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Research Article

EVALUATION OF CARDIOPROTECTIVE ACTIVITY OF ETHANOLIC LEAVES EXTRACT OF *FICUS RELIGIOSA* (PEEPAL PLANT) IN ALBINO WISTAR RATS USING DOXORUBICIN-INDUCED CARDIOTOXICITY

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Abstract

The current study aims to assess the potential cardioprotective efficacy of *Ficus religiosa* leaves extract in rats. In this investigation, one rat model of cardiotoxicity caused by doxorubicin (200 mg/kg, S.C.) was used to assess the cardioprotective efficacy of 70% ethanolic leaf extract of *Ficus religiosa*. Heart weight index (HWI) and biomarkers including ALT, AST, and LDH were considered while determining the cardioprotective property. Animals used in experiments had their hearts subjected to histopathological research as well. *Ficus religiosa* leaf produced 45% extract and was found to have Flavonoids, Alkaloids, Tannins, Phenols, Saponins, Glycosides, and Carbohydrates as a chemical ingredient. The cardiotoxicity that DOX (doxorubicin) caused in test animals was found to be protected by *Ficus religiosa* leaf extract. When compared to the group that had cardiotoxicity induced, it was also discovered that the HWI had decreased due to *Ficus religiosa* leaf extract. Following the estimation of the biomarker enzymes, the histopathology studies were also completed. When tested on albino Wistar rats, it was discovered that the *Ficus religiosa* leaf extract had considerable cardioprotective action.

Keywords: *Ficus religiosa* leaves extract, cardioprotective efficacy, doxorubicin, biomarkers, and histopathological studies.

INTRODUCTION

The occurrence of cardiovascular diseases (CVD) in adults is 48% indicating high risk in both sexes [1]. Heart disease was the leading cause of death in 2015, followed by cancer fatalities (595,930), making cardiovascular diseases (CVD) one of the two biggest causes of death in the US since 1975 (633,842 deaths, or 1 in every 4 deaths) [2]. The World Health Organization (WHO) estimated that 17.7 million people died from CVD in 2015, making it the leading cause of mortality worldwide. The burden of CVD is further enhanced by the fact that it is the most expensive disease, even more so than diabetes and Alzheimer's, with estimated indirect expenses of $368 billion by 2035. Indirect costs for CVD are estimated at $237 billion annually [3]. *Ficus religiosa is* commonly known as the peepal plant. Leaves are 30-meter-long, large, and venerable trees. They are white or brown in appearance and shatter bark. The leaves have 5-7 veins and are slender and glossy. Fruits have a diameter of around a half-inch, which is comparable to an eye pupil. It is compacted and shaped like a circle. It is green when it is fresh and becomes black when it is fully ripe and belongs to the family Moraceae. Pharmacological activities of Ficus religiosa leaves extract include; antimicrobial, anti-parasitic, anti-Parkinson's anticonvulsant, anti-amnesic, anticholinergic, anti-inflammatory, analgesic, hypoglycemic, hypotensive, hepatoprotective, nephroprotective, wound-healing, antiulcer, anti-asthmatic, and reproductive activity [4,5]. Ficus religiosa leaves extract contains phytochemicals such as; flavonoids, phytosterols, tannins, phenols, saponins, sugars, alkaloids, terpenoids, glycosides, proteins, essentials and volatile oils [6].

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There are many drugs available for cardioprotective activities. Flavonoids and phenolic compounds are one of the agents which are extensively used for cardioprotective activities. Antioxidants are substances that chemically react with free radicals and render them harmless while simultaneously breaking the vicious circle, which involves the decomposition of fatty acids and proteins, creating new free radicals and leading to eventual cell death. The antioxidant defense system includes both endogenously and exogenously derived compounds, dietary plant-based antioxidants have recently received great attention. Hence many studies have been performed to identify antioxidant compounds with pharmacological activity and limited toxicity for medicinal plants [7]. Antioxidants may play an important role in chronic disease prevention by arresting oxidative damage caused by reactive oxygen species (ROS) to vital molecules such as DNA, lipids and proteins [8]. Doxorubicin's main mode of action involves its capacity to intercalate inside DNA base pairs, resulting in DNA strand breaks and a suppression of both DNA and RNA production. Topoisomerase II is inhibited by doxorubicin, which damages DNA and triggers death in cells. Doxorubicin, when mixed with iron, also results in free radical-mediated oxidative damage to DNA, reducing DNA synthesis. By reducing doxorubicin's affinity for iron, iron chelators like dexrazoxane can decrease the production of free radicals [9]. However, complementary medicine has grown in popularity for several reasons in recent years. There are limited scientific reports on the cardioprotective activity of the leaf extract of *Ficus religiosa*. Previous studies have only revealed that evaluation of cardioprotective activity has been done for other parts of Ficus religiosa but not with the leaves. So, I intended to carry out the cardioprotective activity of Ethanolic leaf extract of *Ficus religiosa* against doxorubicin-induced toxicities.

MATERIALS AND METHODS

Plant Material

The leaves of *Ficus religiosa* were collected from the surrounding gardens of Mallige College of Pharmacy (MCP), Bengaluru-90, and it was identified and authenticated by a botanist. By rinsing with water, dirt and debris were eliminated. Before extraction, the leaves were crushed to powder and dried in the shade. It was kept for future use, in an airtight container and utilised to make ethanolic extract.

Experimental animals

Male albino Wistar rats (100-120g±10 g) were used for this study. The animals were acclimatized in the animal house of the Mallige College of Pharmacy, Bengaluru-90. Animals were cared for according to CCSEA Guidelines for the Care and Use of Laboratory Animals and allowed free access to animals feed and water (adlibitum). Ethics clearance was obtained from the Mallige College of Pharmacy, Ethics Review Committee with approval number (MCP108/2022-23). Experiments were carried out according to the guidelines of Rajiv Gandhi University of Health Sciences, Jayanagar, Bengaluru, and Karnataka-560041.

Extraction of Ficus religiosa leaves

The dried leaves of *Ficus religiosa* were ground and passed through a sieve (coarse 10/40) before being weighed at 100 g (according to the soxhlet apparatus's capacity) and packed in a timble. They were then processed in the soxhlet apparatus for 72 hours while maintaining a constant temperature. A continuous hot Soxhlet extraction technique was carried out and completely evaporated to dryness [10]. The percentage yield was calculated as follows;

Percentage yield = *weight of dried extract/ weight of powdered onion* leaves × 100%

Figure 1. Soxhlet extraction

Determination of Acute Toxicity (LD_{50})

The acute toxicity test was carried out as stated by the OECD guidelines. The albino rats of both sexes were randomly divided into two groups (n = 5). Group 1 served as a control and received normal saline (10 mL/kg). At the same time, group 2 was administered different doses of the ethanolic leaves extract of *Ficus religiosa* in an increasing concentration, i.e.n1000, 2000, and 3000 mg/kg, i.p. The mortality rate was observed for 24 h, and rats were kept under observation for 24 h for behavioural changes (restlessness, dullness, and agitation) with signs of toxicity and mortality [11]. Hence $1/10^{th}$ of no lethal dose was taken as an effective dose (500mg/kg body weight) for the ethanolic extract of leaves of *Ficus religiosa* in evaluation of cardioprotective potential in rats.

Doxorubicin-induced cardiotoxicity

Male albino Wistar rats (150-200g) were used to evaluate the cardioprotective activity. Rats were treated with *Ficus religiosa* leaf extract daily for 28 days. On the 28^{th} day, myocardial injury was induced in experimental rats by injection of Doxorubicin (DOX) (200 mg/kg, S.C.) twice at an interval of 24 hr. (i.e. on the 28^{th} and 29^{th} day of *Ficus religiosa* leaves extract treatment), while normal control and drug control rats were administration an equivalent volume of the vehicle [12]. The experimental rats were divided into 4 groups of 6 animals each and treated as follows:

Table 1. Effect of *Ficus religiosa* leaves extract against Doxorubicin-induced cardiotoxicity

Sl. No.	Group	Treatment	Duration of treatment
1.	Normal animals	Normal saline	Daily for 28 days
2.	Positive control	DOX (200mg/kg, s.c.)	28th & 29th days
3.	*Ficus religiosa* leaves extract control	*Ficus religiosa* leaves extract (200mg/kg, p.o.)	Daily for 28 days
4.	*Ficus religiosa* leaves extract pretreated	*Ficus religiosa* leaves extract (400mg/kg, p.o.) + DOX (200mg/kg, s.c.)	Dail Daily for 28 days + 28th & 29th day

Biochemical analysis: After a 24-hour treatment period on the 30th day blood was collected from the retro-orbital plexus, and serum was separated by centrifuging at 10000 rpm for 15min. The Separated liquid was subjected to biochemical estimation of cardiac marker enzymes i.e. LDH (Lactate Dehydrogenase), ALT (Alanine Aminotransferase), AST (Aspartate Aminotransferase), CPK (Creatine Phosphokinase).

Heart weight index (HWI): After blood withdrawal, all the rats were sacrificed by cervical dislocation; the hearts were dissected out, washed in ice-cold saline, weighed after blotting with filter paper and heart weight index (HWI) was computed as;

Heart weight index (HWI) = Heart weight (mg) /Body weight (g)

Then myocardial tissue was immediately fixed in a 10% buffered neutral formalin solution and processed for histopathological studies.

Histopathological study: At the end of the study, two rats per group were sacrificed humanely by giving a high dose of anesthesia and the heart was dissected out, and washed in ice-cold saline. Then myocardial tissue was immediately fixed in a 10% buffered neutral formalin solution and processed for histopathological studies.

Table 2. Effect of Ficus religiosa leaves extract on cardiac marker enzymes in the serum ofcontrol and Doxorubicin (DOX) induced oxidative stress and cardiotoxicity in rats

Group	ALT (IU/L)	AST (IU/L)	LDH (IU/L)	CPK (IU/L)
I. Control	24.32 ± 2.35	117 ± 6.35	148 ± 5.18	829.72 ± 95.15
II. Doxorubicin (DOX) (200mg/kg)	44 ± 5.93#	167.34 ± 6.48# #	182.55 ± 3.48# #	1781.67 ± 81.54# #
III. *Ficus religiosa* leaves extract (FRLE) 200mg/kg + DOX	22.14 ± 3.65*	162.37 ± 6.01	57.49 ± 6.25*	1411.43 ± 80.19*
IV. *Ficus religiosa* leaves extract (FRLE) 400mg/kg + DOX	14.47±2.39**	95 ±11.63**	52.77 ± 10.55*	846.34 ± 99.94**

N=6, Values are expressed as Mean ± SEM and analyzed by One way ANOVA test followed by Dunettes post hoc test, where, *P<0.05, **P<0.01 in comparison with ISO only group, #P<0.05, #P<0.01 in comparison with healthy control group.

Table 3. Effect of *Ficus religiosa* leaves extract on Heart weight Index (HWI) of control & Doxorubicin (DOX)-induced oxidative stress and cardiotoxicity in rats

Group	HWI (mg/g)
I.Control	3.49 ± 0.039
II. Doxorubicin (DOX) (10mg/kg)	4.51 ± 0.08###
III. *Ficus religiosa* leaves extract (FRLE)200mg/kg+DOX	3.55 ± 0.006***
IV. *Ficus religiosa* leaves extract (FRLE)400mg/kg+DOX	2.76 ± 0.033***

N=6, Values are expressed as Mean ± SEM and analyzed by One way ANOVA test followed by Dunettes post hoc test, where, *P<0.05, **P<0.01 in comparison with ISO only group, #P<0.05, #P<0.01 in comparison with healthy control group.

Statistical Analysis: All the values are expressed as mean ± SEM. Statistical analysis is performed way analysis of variance (ANOVA) followed by Dunnett's tests. Pralues p: 0.05, p<0.01, p<0.001 are considered as statically significant.

RESULTS

Extraction

The extract obtained was 1.67 gm semi-solid and was green in color. The percentage yield of Ficus religiosa leaf extract was found to be 45%. The ethanolic extract of the leaf contained alkaloids, flavonoids, tannins, phenol, saponins, carbohydrates and glycosides as chemical constituents.

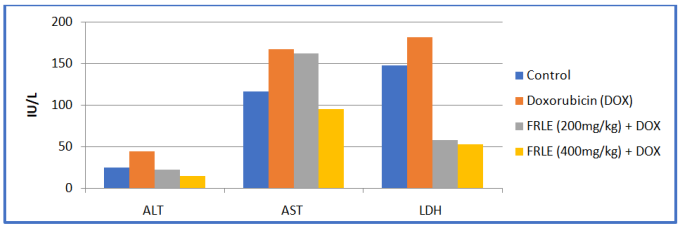

Figure 2. Effect of *Ficus religiosa* leaves extract on cardiac marker enzymes ALT, AST & LDH in serum control and Doxorubicin-induced oxidative stress and cardiotoxicity in rats

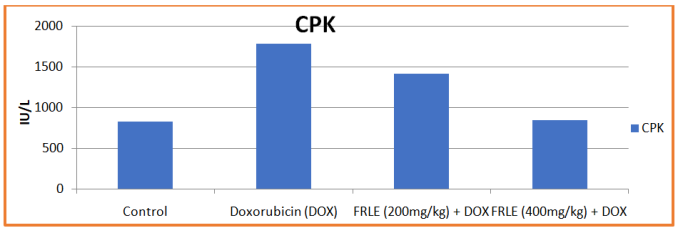

Figure 3. Effect of *Ficus religiosa* leaves extract on cardiac marker enzyme CPK in serum control and Doxorubicin-induced oxidative stress and cardiotoxicity in rats

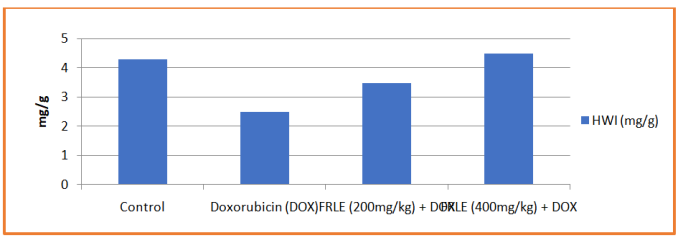

Figure 4. Effect of *Ficus religiosa* leaves extract on Heart weight index (HWI) of control & Doxorubicin-induced oxidative stress and cardiotoxicity in rats

Table 4. Effect of *Ficus religiosa* leaves extract on Heart architecture of control &Doxorubicin (DOX)-induced oxidative stress and cardiotoxicity in rats

Treatment	Inflammation	Myonecrosis	Edema
I. Control	-	-	-
II. Doxorubicin (DOX) (200mg/kg)	+++	+++	+++
III. *Ficus religiosa* leaves extract (FRLE) 200mg/kg + DOX	++	++	+
IV. *Ficus religiosa* leaves extract (FRLE) 400mg/kg + DOX	+	+	-

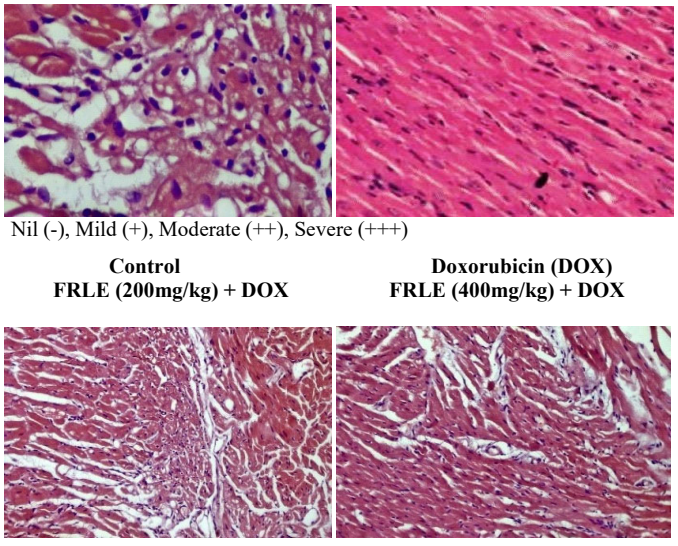

Figure 5. Histopathological findings of ISO-treated groups

DISCUSSION

One of the most prevalent signs of cardiovascular illness is myocardial infarction. Although the exact cause of sudden myocardial infarction is still unknown, research on cardio-toxicity generated by Doxorubicin offers valuable insight into this pathology and unequivocally demonstrates the role of oxidative stress. In the current investigation, we discovered that *Ficus religiosa* possesses a potent cardio-protective effect against myocardial necrosis in rats produced by doxorubicin.
The myocardium is rich in diagnostic marker enzymes for MI. When the myocardium was damaged metabolically, it released its intercellular contents into the extracellular fluid. As a result, changes in membrane integrity and/or permeability are reflected in the serum levels of these marker enzymes. When a

cell membrane ruptures or becomes permeable, cytosolic enzymes such as SGPT, LDH, AST, ALT, and CPK, which are used as diagnostic markers-leak into the bloodstream from the injured tissue. When rats were given doxorubicin (DOX) their serum CPK levels increased. In contrast, the bioactive fractions-pretreated rats had considerably lower serum CPK levels. Nicotinamide adenine dinucleotide (NAD) functions as a coenzyme in the easily reversible reaction that converts lactate to pyruvate, which is catalyzed by the intracellular enzyme lactate dehydrogenase (LDH). Within 24 to 48 hours following a heart attack, this clinically relevant enzyme rises and peaks in the blood in two to three days. In the current investigation, we saw a considerable increase in the LDH levels of rats treated with DOX 48 or 72 hours after the respective therapy, which is consistent with the aforementioned clinical results. The high level of LDH, which indicates a decrease in the severity of MI, was dramatically lowered by the pretreatment with *Ficus religiosa* leaf extract [13]. Although many different mechanisms have been proposed, including oxidative stress, mitochondrial DNA damage, intracellular calcium overload, inhibition of protein synthesis, disruption of myocardial adrenergic function, cytokine release, myofibrillar degeneration, and cardiomyocyte apoptosis, the precise pathogenesis of DOX-induced cardiotoxicity remains unclear. It is generally acknowledged that, of the various mechanisms, the primary cause of DOX-induced cardiac myocyte death is the production of reactive oxygen species in the myocardium, which sets off an intrinsic mitochondria-dependent apoptotic pathway in cardiac myocytes. In this study, we found a significant increase in the activities of cardiac markers LDH, AST, ALT, and CPK-MB in serum of DOX-induced cardiotoxicity. This increase may be due to enhanced susceptibility of the myocardial cell membrane to the DOX-mediated peroxidative damage, which results in increased release of these diagnostic marker enzymes into the systematic circulation. Therefore, it might safeguard membrane integrity by limiting the enzyme's ability to leak.

Conclusion

The results of this investigation showed that ethanolic extracts of Ficus religiosa leaf albino rats against the cardiotoxicity caused by doxorubicin. The larger dose of the two demonstrated the highest protective effect. As a result, the study has produced experimental proof that the Ficus religiosa leaf extract preserved the level of antioxidant enzymes, enhanced heart function, and reduced histological alterations. These results may provide scientific validation for the positive effects of leaf extract from Ficus religiosa as a cardioprotective agent. Based on the aforementioned research, we may infer that Ficus religiosa possesses cardioprotective activities. Before the Ficus religiosa leaf extract utilized in our study is employed in therapy, more clinical research should be done to validate the results and look into any potential mechanisms of action.

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