Discussion
Isoproterenol induced myocardial infarction is well a standardized model to study the effect of many drugs and cardiac function. So the present investigation is aimed to evaluate and explore the cardio protective effect of pretreated mangiferin, a non-nutrient phytochemical extracted from *mangifera indica* Linn, on isoproterenol induced experimental myocardial infarction. Mangiferin, being a polyphenolic antioxidant and a glucosyl xanthone, it has strong antioxidant, anti lipid peroxidation, immunomodulation, cardio tonic, hypotensive, wound healing, anti degenerative and antidiabetic activity. Hence, an attempt has been made to establish the cardio protective role of mangiferin on isoproterenol induced myocardial infarction in rats.

**Effect of mangiferin on heart weight to body weight ratio**

The determination of heart weight and body weight in experimentally induced myocardial infarction is considered to be a positive factor to find out the prognosis of disease.

Group 2 rats showed a significantly increased heart weight to body weight ratio compared to Group 1 control rats (Figure 3). Wexler et al (1978) has reported similar results. The increase in the weight of the myocardium might be due to the edema as well as infiltration of inflammatory cells. These results are in agreement with those of Judd and Wexler (1975) who observed extensive edematous intramuscular spaces after 4 hours of induction of myocardial infarction.

In mangiferin pretreated Group 4 rats, a significant decrease in heart weight to body weight ratio was observed when compared to Group 2
ISPH induced animals. Mangiferin could have protected the myocardium against infiltration by decreasing the water content of the myocardium. Henry and Stephens have reported that the polyphenols do not favor water formation in heart tissues of mice in the study of chronic psychosocial hypertension reduction (Henry and Stephens, 1984). Since mangiferin is a natural polyphenolic antioxidant, the protection offered to the myocardium could be attributed to the presence of polyphenolic activity of the drug.

**Effect of mangiferin on histopathological changes of heart**

Myocardial infarction is defined as myocardial cell death due to prolonged ischemia (Albert and Thygesen *et al.*, 2000). Excessive amounts of catecholamines are associated with typical myocardial pathological changes under stressful conditions (Reichenbach and Benditt, 1970).

In this study, the histopathological observation of heart tissue of Group 2 isoproterenol induced rats (Plate 1) showed myocardial damage and the separation of myocardial fibers with inflammatory mononuclear infiltrate. The cardiac muscle bundles have two areas of foci of extensive inflammatory collections, edema and separated muscle fibers. Rona *et al.* (1959) have described, an infarct like cardiac lesions and cardiomegaly in rats treated with large multiple dose of isoproterenol. They have shown that ISPH is capable of producing gross and microscopic myocardial necrosis, when administered subcutaneously to the rat. Myocytolysis could have resulted in the leakage of the marker enzymes into the serum and the severity of lesions is related to the severity of myocardial necrosis (Paritha *et al.*, 1996).
The heart sections of Group 4 mangiferin pretreated rats showed slightly separated myocardial fibers with small focus of inflammatory collections. The protection offered to the myocardium could be due to free radical scavenging property of the drug mangiferin. Muruganandam et al (2002) has reported that the treatment with mangiferin protected the cardiac tissue with the observation of mild degenerative changes as congestion and less swollen myocardial cell fibers in rats.

**Effect of mangiferin on ECG changes in heart**

The diagnosis of myocardial infarction is dependent on documentation that cardiac necrosis has taken place. The main criteria generally used for the definite diagnosis of myocardial infarction is evolving pattern of electro-cardio graphic abnormalities (Douglas Miller, 1991). Administration of isoproterenol is known to produce electrocardiograph and enzymatic changes suggestive of myocardial ischemia in experimental animals (Dwivedi et al, 1987) (Partha et al, 1996).

An elevation of ST segment was observed (Plate 2) in Group 2 isoproterenol induced rats. Hill et al (1966) have reported similar observations. This could be due to myocardial necrosis accelerated by isoproterenol. Acute ischemic tissue injury when tissue damage has occurred, manifest an ST segment elevation in the region of injured myocardium (Narriander et al, 1979).

Mangiferin pretreated (Group 4) rats exhibited a near normal ECG pattern with a slight elevation in ST segment. Marona et al (1997) have
explained an observed pattern of ECG test on the cardio protective effect of
derivatives of xanthones and reported that these compounds are potential anti
arrhythmic agents. Since mangiferin is one type of glucosyl xanthone
derivative the maintenance of normal ECG pattern might be attributed to the
protective effect of mangiferin in preventing free radical mediated myocardial
damage and thereby eliminating the acute fatal complications by protecting
the membrane damage against isoproterenol mediated infarction

**Effect of mangiferin on serum and heart tissue marker enzymes**

Myocardium contains an abundant concentration of many enzymes
and once metabolically damaged, releases its content into extra cellular fluid
(ECF) (Suchalatha and Devi, 2004). These biomarkers reflect myocardial
damage but do not indicate its mechanism (Albert and Thygesen et al, 2000)
The diagnostic marker enzymes of myocardial infarction are CPK, LDH and

A significant increase in the activities of myocardial marker
enzymes in serum with a corresponding decrease in myocardial tissue was
found in isoproterenol induced Group 2 rats when compared to Group 1
control rats (Table 1).

An increase in the activity of marker enzymes in serum is due to the
leakage of enzymes from heart as a result of isoproterenol induced necrosis
(Suchalatha & Devi, 2004) and the quantity of enzymes that appear in serum
is proportional to the number of necrotic cells. Damage to the myocardium
could be due to the induction of free radical mediated lipid peroxidation by
isoproterenol (Sushmakumari et al, 1989a).
In isoproterenol administered Group 2 rats, the increased activities of the serum marker enzymes accompanied by their concomitant reduction in the heart homogenate confirm the onset of myocardial necrosis (Suchalatha, 2004). Hence the total concentration of the marker enzymes were found to be decreased in heart tissue of isoproterenol administered rats as compared to control which might be the reflection of consequences of cellular injury due to lipid peroxides (Suchalatha, 2004).

In Group 4 mangiferin pretreated animals, serum marker enzymes were found to be significantly decreased where as the heart tissue marker enzymes showed significantly increased level when compared to Group 2 ISPH induced rats.

This could be due to antioxidant and free radical quenching effect of mangiferin as reported by Shibnath Ghosal et al (1996). Mangiferin could have reduced the necrotic damage through anti free radical action and prevented the leakage of enzymes from the tissue. Mangiferin, a principal phenolic compound has been reported to have potent scavenging activity against serum GOT and GPT elevations and TBA-RS formation (Masayuki et al, 2002). Muruganandam et al (2002) have reported that intraperitoneal administration of mangiferin significantly reduce the activity of CPK in heart as well as ameliorates the oxidative stress thereby reducing cardio toxicity and kidney damage. Xanthones have been reported to possess protective action against myocardial ischemia and diminishes the release of AST and LDH enzymes (He, Xu and Peng, 1998). Similar observations have been recorded with mangiferin pretreatment in the present study, which could be attributed to the protective action of mangiferin, a glucosyl xanthone.
Effect of mangiferin on serum (marker) CK-MB isoenzyme

Since the total CK represents the sum of the individual isoenzyme fractions, an elevated total CK does not specify the source of the CK, hence the need for isoenzyme analysis (Edward and Goljan, 1998). Assay of activity of MB isoenzyme of CK (CK-MB) in plasma is the magnitude and persistence of elevations and is useful in estimating the extent of infarction (Sobel, 1992)

A marked increase in this enzyme level was observed in Group 2 isoproterenol induced rats when compared to Group 1 control rats (Figure 4) The heart damage induced by isoproterenol was indicated by the elevated levels of the marker enzymes such as creatine kinase-isoenzyme (CK-MB) as reported by Ahmed et al (2004) The magnitude of CK-MB activity correlates with the size of infarct (Edward and Goljan, 1998)

The Group 4 mangiferin pretreated rats showed a significant decrease in this enzyme level compared to Group 2 isoproterenol administered rats Jiang-De-Jian et al (2003) have reported that the xanthones significantly improves the recovery of cardiac function and decreases the release of creatine kinase in vitro, and reduces infarct size and serum creatine kinase level during reperfusion injury in vivo. Mangiferin, a glucosyl xanthone, could have prevented the damage of heart from isoproterenol, which might be due to the anti free radical scavenging activity of mangiferin (Sanchez et al, 2000).
Effect of mangiferin on serum LDH isoenzymes (Electrophoresis separation)

Measurement of LDH isoenzymes is necessary for greater specificity for cardiac injury and a nonspecific increase of total LD in serum will occur following tissue damage. MI can be differentiated from other tissue damage since LDH isoenzyme begins to rise in 12 to 24 hours following MI, and peaks in 2 to 3 days, gradually dissipating in 5 to 14 days (Jaffe et al, 1996).

Isoproterenol induction caused an increase in LDH isoenzyme bands, predominantly LDH 1 in Group 2 rats compared to Group 1 control rats (Plate 3) Levinson and Hobbs (1994) have also reported similar results. Due to necrosis induced by isoproterenol the heart specific isoenzyme LDH 1 could have been released into the circulation (Voet and Voet, 1995).

The LDH 1 band location in mangiferin pretreated (Group 4) animals was found to be near normal and is almost similar to Group 1 control rats. Previous reports state that some xanthones reduces the activity of lactate dehydrogenase in coronary effluent and protected against ischemic myocardial injury (Jiang De-Jian et al, 2003). Since mangiferin is a polyphenolic xanthone and possess antioxidant activity this could have been attributed to its mechanism of action to protect the myocardium from damage to prevent the leakage of cell marker enzymes in to the blood stream.
Effect of mangiferin on histochemical changes of myocardium (TTC test)

A variety of histochemical approaches have been used to detect myocardial changes compatible with infarction before routine microscopic changes become evident at 6 hours. These include estimation of glycogen, using a periodic acid-schiff stain (PAS) and succinic dehydrogenase activity. This reaction can distinguish infarcted myocardium 6 to 8 hours after the start of infarction (Kloner et al., 1974).

A high percentage of mean infarct size with increased staining was observed in Group 2 isoproterenol administered rats when compared to Group 1 control animals (Plate 4). Damage to the myocardial and aortic tissues could be due to the free radical mediated lipid peroxidation by isoproterenol (Partha et al., 1996; Sushmakumari et al., 1989a). Milei et al. has studied the ISPH induced lesions by means of histochemistry and reported myofibrillar degeneration, and increase of succinic dehydrogenase enzyme (Milei et al., 1978) activity in serum.

Mangiferin pretreated (Group 4) rats showed a moderately low percentage of infarct size with reduced staining when compared to Group 2 ISPH induced rats. Zhang et al. has observed the changes in several myocardial enzymes in rats by enzyme histochemical staining and image analysis and has reported that the polyphenols played a significant role in protecting the myocardium from damage and prevent the leakage of enzymes from the cell (Zhang et al., 2001). Mangiferin possess as polyphenolic activity
and this could be the possible reason for reduction in the mean infarct size, thereby preventing the myocardial necrosis.

**Effect of mangiferin on serum protein, A/G ratio and electrophoresis separation of proteins**

Serum protein electrophoresis (SPEP) is a screening test that measures the major blood proteins by separating them into five distinct fractions: albumin, alpha₁, alpha₂, beta, and gamma proteins (Kathleen, 1998). The fractions form a characteristic band on electrophotogram. Alterations in these patterns are associated with manifestation of chronic diseases.

The serum total protein fractions and albumin: globulin ratios were found to be significantly reduced in Group 2 isoproterenol induced rats when compared to Group 1 control rats (Figure 5). The electrophoresis separation of serum total protein of Group 2 isoproterenol (Plate 5) induced rats showed low bands of protein and albumin fraction zones.

During active necrosis, changes in serum protein levels were reported in isoproterenol induced MI rats (Wexler et al, 1968). A decrease in serum protein is usually as a result of a fall in albumin or some times gamma globulin (Alan et al, 1988). A decrease in albumin with a rise in the alpha₂ globulin usually indicates an acute reaction of the type that occurs in infections, burns, stress or heart attack (David, 1996).

Isoproterenol induced myocardial infarction is a free radical mediated tissue damage and may lead to production of more $O_2$ and $H_2O_2$ ions.
which in turn could bind with albumin and thus destroy it. Similar results have been reported by Halliwell and Gutteridge (1989). Since proteins are SH group moieties they are easily affected by free radicals. Clarke et al (1995) in an in-vivo experiment model of wounded rat heart have reported that the wound sections of heart myocyte had contained only 25% of the cytosolic serum albumin.

Group 4 mangiferin pretreated rats exhibited a significant increase in these values when compared to isoproterenol induced Group 2 rats.

Martinez et al (2001) has reported that the stem bark extract of mangifera indica linn is effective in reducing the hydroxyl mediated oxidation of BSA with antioxidant activity exhibited by its polyphenolic component, mangiferin. Misra et al (2003) have reported that the polyphenol prevented the cigarette smoke (CS) induced oxidative damage of microsomal proteins and BSA in heart both in vivo and in vitro condition. Since mangiferin is a potent polyphenolic antioxidant, it could have neutralized 0₂ and H₂O₂ ions generated by isoproterenol and in turn could have protected the “SH group” and prevented the albumin and total protein damage.

**Effect of mangiferin on serum and heart tissue LPO**

Myocardial infarction, the most dreaded sequel among ischemic heart disease, is invariably followed by several biochemical alterations such as lipid peroxidation, free radical damage, hyperglycemia, hyperlipidemia etc., leading to qualitative and quantitative alterations of the myocardium. Mc Cord et al has reported that an over production of reactive oxygen species
such as super oxide radicals, hydrogen peroxide and hydroxyl radicals during myocardial infarction (Mc Cord, 1988) contribute to myocardial tissue injury. Lipid peroxidation, presumably the result of free radical mediated injury, has been shown to occur during myocardial ischemia (Kloner et al, 1989). Lipid peroxidation is an important pathogenic event in myocardial infarction and the accumulated lipid peroxides reflect the various stages of the disease and its complications (Golikov et al, 1989).

Isoproterenol administered Group 2 rats showed a significant increase in the level of LPO both in serum and heart tissue when compared to Group 1 control rats (Figure 6).

The significant increase observed in the levels of lipid peroxides in serum and heart of isoproterenol administered MI rats compared to control is in accordance with the observation of Sushma Kumari et al (1987) and Jayalakshmi and Niranjali (2004). Isoproterenol is well known to generate free radicals and to stimulate lipid peroxidation, which may be a causative factor for irreversible damage to the myocardium (Subramaniam et al, 2001). The increased levels of TBA reactive substances indicate the excessive formation of free radicals and activation of lipid peroxidation system resulting in the irreversible damage to the heart in animals subjected to isoproterenol stress. Increased levels of lipid peroxides injure blood vessels causing increasing adherence and aggregation of platelets to the injured sites (Sheela & Devi, 2000).
Mangiferin pretreated (Group 4) rats showed a significant decrease in LPO level both in serum and heart tissue when compared to isoproterenol induced Group 2 rats. It showed a decrease in the levels of TBA reactants exhibiting its role in inhibiting lipid peroxidation. Muruganandam et al have reported that the administration of mangiferin significantly reduced the cardiac tissue LPO level and ameliorates the alterations in the biochemical markers which could be one of the reason for the decrease in oxidative damage in the mangiferin treated animals (Muruganandan et al, 2002). It is previously reported that mangiferin is a potent inhibiting agent of LPO in vivo (Sanchez et al, 2000). Previous studies (Yoshikawa et al 2002; Martinez et al, 2001) support the activity of mangiferin as a potent inhibitor of lipid peroxidation. Previous investigations have shown that xanthones have a potent antioxidant activity by inhibiting lipid peroxidation and enhancing the recovery of cardiac function concomitantly with the reduction of LPO products in myocardial tissues (Jiang et al, 2003). Since mangiferin is a polyphenolic antioxidant and a xanthone derivative, it could be able to prevent the lipid peroxidation at different levels of oxidation sequence by decreasing the O2 concentration and generating mangiferin phenoxy radicals (Scartezzini and Speroni, 2000). This could be the reason for the reduced formation of lipid peroxides in the present study.

**Effect of mangiferin on serum non-enzymic antioxidants**

Isoproterenol induced Group 2 rats showed significantly increased level of serum iron with significant decrease in plasma iron binding capacity (Figure 7), while Group 4 mangiferin pretreated animals showed a significant
decrease in serum antioxidant levels such as ceruloplasmin, vitamin E and ascorbic acid with significant increase in serum uric acid levels (Table 2). Similar results have been reported by Sheela & Devi (2000); Sumitra et al (2001).

Heme iron is directly related to the risk of myocardial infarction (Aschero et al, 1994) and total iron-binding capacity is inversely related to the risk of myocardial infarction (Magnusson et al, 1994).

In isoproterenol induced myocardial necrosis, free iron is released from heme dependent proteins like haemoglobin and myoglobin and decreases iron-binding capacity, and thus increases prostaglandin metabolism and *invivo* lipid peroxidation (Halliwell & Gutteridge, 1989). Increased mobilization of iron from ferritin in the heart by xanthine oxidase and over production of hydroxyl and super oxide-radicals result in free radical mediated myocardial damage (Paritha & Devi, 1997) The superoxide radicals generated during the damage are capable of reducing ferritin bound iron to the ferrous state and causing its release (Biemond et al, 1986) in to the circulation.

In Group 4 mangiferin pretreated rats, the free iron concentration was found to be decreased with an increase in plasma iron binding capacity when compared to Group 2 ISPH induced rats. Afsana et al (2004) and Lekse et al (2001) have reported that polyphenol treatment reduces lipid peroxidation and serum iron concentration due to its free radical scavenging and antioxidant activity. Mangiferin a polyphenol has the property to bind
with metal ions like Fe$^{2+}$, Fe$^{3+}$ in state 3 that will not allow the generation of such tissue damaging hydroxy radicals and/or oxoferryl groups (Shibnath Ghosal et al, 1996). Thus the increased plasma iron binding could have prevented hemolysis and iron catalyzed lipid peroxidation. This could be the reason for the decreased level of iron and increased level of plasma iron binding capacity in Group 4 mangiferin pretreated rats.

Ceruloplasmin is an extra cellular antioxidant that can scavenge superoxide - radicals (Altiminin and Dormandy, 1977) and inhibits ferritin dependant lipid peroxidation by catalyzing the oxidative reincorporation of released iron into ferritin (Suresh Kumar & Menon, 1992) and some in vitro studies have shown that ceruloplasmin can inhibit the peroxidation of polyunsaturated fatty acids (Gutteridge, 1978).

Isoproterenol induced myocardial infarction is accompanied by the disintegration of membrane polyunsaturated fatty acids a measure of lipid peroxides (Nirmala and Puvana Krishnan, 1996a). LPO increases iron mediated reaction (Bucher et al, 1983; Minotti and Aust, 1987) and catalyzes more lipid peroxidation (Minotti and Aust, 1987; Braughler et al, 1986). Copper, being a pro-oxidant, could increase the risk of cardiac damage by promoting the oxidation of LDL and elevated serum copper may simply being a marker of the inflammation as in myocardial infarction (Gomez, 2000). Since ceruloplasmin has both ferroxidase and copper binding capacity it could have been used more to neutralize the excess amount of free radicals and hence Group 2 ISPH induced rats showed decreased level of ceruloplasmin.
Mangiferin pretreated rats (Group 4) showed a significant increase in ceruloplasmin level compared to Group 2 isoproterenol administrated rats. Amarakoon *et al* (1995) have reported that polyphenol rich extract inhibits serum ceruloplasmin levels. Mangiferin polyphenol bind metal ions Fe$^{2+}$/Fe$^{3+}$ that will not allow the generation of such tissue damaging hydroxyl radicals/and or oxo-ferryl groups as reported by Shihnath Ghosal (1996). Mangiferin, being a polyphenolic antioxidant by maintaining cellular oxidant-antioxidant balance could have prevented the loss of ceruloplasmin there by reduced iron and copper mediated myocardial damage.

Serum uric acid is considered to be a risk factor in myocardial infarction (Irimia, 1987; Weir *et al*, 2003) The association of serum uric acid level with myocardial infarction, left ventricular dysfunction and elevated inflammatory markers must be interpreted as an association not as a causal relation (Michael Poullis, 2000).

Increase in serum uric acid in an experimental condition could be due to excessive degradation of purine nucleotide and proteolysis as evidenced by Vijayapadma & Devi (2000). McCord has suggested that the dehydrogenase to oxidase conversion occurs in ischemic or hypoxic tissue (Chessbro, 1997). Xanthine oxidase catalyzes the conversion of hypoxanthine to xanthine, uric acid and superoxide (O$_2$-) (Chessbro 1997). This could be one of the reasons for the elevated concentrations of uric acid in isoproterenol treated rats.
Mangiferin pretreated rats (Group 4) showed a significant decrease in uric acid level compared to Group 2 isoproterenol administrated rats. Procyanidins, mangiferin like polyphenols have been reported to possess in vivo urate lowering activities (Ying Wang et al, 2004). Leiro et al (2003) have investigated the effects of mangiferin on superoxide anion, O$_2^-$ production and xanthine oxidase (XO) activity and has reported that the natural polyphenol mangiferin effectively scavenge HX/XO and PMS-NADH systems mediated O$_2^-$ production. This could be the reason in the present study for the decreased levels of uric acid in Group 4 mangiferin pretreated rats.

Alpha tocopherol is a lipid soluble, chain-breaking antioxidant capable of scavenging oxygen, centered free radicals (Halliwell, 1997). Data from human studies have suggested that an inverse correlation exist between plasma levels of vitamin E and mortality from ischemic heart disease (Dutta Roy et al, 1994) Alpha tocopherol is an effective inhibitor of the autocatalyzing process of lipid peroxidation in membrane fatty acids (Tappel, 1980) and can directly react with superoxides, hydroxy radicals and singlet oxygen (Bendich et al, 1986). Ascorbic acid present in aqueous environment has multiple antioxidant properties including the ability to regenerate alpha tocopheryl radicals present at the surface of the membrane (Altimimi and Dormandy, 1977) Due to the above properties of the two antioxidants these two vitamins E and C have been utilized more for the neutralization of isoproterenol mediated free radicals and lipid peroxidation process and hence the decreased level of vitamin E and vitamin C was observed in Group 2 animals.
Mangifera pretreated rats (Group 4) showed a significant increase in vitamin C and vitamin E levels, when compared to isoproterenol administrated Group 2 rats. Goshal et al has reported that mangiferin maintains cellular oxidant antioxidant balance by decreasing the localized O$_2$ concentration, and generating mangiferin phenoxy radicals thereby reducing free radical mediated lipid peroxidation (Ghosal et al, 1996). This could be the reason for the increased levels of vitamin C and vitamin E in the serum of mangiferin pretreated rats. Polyphenols together with other dietary reducing agents such as vitamin C, vitamin E and carotenoids, referred to as antioxidants have been reported to protect the body's tissue against oxidative stress and associated pathologies such as cancers, coronary heart disease and inflammation (Urquida and Leighton, 2000) and carrot (polyphenols) consumption has been reported to improve the antioxidant status by significantly reducing the TBARS levels in heart and increases the plasma vitamin E level (Nicolle et al, 2003). Thus, the observed increase in the levels vitamins C and E in Group 4 mangiferin pretreated rats could be attributed to the polyphenolic effect of mangiferin.

**Effect of mangiferin on glutathione and heart tissue antioxidant enzymes**

Reactive oxygen species are generated from the leakage of electrons onto oxygen from various systems in our body and the endogenous antioxidant enzymatic defense are very important source to neutralize the oxygen free radical mediated tissue injury (Karthikeyan and Rani, 2003) GSH together with GSH dependent enzymes GPX, GST, GSSGR and CAT-SOD couple, efficiently scavenge toxic free radicals (Pohodoro et al,
Glutathione is a low molecular weight thiol which serves as a major endogenous antioxidant with a multitude cellular determining function. It plays an important role in reducing diseases such as atherosclerosis and reoxygenation injury (Uhlig and Wendel, 1992).

In isoproterenol induced Group 2 rats the activity of serum, heart tissue glutathione and heart tissue antioxidant enzymes, were found to be decreased when compared to Group 1 control rats (Table 3). Similar results have been reported by Saravanan & Prakash (2004), Sheela and Devi (2000), Ahmed et al (2004), Mohanty et al (2004) and Sabeeha et al (2004).

Decreased glutathione levels on isoproterenol administration may be due to its increased utilization in protecting SH containing proteins from lipid peroxides. Reduced availability of glutathione also reduces the activity of glutathione peroxidase and glutathione-s-transferase, on isoproterenol administration (Partha and Devi, 1997; Sheela and Devi, 2000). Glutathione reductase and glutathione peroxidase are essential for maintaining constant ratio of reduced glutathione to oxidized glutathione in the cell. Inactivation of glutathione reductase and peroxidase in the heart leads to accumulation of oxidized glutathione (GSSG) (Ferrari et al, 1985a) which in turn inactivates many enzymes containing the SH group and inhibits protein synthesis (Lil et al, 1988).

Group 4 rats exhibited a marked increase in these parameters when compared to Group 2 rats. Abrisarsarkar et al has reported that mangiferin enhances glutathione level almost two-fold than other antioxidants and at the
same time it decreases the levels of GSSG the oxidized product of GSH (Abíra sarkar et al, 2004). The animals pretreated with highest dose of vimang (a mangiferin containing compound) have been reported to protect GPX level and reduced TPA mediated biomolecular oxidation (Sanchez et al, 2000). Goshal et al has reported that mangiferin performs its antioxidant function at different levels of the oxidative sequence by maintaining cellular oxidant and antioxidant balance (Shibnath Ghosal et al, 1996) Since mangiferin possesses antioxidant property, in the present study, it could have been attributed to maintenance of the endogenous GSH antioxidant balance against isoproterenol mediated cellular oxidation thereby protected the GSH related enzymes and in turn inhibited the oxidation of GSH.

SOD is an endogenousy produced intracellular enzymes present in essentially every cell of the body. SOD is considered fundamental in the process of eliminating ROS by reducing (adding an electron to) super oxide to form $H_2O_2$. Catalase is responsible for reducing $H_2O_2$ to H$_2$O. During myocardial infarction, these enzymes are structurally and functionally impaired by free radicals resulting in myocardial damage (Guarnieri et al, 1980)

A significant decrease in the activity of SOD and catalase in isoproterenol administered Group 2 rats is in accordance with the observation of Sabeena et al (2004).

The decrease in SOD and catalase may be due to the involvement of superoxide and hydrogen peroxide free radicals in myocardial cell damage.
mediated by ISPH. The enzymes could have been utilized more to neutralize the ROS particles to protect the tissue from free radical damage. A decrease in the activity of SOD and catalase enzymes can lead to the formation of $O_2$ and $H_2O_2$, which in turn can form hydroxyl radical (OH') which can thus further disturb the cardiac membrane (Sushma kumari et al, 1987).

The decreased levels of superoxide dismutase along with increased levels of lipid peroxides and xanthine oxidase in isoproterenol treated rats may be an important factor in bringing about the loss of function and integrity of myocardial membranes (Surabhi & Kapoor, 1989). Recent investigations have suggested that the xanthine dehydrogenase is converted into xanthine oxidase under ischemic conditions, which produces superoxide radical with the advancement of cardiac cell damage (Biemond et al, 1986, Surabhi & Kapoor, 1989). To neutralize the superoxide and hydrogen peroxide particles the heart tissues could have used more SOD and catalase enzymes as defense and resulted in decreased level of the enzymes in ISPH induced Group 2 rats.

Group 4 rats exhibited a marked increase in these parameters when compared to Group 2 rats. Goshal et al has reported that mangiferin performs antioxidant function at different levels of the oxidation sequence and conceivably acts by decreasing the localized $O_2$ concentration, generating mangiferin phenoxy radicals in concert and regulating polymer chain initiation by interaction with the ROS to produce feebly reactive oxo radical (Ghosal et al, 1996). This could be the reason for the elevation of SOD and catalase enzymes in mangiferin pretreated Group 4 rats.
Mangiferin, a xanthone derivative, has been reported to scavenge O$_2^-$ produced by the HX/XO and PMS-NADH systems (Leiro et al, 2003). He et al (1998) has reported that xanthones elevate the activity of SOD in myocardial ischemic rats. Abirar sarkar et al has reported that mangiferin possesses catalase activity increasing property (Abíra sarkar et al, 2004). Hence the observed elevation in the activity of SOD and catalase might be due to the protective action of mangiferin.

**Effect of mangiferin on serum and heart tissue lipids and lipoproteins**

It was reported that lipids and lipoprotein profile play an important role in myocardial necrosis induced by isoproterenol (Saleena Mathew et al, 1981)

Isoproterenol induced Group 2 rats showed a significant increase in serum and heart tissue total, ester and free-cholesterol levels with concomitant increase in serum VLDL$_C$, LDL$_C$ levels and significant decrease in serum HDL$_C$ lipoprotein fractions (Table 4).

The previous studies (Subramaniam et al, 2001, Sathish et al, 2003; Zakirov et al, 2000) have reported an increase of serum and heart tissue cholesterol in ISPH induced rats. In ISPH induced rats, a significant increase in LDL$_C$ and significant decrease in HDL$_C$ have been reported by Subramaniyam et al (2001). Increased VLDL cholesterol levels have also been observed after MI as reported by John Bernard Henry (2001)
The abnormal cholesterol deposition is favored by the dangerous tendency of cholesterol to passive exchange between the plasma lipoproteins and cell membranes (Brown and Goldstein, 1986). Isoproterenol induces lipid peroxidation (Sushmakumari and Menon, 1987) and peroxidation cause a disturbance in the structural organization of lipoprotein and as a consequence there is an increase of LDL cholesterol donating ability and a decrease of HDL cholesterol accepting ability and it is shown that specific LDL receptor sites exist on the cell surface, LDL binds at these sites and is internalized by endocytosis (Saleena Mathew et al, 1981). The greater the amount of LDL oxidized, the more cholesterol is transported to erythrocytes. LDL carries the bulk of cholesterol in blood and leads to build up of harmful deposits in the arteries and thus favors coronary heart disease (Manjula et al, 1992a). Hence lipid peroxidation can play an important role in lipoprotein modifications, which makes them susceptible to atherogenesis, which could be the reason for acute MI mediated by isoproterenol.

In mangiferin pretreated (Group 4) rats, the level of serum cholesterol showed a significant decrease with a significant decrease in serum LDL, VLDL cholesterol fractions and significant increase in serum HDL cholesterol fraction. Also in heart tissue of mangiferin pretreated (Group 4) rats, the level of total cholesterol, free cholesterol and ester cholesterol showed a significant decrease when compared to isoproterenol induced Group 2 rats.
Miura et al has reported (Miura et al, 2001) that mangiferin decreases blood cholesterol and a significant decrease in serum and heart tissue cholesterol level in mangifera indica flavanoid treated rats (Anila and Vijayalakshmi, 2002). Anila and Vijayalakshmi has also reported that the level of HDL cholesterol remain unchanged while LDL cholesterol and VLDL cholesterol levels show significant reduction in the case of mangifera indica treated groups when compared with control rats. Polyphenols from different plant sources have been reported (Zern et al, 2003; Vinson et al, 2001) to alter the hepatic cholesterol metabolism and protect low density lipoproteins from oxidative modification and affect VLDL secretion rates which result in less accumulation of cholesterol in the aorta and able to significantly inhibit atherosclerosis and decrease the incidence of coronary heart disease. Previous investigations have shown that some xanthones like mangiferin have a potent antioxidant activity and inhibit lipid peroxidation and block the oxidation of low density lipoprotein in vitro and in vivo conditions (Peres et al, 2000; Gonda et al, 2000; Mahabu sarakam et al, 2000). Mangiferin polyphenol is a glucosyl xanthone and possesses antioxidant property it could have inhibited lipid peroxidation due its scavenging lipid peroxy and alkoxy radicals and thereby preventing continued abstraction of hydrogen from cellular lipids (Ghosal et al, 1996). The higher rate of conversion of cholesterol to bile acids and elimination of faecal bile acids and neutral sterols (Anila and Vijayalakshmi, 2000) could also be considered in the present study for the reduction of cholesterol in Group 4 mangiferin pretreated rats.
The increased level of risk factor ratio in isoproterenol induced Group 2 rats in the present study (Figure 8) is in coincidence with the findings of Manjula et al (1992a).

Risk factor ratio is ratio of LDL cholesterol and HDL cholesterol and it is very important to note because if the ratio is high, the risk is more and if the ratio is low, the risk is less. This risk is regarded as better indicators for myocardial infarction (Chaterjee et al, 2002). It has been reported to note importantly that only LDL cholesterol increases significantly at peak period (Saleena et al, 1981; Austin, 1989), while HDL cholesterol decreased, since cholesterol, most of it esterified accounts for about half of LDL mass (John Bernard Henry, 2001). Frick et al (1990) have suggested a negative association of HDL with incidence of coronary heart disease.

A significant reduction in the risk factor ratio was noticed in Group 4 mangiferin pretreated rats when compared to isoproterenol administered (Group 2) rats. A significant reduction in LDL, VLDL cholesterol levels with a significant increase in HDL cholesterol level has been reported in serum of rats given mangifera indica flavanoids (Anila and Vijayalakshmi, 2002). Epidemiological studies on various polyphenols have been reported to reduce the level of LDL$_{C}$ & VLDL$_{C}$, thereby increasing the HDL$_{C}$ and decreasing the RF ratio to near normal levels (Haban et al, 2004; Zhu et al, 1996). The decrease in LDL/HDL ratio could be due to enhanced HDL cholesterol and increased HDL levels could decrease the cellular uptake of LDL (Chaterjee,
2002; Miler et al, 1976). Reduction of risk factor ratio in mangiferin pretreated Group 4 rats could be possibly due to its polyphenolic antioxidant property.

A significant increase (Table 5) in VLDL_{1G}, LDL_{1G} and triglyceride levels of serum and heart tissue with a significant increase in tissue triacyl glycerol lipase activity and concomitant decrease in the activity of tissue lipoprotein lipase in isoproterenol administered (Group 2) rats. Increased level of TG (Manjula et al, 1992a) is associated with cardiovascular disturbances (Freedman et al, 1988). ISPH promotes lipolysis in the myocardium. Enhancement in lipolysis and subsequent elevation of plasma free fatty acid levels may lead to an increase in hepatic VLDL_{TG} synthesis and secretion and elevated plasma triglyceride concentration (Rayssiguier et al, 1986 & 1990). The high level of serum VLDL_{1G} in isoproterenol rats might have been due to low activity of heart and liver lipoprotein lipase (Manjula et al, 1992a). The decrease in the activity of lipoprotein lipase in heart indicates the decreased uptake of triglyceride lipoproteins from the circulation by this organ and while the increased triglyceride lipase activity suggests an increased intracellular hydrolysis of triglycerides (Saleena et al, 1981) and VLDL_{1G}, is increased. Triglyceride lipase is an enzyme involved in the hydrolysis of triglycerides and probably phospholipids in VLDL remnants, which leads to a more efficient uptake of these particles and generation of LDL (Jin et al, 2002; Ramaswamy Subramanian, 2003). Local synthesis of triglycerides in heart tissue is increased at the peak period.
infarction (Saleena Mathew et al, 1981). An increased synthesis of TG in heart tissue registered in the present study could be due to accumulation of acyl coA and an augmented production of glycerol due to an increased glycolytic flux (Bora et al, 1985) and triglyceride could have been transported to tissue by chylomicrons and VLDL from liver (Tall and Small, 1980).

In mangiferin pretreated (Group 4) rats, serum and heart tissue triglyceride levels showed a significant decrease with concomitant decrease in VLDL-TG, LDL-TG and heart tissue TG lipase activity and a significant increase in heart tissue lipoprotein lipase activity when compared to isoproterenol administered Group 2 rats.

Polyphenols have been reported to significantly lower triglycerides and increase the lag time of lipoprotein oxidation and their consumption in foods has been shown to decrease the risk of heart disease in epidemiological studies (Vinson & Jang 2001) Anila and Vijayalakshmi (2002) has reported an elevated activity of lipoprotein lipase in rat heart treated with flavanoids of mangifera indica Linn. They have reported that the stimulation of lipoprotein lipase activity could have increased the transfer of surface material from triglyceride rich lipoproteins to HDL lipoprotein (Anila and Vijayalakshmi, 2002) and the pronounced reduction in the level of triglycerides in serum and tissue has been observed. The improvement of hyperlipidemia may also be due to the significant reduction in the activities of lipogenic enzymes which provide the sole source of NADPH for free fatty acid biosynthesis.
(Anila and Vijayalakshmi, 2002). A decrease in serum and tissue TG levels with an increased lipoprotein lipase and reduced triglyceride lipase activity in Group 4 mangiferin pretreated rats could be due to the protective effect of polyphenol mangiferin.

The levels (Table 5) of free fatty acids both in serum and heart tissue showed a significant increase in Group 2 isoproterenol rats as compared with Group 1 control rats (Sathish et al, 2003c; Sushmakumari et al, 1990; Sushamakumari & Menon, 1987).

As a result of myocardial infarction, marked changes in fatty acid metabolism has been reported to occur in rats (Bora et al, 1985) The increased levels of free fatty acids in the serum may primarily be due to the release of fatty acids from depot fats (Sushmakumari et al, 1990) and the release of fatty acids could be due to increased lipolysis. The cardiac muscle generally utilizes fatty acids as the major source of energy (Neely 1974) (Opie, 1969). Under anoxic conditions, the cardiac muscle is not in a position to oxidize the available fatty acids, as a result of which there is an increase in the levels of these acids and long chain acyl coA derivatives (Whitmer et al, 1978). Accumulation of acyl coA may be deleterious because it inhibits further formation of coA esters of fatty acids. Thus fatty acids entering the cell cannot be esterified and trapped and are therefore prone to egress promptly. Further more, oxidation of fatty acids entering the cell cannot proceed without initial esterification with coA (Eugene Braunwald, 1988)
This could be the reason in the present study for the increased level of fatty acids in serum and heart tissue of Group 2 ISPH induced rats.

In mangiferin pretreated (Group 4) rats, the level of fatty acids in serum and heart tissue showed a significant decrease when compared to Group 2 ISPH induced rats. Anila and Vijayalakshmi have reported the decreased level of free fatty acids in Mangifera indica flavanoid treated rats (Anila and Vijayalakshmi, 2002). The reduced level of free fatty acids both in serum and heart tissue could be due to decreased lipolysis and increased oxidation of FFA.

The level of (Figure 9) heart tissue phospholipid showed a significant decrease in Group 2 isoproterenol rats as compared with Group 1 control rats. Similar observations have been reported earlier (Sureshkumar and Menon, 1992; Remala et al, 1991).

The decrease in heart phospholipid level may be explained due to increase in phospholipase activity (Surabhi & Kapoor, 1989). Kondo have suggested that the activation of phospholipases is mediated through lipid peroxidation with significant decrease in ATP levels during isoproterenol induced myocardial necrosis (Kondo et al, 1987). Accelerated phospholipid degradation could result in cell injury and ultimately cell death and is evidenced by the accumulation of thiobarbituric acid reacting substances and the loss of both extractable phospholipids and their polyunsaturated acyl groups (Sushmakumari et al, 1990).
In mangiferin pretreated (Group 4) rats, the level of serum phospholipid showed a significant decrease with a significant increase in heart tissue phospholipid level when compared to Group 2 isoproterenol induced rats. The increased level of phospholipids in the present study could be due to its antioxidant and anti lipid peroxidative property.

**Effect of mangiferin on heart tissue protein, DNA and RNA**

Isoproterenol induced (Group2) rats showed a significant increase in protein, DNA and RNA content in heart tissues when compared to Group 1 control rats (Table 6)

Kizer *et al* have reported that the amount of DNA is increased during myocardial infarction (Kizer & Howell, 1970). Ravichandran and Puvanakrishnan (1993) have also reported similar result. The increased DNA content in isoproterenol treated rats has been reported to be probably attributable to fibroblast cells since, cardiac muscle cells do not undergo mitotic division (Smits *et al.*, 1992). Lochner *et al* (1971) have reported that the increased protein synthesis following experimental myocardial infarction as a part of repair process may be stimulated after cellular necrosis. The reports of Ravichandran and Puvanakrishnan (1993) support the present study. It has been reported that protein synthesis is preceded and accompanied by enhanced RNA synthesis (Koide and Rabinowitz, 1969). Wood *et al* have also suggested that the early rise in RNA synthesis could be a primary event and leads to hypertrophy at a later phase (Wood *et al.*, 1971). Venugopal *et al* (2001) have reported that the adrenergic agents adrenaline
and isoproterenol exert effects on cardiovascular cells and induces mRNA hybridization signals in the vascular cells of the heart and also in cardiocytes.

Mangiferin pretreatment reduced the myocardial tissue DNA level in the present study. Mangiferin has been reported to inhibit DNA and protein metabolism in animal study. This could be due to antitumor, immunomodulatory and anticancer activity exhibited by mangiferin (Guha et al, 1996) Leiro et al (2004) have also suggested that mangiferin may protect cells against oxidative damage and mutagenesis. Mangiferin pretreatment reduced RNA and tissue protein levels in the present study. Leiro et al (2003) have reported that mangiferin possesses inhibiting activity on mRNA Mangiferin could have protected the myocardium by reducing the cellular DNA and RNA generation thereby reducing the release of protein.

**Effect of mangiferin on heart tissue glycogen and blood glucose**

In isoproterenol induced Group 2 rats, blood glucose level was found to be increased where as heart tissue glycogen level was found to be decreased when compared to Group 1 control animals (Figure 10)

Surabhi & Kapoor (1989) and Zakirov et al (2000) have reported the decreased level of glycogen in isoproterenol induced rats. The observed decrease in the glycogen content of heart could be due to enhanced glycogenolysis and lipolysis. Isoproterenol administration followed by beta-receptor binding activates phosphorylase kinase leading to glycogenolysis and lipolysis (Meenu Agha et al, 1992).
Isoproterenol administration in rats is associated with pronounced metabolic abnormalities such as elevation of glucose (Bora et al., 1985; Suresh Kumar & Menon, 1992). Isoproterenol administered control rats have been shown to increase in total hexose in heart when compared to normal rats at peak period of infarction (Remla et al., 1983). The observed increase in blood glucose could be due to enhanced glycogen break down and less utilization of peripheral tissues.

The Group 4 mangiferin pretreated rats showed a significant decrease in blood glucose level with a significant increase in tissue glycogen level as compared with Group 2 isoproterenol induced rats.

Mangiferin increased the levels of glycogen and decreased the levels of blood glucose in the present study. Miura et al. (2001) & Ichiki et al. (1998) have also reported the decreased level of blood glucose in mangiferin treated rats. The decreased glucose content in blood could be due to anti diabetic activity of mangiferin.

**Effect of mangiferin on serum and heart tissue electrolytes**

In isoproterenol induced rats, sodium level was significantly increased in heart tissue with significant decrease in potassium level when compared to Group 1 control rats (Figure 11).

A number of studies on myocardial ischemia in animals and man have reflected the efflux of potassium (K⁺) and influx of sodium (Na⁺) in the necrotic cells (Asha Devi, 1984). Acute ischemia, congestive heart failure,
electric shock and arrhythmias are some of the factors influencing the efflux of K⁺ from the cardiac muscle (Jennings et al., 1965). Complete loss of potassium ions have been reported (Harris et al., 1954) in the coronary artery of animals.

Sodium and potassium are major extra cellular and intra cellular cations in a free solution with in the myocardial cells (Shporer & Ciran 1972) and the intracellular concentration of sodium is maintained by active transport with in the cell membrane. However, when the metabolism of the cardiac cells are inhibited by ISPH, the cell membrane is made more permeable to sodium and the efflux of it fails to balance with the influx of the ion in to the cells (Whalen et al., 1974). This results in an accumulation of Na⁺ ions and the intracellular electro negativity is reduced and this depolarization of the cell membrane potential leads to an efflux of the K⁺ ions (Jahiluddin et al., 1979).

Isoproterenol induced rats showed low levels of sodium and high levels of potassium in serum (Figure 11) Flear et al have (Flear & Hilton, 1979) suggested a decrease in plasma sodium concentration in myocardial infarction The extent and duration of the fall are indices of severity of the infarction (Caocci et al., 1978). Elevation of seum K⁺ concentration in isoproterenol induced rats could be due to the decreased activity of Na⁺-K⁺ ATPase a critical factor in maintaining and adjusting the ionic gradients on which the nerve impulse transmission and contractility of the heart muscle depend.
In mangiferin pretreated Group 4 rats, the level of serum Na\(^+\) was increased significantly with significant decrease in serum K\(^+\) where as heart tissue Na\(^+\) was observed to be decreased with significant increase in tissue K\(^+\) when compared to Group 2 ISPH induced rat. This could be due to cardio tonic activity (Srinivasan and Subramanlyan, 1981) of the drug, which could have maintained K\(^+\)/Na\(^+\) ratio (Poliment, 1974). The maintenance of activity of Na\(^+\)-K\(^+\) ATPase and provision of oxidative ATP could have corrected the extra cellular Na\(^+\) ion and intracellular K\(^+\) ion in myocardium and the release of excess K\(^+\) ion in to serum might have been reduced.

Mg\(^{2+}\) is a metal cofactor of ATPase, which helps in the normal activity of myocardium and Mg\(^{2+}\) has been implicated in different cardiovascular diseases (Avtar Lal & Rana, 1991) Magnesium ions are responsible for maintaining the functional and structural integrity of myocardium (Avtar Lal & Rana, 1991). Intracellular myocardial magnesium deficiency can be considered as an important biochemical factor in the pathogenesis of electrophysiological changes and is responsible for ventricular fibrillation (Singh et al, 1981).

Isoproterenol induced rats showed decreased level of Mg\(^{2+}\) in heart tissue and increased in serum (Figure 12). Several studies have indicated a potential role of free radical participation in Mg\(^{2+}\) deficient lesions. Moreover, the Mg\(^{2+}\) deficient animals have been reported to show an increased susceptibility to an in vivo oxidative stress (Freedman et al, 1991). The effect of Mg\(^{2+}\) deficiency in rats have been examined and reported that superoxide
anion production is significantly increased in Mg$^{2+}$-deficient rats (Hosakawa et al., 1989). Moreover, an increased concentration of calcium intracellularly have been repeatedly demonstrated in Mg$^{2+}$-deficient animals (Rayssiguier et al., 1991) could theoretically act to enhance lipid peroxidation. The thiobarbituric acid reacting substances, used as a measure for lipid peroxidation, are reported to increase in the plasma of Mg$^{2+}$-deficient rats (Mahfoz and Kummerow, 1989). When the extra cellular Mg$^{2+}$ concentration is low, the basal tension of the isolated coronary artery is increased and its contractile response to vaso constrictive agents is potentiated, where as when the magnesium concentration is high, the basal tension of the artery is depressed causing vasodilatation (Avtar Lal and Rana, 1991). This could be the reason for the increased level of Mg$^{2+}$ in serum and decreased level of Mg$^{2+}$ in tissue of the isoproterenol induced rats.

Mangiferin pretreatment reduced the serum magnesium level and increased the myocardial magnesium level. This could be due to anti lipid peroxidation and antioxidant property of mangiferin which could have further reduced free radical mediated lipid peroxidation thereby the activity of Mg$^{2+}$ ATPase and Na$^+$/K$^+$ ATPase could have been maintained normally and the reduced Ca$^{2+}$ increased the level of Mg$^{2+}$ in tissue and decreased in serum.

Calcium is essential for the normal cardiac contractility function (Ahuja and Ambish, 2002). In the heart, cytosolic Ca$^{2+}$ is carefully controlled and it is the key ion for normal contracture of many enzyme reactions (Hamet, 1995).
Isoproterenol induced rats showed increased level of Ca\(^{2+}\) in tissue with a concomitant decrease in serum calcium (Figure 13). Namikawa et al (1991) have reported the similar results.

Isoproterenol administration causes intracellular calcium over load, which leads to deleterious high energy phosphate deficiency by the inhibition of Ca\(^{2+}\) ATPase (Sathish et al, 2003c). The consequences of raised cytosolic Ca\(^{2+}\) are multifactorial. Events that can be attributed to the early rise in cytosolic Ca\(^{2+}\) include activation of Ca\(^{2+}\) sensitive proteases and phospholipases, stimulation of oxyradical production, energy wastage and elevation of end diastolic resting tension (Nayler, 1993). Each of these events may accelerate cell and tissue necrosis (Nayler, 1993). Intracellular Ca\(^{2+}\) overload changes the activation of Na\(^{+}\)-H\(^{+}\) exchange and Na\(^{+}\)-Ca\(^{2+}\) exchange systems in the ischemic myocardium (Allen and Xiao, 2000). This Na\(^{+}\)/Ca\(^{2+}\) exchange mechanism may increase cellular calcium (Scholz et al, 1993). Ca\(^{2+}\) overload increases generation of oxygen free radicals and exerts a direct toxicity of the myocardium (Marban et al, 1989). The subsequent increase in free intracellular Ca\(^{2+}\) has been shown to be associated with decreased recovery of myocardial contractile function, compromised membrane integrity and a progressive decline in cellular ATP stores (Kaur et al, 1995). Lowered ATP stores reduce extrusion of Ca\(^{2+}\) from cells. The resultant augmented intracellular Ca\(^{2+}\) causes overload, which depresses ATP production further. Finally, a number of calcium activated proteases may destroy critical intracellular structures. Thus high concentrations of
catecholamines may stimulate the slow current carried principally by Ca$^{2+}$, resulting in slow response action potentials (Eugene Braunwald et al, 1988).

Mangiferrin (antioxidant) pretreated rats showed a decreased Ca$^{2+}$ in heart tissue with an increase in serum Ca$^{2+}$ levels. This could be due to antioxidant activity of mangiferin. This could have suppressed the activity of phospholipase or proteases and the activity of Na$^+K^+$ ATPase could be restored and prevented the influx of Ca$^{2+}$ in the cell by blocking the Na$^+ -$ Ca$^{2+}$ exchange and consequently could have increased the store of high-energy phosphate available to the myocyte. Recently, it has been found that some xanthones like mangiferin inhibits the activation of Na$^+Ca^{2+}$ exchange system and decrease Ca$^{2+}$level in myocardial tissues in isolated hearts (He et al, 2000; Cheng & Kang, 1997). Mangiferrin is a xanthone and hence xanthone property of mangiferin could also be attributed in reducing the Ca$^{2+}$ in the myocardium and increased serum calcium level.

**Effect of mangiferin on serum trace elements**

Zinc is a cofactor of protease, alkaline phosphatase, glutamic dehydrogenase and superoxide dismutase. Superoxide dismutase, which is vital for the protection of the cells from oxidative damage and Zinc is an important membrane-stabilizing agent (King and Keen, 1999). Zinc therapy has been found to be beneficial in certain cases of atherosclerosis, a condition of diseased blood vessels affecting arterial perfusion.
In isoproterenol induced rats, the amount of Zinc was reduced in serum (Figure 14), which concurs with the previous findings of Namikawa et al (1993). An abrupt decrease in serum zinc level has been shown to occur after myocardial infarction in both clinical (Low and Ikram, 1976) and experimental conditions (Singh, 1983) Lindermann et al (1973) have also hypothesized the fall of serum zinc level following acute myocardial repetitive process. Significant correlation exists between the fall of serum zinc levels, Creatine Kinase, LDH and SGOT levels (Lil et al, 1988). Prasad and colleagues have observed a decreased activity of several zinc containing and zinc dependent enzymes such as LDH, ADH, MDH and ALP in tissues of zinc deficient animals. The lowered activities of enzymes could be due to direct effects of zinc deprivation because of decreased synthesis due to transcriptional impairment (Jaffery & Jailkhani, 2002)

Mangiferin increased the serum Zinc level, which could be due to its antioxidant and oxidant balance maintaining action that could have prevented the reduction of Zinc as well as Zinc containing enzymes such as SOD, LDH and MDH enzymes thereby prevented myocardial necrosis.

In isoproterenol induced Group 2 rats, the level of copper is high in serum (Figure 14). Khan et al has reported that the patient who have suffered acute MI shows significantly increased level of copper in their serum (Khan et al, 1983). Gomez et al (2000) & Malek et al (2000) have also reported an increase in the values of this trace element in serum of the acute MI patients. Several epidemiological studies have also found that the increased serum copper levels are associated with increased risk of
cardiovascular diseases. Elevated serum copper is reported to be a marker of the inflammation that accompanies atherosclerosis and MI (Khan et al, 1983).

Isoproterenol induced myocardial infarction is accompanied by the disintegration of membrane polyunsaturated fatty acids a measure of lipid peroxides and by the impairment of natural scavenging antioxidants, characterized by the decrease in the levels of superoxide dismutase and ceruloplasmin as reported by Nirmala and Puvana Krishnan (Nirmala Puvanakrishnan, 1996a) Since most copper in the body is associated with ceruloplasmin a decrease in the level of this protein could increase the copper. Copper is known to be a pro-oxidant the frequently generated copper could promote oxidation of LDL protein and increase the risk of cardiac damage by significant increase in the malonaldehyde levels (Malek, 2000)

Mangiferin pretreatment has reduced serum copper in Group 4 rats when compared to Group 1 rats in the present study. Mangiferin is known to chelate a number of metal ions such as Cu^{2+} and Fe^{2+} (Guha et al, 1996) and has been reported to maintain antioxidant oxidant balance (Ghosal et al, 1996).

Effect of mangiferin on heart mitochondrial LPO

Isoproterenol induction caused an increase in the levels of lipid peroxides in the myocardium causing myocardial membrane damage and dysfunction (Sushamakumari et al, 1990). Similar results were observed in the present study (Figure 15), which is also supported by Padma and Devi (2002) & Nirmala (1996a).
Biological membranes are rich in unsaturated fatty acids and are easily susceptible to microsomal peroxidative attack. Lipid peroxidation usually begins with the removal of hydrogen atom from unsaturated fatty acids resulting in the formation of lipid radical. The rearrangement of the double bond results in the formation of conjugated dienes, which forms lipid peroxy radical in the presence of molecular oxygen. The lipid peroxy radical takes up hydrogen atoms to form lipid hydro peroxide or lipid endoperoxides. MDA is a break down product of the unsaturated fatty acids containing 3-methylene interrupted double bonds (Sushamakumari et al, 1987). Lipid peroxidation in the membrane of heart mitochondria with a considerable increase in the content of diene conjugates and accumulation of malondialdehyde has been reported in experimental ischemia. The increase results in a relatively large molecular ordering of the residual phospholipids leading to a decrease in membrane fluidity which is usually associated with myocardial infarction (Sushamakumari et al, 1989) and has been reported to be accompanied by a reduction of mitochondrial respiratory capacity (Ceconi et al, 1988) This could be the reason for an increased LPO activity in mitochondria of isoproterenol induced animals in the present study.

The decreased level of mitochondrial LPO was observed in mangiferin pretreated Group 4 rats. Mangiferin has been reported to reduce mitochondrial (Sanchez et al, 2000) and microsomal (Sato et al, 1992) lipid peroxidation in rats. As for as membrane lipid peroxidation is concerned, mangiferin has been reported to conceivably act by decreasing the localized concentration by generating mangiferin phenoxy radicals and scavenging lipid
peroxy/alkoxy radicals (Gosal et al, 1996). Due to this property, mangiferin could have reduced mitochondrial LPO in Group 4 mangiferin pretreated rats.

**Effect of mangiferin on mitochondrial antioxidant Enzymes**

GSH has direct antioxidant function by reacting with superoxide radicals, peroxy radicals and singlet oxygen (Meister and Anderson, 1983) and protects the mitochondrial membrane from the damaging action of LPO (Tappel, 1973). Isoproterenol induced Group 2 rats showed (Table 7) a decrease in the activities of GSH related enzymes, SOD, catalase and GSH levels in heart tissue mitochondria. Sathish et al have also reported (Sathish et al, 2002) similar results.

In mitochondria, the decreased glutathione level increased GSSG (oxidized product of GSH) during isoproterenol induction and inactivates glutathione related enzymes (Lal et al, 1988). In mitochondria (Shlager et al, 1987) the accumulation of GSSG could result in a respiratory burst with the production of $\cdot O_2^-$ and $H_2O_2$ (Pritsos & Pardini, 1983), which leads to oxidative stress and under conditions of severe oxidative stress glutathione peroxidase reported to be inactivated (Litov et al, 1981).

The decreased activities of mitochondrial SOD and catalase during isoproterenol administration is in accordance with the observation of Manjula et al (1992c). Mitochondrial activities of the major enzymes are responsible for degrading $H_2O_2$ and superoxide anions ($O_2^-$) during ischemia or injury and significantly reduce the activities of SOD and related enzymes in the mitochondrial fraction (Shlager et al, 1987). Evidence have suggested
that myocardial hypoxia induce a significant reduction in the mitochondrial superoxide dismutase and reduced glutathione activity is associated with massive tissue and mitochondrial calcium accumulation, loss of mitochondrial function and severe membrane damage (Ferrari et al, 1985b). SOD depletion has been reported to induce a prolonged modification of membrane lipid composition (Calviello et al, 1988).

Mangiferin pretreatment increased mitochondrial antioxidant GSH, the activities of GSH related enzymes, SOD and Catalase in Group 4 rats. This could be due to the property of mangiferin in reducing the generation of hydroxyl radicals and regulating polymer chain initiation by interaction with the reactive oxygen species to produce feebly reactive oxo radical and maintaining cellular oxidant and antioxidant balance in membranes (Goshal et al 1996). Ochoa et al (1999) has reported that the polyphenol diet prevents lipid peroxidation in rabbit heart mitochondria and protects the mitochondrial antioxidant enzyme from oxidative stress. Since mangiferin is a polyphenolic antioxidant this property could also be attributed to the protection of mitochondrial antioxidant enzymes in Group 4 mangiferin pretreated rats.

**Effect of mangiferin on mitochondrial protein, membrane bound enzymes and electrolyte levels**

ATPases of the cardiac cells are known to be the most important enzymes to maintain the vital reactions by hydrolysis of the terminal high energy phosphate, almost all of which is produced in the mitochondria under
aerobic metabolism. The loss of ATPase activity in the ischemic state may be responsible for causing not only functional damage but also irreversible necrotic changes in the involved myocardial cell.

The activity (Table 8) of mitochondrial membrane bound enzymes such as Na⁺ K⁺ ATPase, Ca²⁺ ATPase were decreased significantly with significant increase in protease and phospholipase activities in ISPH induced MI rats. Similarly the level (Figure 16) of Na⁺ and Ca²⁺ were increased in mitochondria with significant decrease in mitochondrial K⁺ level in ISPH induced MI rats when compared to normal rats.

A significant decrease in the activity of Na⁺ K⁺ ATPase in heart mitochondria of ISPH induced MI rats were comparable with previous reports (Ebenzar et al, 2003 and Vajreswari and Reddy, 1992).

Na⁺ K⁺ ATPase requires lipid domain for its proper functioning (Voet and Voet, 1995) Peroxidation of membrane lipids could inactivate Na⁺K⁺ ATPase because of the oxidation of “SH” groups present in its active site leading to the conformational alteration in the enzymes (Kako et al, 1998). Inactivation of Na⁺ K⁺ ATPase could be due to enhanced lipid peroxidation (Meerson et al, 1983) by free radicals on isoproterenol administration since Na⁺ K⁺ ATPase is a SH group containing enzyme and is lipid dependent Hebel et al (1986) have also correlated inactivation of membrane bound enzymes with peroxidation of membrane lipids.

Isoproterenol induced rats showed increased level of mitochondrial Na⁺ and decreased level of K⁺ in the heart mitochondria. Several studies have
shown that cell injury is associated with alterations in cellular ionic homeostasis. Ischemia leads to an increase in cellular Na\(^+\) with a concomitant decrease in K\(^+\) and elevation of Ca\(^{2+}\) level (Langer, 1982). Elevation of intracellular sodium will operate to depress Ca\(^{2+}\) efflux and augment Ca\(^{2+}\) influx (Katz, 1981). The inhibition of Na\(^+\) K\(^+\) ATPase can activate the Na\(^+\) Ca\(^{2+}\) exchange mechanism in the myocardium. This Na\(^+\) Ca\(^{2+}\) exchange mechanism may play a role in regulating the cellular calcium level (Trump et al., 1984).

Mangiferin increased the activities of Na\(^+\) K\(^+\) ATPase and mitochondrial K\(^+\) level and decreased the mitochondrial Na\(^+\) level. It could be due to its antioxidant property and antilipid peroxidation property. Due to this property, mangiferin could have protected the SH group at the active site of the enzyme from isoproterenol induced free radical attack and there by normal level of mitochondrial Na\(^+\) and K\(^+\) could have been restored by reducing Na\(^+\) Ca\(^{2+}\) exchange.

Ca\(^{2+}\) ATPase serves an important function by actively removing calcium from interior of the cell. A significant decrease in Ca\(^{2+}\) ATPase activity was observed in mitochondria of heart in isoproterenol administered rats. The activity of Ca\(^{2+}\) ATPase is modulated by cellular thiol status and lipid peroxidation (Hers & Manson, 1984). The significant decrease in Ca\(^{2+}\) ATPase activity on isoproterenol administration is attributed to the oxidation of 'SH' groups present in its active site. Inhibition of Ca\(^{2+}\) ATPase in organelles under ischemic condition might be responsible for the deterioration of the
function of mitochondria (Hess et al., 1984). Myocardial ischemia is associated with loss of Ca\(^{2+}\) homeostasis (Nayler, 1992; Nayler, 1993) A significant increase in Ca\(^{2+}\) activity was observed in the isoproterenol induced rat mitochondria. The increase in mitochondrial calcium content following isoproterenol administration is in accordance with the observation of Saestersdal et al (1982). Since there exists a functional interaction between Ca\(^{2+}\) pump and phospholipids (Heramans and Wuytack, 1980) the decrease in the Ca\(^{2+}\) pump activity could also be due to the enhanced phospholipase activity on isoproterenol administration. The activation of phospholipase, during isoproterenol administration could be due to enhanced free radical action and is a result of intracellular Ca\(^{2+}\) accumulation (Hers and Manson, 1984). The subsequent increase in free intracellular Ca\(^{2+}\) is a consequence of ischemic tissue injury and has been shown to be associated with decreased recovery of myocardial contractile function, compromised membrane integrity and a progressive decline in cellular ATP stores (Kaur et al., 1995).

Mangiferin pretreatment decreased the level of Ca\(^{2+}\) ATP-ase, and decreased Ca\(^{2+}\) content in mitochondria of the heart. This could be due to antioxidant, anti-free radical and anti-lipid peroxidative activity of this compound. Due to this property, it could have inhibited phospholipase activity and by keeping reduced amount of Ca\(^{2+}\) it could have reduced calcium overload. The maintenance of Ca\(^{2+}\) ATP-ase activity of mangiferin pretreatment could also be mediated by low membrane permeability of Ca\(^{2+}\) in the inward direction and by an active efflux mechanism catalyzed by the active membrane bound
Na\(^+\)K\(^+\) ATPase. Polyphenols have been reported to normalize the changes in Ca\(^{2+}\) towards the control values and may preserve endothelium integrity in cardiovascular diseases (Martin et al, 2003). Xanthone has been reported to inhibit the Ca\(^{2+}\) influx induced by epinephrine suggesting that xanthone might act as a blocker of Ca\(^{2+}\) channels (Cheng and Kang, 1997). In the present study, the mechanism of action of mangiferin in regulating Ca\(^{2+}\) and inhibiting Ca\(^{2+}\) ATP-ase could be attributed to the antioxidant property of mangiferin.

In the present study, a significant decrease in the mitochondrial protein level with significant increase in mitochondrial protease activity was observed in the myocardium of isoproterenol administered MI rats. The increase in protease activity on isoproterenol administration could result in the degradation of mitochondrial membrane protein of the heart. The decrease in mitochondrial membrane protein depends on the extent of lipid peroxidation and as a result of an alteration in membrane permeability (Diaz et al, 1977). Chagoya et al (1997) has also reported the decrease in mitochondrial proteins after the administration of ISPH. The toxic intracellular free radicals produced by isoproterenol can directly damage protein biosynthesis (Vig, 1979; Friedman and Carter, 1978; Kanter and Schwartz, 1979). Marcillat et al (1988) have reported that mitochondrial protease activity was found to be increased with a concomitant decrease in protein content. Their experiments revealed a distinct elevation of mitochondrial proteolytic pathway, which preferentially degrades oxidatively denatured proteins.
Mangiferin increased the level of mitochondrial protein and thereby reduced protease activity. Since mangiferin is a potent antioxidant it could have protected the mitochondria from free radical attack and the degradation of proteins could have been prevented due to inhibition of protease activity in mitochondria.

The increase in the activity of phospholipase on isoproterenol administration indicates enhanced degradation of mitochondrial membrane phospholipids by the activation of phospholipase (Farber and Young, 1981). The activation of phospholipase caused by biologically active $O_2$ free radicals (Manjula et al, 1994) since isoproterenol causes excessive production of free radicals and lipid peroxidation. Thus a significant decrease in mitochondrial phospholipid content could account for impaired mitochondrial function, following changes in mitochondrial structures (Dass et al, 1986).

Mangiferin reduced the activity phospholipase. It could be due to antioxidant and anti lipid peroxidative and anti free radical property of mangiferin thereby prevented the degradation of phospholipids. Due to decreased degradation of phospholipids the mitochondrial membrane was protected from ISPH induced free radical mediated attack.

**Effect of mangiferin on mitochondrial TCA cycle enzymes**

The activity of TCA cycle enzymes such as isocitrate dehydrogenase, alpha keto glutarate dehydrogenase, succinate dehydrogenase and malate dehydrogenases were recorded to be decreased in isoproterenol
induced Group 2 rats (Table 9). Similar results have been reported previously (Padma and Devi, 2002; Sathish et al, 2002) in heart mitochondrial studies. The dehydrogenases of TCA cycle enzymes could have been affected by the free radicals produced during isoproterenol administration (Padma and Devi 2002). Dehydrogenases are "SH group" containing enzymes and are readily inactivated on exposure to free radicals (Manjula et al 1993a), and accumulation of reducing equivalents (NADH₂) and metabolites (hydrogen ions) in the cytosol generates more protons so that intracellular acidosis is promoted. Pyruvate dehydrogenase located on the mitochondrial membrane is inhibited by NADH₂ and that entry to citrate cycle is inhibited (Padma and Devi, 2002). This could be the reason in the present study for the decreased activity of mitochondrial TCA cycle enzymes in Group 2 isoproterenol administered rats.

Reduced activities of cytochrome-c-oxidase and NADH dehydrogenase in isoproterenol administered Group 2 rats observed in the present study (Table 9) could be due to enhanced free radical mediated lipid peroxidation induced phospholipid degradation resulting in the unavailability of cardiolipin for their functional activities. Cytochrome-c-oxidase and NADH dehydrogenase have an absolute requirement of cardiolipin phospholipid (Nicolay et al, 1985) for their activity. Accelerated degradation of membrane phospholipids by phospholipases has been proposed to be the cause for irreversible ischemic injury (Farber and Young, 1981).

In the present study, mangiferin pretreated rats showed an increased activity of all the enzymes involved in TCA cycle including NADH
dehydrogenase and cytochrome oxidase in Group 4 rats. Mangiferin like polyphenols has been reported to increase the activity of mitochondrial SDH activity in experimental studies (Zhang et al., 2001; Hsu et al., 2003). Mangiferin polyphenol has anti lipid peroxidative and anti free radical activity and this property of the drug could have helped in protecting ‘SH’ group of dehydrogenases from free radical attack and thus lowered the accumulation of reducing equivalents (NADH₂), thereby oxygen consumption could be maintained to normalize the TCA cycle. Due to anti lipid peroxidative activity, mangiferin could have protected phospholipid degradation by inhibiting the activity of phospholipase and thus could be able to restore cardiolipin for the activity of cytochrome oxidase and NADH dehydrogenase.

**Effect of mangiferin on plasma lactate**

In the present investigation, isoproterenol induced Group 2 rats showed an increase in plasma lactate concentration (Figure 17) The increased levels of plasma lactate have already been reported (Sathish et al., 2002; Padma and Devi, 2002) Lactate released into coronary sinus blood, is used as a sign of myocardial ischemia (Gertz et al., 1980) Isoproterenol induces myocardial ischemia and leads to hypoxia, which further creates anaerobic environment. Lactate is taken up by the aerobic heart and is produced during anaerobiosis. (Gertz et al., 1980). The increasing concentrations of lactic acid within the cell decline pH and the accumulation of other metabolites inhibit glycolytic flux at the phosphofructokinase and glyceraldehyde-3-phosphate dehydrogenase steps and others (Rovetto et al., 1975; Sobel & Mayer, 1973). The decrease in internal pH leads to acidosis which further increases cytosolic
calcium by exchange of Na\(^+\)/H\(^+\) and Na\(^+\)/Ca\(^{2+}\). On the other hand, acidosis itself inhibits glycolytic flux and the malate-aspartate cycle (Williamson et al., 1976), and accumulation of reducing equivalents (NADH\(_2\)) and metabolites (hydrogen ions) in the cytosol. NADH\(_2\) accumulation in the cytosol generates more protons so that intracellular acidosis is promoted. Pyruvate dehydrogenase located on the mitochondrial membrane is inhibited by NADH\(_2\) and that entry to citrate cycle is inhibited and more lactate forms (Padma and Devi, 2002).

Mangiferin decreased plasma lactate content in mangiferin pretreated Group 4 rats in the present study. This could be due to antioxidant activity and anti lipid peroxidative property of mangiferin. Due to this activity mangiferin might have protected mitochondria from ischemic hypoxia and hence anaerobic glycolysis could be prevented, which could be able to reduce both acidosis and lactate accumulation.

**Effect of mangiferin on mitochondrial oxidative phosphorylation and ETC**

In the present study, isoproterenol induced rats showed decreased levels of ATP, State 3 (+ADP), State 4(-ADP) levels (Table 10) and ADP/O and RCR ratio (Figure 18) in the heart mitochondria. Previous reports supported (Sathish et al., 2002; Paritha and Devi, 1998; Chagoya de Sanchez et al., 1997; Padma and Devi, 2002) the results of the present study.

Oxidative phosphorylation is regulated to a large extent by the phosphate potential. i.e. ATP/ADPxPi. Under normal aerobic conditions,
myocardium derives its energy primarily from oxidative phosphorylation, a process localized to the mitochondria (Taegtmeyer, 1985). Isoproterenol induced MI favors lipolysis and hyperlipidemia. The impaired beta-oxidation of fatty acids increases free fatty acids and other lipid metabolites, which may further inhibit the activity of adenine nucleotide translocase resulting in the deprivation of ADP for oxidative phosphorylation (Singal et al, 1983). The decreased oxygen uptake may be due to impairment in myocardial oxygen production (Jarmakani et al, 1978). Decrease in ADP/O ratio shows that the utilization of oxygen is delinked from oxidative phosphorylation and thus there will be decreased synthesis of ATP (Sathish et al, 2003c; Padma and Devi, 2002).

Isoproterenol induced rats showed decreased level of ATP in the heart mitochondria. The result of Chagoya de Sanchez et al (1997) concurs with the present findings. If oxygen delivery is interrupted and the balance between ATP production and consumption is disturbed, ATP concentration will decline and ADP, AMP, adenosine and Pi levels increase shifting non-aerobic respiration to anaerobic glycolysis (Zarco and Henas Zarco, 1996). When oxygen availability is limited, the rate of ATP synthesis declines and high-energy phosphate stores decline. In an attempt to maintain ATP stores, creatine phosphate is depleted and transferring its high energy phosphate to ADP (Ugurbil, 1985). The reduction in phosphate potential alters the activity of enzymes involved in the intermediary metabolism. There is evidence for functional compartmentalization of oxidative metabolism that energy derived from oxidative phosphorylation preferentially supports
contractile function (Weiss and Hiltbrand, 1985). Isoproterenol induces myocardial contractility, cyclic AMP and increased heart rate. The combination of reduced myocardial high energy phosphate concentration and cell swelling results in damage to the sarcolemma, which may play a key role in cell death during myocardial ischemia (Steenberger et al, 1985 and Reimer et al, 1981).

Isoproterenol induced rat showed the decreased level of State 3, State 4 and RCR in the present study. A decrease in respiratory rate (State 3 and State 4) and RCR has been reported in ischemic myocardium (Sathish et al, 2003c). This respiratory control ratio is a useful measure of the integrity of isolated mitochondria. The phenomenon in which the rate of electron transport is controlled by the concentration of ADP is called respiratory control. Respiratory control ratio is the ratio of the rate of respiration of mitochondria in the presence of ample ADP (State 3) to the ratio of respiration in the absence of ADP (State 4). The most dramatic decrease in respiratory activity during State 3 respiration indicates an impairment of electron transport coupling and loss in the mitochondrial energy production on isoproterenol treatment (Sathish et al, 2003c).

Mangiferin pretreated rats showed increased ATP level, ADP/O Ratio, State 3 level, State 4 level and RCR in the heart mitochondria. Ghosal et al (1996) has reported that mangiferin has the property of regulating polymer chain initiation by interaction with the reactive oxygen species to produce feebly reactive oxoradical and scavenging lipid peroxy/alkoxy radicals on membrane. This property could be attributed in the enhancement
of beta-oxidation of free fatty acids and the reduction of polyunsaturated free fatty acid concentration could increase the activity of adenine translocase, thus able to synthesize more ADP for oxidative phosphorylation with increased uptake of myocardial oxygen. This could have normalized the flow of electrons to maintain ETC and mitochondrial ATP production was increased.

Decreased cytochrome content (Figure 20 & 21) and increased NADH oxidation (Figure 19) in heart tissue mitochondria was observed in isoproterenol induced Group 2 rats.

NADH oxidized easily since NADH dehydrogenase is an autooxidisable electron carrier responsible for a portion of free radical production in mitochondria. The increase in NADH oxidation on isoproterenol administration clearly indicates the increased permeability of mitochondrial membrane to NADH and increase in NADH oxidation in membrane results in the peroxidation of membrane lipids (Rickwood et al., 1987). Lipid peroxidation in mitochondria has been reported to accompany by a reduction of mitochondrial respiratory capacity and Ca\(^{2+}\) transport (Ceconi et al 1988) A decrease in mitochondrial cytochrome content in ischemic myocardium causes loss of oxidative phosphorylation (Bush et al., 1980). During respiratory process the transport of electrons occur via cytochromes (electron carriers) to molecular oxygen Variations in the cytochrome content may affect the transport of electrons via electron transport chain and thereby alter the energy production. Cytochorme aa\(_3\) is the terminal cytochrome in electron transport chain and a decrease in its concentration will
lead to a decrease in the uptake of oxygen, resulting in low respiratory rate (Bush et al, 1980). The close correlation between the decline of mitochondrial respiration and the activity of complex II strongly suggests that an impairment of the respiratory chain in the b-c1 region represents one of the functional events in the causal sequence of peroxidative reaction preceding the phase of massive malondialdehyde production (Trumper et al, 1988).

Mangiferin pretreatment increased cytochrome level and decreased NADH oxidation in Group 4 rats. This could be due to antioxidant and anti lipid peroxidative property of the drug, which might have reduced the oxidation of NADH and thus prevented the inhibition of cytochromes and protected the myocardial mitochondrium from free radical attack mediated by isoproterenol.

Effect of mangiferin on heart mitochondrial structure (Electron microscopic study)

The alterations in cardiac ultrastucture is based on animal experiments and provide important information concerning the process of myocardial infarction In the present study, the transmission electron micrography of mitochondria from the heart of Group 2 isoproterenol induced rats showed swollen morphology (Plate 6). The mitochondrial cristae were also disrupted and fragmented. These observations in isoproterenol induced myocardial ischemic injury, is in accordance with the observation of Bhimji et al (1986). Namikawa et al (1991) & Chagoya de Sanchez et al (1997) have also reported similar results.
The swollen morphology is typical for mitochondria that have been subjected to ischemic and hypoxic conditions (Khariu et al., 1984). The increased mitochondrial swelling could be due to the accumulation of lipid peroxide products as a result of GSH depletion (Bors et al., 1978). A strong correlation exists between mitochondrial swelling and ATP concentration in ischemic heart (Noronha & Steen, 1982). Vacuolization and electron lutency at the mitochondria is due to an inability of the mitochondrial membrane to produce high energy phosphate, which primarily induces lack of ATP for cardiac mitochondrial activity and membrane integrity which in turn leads to the impairment of myocardial function (Nakahara and Takeo, 1986).

Electron microscopic observation of mangiferin pretreated Group 4 rats showed reduced swelling of mitochondria, without any disturbances in cristae and fragmentation was not seen. The vacuolization was also absent and this could be due to the anti lipid peroxidative and antioxidant property of mangiferin which could have reduced mitochondrial lipid peroxidation to maintain the ETC and ATP concentration for the normal functioning of heart mitochondria.

**Mitochondrial Lipids**

Isoproterenol induced rat showed increased level of mitochondrial cholesterol, free fatty acids, triacylglycerol with decreased level of mitochondrial phospholipids (Table11). A significant rise in the mitochondrial cholesterol content suggests a redistribution of cholesterol in the ischemic cell (Sathish et al., 2003a). Manjula et al (1993b) have also
reported the similar results. An alteration in mitochondrial membrane cholesterol content affects its fluidity, permeability of ions, activities of membrane bound enzymes and increased degradation of membrane PL. Changes in membrane cholesterol content affects the molecular motion of hydrocarbon chain of lipids in bilayers and cell membranes (Sushamakumari et al, 1990). The increased cholesterol viscosity of the chains is associated with decreased phospholipids, which may be an important factor contributing to the membrane dysfunction (Sushamakumari et al. 1990).

Biological membranes are rich in phospholipids. In myocardial mitochondria, the primary role of PL is to stabilize the conformation of membrane bound enzymes (Laird et al, 1986). Myocardial ischemia is associated with free radical mediated tissue injury involving peroxidation of membrane phospholipids. Accelerated phospholipid degradation could produce membrane dysfunction resulting in cell injury and ultimately cell death. The increased peroxidation of phospholipids in the mitochondrial membrane results in the release of polyunsaturated fatty acids by enhanced activity of phospholipase (Manjula et al, 1992b). Accelerated degradation of membrane PL by phospholipases and lysophospholipases has been related to membrane dysfunction and irreversible ischemic injury (Farber and Young, 1981).

A significant increase in free fatty acids with increase in triacylglycerol content were observed in the myocytes of isoproterenol administered rats. Vorbeck et al (1975) and Manjula et al (1993b) have
reported the same. The accumulation of free fatty acids is a consequence of changes in myocardial mitochondrial lipid metabolism. This could be due to the accelerated degradation of membrane phospholipids by phospholipases. When supply of oxygen is reduced, as in myocardial infarction, beta-oxidation of free fatty acids ceases, leading to increased synthesis of triglyceride due to excess free fatty acid accumulation (Taegtmeyer, 1998). The subsequent increase in the levels of fatty acids may be closely associated with the breakdown of the membrane structure caused by lipid peroxidation and decreased uptake by the damaged cells (Sushmakumari menon, 1987). Excess free fatty acids inhibit respiratory activity and depress cardiac function in ischemic condition (Jackson, 1985).

Mangiferin decreased the levels of cholesterol, triglyceride, free fatty acids and increased the levels of phospholipids. This could be due to its antioxidant, anti lipid peroxidative and free radical scavenging activity. Ghosal et al (1996) has reported that mangiferin performs its antioxidant function at different levels of the oxidative sequence. As far as membrane lipid peroxidation is concerned, it conceivably acts by decreasing the localized O₂ concentration, generating mangiferin phenoxy radicals, to produce feebly reactive oxo radical and scavenging lipid peroxy / alkoxy radicals and thereby preventing continued abstraction of hydrogen from cellular lipids. Mangiferin could have decreased phospholipase activity and thereby phospholipid degradation could have been avoided and the increased level of phospholipid was noted in the mitochondria of mangiferin pretreated Group 4 rats.
Lysosomal lipid peroxidation and its enzymes in serum and heart lysosomes

Lysosomes are a distinct group of cytoplasmic organelles, known to occur in numerous animal tissues and characterized by their content of a variety of acid hydrolases (De Duve, 1959). In addition, lysosomes play a major role in secretion and transport processes. It has been postulated that the intracellular release of lysosomal enzymes and their subsequent extra lysosomal activity may exercise a pivotal role in the progressive modifications that lead from reversible myocardial ischemia to irreversible infarction (Decker et al, 1977).

In isoproterenol induced Group 2 rats, the level of lysosomal LPO increased with concomitant increase in the activities of serum hydrolases (Table 12) and significant decrease in lysosomal subcellular fraction hydrolase enzyme activities. These observations are in accordance with that of (Ravichandran et al, 1991) Elevated levels of lysosomal hydrolases have been reported in the serum of patients with MI (Welman 1978) and in myocardial infarction induced experimental animals (Mathew et al 1982 a,b). Sathish et al (2003b) and Ebenezar et al (2003) have also reported similar results.

Lysosomal hydrolases are important mediators of myocardial infarction and its release into cytoplasm stimulate the inflammatory mediators like oxygen radicals, prostaglandin's etc (Mathew et al, 1982a,b). It has been suggested that oxygen free radicals generated during ischemia in addition to
the direct myocardial damaging effect may also be responsible for the cardiac
damage through the release of lysosomal enzymes (Kalra and Prasad, 1994).
Altered membrane integrity has been suggested as a major factor in the
development of cellular injury during myocardial ischemia (Burton et al,
1980). The membrane deterioration of lysosomes by isoproterenol induced
lipid peroxidation could have resulted in the leakage of enzymes from the
enclosed sacs (Hîrchhora, 1974) leading to intracellular digestion and
autolysis of myocardial cells. The increase in LPO on isoproterenol
administration indicate enhanced LPO as a result of phospholipase activation
in lysosomal membrane, leading to decreased membrane fluidity
(Mathew et al, 1982a,b). Elevated lysosomal enzymes in the extra cellular
fluid occur as a result of decreased lysosomal stability (Sathish et al, 2003b)
Decker et al (1977) has reported that the decreased stability of the lysosomal
membrane results in irreversible damage. Kennett and Weglicki (1978) has
reported that the cytosolic acid hydrolases released from lysosomes and from
the sarcoplasmic reticulum induce the dysfunction and distribution of
mitochondria, sarcolemma and other organelle It has been shown that
ischemic injury and consequent intracellular acidosis permit lysosomal
hydrolases to gain access to the cytosol and extra cellular milieu (Hîrchhora,
1974). Isoproterenol induced myocardial infarction results in increased
lysosomal hydrolase activity that may be responsible for tissue damage and

Mangifera significantly reduced the level of lysosomal LPO and
increased the activities of hydrolase enzymes with significant decrease in
serum hydrolase enzyme activities. Due to antioxidant, anti lipid peroxidative and free radical scavenging property of mangiferin (Ghosal et al 1996), it could have reduced the activity of phospholipase and protected the lysosome from free radical mediated damage induced by isoproterenol. Hence the release of lysosomal hydrolases from its native site (sub cellular fraction) to circulation could have been reduced.

HAEMOTOLOGY

The techniques of hematology are concerned mainly with the cellular formed elements of blood, their number or concentration, the relative distribution of various type of cells and the structural or biochemical abnormalities that promote disease.

Based on the close relationship between cardiovascular disorders and haemorheology it has been postulated that both atherogenesis and blood rheology might have some common denominator (Ernst et al, 1986). Adsorption phenomena taking place on surfaces, which are in contact with blood plasma might constitute such a common denominator. These phenomena are a basic mechanism of early atherogenic changes and are the cause for abnormality in blood therapy. A positive correlation is observed between blood viscosity and parameters like haemotocrit, fibrinogen, globulin as well as total lipid concentration (Schabitz et al, 1983)

Tarasov (1976) has reported that the alteration in red blood cell indicators during acute myocardial infarction could be due to the impairment in the circulation of blood to the myocardium, resulting in hypoxia, a
condition that stimulates erythrocytosis. Increased erythrocytosis during acute myocardial infarction can lead to haemolysis. In response to increased haemolysis, there is an increase in erythropoiesis, which is a compensatory mechanism of O₂ insufficiency normally accompanying myocardial infarction.

During acute myocardial infarction, there is an increase in the number of erythrocytes, Hb content, haemtocrit indices, mass of circulating erythrocytes, reticulocyte quantity and intensity of erythrocytosis. A significant increase in red blood cell count, haemoglobin content and haemtocrit values, following isoproterenol administration when compared to control is in accordance with the observation of Tarasov (1976) during myocardial infarction (Table 13). The enhanced haemtocrit value during acute myocardial infarction is a consequence of haemo concentration and increased blood viscosity (Hershberg et al., 1972). Kostis et al (1984) have also suggested an increase in RBC count, as a biochemical indicator in coronary artery disease.

Erythrocyte sedimentation rate is not dependent upon anyone factor but is the release of a complex interplay among various influences. Isoproterenol induced rats showed decreased level of ESR in serum (Table13). Plasma fibrinogen is the major determinant of platelet aggregation and blood viscosity (Maresca et al., 1999). Plasma fibrinogen level (Table 13) has been reported to be elevated, following experimental as well as clinical myocardial damage (Saxena et al., 1979). Under stressful conditions increased fibrinogen synthesis by liver is a well documented (Saxena et al., 1979).
Following tissue injury (Chakrabarty, 1969) and myocardial infarction, plasma fibrinogen has consistently been shown to be elevated (Chakrabarty, 1969). An experimental study conducted by Saxena et al, 1979 has reported that the animals, which developed myocardial necrosis as confirmed on autopsy, exhibits significant elevation in the levels of plasma fibrinogen. Epidemiological studies have suggested that high clotting factor levels, especially factor VII and fibrinogen may be of significance in coronary heart disease (Meade et al, 1980). The relationship between lipid peroxidation and plasma fibrinogen analyzed by Rankenen et al (2000) has suggested that increased lipid peroxidation is associated with elevated plasma fibrinogen level

A significant increase in platelet count (Table 13) was observed in ISPHanderminated rats and similar results on isoproterenol administration have been reported (Manjula et al, 1992c). This could be due to the synthesis of higher percentage of small platelets and rapid consumption of medium and large sized platelets following acute myocardial infarction resulting in the net increase in platelet volume (Sewel et al, 1984). In vitro studies have indicated that large platelets are more reactive heamostatically than small ones (Thompson et al, 1982) and that large platelets produce more thromboxane B₂ in response to stimulation with collagen or thrombin (Jakubowski et al, 1983).

A significant decrease was observed in bleeding time and clotting time (Figure 22) following isoproterenol administration compared to control is in accordance with the observation made in earlier studies (Kristensen et al, 1988). The cutaneous bleeding time provides an over all estimate of the
platelet vessel wall interaction (Harker and Slichter, 1972). Millner et al (1985) has reported that the bleeding time was found to be shortened in the acute phase of myocardial infarction. The shortened bleeding time may be an indicator of an increased prothrombotic tendency in MI and this effect appear to be mediated by both thromboxane A₂ and adrenaline (Kristensen et al 1988). Several factors control bleeding time and these include platelet count, packed cell volume, blood pressure and vascular reactivity. Mean platelet value has shown to be inversely correlated with the bleeding time in patients with ischemic heart disease (Kristensen et al, 1988). Studies have shown that the mean platelet volume is increased in acute MI. Shortened clotting time following isoproterenol administration is in accordance with the reports of Meenu et al (1992). Acute Myocardial infarction is associated with the pathological reduction in clotting time (Eastham et al, 1992). Increased beta adrenergic activity also caused a marked leucocytosis and decreased blood-clotting time, which is in agreement with the work of Meenu et al (1992).

Prothrombin time was significantly decreased in Group 2 isoproterenol administered rats and this is accordance with the results (Manjula et al, 1992c). Acute myocardial infarction is associated with a decrease in prothrombin time (Figure 20).

Leukocytes are functionally important cells in inflammation and monocytes have now been known to be involved in the initial stages of atherosclerosis (Mitchinson et al, 1987). High levels of circulating neutrophil leukocytes may be a risk factor for the development of atherosclerotic
complications (Ernst et al, 1987) and neutrophil activity may be an important determinant of infarct size in AMI. Neutrophils may contribute to the tissue injury following AMI possibly by release of proinflammatory mediators such as leukotrienes, free oxygen radicals and hydrolytic enzymes.

Cannon et al (2001) have suggested the WBC count as a new inexpensive tool for risk stratification in acute coronary syndromes. Ernst et al (1987) have suggested that the correlation between high leukocyte amount and the risk of MI. Among the leukocytes, the neutrophil count showed a significant increase. The increase in leukocyte could be due to leucocytosis, which is related to the necrotic process and its magnitude (Table 13). Beta-adrenergic activation following isoproterenol administration results in leucocytosis (Gryglewski et al, 1971).

Mangiferin pretreatment reduced the level of RBC cells, Hb content, Packed cell volume (Haemocrit), neutrophils, platelet count, fibrinogen level and increased the level of lymphocyte content, eosinophil content, basophil amount, ESR, prothrombin time, bleeding time and clotting time. Mangiferin being an antioxidant could have reduced haemolysis and thus could have increased ESR. The formation of neutrophils and fibrinogen could have been reduced by anti free radical and antioxidant scavenging activity of mangiferin and thereby reduced platelet count, which in turn could have increased prothrombin time and thus clotting time and bleeding time could have been increased.
Conclusion

Based on the data presented in the present study, it could be stated that the intraperitoneal administration of mangiferin at a dose of 10 mg / 100 g body weight has potent cardio protective action against isoproterenol induced experimental myocardial infarction and the possible daily consumption of mangiferin might be considered as a cardio tonic drug for human beings in future.