Original Research Article (Experimental)

Mangiferin protects rat myocardial tissue against cyclophosphamide induced cardiotoxicity

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ABSTRACT

Background: Mangiferin is a highly potent antioxidant present in mango leaves which is utilized for therapeutic purposes.
Objective: The present study was undertaken to evaluate the cardioprotective effect of mangiferin against cyclophosphamide induced cardiotoxicity.
Materials and methods: Rats were treated with 100 mg/kg of mangiferin in alone and interactive groups for 10 days. Apart from normal and mangiferin control groups, all the groups were subjected to cyclophosphamide (200 mg/kg, i.p.) toxicity on Day 1 and effects of different treatments were analyzed by changes in serum biomarkers, tissue antioxidant levels, electrocardiographic parameters, lipid profile and histopathological evaluation.
Results: Mangiferin treated group showed decrease in serum biomarker enzyme levels and increase in tissue antioxidant levels. Compared to cyclophosphamide control group, mangiferin treated animals showed improvement in lipid profile, electrocardiographic parameters, histological score and mortality.
Conclusion: The present findings clearly suggest the protective role of mangiferin as a powerful antioxidant preventing cardiotoxicity caused by cyclophosphamide.
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1. Introduction

Evolution of cancer therapy has made cancer a manageable disease today than ever before. However, chemotherapy-induced cardiac complications are an important cause of morbidity and mortality. Patients are likely to suffer from a cardiac disease rather than recurrent cancer. It is therefore necessary to manage both, the neoplasm and associated toxicities of anti-neoplastic drugs, especially cardiotoxicity [1,2].

Cyclophosphamide (CYP) is perhaps the most widely used anti-neoplastic agent [3]. It is used for the treatment of chronic and acute leukemias, myelomas, lymphomas, and for bone marrow transplantation [4]. CYP is attributed to also possess highly potent immunosuppressant activity [3]. Apart from having tumor selective action, it also possesses many highly toxic side-effects. Dose-mediated cardiotoxicity is one of the most important toxic effects [5]. The incidence of fatal cardiomyopathy due to a single high dose of CYP is up to 17%, depending on the different regimens and patient populations [6]. In contrast to the delayed cardiotoxic effects of other anti-neoplastic drugs, CYP causes lethal cardiomyopathy within 1–10 days after first administration of the dose (180–200 mg/kg) [7,8]. The cardiotoxic effects of CYP consists of acute, dose-dependent cardiac damage, morphologically characterized by necrosis, hemorrhage and later development of fibrosis [4,9].

The anti-neoplastic activity of CYP is due to phosphoramide mustard, the therapeutically active metabolite, which possesses significant DNA-alkylating activity [10,11]. The other metabolite, acrolein interferes with antioxidant system producing highly reactive oxygen-free radicals – superoxide radicals and hydrogen peroxide [12]. These Reactive Oxygen Species (ROS) cause damage to the inner mitochondrial membrane of the heart, diminishing the oxygen radical detoxifying capacity of cardiac mitochondria [13].

Natural products are known to possess wide range of biological activity. Flavonoids and polyphenolic compounds are the active antioxidant principles found in large number of natural products.

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Apart from strong antioxidant activity, they also demonstrate a number of other biological activities like, hepatoprotective, anti-diabetic, anti-bacterial, and anti-cancer to name a few [14–16]. These phenolic compounds have the ability to suppress lipid per-oxidation, prevent DNA oxidative damage, and scavenge free radi-cals. Free radicals cause depletion of the immune system antioxidants, change in gene expression, and induce abnormal proteins resulting in degenerative diseases and aging [17].

Mangifera indica L. (Mango) is an important plant of the Ayur-vedic and other indigenous systems of medicine. Different parts of the mango tree are known to possess different bioactivities and have been extensively used in Ayurvedic system of medicine. The fruit and its juice are used as a restorative tonic, while the seeds are used in asthma. The bark finds use in diphtheria and rheumatism and the smoke of the dried leaves in prevention of hiccups and throat infections [18]. Leaves of *M. indica* L. are a rich source of phenolic compounds. Mangiferin, obtained from the leaves and bark of the tree, is a xanthone with wide range of pharmacological effects [19]. Bioactivity of mangiferin is attributed to its ability to decrease localized O_2 concentration and generation of mangiferin phenoxy radicals and metal–ligand complexes with iron, that prevent the formation of OH radicals and oxo-ferryl groups that cause tissue damage. It also helps in maintaining the oxidant-antioxidant balance necessary for normal cellular function [20].

Research suggests that mangiferin possesses anti-diabetic effect on streptozotocin induced diabetes in mice and rats, possibly by reducing intestinal absorption of glucose [21,22]. It is known to prevent isoproterenol induced myocardial infarction in rats [23]. Previous studies have also revealed protective effects of mangiferin on rat brain by inducing peroxidation of phospholipids and pre-vention of DNA damage by bleomycin [24]. Immunomodulatory activity of mangiferin is evident in its action to inhibit TNF-induced activation of NF-κB in mice [25]. Apart from these bioactivities, mangiferin is also attributed to possess antimicrobial [26], anti-allergenic [27], anti-inflammatory, analgesic [28], and hep-atoprotective [29] activities among many others.

However, till now, there have been no studies carried out to demonstrate the protective effect of mangiferin against cardiotox-icity caused by anti-cancer drugs. This study is designed to evaluate the protective effect of mangiferin on cardiotoxicity caused by CYP.

2. Materials and methods

2.1. Animals

Wistar rats of both sexes, weighing between 200 and 250 g, were obtained from the animal facility of Shree Devi College of Pharmacy, Mangalore, India. The rats were maintained in an animal house with standard facilities. The animals were housed in clean cages and maintained at 25 ± 5 °C and humidity at 30–70% under 12 h light–dark cycles, and were fed with standard feed with free access to purified drinking water. Animals were acclimatized for one week to laboratory conditions before starting the experiment. All experiment protocols were conducted according to the guide-lines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), the Ministry of Social Justices and Empowerment, Government of India. Prior to the commencement of the experiment Institutional Animal Ethics Committee (SDCP/IAEC-07/2012-13) approval was obtained.

2.2. Isolation of mangiferin

M. indica L., leaves were obtained from cultivated trees from Mangalore area. The plant was identified at the Herbarium, Department of Pharmacognosy, Shree Devi College of Pharmacy,

Mangalore. The leaves were shade dried and powdered. The powdered material was defatted with petroleum ether (60–80 °C). Defatted powdered leaves were extracted in a Soxhlet apparatus with required quantity of ethanol for 21 h and concentrated under reduced pressure to yield semisolid mass. The ethanolic mass was then subjected to hydrolysation and followed by treatment with ethyl acetate. The ethyl acetate fraction was then precipitated with ethanol and crystallized. Briefly, the compound purity was confirmed through high performance liquid chromatography (HPLC) (Data not shown). The product was stored in a desiccator to prevent humidification and the weighed dose was dissolved in Dimethyl sulfoxide (DMSO) and used for the present study.

2.3. Experimental protocol

After the end of 1 week acclimatization, animals were divided into 4 groups of 6 animals each.

- Group I (*Normal Control*) served as normal control and received 1% Dimethyl Sulfoxide (DMSO) (i.p.) for 10 days.
- Group II (*CYP Control*) served as toxic control, in which the an-imals received single injection of CYP (200 mg/kg, i.p.) on the first day of experimental period to induce cardiotoxicity [30].
- Group III (*MANG*) received mangiferin (100 mg/kg body weight i.p.) for 10 days, dissolved in 1% DMSO [23].
- Group IV (*MANG + CYP*) received CYP (200 mg/kg, i.p.) on the first day and mangiferin (100 mg/kg body weight, i.p.) for 10 days.

Acute & chronic toxicity studies [31] and pharmacological studies [23] conducted on mangiferin were used as reference in deriving the current dose of 100 mg/kg.

2.4. Biochemical analysis

At the end of the experimental period, all the rats were anaes-thetized under light ether anesthesia and blood was collected by the retro-orbital route using microcapillaries. Serum was then separated from blood and used for the estimation of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphate (ALP), creatine kinase-MB (CK-MB), creatine kinase-NAC (CK-NAC) and lactate dehydrogenase (LDH). Then, the animals were sacrificed by mild ether anesthesia and four hearts from each group were homogenized with ice cold 0.25 M sucrose solution [32,33] for estimation of superoxide dismutase (SOD), catalase and Reduced Glutathione (GSH). SOD activity was determined on the capacity of the enzyme to reduce nitro blue tetrazolium [34]. The absorbance was measured at 560 nm. Ellman method was followed for the estimation of GSH [35], while method of Aebi was followed to es-timate catalase [36].

2.5. Electrocardiographic studies

Twenty-four hours after the last treatment, the animals were anesthetized with the combination of ketamine (75 mg/kg, i.p.) and xylazine (8 mg/kg, i.p.). The leads were attached to the dermal layer of both the front paws and the hind legs and recordings were made with the help of a digital physiograph (Model number – DI-2, INCO, Ambala, India). The changes in heart rate, QRS, QT, PR and RR in-tervals were determined.

2.6. Lipid profile assay

Serum cholesterol and triglyceride levels were measured by commercial kits with the help of a semi-autoanalyzer.

Table 1
Effect of Mangiferin on body weight

Treatment	Body weight (g)		
	Day 1	Day 10 (Terminal)	Relative weight gain
Normal control	244.75 ± 4.23	255.00 ± 4.99	10.25 ± 2.36
CYP control	243.14 ± 7.17	191.00 ± 4.72	−52.14 ± 8.83[***]
MANG	225.43 ± 3.49	235.33 ± 4.20	9.90 ± 1.61
MANG + CYP	240.74 ± 3.15	225.00 ± 6.45	−15.74 ± 6.78[***###]

All the values are in Mean ± SD, n = 6. [***]$p < 0.001$ when compared to normal control group, [###]$p < 0.001$ when compared to CYP control group. CYP − Cyclophosphamide; MANG − Mangiferin; g − grams.

2.7. Histopathological analysis

Hearts were immediately removed from the sacrificed animals and were fixed in 10% formalin before being processed for histopathological analysis. Histological sections of the heart were stained with hematoxylin and eosin. Myocardial damage and its severity were reported for specimen. The sections were given scores as follows: No Changes = 0; Mild = + (myocytes damage or small multifocal degeneration with slight degree of inflammation); Moderate = ++ (extensive myofibrillar degeneration); Marked = +++ (necrosis with diffuse inflammatory process).

2.8. Statistical analysis

The results are expressed as mean ± SD. Statistical significance was assessed using one-way analysis of variance (ANOVA), followed by the Tukey−Kramer multiple comparison tests. $p < 0.05$ was considered significant.

3. Results

Effect of mangiferin on CYP induced cardiomyopathy was evaluated by measuring the cardiac biomarker enzymes and antioxidant enzymes levels as well as evaluating electrocardiographic parameters and myocardial tissue histopathology. The observations recorded were terminal and inter-group comparison was performed.

3.1. General observations and mortality

General observations were recorded daily for the study period in all the animals. Abnormal changes like watery faeces, scruffy hair coat with slight alopecia and a pink tinge were observed in the CYP-treated group. However, these changes were remarkably reduced in mangiferin-treated group. Mortality was found in 3 animals (50%) of the CYP-treated group (Table 2).

3.2. Body weight and heart weight

Effect of CYP on heart weight, body weight and ratio of heart weight to body weight is shown in Table 1. In the CYP-treated group,

Table 2
Effect of Mangiferin on physical parameters

Treatment	Heart weight (g)	Heart/body Wt ratio ($\times 10^{-4}$)	% Mortality
Normal control	0.72 ± 0.02	28.23	0%
CYP control	1.03 ± 0.07[***]	53.92	50%
MANG	0.73 ± 0.05	31.02	0%
MANG + CYP	0.84 ± 0.02[*##]	37.33	33.33%

All the values are in Mean ± SD, n = 6. [*]$p < 0.05$, [***]$p < 0.001$ when compared to normal control group, [##]$p < 0.01$ when compared to CYP control group. CYP − Cyclophosphamide; MANG − Mangiferin; g − grams.

heart weight and heart weight to body weight ratio significantly increased compared to normal control group. The increase may be due to hypertrophy of the heart. In the MANG + CYP group, the heart weight and heart weight to body weight ratio was significantly less compared to CYP-treated group. Body weight was significantly reduced in the CYP-treated group animals, which was found to increase in the MANG + CYP treated group animals (Tables 1 and 2).

3.3. Effect on electrocardiographic parameters

The CYP control group demonstrated a significant increase in RR segment, QT segment, PR interval and QRS interval and significant decrease in heart rate compared with the normal control. Mangiferin pre-treatment normalized almost all of the parameters (Table 3).

3.4. Serum enzyme biomarkers

The CYP-treated group demonstrated significant increase in serum AST, ALT, ALP, CK-MB, CK-NAC and LDH values compared with the normal control. Treatment group MANG + CYP showed a significant decrease in AST, ALT, ALP, CK-MB, CK-NAC and LDH values compared with toxic control (Table 4).

3.5. Effect on SOD, catalase and GSH

The SOD, catalase and GSH activities were significantly reduced in the CYP control group compared to normal control group. However, the activities of these enzymes were significantly increased in the mangiferin treated (MANG + CYP) group compared to CYP control group, indicating the tissue protecting activity of mangiferin (Table 5).

3.6. Effect on lipid profile

Significant incremental values were found for triglycerides and cholesterol levels in the CYP control group compared to normal control. Treatment MANG + CYP group showed significant decreased values of both triglyceride and cholesterol (Table 5).

3.7. Effect on histological score

Heart tissue of normal control group and MANG group showed healthy myocardial cells with normal texture. CYP control group showed vacuolization of the cardiomyocytes, infiltration of inflammatory cells, myocardial tissue separation and myofibril loss. MANG + CYP group demonstrated protective efficiency of mangiferin. The group showed decreased infiltration of leukocytes and lesser defragmentation of myofibrils. Intracellular spaces within the myocardium also decreased (Table 6; Fig. 1).

4. Discussion

Plants containing flavonoids and polyphenolic compounds possess strong antioxidant activity. Mangiferin, the major bioactive constituent of *M. indica* L., is a glycosyl xanthone. Epidemiological studies have found that antioxidants and similar polyphenolic compounds can successfully prevent cardiomyopathy [19]. In the present study, we studied the possible role of mangiferin from mango leaves as a cardioprotective compound in CYP-induced cardiomyopathy.

CYP treatment caused an increase in heart weight and decrease in body weight of the animals. There was also a significant rise in mortality rate. This indicates that the general state of the animals was deranged, an indication of CYP toxicity. Increase in heart

Table 3
Effect of Mangiferin on electrocardiographic parameters

Treatment	Heart rate (beats/min)	RR interval (ms)	QT interval (ms)	QRS interval (ms)	PR interval (ms)
Normal control	182.33 ± 15.12	191.33 ± 12.81	195.66 ± 7.79	143.66 ± 10.02	82.66 ± 10.61
CYP control	87.66 ± 7.79***	289.66 ± 7.79***	297.33 ± 9.92***	213.66 ± 9.19***	199.33 ± 13.43***
MANG	182.66 ± 10.02	194.00 ± 16.29	196.00 ± 9.26	148.66 ± 9.19	84.66 ± 11.86
MANG + CYP	150.33 ± 9.92***###	256.33 ± 9.92***##	263.66 ± 12.03***###	184.66 ± 6.37***##	150.33 ± 14.16***###

All the values are in Mean ± SD, n = 6. ***$p < 0.001$ when compared to normal control group, ##$p < 0.01$, ###$p < 0.001$ when compared to CYP control group. CYP − Cyclophosphamide; MANG − Mangiferin; min − minutes; ms − milliseconds.

Table 4
Effect of Mangiferin on serum enzyme biomarkers

Treatment	Blood serum level (U/L)					
	CK-MB	CK-NAC	LDH	AST	ALT	ALP
Normal control	146.30 ± 5.24	82.18 ± 3.45	399.64 ± 11.10	118.95 ± 7.03	46.81 ± 9.82	98.84 ± 11.32
CYP control	499.09 ± 9.87***	296.70 ± 8.84***	757.75 ± 11.29***	562.77 ± 10.49***	254.66 ± 8.72***	452.21 ± 10.76***
MANG	166.34 ± 4.90	86.93 ± 3.36	405.21 ± 5.32	134.67 ± 6.84	78.09 ± 7.20	109.97 ± 6.39
MANG + CYP	304.80 ± 6.30***###	192.63 ± 4.04***###	609.40 ± 11.49***###	538.33 ± 3.85***##	229.41 ± 4.58***##	230.19 ± 16.46***###

All the values are in Mean ± SD, n = 6. ***$p < 0.001$ when compared to normal control group, ##$p < 0.01$, ###$p < 0.001$ when compared to CYP control group. CYP − Cyclophosphamide; MANG − Mangiferin.

Table 5
Effect of Mangiferin on Total Cholesterol & Triglycerides and heart tissue homogenate levels of SOD, Catalase and GSH

Treatment	Blood serum level (mg/dl)		Heart tissue homogenate (U/L)		
	TC	TG	SOD	Catalase	GSH
Normal control	21.76 ± 3.33	79.61 ± 9.33	87.46 ± 9.75	57.78 ± 12.74	87.90 ± 11.71
CYP control	74.46 ± 8.82***	205.51 ± 17.52***	17.99 ± 4.92***	16.24 ± 5.76***	24.49 ± 8.55***
MANG	33.58 ± 6.64	75.94 ± 9.19	63.34 ± 7.47	40.39 ± 7.99	65.29 ± 8.04
MANG + CYP	43.81 ± 6.88***###	177.75 ± 10.63***##	51.09 ± 14.01***##	39.34 ± 4.53**###	52.97 ± 10.12***##

All the values are in Mean ± SD, n = 6. **$p < 0.01$ ***$p < 0.001$ when compared to normal control group, ##$p < 0.01$, ###$p < 0.001$ when compared to CYP control group. CYP − Cyclophosphamide; MANG − Mangiferin; mg − milligrams; dl − deciliter.

weight may be due to extensive necrosis of cardiac muscles and invasion of inflammatory cells to the damaged site. Edema of the tissue may also have occurred [37]. Treatment with mangiferin (100 mg/kg body weight) showed significant improvement in the body state of the animals.

Of the many actions of CYP, one is direct damage to myocardial endothelium, leading to death of myocardial cells. Enzymes like CK-NAC, CK-MB, LDH, ALT, AST and ALP, otherwise trapped inside the myocardial cells, are released into the blood stream due to damage to the endothelium. Quantitative estimation of these enzymes (hence called, biomarker enzymes) can be associated with the extent of damage to the myocardial tissue [38]. Administration of mangiferin showed decrease in the levels of these biomarker enzymes, indicating a reparative or membrane stabilizing action of the xanthone, preventing further damage to the myocardium.
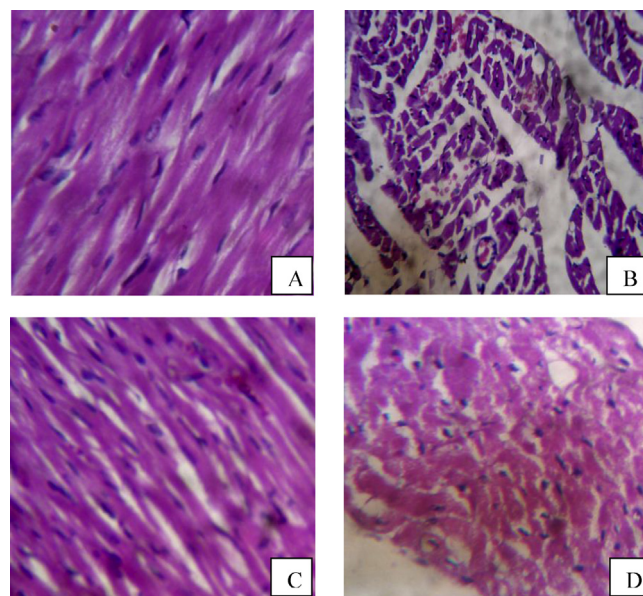
As discussed earlier, CYP causes injury to the myocardial membrane. This injury is a result of generation of OH⁻ free radicals during CYP metabolism. These free radicals induced lipid peroxidation, resulting in loss of integrity of the myocardial membrane and thus its function. Reduced Glutathione (GSH), Catalase and Superoxide Dismutase (SOD) are important inhibitors of free

radicals generated by lipid peroxidation; CYP induces decline in the amount of these enzymes leading to myocardial injury [39,40]. Our study showed marked increase in the values of these antioxidant enzymes after treatment with mangiferin, thus improving the antioxidant status and prevention of damage to the heart.

Fig. 1. Histopathological evaluation of heart tissue in (A) Normal (B) CYP Control (C) MANG (D) MANG + CYP.

Table 6
Histological changes in myocardial tissues

Groups/scores	0	+	++	+++
Normal control	6	0	0	0
CYP control	0	0	1	5
MANG	6	0	0	0
MANG + CYP	0	1	3	2

Photomicrographs were used to evaluate the damage in the heart tissue: (0) no changes; (+) mild changes; (++) moderate changes; (+++) marked changes.

Mangiferin treated group also showed decreasing amounts of cholesterol and triglycerides. CYP induced free radicals cause accumulation of cholesterol by decreasing its uptake and increase in biosynthesis [40]. Triglycerides are converted to fatty acids in the body by lipoprotein lipase enzyme, alteration in the activity of which, results in increase in serum triglycerides levels [39]. Mangiferin shows antihyperlipidemic action, protecting against CYP induced hyperlipidemic cardiomyopathy.

CYP treated group showed changes in ECG pattern such as decrease in heart rate, and prolongation of QT interval, QRS interval, PR interval and RR interval in this study [41]. Release of higher quantities of neurotransmitter, acetylcholine, during myocardial damage results in the bradycardia, as observed in the present study. Mangiferin prevents the CYP induced bradycardia. CYP also induces changes in parasympathetic tone and conduction system. This leads to an AV block causing PR interval elongation [42]. CYP is also known to cause hyponatremia and hypokalemia, in turn resulting in elongation of QT interval [43–45]. Treatment with mangiferin showed normalization in the values of these intervals, describing the protective effect.

Histopathologically, treatment with CYP showed vacuolization of the cardiomyocytes, infiltration of inflammatory cells, myocardial tissue separation and myofibril loss. Mangiferin treated group showed reversal of such toxic changes (Fig. 1), an effect of possible vasodilatation.

5. Conclusion

Cyclophosphamide induced oxidative stress and myocardial damage can be prevented to a greater extent by mangiferin. This finding is very important for cancer patients suffering from chemotherapy-induced complications as there are no established guidelines for prevention or treatment of cyclophosphamide-associated cardiomyopathy. Further clinical studies are needed to establish the protective effect of mangiferin.

Conflict of interest

None declared.

Acknowledgement

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