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

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ABSTRACT

Objective: The current study aims to assess the potential cardioprotective efficacy of *Ficus religiosa* leaves extract in rats.

Methods: In this investigation, one rat model of cardiotoxicity caused by isoproterenol (200 mg/kg, S.C.) was used to assess the cardioprotective efficacy of 70% ethanolic leaves 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.

Result: *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 ISO (isoproterenol) 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. In accordance with the estimation of the biomarker enzymes, the histopathology studies were also completed.

Conclusion: 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, isoproterenol, biomarkers, histopathological studies

INTRODUCTION

The prevalence of cardiovascular diseases (CVD) in adults (20 years and older) is 48%, with risk rising with age in both sexes. Adults are at increased risk for CVD **[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) estimates 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 (Peepal Plant) of Genus Ficus, Family Moraceae is used in India as a religious tree for Hindus and medicinal purposes. It is commonly originated in south Asian countries such as India, Nepal, Bhutan, Srilanka, Bangladesh, Iran, china, and Pakistan, Furthermore grown in sub-Himalayan and tropical areas **[4, 5]**. In the past, it was found that Homeopathy, Ayurvrda and Unani had utilized *Ficus religiosa* in the preparation of medicine **[6]**, used for the treatment of variety of diseases like diabetes, bronchitis, asthma, stomach ulcer, sexual dysfunction, skin and central nervous diseases **[7]**. Pharmacological activities of *Ficus religiosa* leaves extract includes; antimicrobial, anti-parasitic, anti-parkinson's anticonvusant, anti-amnesic, anticholinergic, anti-inflammatory, analgesic, hypoglycemic, hypotensive, hepatoprotective, nephroprotective, wound-healing, antiulcer, anti-asthmatic, and reproductive activity **[4, 8]**. *Ficus religiosa* leaves extract contain phytochemicals such as; flavonoids, phytosterols, tannins, phenols, saponins, sugars, alkaloids, terpenoids, glycosides, proteins, essentials and volatile oils **[9, 10]**.

According to reports, Isoproterenol (ISO) produce cardiotoxicity by formation of myocardial infarction, necrosis, increase in oxidative stress and lipid per-oxidation, decreases in antioxidant enzymes and an oxygen delivery as well as activation of phospholipase, ATPase, NF-B and mitogen-activated protein kinases (MAPKs) enzymes. All of these events combine to produce the recognizable signs of ISO-induced toxicity, which, if they persist over time, may lead to cardiac fibrosis **[11, 12]**.

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 the substances which chemically react with

free radicals and render them harmless and at the same time break the viscous circle, which involve in the decomposition of fatty acids and proteins, the creation of new free radicals and leads to eventual cell death. The antioxidant defense system includes both endogenously and exogenously derived compounds, dietary plant-based antioxidant have recently received a great attention. Hence many studies have been performed to identify antioxidant compounds with pharmacological activity and a limited toxicity for medicinal plants **[13]**. 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 **[14]**.

However, for several reasons, complimentary medicine has grown in popularity in recent years. Dietary measures and traditional plant therapies as prescribed by Ayurvedic, and other indigenous systems of medicine are commonly used in India. Many indigenous Indian medicinal plants have been found to be useful to successfully manage cardio and some of them have been tested and their active ingredients isolated. The World Health Organization (WHO) has also recommended evaluation of the plants for medical importance.

There are limited scientific reports on the cardioprotective activity of the leaves extract of *Ficus religiosa*. Previous studies have only revealed that evaluation of cardioprotective activity has done for other parts of *Ficus religiosa* but not with the leaves and it also contains good amount of flavonoids, terpenoids, alkaloids, phenol, saponins, carbohydrates, saponins and chlorophylls. So, I intended to carry out cardioprotective activity of Ethanolic leaves extract of *Ficus religiosa* against isoproterenol and doxorubicin induced toxicities.

1. MATERIALS AND METHODS

2.1 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 Mrs. Norunnisa Begum, curator from heading center for herbal gardens of the university of Trans disciplinary health science and technology, Karnataka state private university, Bengaluru, Karnataka. By rinsing with water, dirt and debris were eliminated. Before extraction, the leaves were crushed to powder and dried in the shade. For future use, the powder was kept in an airtight container and utilised to make ethanolic extract.

2.2 Drugs and Chemicals

Ethanol, Isoproterenol (ISO), Doxorubicin (DOX), Formalin, Ether, Sodium hydroxide, HCL etc. (MCP, Laboratory)

2.3 Experimental animals

Male albino wistar rats (100-120g±10 g) were used for this study. The animals were acclimatized in 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 Rajhiv Gandhi University of Health Sciences, Jayanagar, Bengaluru, Karnataka-560041.

2.4 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. Continuous hot Soxhlet extraction technique was carried out and completely evapourated to dryness **[15]**. The percentage yield was calculated as follows;

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

Figure 1: Soxhlet Extraction

2.5 Determination of Acute Toxicity (LD_{50}):

The acute toxicity test was carried out as stated by the OECD guidelines **[16]**. 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 mice were kept under observation for 24 h for behavioral changes (restlessness, dullness, and agitation) with signs of toxicity and mortality **[17]**. Hence $1/10^{th}$ of no lethal dose was taken as effective dose (500mg/kg

body weight) for the ethanolic extract of leaves of *Ficus religiosa* in evalution of cardioprotective potential in rats.

2.6 Isoproterenol induced cardiotoxicity:

Male albino wistar rats (150-200g) were used to evaluate the cardioprotective activity. Rats were treated with *Ficus religiosa* leaves extract daily for 28 days. On 28th day, myocardial injury was induced in experimental rats by injection of Isoproterenol (ISO) (200 mg/kg, S.C.) twice at an interval of 24 hr. (i.e. on 28th and 29th day of *Ficus religiosa* leaves extract treatment), while normal control and drug control rats were administration an equivalent volume of the vehicle **[18, 19]**. The experimental rats were divided into 4 groups of 6 animals each and treated as follows **(Table 1)**:

Table 1: Effect of *Ficus religiosa* leaves extract against Isoproterenol induced cardiotoxicity.

Sl. No.	Group	Treatment	Duration of treatment
1.	Normal animals	Normal saline	Daily for 28 days
2.	Positive control	ISO (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.) + ISO (200mg/kg, s.c.)	Daily for 28 days + 28th & 29th day

2.6.1 Biochemical analysis: After 24-hour treatment period on 30th day blood was collected from retro-orbitalplexus, serum was separated by centrifuging 10000 rpm for 15min. The Separated liquid was subjected for biochemical estimation of cardiac marker enzymes;

- LDH (Lactate Dehydrogenase)
- ALT (Alanine Aminotransferase)
- AST (Aspartate Aminotransferase)
- CPK (Creatine Phosphokinase)

2.6.2 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;

$$\text{Heart weight index (HWI)} = \frac{\text{Heart weight (mg)}}{\textit{Body weight (g)}}$$

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

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

2.7 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.

2. RESULTS

2.1 Cardioprotective activity of *Ficus religiosa* leaves against ISO-induced Cardiotoxicity:

Three cardiac marker enzymes were measured in albino wistar rat serum to assess the heart activity, and the results are shown in **Table 2 and Figure 2 & 3**. Animals exposed to isoproterenol for just two days were found to have higher levels of cardiac marker enzymes than the Control group. All of the heart's marker enzymes, including AST, ALT, LDH, and CPK have been found to be high. The levels of AST (from 25.21 to 72 IU/L), ALT (from 118 to 165 IU/L), LDH (from 149 to 172.34 IU/L) and CPK (from 825.76 to 1721), had all increased. Surprisingly, biomarker considerably increased in the group of rats given 200 mg/kg for 28 days, followed by isoproterenol for 2 days, compared to the control. Although ISO was employed to reduce cardiotoxicity in a group of two rats, the level of biomarkers was shown to be lower. Animals given a 400mg/kg leaf extract of *Ficus religiosa* for 28 days, followed by 2 days of ISO, had considerably lower levels of the biomarkers. Additionally, an increase in the Heart Weight Index (HWI) suggests cardiac damage. In the study, as shown in **Table 3 and Figure 4**, the HWI of the animal group that received only isoproterenol treatment shows a considerable increase, whereas the animal group that received a low dose of the extract also experiences an increase in HWI but at a lower rate than Group II does. The animals in Group IV that received a greater dose of the *Ficus religiosa* leaf extract also exhibit a slight increase in HWI, while it is still below that of the Group III animals and close to that of the Normal group. Additional histopathological examinations of the animals, as depicted in **Figure 5 and Table 4**, showed evidence of cardiac injury and the impact of an extract from *Ficus religiosa* leaves on that injury. The animals in Group II have severe inflammation, myonecrosis, and edema while Group I, the healthy control group, displays no signs of any heart muscle damage. The group of rats who received the *Ficus religiosa* leaf extracts exhibit relatively modest levels of inflammation, necrosis, and edema, with only very minor edema. The extract has a noticeable impact on Group IV animals since there is less necrosis, inflammation, and edema. As a result, when compared to the ISO alone treated group, this group exhibits very less heart harm caused by ISO. Consequently, the Extract of *Ficus religiosa*

leaves was discovered to be cardioprotective in the albino wistar rats based on the aforementioned reported parameters and histological examinations.

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

Group	ALT (IU/L)	AST (IU/L)	LDH (IU/L)	CPK (IU/L)
I. Control	25.21 ± 2.26	118 ± 6.81	149 ± 5.18	825.76 ± 94.27
II. Isoproterenol (ISO) (200mg/kg)	72 ± 8.94$^{\#\#}$	165 ± 9.12$^{\#\#}$	172.34 ±5.41$^{\#}$	1721 ± 51.66$^{\#\#}$
III. *Ficus religiosa* leaves extract (FRLE) 200mg/kg + ISO	33.67 ± 5.24*	133 ± 6.67*	143 ± 5.11**	1224 ± 242.1
IV. *Ficus religiosa* leaves extract (FRLE) 400mg/kg + ISO	21 ± 4.16**	96.67 ±8.01**	136.29±5.53**	742.34 ±102.24**

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.

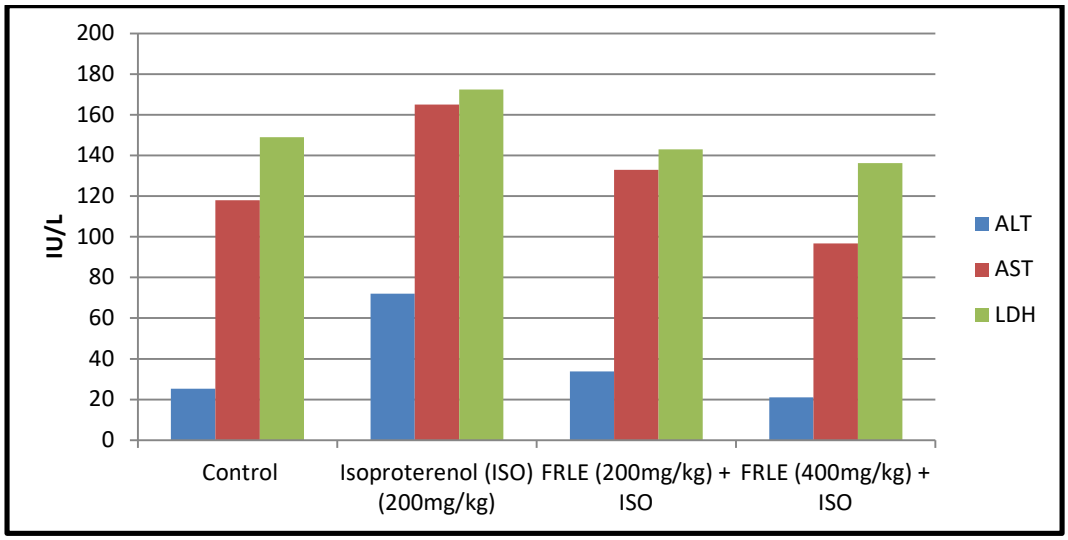


Figure 2: Effect of *Ficus religiosa* leaves extract on cardiac marker enzymes ALT, AST & LDH in serum control and Isoproterenol induced oxidative stress and cardiotoxicity in rats

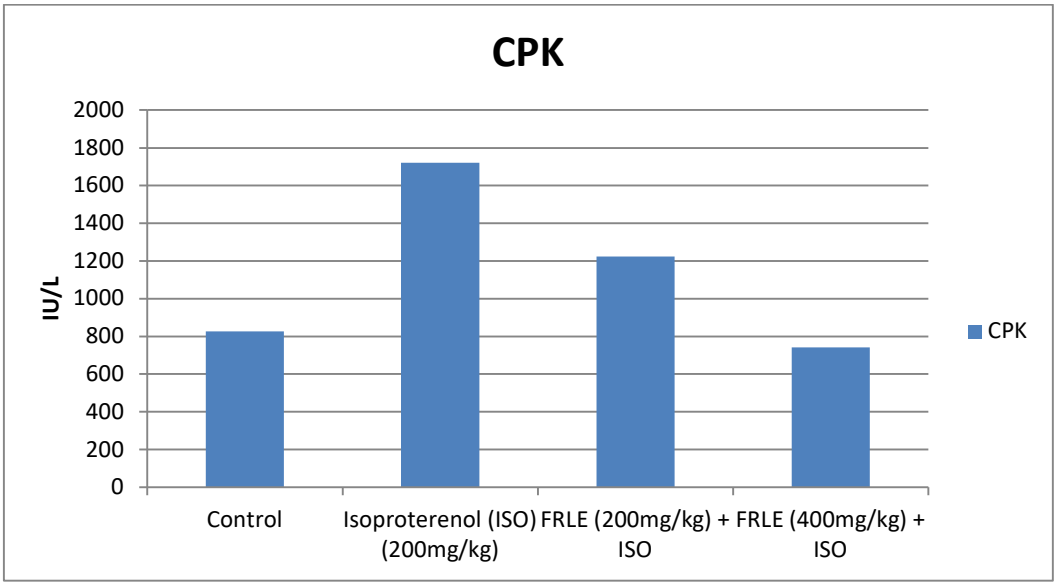


Figure 3: Effect of *Ficus religiosa* leaves extract on cardiac marker enzyme CPK in serum control and Isoproterenol induced oxidative stress and cardiotoxicity in rats

Table 3: Effect of *Ficus religiosa* leaves extract on Heart weight Index (HWI) of control & isoproterenol (ISO) induced oxidative stress and cardiotoxicity in rats

Group	HWI (mg/g)
I. Control	3.17 ± 0.09
II. Isoproterenol (ISO) (200mg/kg)	4.52 ± 0.04$^{\#\#\#}$
III. *Ficus religiosa* leaves extract (FRLE) 200mg/kg + ISO	3.71 ± 0.04**
IV. *Ficus religiosa* leaves extract (FRLE) 400mg/kg + ISO	2.44 ± 0.17**

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

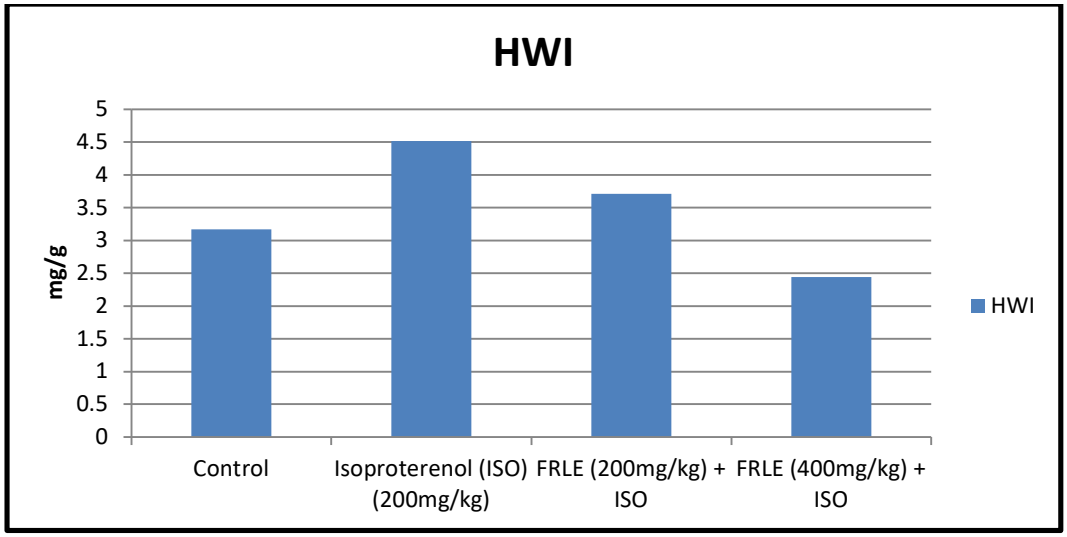
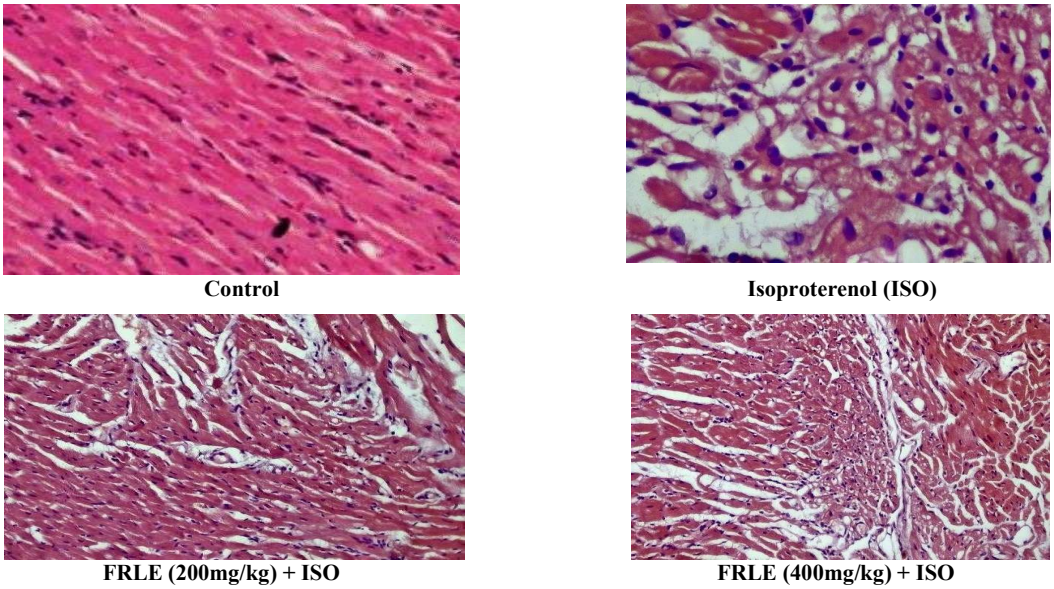


Figure 4: Effect of *Ficus religiosa* leaves extract on Heart weight index (HWI) of control & Isoproterenol induced oxidative stress and cardiotoxicity in rats

Table 4: Effect of *Ficus religiosa* leaves extract on Heart architecture of control &isoproterenol (ISO) induced oxidative stress and cardiotoxicity in rats

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

Nil (-), Mild (+), Moderate (++), Severe (+++)



Control

Isoproterenol (ISO)

FRLE (200mg/kg) + ISO

FRLE (400mg/kg) + ISO

Figure 5: Histopathological findings of ISO treated groups

DISCUSSION

After extraction, it was discovered that the percentage yield of the *Ficus religiosa* leaf extract was 45%. The chemical components of the leaves ethanolic extract included flavonoids, alkaloids, tannins, phenols, saponins, glycosides, and carbohydrates. These findings concur with those reported in a typical research publication **[20]**.

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 isoproterenol 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 isoproterenol.

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. The measurement of CPK activity in serum is a crucial diagnostic procedure because to the significant concentration of this enzyme in cardiac tissue, it's near complete absence from other tissues, and its resulting sensitivity. For any kind of myocardial damage, CPK isoenzyme activity is helpful as a gauge for early MI diagnosis as well. When rats were given isoproterenol (ISO) 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 ISO 48 or 72 hours after the respective therapy, which is consistent with the aforesaid 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. The widely used standard model for increasing the potency of medications operating on the cardiovascular system is ISO-Induced Myocardial Infarction. At larger doses, ISO, a synthetic catecholamine and beta-adrenergic agonist, has been shown to cause significant stress in the

myocardium, which results in infarct-like necrosis of the heart muscle. ISO has been documented to cause myocardial infarction. It has been revealed that myocardial infarction generated by ISO exhibits numerous metabolic and morphologic abnormalities in the experimental animal's heart tissue, which are comparable to those seen in myocardial infarction in humans. The interventricular septum and the left ventricle subendocardial area experience the greatest levels of ISO-induced necrosis.

Isoproterenol (ISP) causes oxidative stress in cardiac tissue, which depletes antioxidant enzymes and causes toxicological alterations. ISP also causes a deficiency in the oxygen supply, which causes hypoxia in the heart and subsequently cardiac necrosis. Elevated peroxidation of lipids leads to cellular leaking and stimulates hypertrophy of the heart. When phospholipase is activated, there is a significant increase in inflammation and direct ST-segment elevation, which can lead to myocardial ischemia and severe heart damage. Furthermore, activation of several signaling pathways such as NFκB and mitogen activated protein kinases (MAPK), enhances cellular death. Collectively, these occurrences constitute the characteristic traits of ISO-induced toxicity, which, if prolonged, may result in cardiac fibrosis **[21]**.

One could argue that among the various theories put forth to explain the myocardial damage caused by isoproterenol is an imbalance between the internal supply and demand of oxygen for cardiomyocytes. This imbalance is linked to myocardial hyperfunction because it increases both chronotropism and inotropism and causes hypotension in the coronary bed. Furthermore, it is asserted that there is an increase in Ca^{++} overcharge within the cell. Furthermore, during the course of the events, that ion is connected to the adenylate cyclase enzyme's activation and the depletion of ATP levels. In the end, there is an increase in oxidative stress due to the production of free radicals and various metabolic products derived from isoproterenol **[22]**.

Cardiac indicators from heart tissue detected in blood, such as ALT, AST, LDH, CPK, TG, and TC protein. Measuring the quantity of non-functional plasma enzymes in a tissue or organ can help diagnose disorders related to those organs. All of the reports and studies mentioned in the results show that *Ficus religiosa* leaf extract decreases the release of these marker enzymes when compared with the ISO groups.

In this study, we found a significant increase in the activities of cardiac markers LDH, AST, ALT, and CPK-MB in serum of ISO induced cardiotoxicity. This increase may be due to enhanced susceptibility of myocardial

cell membrane to the ISO 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 enzymes ability to leak.

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