

Cardioprotective effect of mangiferin on isoproterenol induced myocardial infarction in rats

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Isoproterenol (ISPH) induced myocardial infarction was confirmed by disturbances in serum and heart tissue marker enzymes such as lactate dehydrogenase (LDH), creatine phospho kinase (CPK), aspartate transaminase (AST) and alanine transaminase (ALT), increased level of lipid peroxidation and histopathological changes in the heart of ISPH administered rats. Pretreatment with mangiferin (10 mg/100 g body weight) for 28 days was found to ameliorate the effect of ISPH-induced pathological changes, reduced the lipid peroxide formation and retained the myocardial marker enzyme activities at near normal level. The above results indicate the cardioprotective effect of mangiferin against ISPH-induced myocardial infarction in rats.

Keywords: Isoproterenol, Lipid peroxides, Mangiferin, Marker enzymes, Myocardial infarction

Myocardial infarction (MI) is the acute condition of necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand¹. There is substantial evidence that ischemic tissue generates oxygen-derived free radicals (oxygen radicals), i.e., oxygen molecules containing an odd number of electrons, making them chemically reactive and often leading to chain reactions^{2,3}. Free radicals and reactive oxygen species have been implicated in cardiac diseases and metabolic disorders, which result due to exposure to chemicals and environmental agents. Isoproterenol (ISPH), a synthetic catecholamine and beta-adrenergic agonist, has been found to cause a severe stress in the myocardium resulting in infarct like necrosis of the heart muscle and is also well known to generate free radicals and stimulate lipid peroxidation, which may be a causative factor for irreversible damage to the myocardial membrane in experimental myocardial infarction⁴. Millions of adults are taking beta-adrenoreceptor blocker drugs to lower blood pressure, lower cholesterol, and/or to reduce platelet aggregation and the prescribed regimen must be adjusted for individual needs by modulating the drug

dosage and selecting from a collection of possible drugs to yield the desired response while keeping serious side effects to a minimum.

Recently, attention has been focused on non-nutrient phytochemicals and polyphenols such as the flavonoids, alkaloids and xanthenes derived from different plant species as potential therapeutic agents in the prevention and management of cardiovascular diseases due to their antioxidant nature⁵. With technological advancement of science, the isolation, identification and elucidation of the chemical principles from medicinal plants have become much simpler and have contributed significantly to the development of new drugs for almost all type of diseases including cardiovascular, cancer and hepatic diseases⁶.

Mangiferin is a naturally occurring C-glycosyl xanthone extracted from the leaves of *Mangifera indica* Linn. (Mango; Anacardiaceae)⁷. Mangiferin is present in leaves⁸, fruits, stem and roots⁹ of *M. indica*. It has cardiogenic and diuretic properties¹⁰. Mangiferin rich plants are widely used medicinal plants in India for the treatment of immuno-deficiency diseases such as arthritis, diabetes, hepatitis, cardiac and mental disorders¹¹. Being a polyphenolic antioxidant, mangiferin has strong antioxidant, anti-lipid peroxidative, immunomodulatory, cardiogenic, hypotensive, wound healing, antidegenerative and antidiabetic activities¹².

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Experimental evidence on the biochemical role of mangiferin on myocardial infarction induced by ISPH is lacking. In this context, an attempt has been made to elucidate the maintenance of myocardial integrity in presence of mangiferin on ISPH induced cardiac damage in rats with reference to biochemical markers, lipid peroxidation and histology.

Materials and Methods

Animals — Adult male albino rats of Wistar strain weighing 150-200 g were obtained from Tamil Nadu Veterinary and Animal Sciences University, Chennai. They were fed with standard diet and water *ad libitum* and housed under standard environmental conditions. All experiments were carried out according to the guidelines of Institutional Animal Ethics Committee (IAEC No: 01/046/04).

Chemicals — Isoproterenol, dimethyl sulphoxide, reference mangiferin and bovine serum albumin were purchased from Sigma Chemical Co., USA. Nicotinamide adenine dinucleotide phosphate, nicotinamide adenine dinucleotide reduced and pyruvate were obtained from Loba Chemie Co., Bombay, India.

Mangiferin — The mangiferin powder from the leaves of *M. indica* was obtained as a gift from Herbo Organic Ltd., Chennai. As recommended by Sigma Aldrich Company (Invoice Number and Date: 132465, 13-12-2001), 5 g of mangiferin was dissolved in 100 ml of dimethyl sulphoxide (DMSO) solvent as a standard dissolving concentration. Based on this concentration/dilution ratio and dosage fixation assessment, 10 mg of mangiferin was dissolved in 0.2 ml DMSO and administered ip to 100 g body weight of each animal for the subsequent experimental studies.

Toxicity studies — Mangiferin (100, 250, 500 and 1000 mg/kg bodyweight/day) dissolved in an appropriate volume of (2, 5, 10 and 20 ml) DMSO was administered ip to rats for 60 days for chronic toxicity study. Another group of animals, which received DMSO vehicle alone (without mangiferin) through ip injection were served as control. Morphological behavior and toxic symptoms of the animals were also checked for 24, 48 and 72 hr and the animals were weighed biweekly for the whole treatment period for delayed toxicity.

Dosage fixation — Assessment of the effective dose of mangiferin and duration of treatment against ISPH induced myocardial injury in rats was done

based on the activities of serum lactate dehydrogenase (LDH) and creatine phospho kinase (CPK) enzymes. Different doses of mangiferin (5, 10 and 20 mg/100 g body weight) dissolved in an appropriate volume (0.1, 0.2 and 0.4 ml) of DMSO were administered daily for 7, 14, 28 and 35 days, ip to rats followed by sc administration of ISPH for 2 days. Serum LDH and CPK enzyme activities of all the doses of mangiferin pre treated groups of rats were compared with the enzyme activities of ISPH myocardial infarcted group of rats. Based on the comparison, mangiferin pretreatment at a dose of 10 mg/100 g bodyweight/day for 28 days in ISPH administered rats showed a significant reducing effect in serum LDH and CPK enzymes activity as compared to ISPH myocardial infarcted group of rats and hence this was fixed as a optimum dosage for the subsequent biochemical analysis.

Experimental design — Animals were grouped into following 4 group of six animals each:

- Group 1: Control rats received DMSO (0.2 ml/ 100 g body weight) as a vehicle, ip, for 28 days. Group 1 rats were referred as positive control rats.
- Group 2: Rats were administered with ISPH (20 mg/100 g body weight suspended in 0.1 ml of 0.9% saline), sc, twice daily at an interval of 24 hr¹³. Group 2 rats were referred as ISPH myocardial infarcted rats.
- Group 3: Rats treated with mangiferin alone (10 mg/100 g body weight, ip suspended in 0.2 ml of DMSO) for 28 days². Group 3 rats were referred as drug control rats.
- Group 4: Rats pretreated with mangiferin (10 mg/100 g body weight suspended in 0.2 ml of DMSO) given ip for 28 days and ISPH was administered as in Group 2. Group 4 rats were referred as mangiferin pretreated rats.

Since DMSO is used as a vehicle in the above experimental design, the negative or positive interference of DMSO with the experimental group of rats was tested using another separate group (Group 5) of animals. The Group 5 rats fed with only standard diet and water *ad libitum* were experimentally monitored for the same period of duration and compared with DMSO treated Group 1 rats. Since no specific significant changes were observed in marker enzyme parameters of these two groups of rats, the Group 1 DMSO treated rats were

considered as control rats and Group 5 rats were dropped from the experimental design¹⁴.

After 28 days of experimental period, Group 1 and 3 rats were not provided any experimental drugs such as DMSO or mangiferin other than water *ad libitum* and standard diet. Group 2 rats were not provided any experimental drugs other than standard diet and water *ad libitum* for initial 28 days and administered ISPH, sc, on 29th and 30th day (2 days only). Group 4 rats were received mangiferin as a pretreated drug for 28 days and were administered ISPH, sc, for 2 days to induce experimental myocardial infarction as in Group 2 rats. After 30 days of experimental period, on the next day, animals of all the groups were anaesthetized with pentobarbital sodium (35 mg/kg, ip), blood was drawn from the external jugular vein of the rat and serum was separated by centrifugation. The heart tissues were dissected out immediately and washed in ice-cold saline. The tissue (100 mg) was weighed accurately and homogenized in 5 ml of 0.1 M Tris-HCl buffer (pH 7.4) in ice-cold condition. The homogenate was centrifuged at 2500 g and the clear supernatant solution was taken for the assay of marker enzymes such as lactate dehydrogenase (LDH)¹⁵, creatine phospho kinase (CPK)¹⁶, aspartate transaminase (AST)¹⁷, alanine transaminase (ALT)¹⁷, lipid peroxides (LPO)¹⁸ and protein¹⁹ in tissues and serum.

Histological studies — Histological evaluation was performed on lower portion of the heart tissue. Fresh heart tissues were excised and then fixed in 10% formalin for 24 hr. The fixative was removed by washing through running tap water for overnight. After dehydration through a graded series of alcohols, the tissues were cleaned in methyl benzoate, embedded in paraffin wax. Sections were cut into 5 μ m thickness and stained with hematoxylin and eosin. After repeated dehydration and cleaning, the sections were mounted and observed under light microscope with magnification of 100 \times for histological changes.

Statistical analysis — Results are presented as mean \pm SD. The significance of difference among the groups was assessed using one-way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) multiple comparison test. Significance was set at $P < 0.05$, < 0.01 and < 0.001 .

Results

The results of the study assessing the toxicological effect of mangiferin have shown that a small increase

in body weight may be considered as variation that is within the normal range and appeared to be non-toxic as monitored by survival outcome. Mangiferin showed no lethal effect during chronic period of (60 days) toxicity study at least up to an ip dose of 1000 mg/kg body weight indicating that LD₅₀ if any should be higher than this dose.

The effective dosage was fixed by assessing the activities of serum cardiac marker enzymes such as LDH and CPK (Figs 1a and 1b). Dose-dependent protection was observed upon mangiferin pretreatment at different dose levels (5, 10 and 20 mg/100 g body weight, daily) for 7, 14, 28 and 35 days in ISPH administered rats as compared with ISPH myocardial infarcted rats. A significant ($P < 0.001$) cardioprotection was observed both in 10 and 20 mg/100 g body weight of mangiferin for 28 days and since it is safe to take minimum dose of drug for the treatment of clinical disorders, a minimum dose of 10 mg/100 g body weight of mangiferin daily

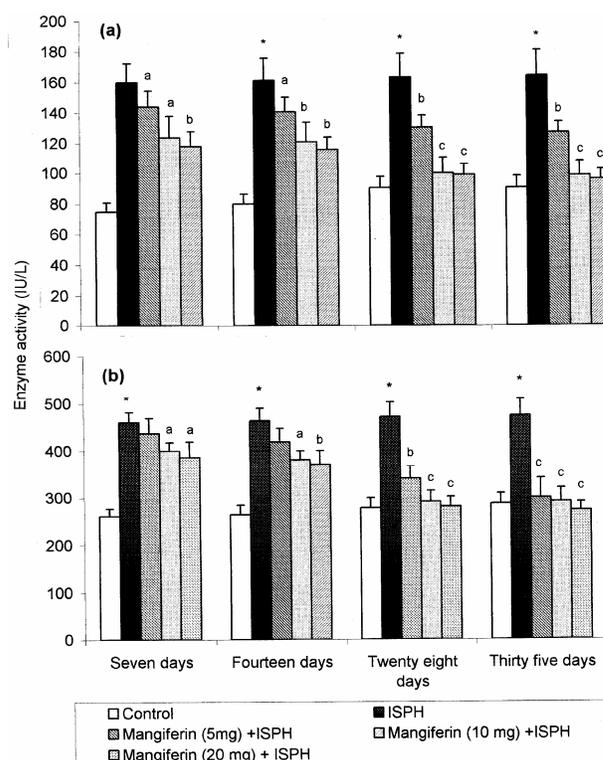


Fig. 1— Effect of different doses of mangiferin on the activity of lactate dehydrogenase (a) and creatine phosphokinase (b) enzymes for different treatment period in isoproterenol (ISPH) induced myocardial infarcted rats. [Results are expressed as mean \pm SD for 6 animals in each group. P : * < 0.001 statistically significant when compared with control; ^a < 0.05 , ^b < 0.01 , ^c < 0.001 statistically significant when compared with ISPH administered group].

for 28 days was chosen as optimum dosage and optimum duration to protect the myocardium effectively from the necrotic damage induced by ISPH and further biochemical analysis were carried out with this dosage alone.

A significant elevation of ($P<0.001$) lipid peroxides (both in serum and heart tissue) with concomitant increase in serum ALT and AST activity was observed in ISPH myocardial infarcted (Group 2) rats as compared to Group 1 control rats (Table 1). Mangiferin pretreatment (Group 4) significantly reduced ($P<0.001$) the level of serum and heart tissue lipid peroxides and the activities of the serum marker enzymes (ALT and AST) as compared to ISPH-myocardial infarcted Group 2 rats. Treatment with mangiferin alone (Group 3) did not show any significant change as compared with Group 1 control rats.

A significant reduction ($P<0.001$) in the activities of marker enzymes (AST, ALT, CK and LDH) was observed in heart tissue of (Group 2) ISPH myocardial infarcted rats as compared to Group 1 control rats (Table. 2). Pretreatment with mangiferin (Group 4) retained the activities of these enzymes to near normal levels ($P<0.001$) in heart tissue as compared to (Group 2) ISPH myocardial infarcted rats. No altered variation was observed in these enzymes activity in heart tissue of mangiferin alone

treated (Group 3) animals as compared with Group 1 control rats.

Microscopic examination of heart tissue of the Group 1 control rats (Fig. 2a) showed normal myocardial fibers and muscle bundles with normal architecture. Mangiferin alone treated Group 3 rats (Fig. 2b) showed normal myocardial fibers with no pathological changes. Heart tissue of Group 2 ISPH myocardial infarcted rats (Fig. 2c) showed separation of myocardial fibers with inflammatory mononuclear collections, edema and myocardial necrosis. Myocardial section of mangiferin pretreated Group 4 rats showed slightly separated myocardial fibers with small focus of inflammatory mononuclear collections (Fig. 2d) with absence of necrotic damage.

Discussion

The present investigation is aimed to evaluate and explore the cardioprotective effect of mangiferin, a non-nutrient phytochemical extracted from *M. indica* on ISPH induced myocardial infarction in rats.

Myocardium contains an abundant concentration of diagnostic marker enzymes of myocardial infarction viz., CPK, LDH and transaminases and once metabolically damaged, releases its content into the extra cellular fluid (ECF)²⁰. In ISPH myocardial infarcted (Group 2) rats, the increased activities of the serum marker enzymes accompanied by their

Table 1— Effect of mangiferin on the activity of AST and ALT in serum and LPO content both in serum and heart tissue of isoproterenol (ISPH) induced rats

[Values are expressed as mean \pm SD for 6 animals in each group]

Parameters	Control (Group 1)	ISPH (Group 2)	Mangiferin (Group 3)	Mangiferin + ISPH (Group 4)
AST (IU/L)	27.83 \pm 1.89	44.70 \pm 3.40*	27.05 \pm 1.88	30.92 \pm 1.79 ^a
ALT (IU/L)	19.46 \pm 1.65	35.94 \pm 3.20*	18.05 \pm 1.76	22.43 \pm 1.57 ^a
Serum LPO (nmoles of TBARS/dl)	3.39 \pm 0.28	5.49 \pm 0.41*	3.14 \pm 0.22	3.80 \pm 0.31 ^a
Heart LPO (nmoles of TBARS/mg of protein)	6.7 \pm 0.51	9.49 \pm 0.76*	6.63 \pm 0.48	6.96 \pm 0.62 ^a

P values: * <0.001 statistically significant when compared with Group 1; ^a <0.001 statistically significant when compared with Group 2

Table 2— Effect of mangiferin on heart tissue marker enzymes in control and experimental group of rats

[Values are expressed as mean \pm SD for 6 animals in each group]

Parameters	Control (Group 1)	ISPH (Group 2)	Mangiferin (Group 3)	Mangiferin + ISPH (Group 4)
AST (nmoles of pyruvate liberated/min/mg protein)	53.69 \pm 3.88	38.03 \pm 2.75*	52.78 \pm 2.94	48.16 \pm 3.20 ^a
ALT (nmoles of pyruvate liberated/min/mg protein)	25.97 \pm 1.79	12.02 \pm 0.79*	26.13 \pm 1.55	23.41 \pm 1.83 ^a
LDH (nmoles of pyruvate liberated/min/mg protein)	139.79 \pm 11.21	81.83 \pm 5.36*	140.20 \pm 9.11	126.21 \pm 9.25 ^a
CPK (μ moles of phosphorous liberated/min/mg protein)	17.61 \pm 1.23	8.49 \pm 0.70*	18.22 \pm 1.18	16.12 \pm 1.08 ^a

P values: * <0.001 statistically significant when compared with Group 1; ^a <0.001 statistically significant when compared with Group 2

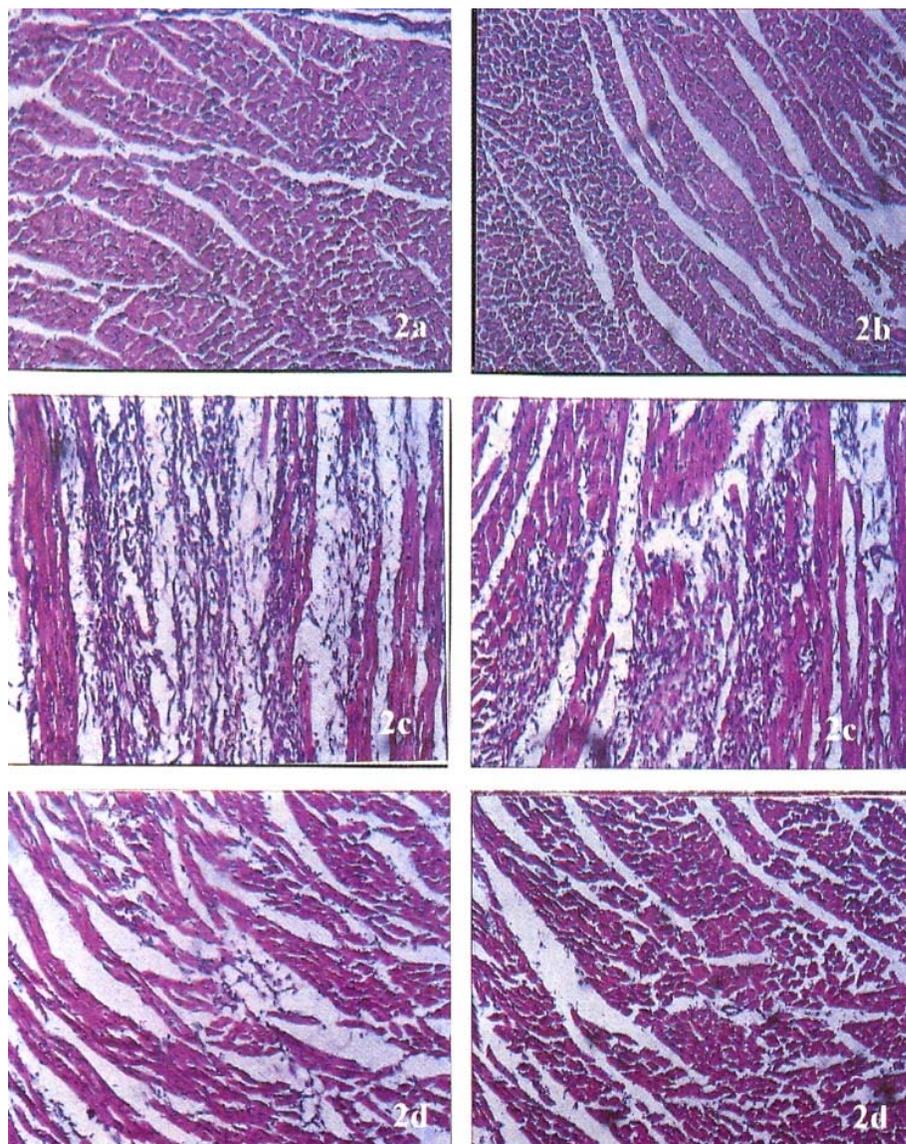


Fig. 2—Histological examination of heart tissue sections in control and experimental animals (Hematoxylin and Eosin, 100 \times). Section of heart tissue from (a) control rat showing normal architecture; (b) mangiferin treated rat showing apparently normal architecture with no pathologic changes (c) ISPH-myocardial infarcted rat showing degenerative changes, hyalinization of muscle fibres and cellular infiltration; (d) mangiferin pretreated rat reveals less cellular infiltration, normal muscle fibres and the cardioprotective effect are evident from reduced myocardial damage even after ISPH administration.

concomitant reduction in the heart homogenate confirm the onset of myocardial necrosis. Hence the total concentration of the marker enzymes were found to be decreased in heart tissue of ISPH infarcted rats as compared to control, which may be the reflection of consequences of cellular injury due to lipid peroxides. ISPH is well known to generate free radicals and to stimulate lipid peroxidation, which may be a causative factor for irreversible damage to

the myocardium⁴. The increased levels of TBA reactive substances indicate the excessive formation of free radicals and activation of lipid peroxidation system resulting in irreversible damage to the heart in animals subjected to ISPH stress. The significant increase observed in the levels of lipid peroxides in serum and heart of ISPH infarcted rats compared to control, was in accordance with the observation of previous reports²¹.

Upon mangiferin pretreatment serum marker enzymes of (Group 4) rats were found to be significantly decreased whereas the heart tissue marker enzymes showed significantly increased level as compared to ISPH myocardial infarcted rats and this could be due to antioxidant and free radical quenching effect of mangiferin as reported by Ghosal *et al*¹⁰. Mangiferin, a principal phenolic compound has potent free radical scavenging activity and protective effect against altered changes in AST and ALT activities caused by toxicant²². Muruganandan *et al.*² have reported that ip administration of mangiferin significantly reduce the activity of CPK and LDH in heart as well as ameliorates the oxidative stress thereby reducing cardiotoxicity and kidney damage. Xanthones have been reported to possess protective action against myocardial ischemia and diminishes the release of AST and LDH enzymes²³. Similar observations have been recorded with mangiferin pretreatment in the present study, which could be attributed to the cardioprotective action of mangiferin, a glucosyl xanthone. Mangiferin pretreated (Group 4) rats showed a significant decrease in LPO level both in serum and heart tissue as compared with ISPH myocardial infarcted rats. Previous investigations have shown that xanthones exhibit a cardioprotective effect against myocardial ischemic injury by inhibiting lipid peroxidation and thus enhancing the recovery of cardiac function³. This could be the reason for the reduced formation of LPO in mangiferin-pretreated rats.

Histological examination of heart tissue of Group 2 rats showed myocardial necrosis and separation of myocardial fibers with inflammatory mononuclear infiltrate whereas the examination of heart tissue of mangiferin pretreated (Group 4) rats showed maximum protective effect by reduced histological changes as compared to ISPH myocardial infarcted rats. Muruganandan *et al.*² have reported that the treatment with mangiferin protected the cardiac tissue in diabetic rats with the observation of mild degenerative changes as congestion and less swollen myocardial cell fibers in rats which is in accordance with the present report.

The therapeutic efficacy of mangiferin may be due to its antioxidant, antilipidperoxidative, free radical scavenging, immunomodulatory and cardiotoxic property that could have prevented ISPH-induced tissue injury. Thus it could be concluded that mangiferin protects experimental myocardial

infarction as revealed by the amelioration of histological changes and biochemical markers of cardiac tissue damage without any adverse effect which merit further detailed studies to develop it as a cardioprotective drug.

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