



Mangifera indica L. leaf extract alleviates doxorubicin induced cardiac stress

Laxit Bhatt^{1,2}, Viraj Joshi³

ABSTRACT

Aim: The study was undertaken to evaluate the cardioprotective effect of the alcoholic leaf extract of *Mangifera indica* L. against cardiac stress caused by doxorubicin (DOX). **Materials and Methods:** Rats were treated with 100 mg/kg of *M. indica* leaf extract (MILE) in alone and interactive groups for 21 days. Apart from the normal and MILE control groups, all the groups were subjected to DOX (15 mg/kg, i.p.) toxicity for 21 days and effects of different treatments were analyzed by changes in serum biomarkers, tissue antioxidant levels, electrocardiographic parameters, lipid profile, and histopathological evaluation. **Results:** The MILE treated group showed decrease in serum biomarker enzyme levels and increase in tissue antioxidants levels. Compared to DOX control group, MILE treated animals showed improvement in lipid profile, electrocardiographic parameters, histological score, and mortality. **Conclusion:** These findings clearly suggest the protective role of alcoholic leaf extract of *M. indica* against oxidative stress induced by DOX.

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INTRODUCTION

Doxorubicin (DOX) is an anticancer antibiotic widely used in the treatment of hematopoietic, lymphoblastic, and solid tumors in humans. However, its use is limited because of its capacity to cause dose-dependent cardiotoxicity [1]. DOX acts by different mechanisms: Free radicals induced cell injury, iron-dependent oxidative damage, lipid peroxidation [2], release of vasoactive amines [3], mitochondrial damage, and free radicals induced cellular apoptosis [2]. However, it should be noted that the release of reactive oxygen free radicals and increased oxidative stress play a major role in DOX-induced cardiotoxicity [4].

Billingham *et al.* [5] and Mackay *et al.* [6], along with their groups, have quantitated the importance of cumulative dose of DOX in development of cardiomyopathy by the anticancer drug. Cumulative dose dependence involves incremental and partially irreversible cardiac damage, which only worsens after a second administration. Sequential administration of the drug causes compromise of the cardiac activity, ultimately resulting in cardiac failure and death. This concept is supported

by observation of ultrastructural changes such as vacuoles formation, sarcomere disruption, and necrosis of myocytes in DOX-treated heart tissues [7].

The prevalence of cardiotoxicity, even on lower cumulative doses of DOX, demands the development of cardioprotective regimens that not only prevent the generation of toxic effects but also not produce adverse effects of their own. There has been a growing interest in the therapeutic potential of natural antioxidants in cardiovascular related problems as they are widely known to possess lesser side effects than their synthetic counterparts [4,8,9].

Mangifera indica L., also known as Mango is an important herb of indigenous medical systems of the world. Mango belongs to the family Anacardiaceae, and the genus *Mangifera* consists of about 30 species. Ayurvedic system of medicine attributes a variety of medicinal properties to Mango, wherein different parts of the tree possessing different pharmacological activities. Leaves of mango trees have been used since generations in therapeutic purposes ranging from asthma to hiccups [10]. The plant contains different chemical constituents: Polyphenolics,

flavonoids, and triterpenoids. Mangiferin, a xanthone glycoside is the major bioactive constituent; isomangiferin, galloyl, hydroxy benzoyl esters, epicatechin, tannins, and gallic acid derivatives are other chief constituents of the leaves [11]. The leaf extract exerts different biological activities, namely, antidiabetic [12], immunomodulatory [10], antimicrobial [13], antiallergenic [14], anti-inflammatory, analgesic [15], and hepatoprotective [16] activities among many others. There have been previous studies that prove higher biological activities of an extract compared to that of an active, isolated constituent, like Mangiferin [11]. Research exists regarding the role of isolated mangiferin on protection of rat myocardium against oxidative stress [17,18].

However, there have been no studies on the protective role of the alcoholic extract of mango leaves on DOX-induced oxidative stress. Therefore, this study is designed to evaluate the cardioprotective role of alcoholic extract of *M. indica* leaves against cardiotoxicity induced by DOX.

MATERIALS AND METHODS

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 animals were housed in clean cages and maintained at $25 \pm 5^\circ\text{C}$ 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 1 week to the laboratory conditions before starting the experiment. All experiment protocols were conducted according to the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals, the Ministry of Social Justice and Empowerment, Government of India. Before the commencement of the experiment, approval was obtained from the Institutional Animal Ethics Committee (SDCP/IAEC-07/2012-13).

Preparation of *M. indica* Leaf Extract (MILE)

The leaves of *M. indica* (Family: Anacardiaceae) were collected from Mangalore region in the month of May and authenticated at the Herbarium, of the Department of Pharmacognosy, Shree Devi College of Pharmacy, Mangalore. The leaves were dried under shade and powdered. The powdered material was defatted with petroleum ether (60-80°C). Defatted powdered leaves were extracted by Soxhlet apparatus with required quantity of ethanol for 21-h and concentrated under reduced pressure to yield semisolid mass. Required quantity of the extract was suspended in purified water and used for the experiment.

Experimental Design

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

- Group I (normal control) served as normal control and received purified water p.o., for 21 days.
- Group II (DOX) served as toxic control, in which the animals

received a total cumulative dose of 15 mg/kg, i.p. of DOX for 2 weeks in six divided dosages to induce cardiotoxicity.

- Group III (MILE) received MILE (100 mg/kg body weight, p.o.) for 21 days, suspended in purified water.
- Group IV (DOX + MILE) animals received the same treatment as Group II along with MILE suspended in purified water (100 mg/kg body weight, p.o.) for 21 days.

100 mg/kg dose of MILE was selected on the basis of different pharmacological and toxicity studies conducted on the extract [16,19]. Groups II and IV received DOX at alternate days for 2 weeks. The days selected for DOX administration were 8th, 10th, 14th, 16th, 18th, and 21st day after 7 days pre-treatment with MILE.

Biochemical Analysis

At the end of the experimental period, all the rats were anesthetized under light ether anesthesia and blood was collected by the retro-orbital route using microcapillaries. Serum was separated 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 0.9% buffered KCl (pH 7.4) for estimation of superoxide dismutase (SOD), catalase and reduced glutathione (GSH).

SOD activity [20] was determined and measured at 560 nm. Ellman method was followed for the estimation of GSH [21], while the method of Aebi was followed to estimate catalase [22].

Electrocardiographic Studies

24 h 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.). 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 intervals were determined.

Lipid Profile Assay

Serum cholesterol and triglyceride levels were measured by commercial kits (Prietest, Robonik (India) Pvt. Ltd.) with the help of a semi auto analyzer (Prietest TOUCH, Robonik (India) Pvt. Ltd.).

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).

Statistical Analysis

Results are expressed as a mean ± standard error. 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.

RESULTS

General Observations and Mortality

All animals were observed daily for any clinical signs. Most evident abnormal signs observed were chromodacryorrhea, piloerection and scruffy and pinkish hair coat. Along with these signs, mortality was observed in 3 animals of DOX-treated group (toxic control group) [Figure 1 and Table 1].

Body Weight, Heart Weight, and Ratio of Heart Weight to Body Weight

A significant decrease in body weight was observed in the DOX group when compared to the control. The MILE pre-treatment prevented such decrease in body weight in animals of DOX + MILE group, where a moderately significant increase in body weight was observed when compared to the DOX group.

A reverse effect was seen on the heart weight. Treatment with DOX caused an extremely significant increase in heart weight of the animals on DOX group when compared to control group animals. Similar was the effect of DOX on heart weight/body weight ratio, which showed a marked, extremely significant increase. MILE treatment normalized both these parameters in

DOX + MILE group where animals had moderately significant decrease in heart weight and heart weight/body weight ratio [Table 1].

Serum Enzyme Biomarkers

The DOX-treated group demonstrated a significant increase in serum AST, ALT, ALP, CK-MB, CK-NAC, and LDH values when compared with the normal control group. Treatment group DOX + MILE showed a significant decrease in biomarker enzymes values compared with toxic control, indicating normalization in their values [Table 2].

Effect on Electrocardiographic Parameters

The DOX control group demonstrated a significant increase in heart rate, RR segment, QT segment, PR interval, and QRS interval compared with the normal control. MILE pre-treatment



Figure 1: (a and b) Chromodacryorrhea observed in Group-II doxorubicin control rats

Table 1: Effect on general parameters

| Treatment | Body weight (g) | Heart weight (g) | Heart weight/body weight ratio (10 ⁻⁴) | Mortality (%) |
|----------------|-----------------|------------------|--|---------------|
| Normal control | 252.00±7.63 | 0.75±0.01 | 29.76 | 0 |
| DOX | 190.00±4.93*** | 1.13±0.03*** | 59.47*** | 50 |
| MILE | 220.00±2.88* | 0.71±0.02 | 32.27 | 0 |
| DOX+MILE | 224.66±3.18*** | 0.87±0.01*** | 38.72*** | 0 |

All the values are in mean±SEM, n=6. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ when compared to normal, ** $P < 0.01$ when compared to DOX.

DOX: Doxorubicin-treated group, MILE: *Mangifera indica* leaves extract treated group, DOX+MILE: Pre-treatment with *Mangifera indica* extract followed by DOX treatment

Table 2: Effect on serum biomarker enzymes

| Treatment | Blood serum level (U/L) | | | | | |
|----------------|-------------------------|----------------|----------------|----------------|----------------|----------------|
| | CK-MB | CK-NAC | LDH | AST | ALT | ALP |
| Normal control | 144.25±2.14 | 80.21±1.41 | 397.69±4.53 | 119.09±2.87 | 44.97±4.01 | 97.87±4.62 |
| DOX | 457.11±2.45*** | 267.59±3.31*** | 685.19±2.79*** | 457.86±5.18*** | 201.71±4.72*** | 361.23±4.10*** |
| MILE | 175.94±1.91* | 88.02±1.74 | 412.91±2.47 | 135.39±2.29 | 79.72±4.34* | 115.24±3.21 |
| DOX+MILE | 254.31±1.72*** | 128.98±3.54*** | 456.11±3.45*** | 338.21±4.32*** | 124.72±3.22*** | 216.91±6.74*** |

All the values are in mean±SEM, n=6. * $P < 0.05$, *** $P < 0.001$ when compared to normal, *** $P < 0.001$ when compared to DOX.

DOX: Doxorubicin-treated group, MILE: *Mangifera indica* leaves extract treated group, DOX+MILE: Pre-treatment with *Mangifera indica* extract followed by doxorubicin treatment, CK-MB: Creatine Kinase myocardial b fraction, CK-NAC: Creatine kinase N-acetylcysteine, LDH: Lactate dehydrogenase, AST: Aspartate aminotransferase, ALT: Alanine transaminase, ALP: Alkaline phosphatase

Table 3: Effect on ECG parameters

| Treatment | Heart rate | RR | QRS | QT | PR |
|----------------|-----------------|-----------------|----------------|-----------------|-----------------|
| Normal control | 180.29±6.17 | 192.29±5.23 | 142.47±4.09 | 194.60±3.18 | 81.59±4.33 |
| DOX | 269.33±5.20*** | 269.33±5.20*** | 197.33±1.45*** | 268.33±5.23*** | 178.00±4.35*** |
| MILE | 184.00±3.18 | 193.66±5.23 | 144.33±5.81 | 197.33±4.05 | 87.66±4.91 |
| DOX+MILE | 219.33±5.78**** | 228.66±5.78**** | 165.33±3.18*** | 226.66±4.41**** | 115.33±3.75**** |

All the values are in mean±SEM, $n=6$. *** $P<0.001$, ** $P<0.01$, * $P<0.05$ when compared to normal, **** $P<0.001$ when compared to DOX.

DOX: Doxorubicin-treated group, MILE: *Mangifera indica* leaves extract treated group, DOX+MILE: Pre-treatment with *Mangifera indica* extract followed by DOX treatment, ECG: Electrocardiogram

Table 4: Effect on tissue antioxidants and lipid profile

| Treatment | Blood serum level (mg/dl) | | Heart tissue homogenate (U/L) | | |
|----------------|---------------------------|-----------------|-------------------------------|---------------|---------------|
| | TC | TG | SOD | Catalase | GSH |
| Normal control | 20.44±1.36 | 78.99±3.81 | 86.97±3.98 | 58.00±5.20 | 88.01±4.78 |
| DOX | 61.07±3.42*** | 188.23±4.12*** | 22.73±2.09*** | 22.39±1.70*** | 38.02±4.64*** |
| MILE | 31.01±2.27 | 88.73±3.38 | 67.83±5.21* | 38.68±3.89* | 64.98±3.27 |
| DOX+MILE | 39.65±3.16**** | 110.58±4.11**** | 50.21±2.79**** | 41.63±2.27*** | 65.78±2.83*** |

All the values are in mean±SEM, $n=6$, *** $P<0.001$, ** $P<0.01$, * $P<0.05$ when compared to normal, **** $P<0.001$, *** $P<0.001$, ** $P<0.01$ when compared to DOX-treated. DOX: Doxorubicin-treated group, MILE: *Mangifera indica* leaves extract treated group, DOX+MILE: Pre-treatment with *Mangifera indica* extract followed by DOX treatment. TC: Total cholesterol, TG: Triglycerides, SOD: Superoxide dismutase, GSH: Glutathione

almost normalized all of the parameters [Table 3].

Effect on SOD, Catalase and GSH

The SOD, catalase and GSH activities were significantly reduced in the DOX control group compared to normal control group. However, the activities of these enzymes were significantly increased in the MILE treated (DOX + MILE) group compared to DOX control group [Table 4].

Effect on Lipid Profile

Significant incremental values were found for triglycerides and cholesterol levels in the DOX control group compared to normal control. Treatment DOX + MILE group showed significantly decreased values of both triglyceride and cholesterol [Table 4].

Effect on Histological Score

Myocardial cells of the animals in normal and MILE group showed normal texture and intact cell membranes. As expected, DOX control group showed separation of myocardial tissue, vacuolization of myocardial cells, and accumulation of inflammatory cells and loss of myofibril. Treatment with MILE in the DOX + MILE group, showed decreased infiltration of inflammatory cells, lesser defragmentation, vacuolization, and myofibril loss [Table 5 and Figure 2].

DISCUSSION

Products of plant origins with flavonoids and polyphenolic contents are in great demand in recent times due to their strong antioxidant effects [9,18,23]. *M. indica* leaves contain a complex mixture of mangiferin, isomangiferin, galloyl, hydroxy benzoyl esters, epicatechin, tannins, and gallic acid derivatives. These constituents are of flavonoid and phenolic origin and possess antioxidant activity as high as vitamins C

Table 5: Effect on histological score

| Groups | 0 | + | ++ | +++ |
|----------------|---|---|----|-----|
| Normal control | 6 | 0 | 0 | 0 |
| DOX | 0 | 0 | 1 | 5 |
| MILE | 6 | 0 | 0 | 0 |
| DOX+MILE | 0 | 1 | 3 | 2 |

Photomicrographs were used to evaluate the damage in the heart tissue: (0) No changes; (+) mild changes; (++) moderate changes; (+++) marked changes. Numbers in the table represent the total number of animals in the groups, DOX: Doxorubicin-treated group, and MILE: *Mangifera indica* leaves extract treated group

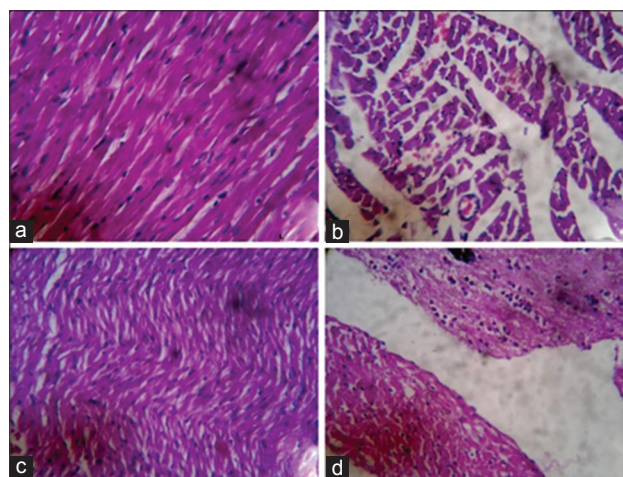


Figure 2: Histopathological evaluation of heart tissue stained in H and E. (a) Normal control, (b) doxorubicin (DOX), (c) *Mangifera indica* leaves extract (MILE), and (d) DOX + MILE

and E [11]. Reports exist that demonstrate higher potency these constituents collectively than any one isolated, active constituent [11]. In this study, we studied a possible potent cardioprotective role of the MILE on DOX-induced oxidative stress on rat myocardium.

DOX-treated animals have shown scruffy hair coat and pinkish tinge to the fur as well as significant decrease in body weight. The decrease in body weight in this study is in accordance with other studies, and it may be attributed to reduced food intake and inhibition of protein synthesis due to DOX treatment compared to normal group [24]. Increase in heart weight may be attributed to necrosis of myocardial tissue possibly due to increased ROS, mitochondrial swelling and dysfunction, and ATP depletion [25]. Our study demonstrated a significant increase in the heart weight and heart/body weight ratio when compared with the control group. DOX + MILE treated group showed normalization in the heart weight. This effect may be due to the direct free radical scavenging ability of the extract [26].

DOX treatment induces a sharp increase in the amplitude of P wave, QT interval and RR interval and a dose-dependent, reversible increase in QRS complex while reduces cardiac cycle. DOX also caused changes in ST segment which may link to its degenerative effect on cell membrane [27,28]. Treatment with MILE caused reduction in P-wave amplitude, QRS complex, QT interval and RR interval while cardiac cycle was increased, the ST segment was also near to normal. These changes in the electrocardiogram pattern induced by MILE may be due to its membrane stabilizing action.

One of the major toxic effects of DOX is inducing a decreased supply of oxygen to the myocardial cell leading to hypoxia. DOX is also a known agent to cause lipid peroxidation [25,29]. Lipid peroxidation under hypoxic conditions leads to acute membrane damage, causing rupture of the cell membrane and leakage of cellular enzymes [30]. These enzymes can be estimated in serum and used as biomarkers to check the damage caused to the myocardium [31]. Studies have demonstrated that DOX causes elevation in levels of these biomarker enzymes [32]. Treatment with MILE caused a significant decrease in the levels of CK-MB, CK-NAC, LDH, AST, ALP, and ALT enzymes suggesting the membrane stabilizing and reparative action of the extract preventing damage to the rat myocardium.

Overproduction of reactive oxygen species leads to peroxidation of membrane phospholipids and generation of reactive aldehydes. DOX induces strong oxidative stress in myocardium that leads to decrease in antioxidant stores, viz., SOD, catalase, and GSH [33]. The MILE treated groups showed an increase in levels of these antioxidant enzymes when compared to DOX control. This protective effect may be due to the collective free radical scavenging effect of the constituents of the extract [26].

The DOX + MILE treated groups showed a decrease in the amounts of serum cholesterol and triglycerides. One of the many toxic manifestations of DOX is its interference in biosynthesis and metabolism of lipids [34]. DOX inhibits adipogenesis by downregulating peroxisome proliferator-activated receptor gamma, causing inhibition of lipid clearance and hyperlipidemia [35]. DOX control group showed increase in the levels of total cholesterol and triglycerides confirming the toxicity of DOX. Treatment with MILE showed a concomitant

decrease in the blood lipid profile levels describing the antihyperlipidemic action of the leaf extract [26].

Histopathological examination of the normal animals displayed intact myocardial cells. Tissues of animals treated with DOX alone showed loss of myofibrils, vacuolization, lysosomal bodies, and dilatation; a clear indication of DOX toxicity [36,37]. MILE showed protective effect in these animals and tissues displayed less disruption and more intact myofibrils, decreased lysosomal bodies, and lesser vacuolization.

CONCLUSION

It can be concluded from this study that DOX-induced cardiac stress is prevented by the alcoholic extract of *M. indica* leaves to a greater extent. There are no preset guidelines on prevention of cardiotoxicity caused by anticancer agents. This renders the finding from this study a great value for patients suffering from chemotherapy-related complications of the heart. Further studies can be designed and conducted clinically.

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