Pharmacognosy Magazine
ISSN: 0973-1296

PHCOG MAG.
An official Publication of Phcog.Net

PHCOG MAG.: Research Article

# A Study on the Beneficial Effect of Mangiferin on Isoproterenol Induced Mycocardial Infarction Rat Model

S. Prabhu*, Mallika Jainu, K. E. Sabitha and C.S. Shyamala Devi

*Department of Biochemistry, University of Madras, A.C.tech, Guindy Campus, Chennai-600 025, India.*
*E-mail: prabhubio2k@yahoo.co.in.*

**Abstract**
Over the past last decades there exist a relationship between diet and the development of major chronic diseases such as chronic heart diseases, hypertension and cancer. Mangiferin, the important phytochemical constituent of Mangifera indica L. has been used in the treatment of various human ailments. In this article an attempt has been made to evaluate the beneficial effect of mangiferin on isoproterenol induced myocardial infarction in rats. The ratio of heart weight to body weight was monitored as an index to study the cardioprotective property in the animal model. The following hematological parameters, RBC counts, haemoglobin amount, packed cell volume, total WBCs, neutrophil, eosnophil counts and platelet count, fibrinogen content, ESR, the percentage of lymphocytes, eosinophil counts, clotting time, bleeding time and prothrombin time were studied. The pretreatment with mangiferin prevented the alterations induced by isoproterenol and retained the hematological changes to near normal.

**Key words:** Myocardial infarction, Mangiferin, Hematological alterations, cardioprotective

## INTRODUCTION

Among the many health predictions for the new millennium, the most alarming is cardiovascular disease (CVD) - heart disease and stroke (1,2). 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 demand (3). Myocardial necrosis results in and can be recognized by the appearance in the blood of different proteins released into the circulation due to the damaged myocytes (4).

Isoproterenol, (ISPH) a synthetic catecholamine and beta-adrenergic agonist, has been found to cause severe stress in the myocardium resulting in infarct like necrosis of the heart muscles by generating free radicals and stimulating lipid peroxidation, resulting in irreversible damage to the myocardial membrane in experimental myocardial infarction (5). 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. Among the side effects commonly reported are fatigues and lowered libido from some anti hypertensive drugs such as beta blockers (eg: Bisoprolol and hydrochlorothiazide) and liver damage from cholesterol reducers.

Consumption of food produced with natural essential oil or aromatic plant extracts are proposed to prevent the risk of many free radical mediated diseases including cardiovascular diseases. They safely interact with free radicals and terminate the chain reaction before vital molecules are damaged (6). With technological advancement of science, the isolation, identification

and elucidation of chemical principles from natural sources have become much simpler and have contributed significantly to the development of new drugs.

*Mangifera indica* Linn. (7) belongs to the family of anacardiaceae,. The major and important phytochemical in *Mangifera indica* is mangiferin (8). Mangiferin has been reported to be present in various parts of *Mangifera indica* viz. leaves (9), fruits (10), stem bark (11), and roots (12). Mangiferin has cardiotonic activity and diuretic properties (13). Mangiferin rich plants are widely used medicinal plants in the traditional Indian systems of medicine for the treatment of immuno-deficiency diseases such as arthritis, diabetes, hepatitis, cardiac and mental disorders (14). Mangiferin, being a polyphenolic antioxidant and a glucosyl xanthone, it has strong antioxidant, anti lipid peroxidation, immunomodulation, cardiotonic, hypotensive, wound healing, antidegenerative and antidiabetic activities (15). We here in attempted to study the cardioprotective role of mangiferin on ISPH-induced myocardial infarction in rats.

**Materials and Methods**

**Drug**

The mangiferin powder from *Mangifera indica* Linn. was isolated and purified by HPLC according to the method of Ghosal and Rao (13) with slight modifications. The shade dried and powdered plant material of *Mangifera indica* leaves was soxhleted for 12h with petroleum ether at 60-80°C. The defatted plant material was extracted with ethanol under reflux for 16h and concentrated under reduced pressure to yield a semi-solid mass. The semi-solid mass was defatted repeatedly and finally dissolved in ethanol at room temperature, it yielded an amorphous white powder after 2 weeks. After repeated crystallization of the powder in aqueous ethyl acetate, pale-yellow needle shaped crystals of mangiferin were obtained as a gift from Herbo Organic Ltd., Chennai. Purity of the compound was confirmed by a HPLC method as described by Geodakyan et al (16), using C18 column for separation. An isocratic mobile phase consisting of acetonitrile and 3% acetic acid (16: 84) was used with a flow rate of 0.5 ml/min and photodiode array detector wavelength was set at 254 nm. HPLC analysis of reference mangiferin purchased from Sigma Aldrich company showed a single peak (95.5%) which closely matched to its specified purity of isolated mangiferin (greater than 90% w/w). As recommended by Sigma Aldrich Company, 5g of mangiferin was dissolved in 100 ml of dimethyl sulphoxide (DMSO) solvent as a standard dissolving concentration. For the experimental study, rats were administered standard mangiferin solution at a dose of 0.2 ml/100g body weight of each rat.

**Animals**

Animals were obtained from Tamil Nadu Veterinary and Animal Sciences University, Chennai. Adult male albino Wistar rats weighing 150-200 g were selected for the study. 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 (No: 360/01/a/CPSEA/2001).

**Dosage fixation**

Different doses of mangiferin (2.5 mg, 5.0 mg, 7.5mg, 10mg and 20mg/100g body weight) were dissolved in DMSO and pretreated at different time intervals of 7, 14, 21, 28 and 35 days i.p. to assess the effective dose of mangiferin and duration of treatment against ISPH induced myocardial injury based on the activities of serum lactate dehydrogenase (LDH) and creatine kinase (CK) enzymes. Pretreatment with mangiferin at a dose of 10mg/100g body weight for 28 days was found to be effective against ISPH induced myocardial infarction and hence this was fixed as optimum dosage for the subsequent biochemical analysis (Prabhu, 2005).

EXPERIMENTAL DESIGN

Animals were grouped into 4, each group consisting of six animals.

Group 1: Control rats received DMSO (0.2 ml/ 100 g body weight) as a vehicle intraperitonially.

Group 2: Rats were administered with ISPH (20 mg/100 g body weight suspended in 0.1 ml of 0.9% saline) subcutaneously twice at an interval of 24 hours (17).

Group 3: Rats pretreated with mangiferin alone (10 mg/100g body weight intraperitonially suspended in 0.2 ml of DMSO) for 28 days (18).

Group 4: Rats pretreated with mangiferin (10 mg/100 g body weight suspended in 0.2 ml of DMSO) given intraperitonially for 28 days and ISPH was administered as mentioned in Group II.

After the experimental period, the animals were weighed and body weights were recorded. The animals were sacrificed by over dose of ether anesthesia. The heart tissue was dissected out immediately and washed in ice-cold saline. The heart tissue of each animal from respective groups were weighed accurately and the heart weight to body weight ratio (HW / BW ratio) was calculated for each group and compared with that of normal rat. The blood collected from each animal was used to analyse hematological parameters. For plasma separation, anticoagulant (EDTA) was added to the blood samples and then analysis was done. Red blood corpuscles count (RBC), hemoglobin (Hb), packed cell volume (PCV), white blood corpuscles (WBC), neutrophil count, lymphocytes, eosnophil count, platelet count and plasma fibrinogen levels were measured with the use of autoanalyzer (Hitachi 911, German). Erythrocyte sedimentation rate (ESR) was assayed by the method of Bottiger and Svedberg (19). The determination of bleeding time (20), clotting time (21) and plasma prothrombin time

(22) were carried out in the control and experimental animals.

**Statistical analysis**

Results are presented as mean ± 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 $P<0.05$, $P<0.01$ and $P<0.001$.

**Results and Discussion**

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 ISPH induced experimental myocardial infarction (MI) in rat model.

The determination of heart weight and body weight ratio is considered to be an index of the prognosis of heart disease in experimental animals. Group 2 rats showed a significantly increased heart weight to body weight ratio compared to Group 1 rats (Figure 1). 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 the reports of Stephanie (23).

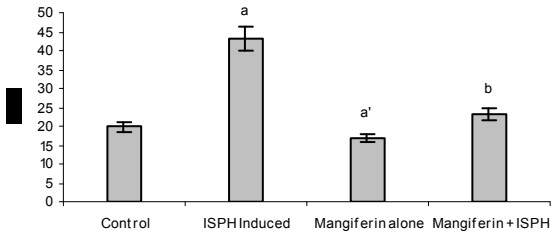


*FIGURE 1: Effect of mangiferin on heart weight to body weight ratio in isoproterenol induced rats. Results are expressed as mean ± SD for 6 animals in each group. [a]p < 0.001 statistically significant when compared with Group 1; [b]p < 0.001 statistically significant when compared with Group 2.*

In mangiferin pretreated rats (Group 4), 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 edema in the myocardium. Henry and Stephens (25) have reported that the polyphenols do not favor edema formation in heart tissues of mice in the study of chronic psychosocial hypertension reduction. Since mangiferin is a natural polyphenolic antioxidant, the protection offered to the myocardium could be attributed to the presence of polyphenols and anti-inflammatory activity of the drug (25). Group 3 rats showed a non-significant change in heart weight to body weight ratio when compared to control rats.

Hematological parameters are concerned mainly with the cellular formed elements of blood, their number or concentration, the relative distribution of various types of cells and the structural or biochemical abnormalities that promote disease. Based on the close relationship between cardiovascular disorders and hematology it has been postulated that both atherogenesis and blood rheology might have some common denominator (26). A positive correlation is observed between blood viscosity and parameters like haemotocrit, fibrinogen, globulin as well as total lipid concentration (27). Tarasov (28) has reported that the increase in RBC observed during AMI could be due to the impairment in the circulation of blood to the myocardium, resulting in hypoxia, a condition that stimulates erythrocytosis. In response to increased haemolysis in acute myocardial infarction (AMI) condition, there is an increase in erythropoiesis, which is a compensatory mechanism of $O_2$ insufficiency normally accompanying myocardial infarction. During AMI, a significant increase in RBC count, Hb content and haemotocrit values, following ISPH administration when compared to control which is in accordance with the observation of Kostis *et al* (29) (Table 1).

ESR is not dependent upon single factor but is the reflection of a complex interplay among various factors. ISPH induced rats showed decreased ESR in the blood as compared with control group (Table 1). Plasma fibrinogen is the major determinant of platelet aggregation and blood viscosity (30). Plasma fibrinogen level (Table 1) has been reported to be elevated, following experimental as well as clinical myocardial damage (31). Epidemiological studies have suggested that high clotting factor levels, especially factor VII and fibrinogen may be of significance in coronary heart disease (32). A significant increase in platelet count (Table 1) was observed in ISPH administered rats and similar results have been reported in the present study (33). This could be due to the synthesis of higher percentage of small platelets and rapid consumption of medium and large sized platelets following AMI resulting in the net increase in platelet volume (34).

Leukocytes are functionally important cells in inflammation and monocytes have now been known to be involved in the initial stages of atherosclerosis. Neutrophils may contribute to the tissue injury following AMI possibly by release of proinflammatory mediators such as leukotrienes, free oxygen radicals and hydrolytic enzymes (35). Cannon *et al* (36) have suggested the WBC count as a new inexpensive tool for risk stratification in acute coronary syndromes. Among the leukocytes, the neutrophil count showed a significant increase in myocardial infracted rats. The increase in leukocyte could be due to leucocytosis, which is related to the necrotic process and its magnitude (Table 1). A significant decrease was observed in bleeding time and clotting time (Figure 2) following ISPH administration compared to control that is in accordance with the observation made in earlier studies. The shortened bleeding time may be an indicator of an increased prothrombotic tendency in MI and this effect appear to be mediated by both thrombaxane $A_2$ and adrenaline

(37). Several factors control bleeding time and these include platelet count, PCV, blood pressure and vascular reactivity. Mean platelet value was inversely correlated with the bleeding time in patients with ischemic heart disease (37). AMI is associated with the pathological reduction in clotting time (38). 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* (39). Prothrombin time was significantly decreased in Group 2 ISPH administered rats (Figure 2), which is consistent with the reports of Manjula *et al* (33).

Mangiferin pretreatment reduced the level of RBC cells, Hb content, PCV (haemotocrit), neutrophils, platelet count, fibrinogen level and increased the level of lymphocyte content, eosinophil content, ESR, prothrombin time, bleeding time and clotting time in infracted rats. No alteration was observed in Group 3 drug alone treated rats when compared to control animals for all these parameters (Table 1).

***TABLE 1: Effect of mangiferin on the level of hematological parameters in isoproterenol induced rats***

| Parameters | Control (Group 1) | ISPH induced (Group 2) | Mangiferin alone treated (Group 3) | Mangiferin + ISPH (Group 4) |
|---|---|---|---|---|
| RBC (millions/cu.mm) | 9.13 ± 0.41 | 12.00 ± 0.70[a] | 8.88 ± 0.24 | 10.00 ± 0.70 [b] |
| ESR (mm/hr) | 20.66 ± 1.86 | 10.00 ± 0.89[a] | 21.66 ± 0.51 | 19.00 ± 1.41[b] |
| Hb (g/dl) | 13.01 ± 0.28 | 20.08 ± 1.28[a] | 12.58 ± 0.80 | 14.19 ± 0.81[b] |
| PCV (%) | 20.11 ± 1.05 | 38.00 ± 2.12[a] | 18.96 ± 0.76 | 22 ± 1.41[b] |
| WBC (cells / cu.mm) | 7375 ± 440.17 | 11813.33 ± 1120.47[a] | 7000 ± 494.97 | 8249.83 ± 583.86[b] |
| Neutrophil (%) | 54.66 ± 4.80 | 74.83 ± 1.87[a] | 53.21 ± 1.41 | 63.25 ± 1.87[b] |
| Lymphocyte (%) | 39.08 ± 1. 42 | 25.89± 4.02[a] | 40.21 ± 4.14 | 33.16 ± 5.52[b] |
| Eosinophil (%) | 7.0 ± 0.48 | 2.3 ± 0.14[a] | 7.09 ± 0.46 | 5.50 ± 0.35[b] |
| Platelet count ($10^5$ cells/cu.mm) | 1.52 ± 0.05 | 2.81 ± 0.10[a] | 1.5 ± 0.037 | 1.90 ± 0.026[b] |
| Fibrinogen count (mg/dl) | 227 ± 15.49 | 340.83 ± 23.54[a] | 219.5 ± 14.33 | 269.16 ± 15.30[b] |

*Values are expressed as mean ± SD for 6 animals in each group. [a]p < 0.001 statistically significant when compared with Group 1; [b]p < 0.001 statistically significant when compared with Group 2.*

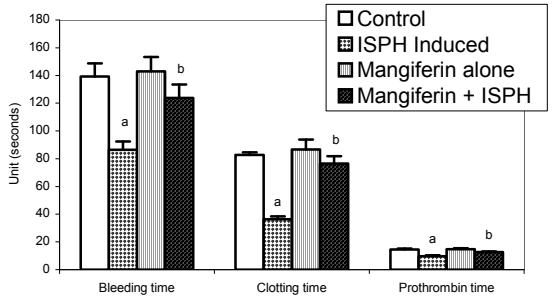


*FIGURE 2: Effect of mangiferin on bleeding time, clotting time and prothrombin time in isoproterenol induced rats. Results are expressed as mean ± SD for 6 animals in each group. [a]p < 0.001 statistically significant when compared with Group 1; [b]p < 0.001 statistically significant when compared with Group 2.*

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 free radical scavenging and antioxidant 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. In addition, previous finding suggests that this polyphenol, mangiferin might also be of value in the prevention of atherosclerosis and coronary heart disease (40).

As per the results described above, mangiferin exhibited excellent cardioprotective activity in ISPH induced rats. Further studies are required to understand the mechanism underlying protective actions of mangiferin on myocardial infarction.

**References**

1. S. Padmavati, ***Ind. Heart J.***, 54, 99-102 (2002).
2. J.E. Manson, J.M. Gaziano, M.A. Jonas and C.H.J. Hennekens, ***J. Am. Coll. Nutr.***, 12, 426-432 (1993).
3. K. Nageswari, R. Banerjee and V.P. Menon, ***J. Nutri. Biochem.***, 10, 338-344 (1999).
4. J.S. Albert and T. Thygesen, ***JACC.***, 3, 959-969 (2000).
5. A. Paritha Ithayarasi and C.S. Shyamala Devi, ***Ind. J. Physiol. Pharmacol.***, 41, 369-376 (1997).
6. K. Chitra and M. Pillai, ***Ind. J. Physiol. Pharmacol.***, 46, 1-5 (2002).
7. P. Scartezzini and E. Speroni, ***J. Ethnopharmacol.***, 71, 23-27 (2002).
8. M.A. Ansari, S. Rajadurai and Y. Nayudamma, ***Leather Science*** 14, 247–251 (1967).
9. P.D. Desai, A.K Ganguly, T.R. Govindachari, B.S. Joshi, V.N. Kamat, A.H. Manmade, P.A. Mohamed, S.K. Nagle, R.H. Nayak, A.K. Saksena, S.S. Sathe

and N. Vishwanathan, ***Ind. J. Chem.***, 4, 457-459 (1966).
10. M.A. Ansari, K.K. Reddy, K.N.S. Sastry and Y. Nayudamma, ***Phytochemistry*** 10, 2239-2241 (1971).
11. S.K. Nigam and C.R. Mitra, ***Ind. J. Chem.***, 2, 378-379 (1964).
12. K.K. Srinivasan and S. Sankara Subramanian, *Arogya* ***J. Health Sci.***, 7, 140-141 (1981).
13. S. Ghosal, G. Rao, V. Saravanan, N. Misra and D. Rana, ***Ind. J. Chem.***, 35, 561-566 (1996).
14. H. Ichiki, T. Miura, M. Kubo, E. Ishihara, Y. Komatsu, K. Tanigawa and M. Okada, ***Biol. Pharm. Bull.***, 12, 1389-1390 (1998).
15. G.P. Andreu, R. Delgado, J.A. Velho, C. Curti and A.E. Vercesi, ***Eur. J. Pharmacol.***, 513, 47-55 (2005).
16. S.V. Geodakyan, I.V. Voskoboinikova, J.A. Kolesnik, N.A. Tiukavkina, V.I. Litvinenko and V.I. Glyzin, ***J Chromatogr***. 577, 371-375 (1992).
17. J.T. Judd and B.C. Wexler, ***Am. J. Physiol.***, 228, 212-216 (1975).
18. S. Muruganandam, S. Gupta, M. Kataria, J. Lal and P.K. Gupta, ***Toxicology*** 176, 165-173 (2002).
19. L.E. Bottiger and C.A. Svedburg, ***Br. Med. J.***, 2, 85-88 (1967).
20. I. Caocci, M. Alberti, P. Burrai and R. Corda, ***Acta Haematol.***, 60, 358-364 (1978).
21. K.M. Samuel. *Notes on Clinical Lab techniques*, 4th Ed., (1980), pp.163-175.
22. A.J. Quick, in Haemorhagic diseases and thrombosis, L. Febiger, 2nd Ed. (Philadelphia, 1966), pp.106-112.
23. H. Stephanie, H. Michael, M.D. Groot, H. Remkes, C. Dienesch, K. Hu, G. Ertl and S. N. Stephanie, Am. ***J. Physiol. Heart Circ. Physiol.***, 277, H2167-H2175 (1999).
24. A.E. Beltran, Y. Alvarez, F.E. Xavier, R. Hernanz, J. Rodriguez, A.J. Nunez, M.J. Alonso and M. Salaices, ***Eur. J. Pharmacol.***, 499, 297-305 (2004).
25. J.P. Henry and L.P. Stephens, ***Hypertension*** 6, 437-44 (1984).
26. E. Ernst, T. Weihmayr, M. Schmid, M. Banmann and A. Matria, ***Atherosclerosis*** 59, 263-269 (1986).
27. J. Schabitz and H. Krosch, ***Cardiovas. Res.***, 25, 161-167 (1983).
28. A.N. Tarasov, ***Kardiologica*** 16, 129-131 (1976).
29. J.B. Kostis, D. Turkevich and J. Sharp, ***Am. J. Cardiol.***, 53, 997-999 (1984).
30. G. Maresca, A. Diblasio, R. Marchiol and G. Diminno, ***Thromb. Vasc. Biol.***, 19, 1368-1377 (1999).
31. T. Rankinen, E. Hietanen, S. Vasisanen, M. Lehito, I. Penttila, C. Bouchard and R. Rauramma, ***Thromb. Res.***, 99, 453-459 (2000).
32. T.W. Meade, W.R.S. North, R.R. Chakrabarth, Y. Stirling, A.P. Hasines and S.G. Thompson, ***Lancet*** **1**, 1050-1054 (1980).
33. T.S. Manjula, S. Shyama and C.S. Shyamala Devi, ***Indian J. Comp. Anim. Physiol.***, 10, 109-13 (1992).
34. R. Sewel, R.M. Ibbotson, R. Philips and P. Ceusson, *Br. Med. J. Clin. Res. Edn.*, 15, 76-78 (1984).
35. N.J. Mitchinson and R.Y. Ball, ***Lancet*** 11, 146-149 (1987).
36. C.P. Canon, C.H. Mccabe, R.G. Wilcox, J.H. Bentley and E. Braunwald, ***Am. J. Cardiol.***, 87, 636-639 (2001).
37. S.D. Kristensen, P.C. Milner and J.F. Martin, ***Thromb. Haemo.***, 59, 353-356 (1988).
38. R.D. Eastham, in Bleeding, clotting and transfusion, In: *"Clinical haemotology"*, R. Slaze, Ed. (Bath Press Avon, Great Britain, 1992), pp.131-216.
39. M. Aghi, Z. Husain, K K Pillai, J.S Qadry, D.K. Balani and M. Imran, ***Indian J. Exp. Biol.***, 30, 611-614 (1992).
40. J.M. Leiro, E. Alvarez, J.A. Arranz, I.G. Siso, and F. Orallo, ***Biochem. Pharmacol.***, 65, 1361-1371 (2003).

**CALL FOR NEWS ITEM FROM THE RESEARCHERS IN NATURAL PRODUCTS**

Pharmacognosy Magazine - (ISSN 0973-1296: PHCOG MAG.) is a quarterly publication of Phcog Net, the first issue was released on January 1st, 2005 (for more details, visit http://www.phcog.com/content). In view of enormous response, Phcog Mag also invites News Item, reports on conference/symposium/workshop, individual awards, honours, acheivements, grants received, international presentations, or any other related matter from researchers in the field of natural products.

Kindly send reports or write-ups along with scanned copy of photograph with a subject line of "News Item" to
mueen.ahmed@phcog.net
or
arun.kumar@phcog.net