4 \( p < 0.001 \) and 6 \( p < 0.05 \) (Figure 11.4).
Figure 11.1. Effect of hydroalcoholic extract of *Cytisus scoparius* and *Asclepias curassavica* on myocardial level of TBARS (nM/g of tissue) and myocardial level of CAT (U/mg of protein) during isoproterenol induced oxidative stress in rats. Values are mean ± s.d (n=6). a Group-2 (isoproterenol induced) compared with group-1 (control rats). b Groups- 3, 4, 5 and 6 (plant extracts) compared with group-2 (isoproterenol induced rats). c P<0.001, d P<0.01, e P<0.05.

Figure 11.2. Effect of hydroalcoholic extract of *Cytisus scoparius* and *Asclepias curassavica* on plasma TBARS (nM/mL) and myocardial level of SOD (U/mg of protein) during isoproterenol induced oxidative stress in rats. Values are mean ± s.d (n=6). a Group-2 (isoproterenol induced) compared with group-1 (control rats). b Groups- 3, 4, 5 and 6 (plant extracts) compared with group-2 (isoproterenol induced rats). c P<0.001, e P<0.05
Figure 11.3. Effect of hydroalcoholic extract of *Cytisus scoparius* and *Asclepias curassavica* on myocardial level of GSH (µg/g of tissue) and myocardial level of GPX (mU/mg of protein) during isoproterenol induced oxidative stress in rats. Values are mean ± s.d (n=6). *Group-2* (isoproterenol induced) compared with *group-1* (control rats). *Groups-3, 4, 5 and 6* (plant extracts) compared with *group-2* (isoproterenol induced rats). *P< 0.001, *P< 0.01, *P< 0.05

Figure 11.4. Effect of hydroalcoholic extract of *Cytisus scoparius* and *Asclepias curassavica* on Plasma-LDH (mU/mL) during isoproterenol induced oxidative stress in rats. Values are mean ± s.d (n=6). *Group-2* (isoproterenol induced) compared with *group-1* (control rats). *Groups- 3, 4, 5 and 6* (plant extracts) compared with *group-2* (isoproterenol induced rats). *P< 0.001, *P< 0.05
11.3.8. Light microscopical changes

Figure 11.5 (A), shows the H&E light micrograph of control heart showing normal architecture. There was confluent necrosis of cardiac muscle fibers with infiltration of acute and chronic inflammatory cells along with extravasation of red blood cells in isoproterenol induced group (Figure 11.6, B). In groups 3 and 5, there was focal necrosis of muscle fibers with acute and chronic inflammation (Figure 11.7, C and 11.9, C₁). In groups 4 & 6, there was only occasional loss of myofibers and inflammation was minimal in comparison to other groups (Figure 11.8, D and 11.10 D₁). The myonecrosis was also unremarkable in these groups.

11.4. Discussion

During myocardial infarction, reactive oxygen species like superoxide, hydrogen peroxide and hydroxyl radicals are produced in enormous amount which contributes to myocardial tissue injury (McCord, 1988). Some factors proposed to explain the mechanisms of isoproterenol-induced damage to cardiac myocytes include hypoxia due to myocardial hyperactivity and coronary hypotension, calcium overload, depletion of energy reserves, and excessive production of oxygen-free radicals resulting from the oxidative metabolism of catecholamines. The oxidation products of catecholamines depress cardiac contractile activity and affect the cardiac myocyte membrane, which is followed only later by damaged mitochondria, the sarcotubular system, and contractile elements (Rona, 1985). Isoproterenol-induced cardio toxicity was explained by the formation of oxygen-free radicals and sulfhydryl reactivity through a variety of its oxidation products (Singal et al., 1981). Myocardial antioxidants inhibit or delay the oxidative damage to sub cellular proteins, carbohydrates, lipids and DNA. Although the exact mechanisms and interactions among various antioxidants are not fully understood, it is possible that one antioxidant may equilibrate with another to establish a cellular redox potential and thus all endogenous antioxidants may act in concert to protect against oxidative insult (Naranjan et al., 2000).

Lipid peroxidation is an important pathogenic event in myocardial infarction and the accumulated lipid peroxides reflects the various stages of the disease and its complications (Golikov et al., 1989). It is reported that enhanced lipid peroxidation in serum/plasma and heart of isoproterenol treated rats is increased when compared to control. Increased levels of lipid peroxides injure blood vessels, causing increasing adherence and aggregation of platelets to the injured sites (Grylewski, 1980). Significant rise observed in the level of lipid peroxides in the heart tissue of isoproterenol-administered rats, and has suggested an enhanced oxidative stress in experimentally induced myocardial infarction condition (Padma and Devi, 2002). The rats fed with extract of Cytisus scoparius and Asclepias curassavica at two different doses of 250 and 500 mg/kg body weight showed significant (p < 0.05) decrease in the level of lipid peroxidation in the heart tissue as compared to that of isoproterenol-treated rats, indicating that the extracts possessed some antioxidant property.
Figure 11.5. (Normal) (A) Light micrograph of control rat heart. Normal architecture of myocytes (H&E, 10X)

Figure 11.6. (Induced) (B) ISO (isoproterenol) group showing focal confluent necrosis of muscle fiber with acute and chronic inflammation and myophagocytosis along with extravasation of red blood cells (10X, H&E).

Figure 11.7. (C) *Cytisus scoparius* extract (250 mg/kg) +ISO (isoproterenol) group, showing focal necrosis of muscle fiber with acute and chronic inflammation (10X H&E)
Figure 11.8. (D) *Cytisus scoparius* extract (500 mg/kg) +ISO (isoproterenol) group, showing occasional loss of muscle fiber with focal acute and chronic inflammation (10X H&E).

Figure 11.9. (C₁) *Asclepias curassavica* extract (250 mg/kg) +ISO (isoproterenol) group, showing focal necrosis of muscle fiber with acute and chronic inflammation (10X H&E).

Figure 11.10. (D₁) *Asclepias curassavica* extract (500 mg/kg) +ISO (isoproterenol) group, showing occasional loss of muscle fiber with focal acute and chronic inflammation (10X H&E).
SOD and CAT activities were decreased upon isoproterenol administration in accordance with the observation of Manjula and Shymaladevi (1994). During myocardial infarction/necrosis, superoxide radicals generated at the site of damage, modulates SOD and CAT, resulting in the loss of activity and accumulation of superoxide radical, which damages myocardium. In many other tissues, catalase is the major antioxidant enzyme that converts hydrogen peroxide to water and oxygen, which prevents the formation of the highly reactive hydroxyl radical (Henry et al., 2000). Pretreatment with extract of both plants at 500 mg/kg body weight significantly increased the activity of SOD and CAT and scavenged superoxide radicals and reduced myocardial damage caused by free radicals.

Isoproterenol treated rats showed significant increase in the level of heart GSH, when compared to control group. A similar observation was also reported previously (Rathore et al., 1998), the mechanism of which is unclear. Increased glutathione levels upon isoproterenol administration may be due to its decreased utilization in protecting SH containing proteins from lipid peroxides The glutathione is involved in many important cellular functions, ranging from the control of physico chemical properties of cellular proteins and peptides to the detoxification of free radicals (Meister and Anderson, 1983). The administration of extracts of Cytisus scoparius (500 mg/kg) and Asclepias curassavica (500 mg/kg) for 30 days resulted in significantly reducing the GSH level, which protects the myocardial membrane from oxidative damage.

GPx was significantly (p < 0.05) lower in the heart tissue of group- 2 rats (isoproterenol induced) as compared to normal controls, which was in accordance with the report of Nirmala and puvanakrishnan, (1996b), reflecting an increased oxidative stress in isoproterenol-induced myocardial necrosis. GPx offers protection to the cellular and subcellular membranes from the peroxidative damage by eliminating hydrogen peroxide and lipid peroxide. Inhibition of this enzyme leads to the accumulation of these oxidants and makes myocardial cell membranes more susceptible to oxidative damage (Sabeena Farvin et al., 2004). Pretreatment with the plant extracts increased the activities of GPX, but significant change was observed in high doses from both the plants.

Significant elevation was observed in the levels of LDH and TBARS in plasma of isoproterenol induced rats, which is in line with earlier reports (Sathish et al., 2002; Ithayarasi et al., 1996). Geetha et al (1990) have reported that the amount of enzymes present in plasma is directly proportional to the number of necrotic cells present in the cardiac tissue. In the present study, administration of hydroalcoholic extract of both plants were found to significantly prevent the elevation of plasma TBARS in groups 3, 4, 5 and 6 and plasma LDH in groups-4 and 6 animals. The results of the present study indicated that pretreatment with hydroalcoholic extract of Cytisus scoparius and Asclepias curassavica for 30 days prevented the oxidative stress induced by isoproterenol. The overall cardioprotective effect is probably related to a counteraction of free radicals by its antioxidant property of plant extracts.

Based on this work two research papers have been communicated for publication in Chemical and Pharmaceutical Bulletin, Japan and Phytotherapy Research, UK.