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## Cardioprotective effect of *Ficus religiosa* on isoprenaline induced myocardial infarction in Wistar rats

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### ABSTRACT

In Indian traditional medicine, *Ficus religiosa* (*F. religiosa*) possesses numerous therapeutic properties, including its use in treating heart problems. This study was designed to elucidate its effects on the heart, with the objective of assessing the cardioprotective effect of *F. religiosa* (FR) in isoprenaline (ISO)-induced myocardial infarction in Wistar rats. Thirty male wistar rats (200-250 g) were equally divided into five groups, group I (control), group II (ISO 150 mg/kg BW on 27th and 28th day at 24 h interval), group III (FR 200 mg/kg BW), group IV (FR 100 mg/kg BW + ISO 150 mg/kg BW on 27th and 28th day at 24 h interval) and group V (FR 200 mg/kg BW + ISO 150 mg/kg BW on 27th and 28th day at 24 h interval). At the end of experiment ECG, HW/BW ratio, percent infarcted area, haematobiochemical estimations and microscopic examination of heart tissue were assessed. In positive control rats administration of ISO altered ECG indices viz. increase in heart rate, prolonged QT interval and ST segment whereas QRS complex was decreased. ISO caused significant rise in percent infarcted area of heart, HW/BW ratio and alterations in Hb, TEC, TLC, PCV, ALT, AST, total cholesterol (TC), triglycerides (TG), HDL, LDL, LDH, CK-MB and histopathological architecture of heart tissues in positive control rats. FR treated animals showed significant ( $p < 0.05$ ) minimal alterations in ECG, hematobiochemical parameters and histology of heart tissue. In conclusion the hydroethanolic extract of leaves of *F. religiosa* showed cardioprotective effect in ISO induced myocardial infarction. Thus, present findings may qualify *F. religiosa* as a potential cardioprotective agent.

**Keywords:** Isoprenaline, *Ficus religiosa*, Myocardial Infarction, Cardioprotective, Wistar rats.

### INTRODUCTION

Medicinal plants have played a vital role in improving and maintaining human health from the past thousands of years. *Ficus religiosa* (FR) belongs to *Moraceae* family, known as Pipal tree in India, gaining significant attention due to its beneficial effect on heart diseases. In Indian Traditional medicine it is reported that leaves of the plant soaked overnight in water, distilled and a consumption of this extract has the curative effect on the palpitation of heart diseases and the cardiac weakness [1, 2]. The literature reviewed indicates that FR has remarkable analgesic, antioxidant, anticonvulsant, antimicrobial, wound healing, anti-acetyl cholinesterase and proteolytic activities [3-5].

Myocardial infarction (MI) is a principal cause of death and the major public health concern throughout the world. Acute myocardial infarction is the most severe manifestation of coronary artery disease, which causes more than 2.4 million deaths in the USA, more than 4 million deaths in Europe and northern Asia, and more than a third of deaths in developed nations annually [6]. An acute myocardial infarction is characterized by the chest pain, sweating, weakness, vomiting, arrhythmia, loss of consciousness and sudden death. Several factors increase the risk of developing atherosclerosis and the heart attack which include an elevated level of low-density lipoprotein, triglycerides, reduced high density lipoprotein levels, increase blood cholesterol, high blood pressure, use of tobacco, diabetes mellitus, family history of coronary heart disease and change in lifestyle [7]. An increasing level of circulating catecholamine has been reported in the early phase of myocardial ischemia [8]. Animal model of induced myocardial infarction is highly useful tool to study the diagnostic, preventive and therapeutic approaches for human myocardial infarction. The rat model of isoprenaline induced myocardial infarction offers several advantages over the surgical model due to its effectiveness, practicability, reliable, non-invasive, reproducible and low mortality [9-11]. Experimental induction of myocardial infarction in Wistar rats by isoprenaline (ISO), a synthetic catecholamine and beta adrenoceptor agonist is associated with numerous pathological and biochemical changes that are believed to be comparable to that taking place in human myocardial damage [12-14].

Although having beneficial myocardial activities, protective effect of FR against heart failure or ischemic condition have not been properly studied as per the earlier literature. Therefore, the present study was designed to evaluate the cardioprotective potential of ethanolic extract of FR in ISO induced myocardial infarction in Wistar rats.

## MATERIALS AND METHODS

### Experimental Animals

Thirty healthy adult male Wistar rats (200-250g) were procured from CCSEA registered animal house of PGIVAS, Akola (M.S., India). The experimental animals were acclimatized for 5 days before the start of the experiment, under hygienic and standard management conditions in the Department of Veterinary Pharmacology and Toxicology of the Institute. All rats were housed in polypropylene cages (47×34×18 cm) under a 12-12 h dark-light cycle. The maximum number of animals in each cage was restricted to three. All animals were fed with standard rat chow procured from Nutrivet Life Sciences, Pune (M.S.), alongside unrestricted access to potable drinking water.

### Experimental study

**Table 1:** Different groups of animals, their treatments, doses and the duration of the experiment

| Groups | Treatment  | No. of animals | Duration of experiment |
|--------|--|----------------|------------------------|
| I      | Control – Distilled water p.o. for 4 weeks   | 6              | 4 weeks                |
| II     | Positive control - Isoprenaline (ISO)150 mg/kg i.p. on 27 <sup>th</sup> and 28 <sup>th</sup> day at 24 h interval            | 6              | 4 weeks                |
| III    | <i>Ficus religiosa</i> 200 mg/kg /day, p.o. 4 weeks  | 6              | 4 weeks                |
| IV     | FR 100 mg/kg/ day, p.o. from day 1 to 28 <sup>th</sup> day + ISO 150 mg/kg i.p. on 27 <sup>th</sup> and 28 <sup>th</sup> day | 6              | 4 weeks                |
| V      | FR 200 mg/kg/day, p.o. from day 1 to 28 <sup>th</sup> day + ISO 150 mg/kg i.p. on 27 <sup>th</sup> and 28 <sup>th</sup> day  | 6              | 4 weeks                |

### Experimental reagents

ISO was procured from Sigma Aldrich, Co. St. Louis, Mo, USA. All remaining drugs and chemicals utilized in the study were of analytical grade quality. ISO stock solution was prepared in sterile normal saline solution (100mg/ml) and required concentration of ISO prepared a fresh before administration to animals. Hydro-ethanolic extract of plant FR was dissolved in double distilled water to make a suspension of required concentration. FR extract of exact dilution was prepared a fresh every day before administration to animals.

### Extraction of plant material

Fresh leaves of FR were collected from the campus of the Post Graduate Institute of Veterinary and Animal Sciences, Akola and identified by Prof. Dr. S. P. Rothe, expert taxonomist, Principal Meherbanu, Science Collage, Akola (M.S.) and the voucher specimen No. 11 (VPT/VS/11/2020) has been deposited in the departmental herbarium. The leaves were collected, washed with clean water, dried, powdered and FR powder (100g) was soaked in 500ml of solvent solution (60% ethanol and 40% distilled water) and was kept in an orbital shaker for 24 h at 110 rpm at room temperature. Then the extract was filtered through Whatman's filter paper no.1. The filtrate obtained was air-dried and concentrated to semisolid mass. The extract thus obtained was further used in the experiment.

### Phytochemical Analysis

For different groups of phytoconstituents, qualitative phytochemical analysis was done for the presences of alkaloids, flavonoids, saponins, tannins, sterols, carbohydrates and glycosides<sup>[15]</sup>.

### Induction of myocardial infarction

To induce myocardial infarction, a freshly prepared stock solution of ISO was used, dissolved in sterile normal saline (0.9%). Rats injected with ISO @ 150 mg/kg for two consecutive days (27<sup>th</sup> and 28<sup>th</sup>) via intraperitoneal route at 24 h interval. Animals were sacrificed post 24 h of the last dose of ISO. Before sacrificing the rats, the live body weights were taken. After blood collection, animals were sacrificed using thiopental sodium and heart was removed carefully and the dry weight of heart was recorded. The heart weight to body weight ratio was calculated as a number of grams of heart tissues for every kilogram of body weight.

### Measurement of infarcted area of heart tissue

The infarcted areas of heart tissue were estimated by using TTC (2, 3, 5 Tri-phenyl Tetrazolium Chloride) staining. Heart tissues were snap frozen in liquid nitrogen for 1-2 h and sliced transversely into 2 mm thickness using a sharp razor. These slices were incubated in 1% TTC solution for one hour at 37°C. Excess stains were washed off using continuous rinsing in a normal saline solution. Infarcted areas were detected as white, unstained areas, which were measured with image J software (NIH, Maryland, USA). Normal live area and infarcted area were compared and expressed as percent infarcted area<sup>[16]</sup>.

### Haemato-biochemical estimates

Hematological parameters, including hemoglobin (Hb), total erythrocyte count (TEC), total leukocyte count (TLC), differential leukocyte count (DLC), and packed cell volume (PCV), were measured. In serum biochemical parameters, ALT, AST and serum lipid parameters such as, total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) and low-density lipoprotein (LDL) were estimated using commercial kits of AGD chemicals on Semi-Autoanalyzer (AGD biochemical analyzer, India). Myocardial enzymes, Lactate Dehydrogenase (LDH) and Creatine Kinase-myocardial band (CK-MB) in serum and heart tissue homogenate were estimated separately using modified UV kinetic kit method on Semi-Autoanalyzer. The extent of lipid peroxidation was estimated in heart tissue homogenate in the form of malonaldehyde (MDA) production and was determined by method as described by Shafiq-ur-Rehman<sup>[17]</sup>. Heart tissue homogenate was prepared by using phosphate buffer (PBS) solution, having pH 7.4 under cold condition.

### Electrocardiography (ECG)

Rats from the different groups were anesthetized using diethyl ether and kept in a prone position, and the ECG electrodes were placed behind the elbow in the forelimb and at the front of stifle in the hind limb and ECG were recorded at speed of 50mm/sec and amplitude of 20mV, using a single channel Cardiar 108T-DiGi ECG machine (BPL, India). Electrocardiographic parameters such as ST segment, QRS complex, QT interval, R-R interval and heart rate were recorded.

### Histopathological examination

Heart tissues from various groups were collected and preserved in a 10% formalin solution for histopathological examination. Fixation and impregnation in paraffin wax (Qualigen) as per routine method and sections of 5-6µm were cut and stained with H & E stain<sup>[18]</sup> for recording histopathological observations.

### Statistical analysis

The mean value from different groups were analyzed by using one-way analysis variation (ANOVA) followed by Tukey's post hoc tests in IBM SPSS Statistics, Version 22 Application/ Software (2013). Data were expressed as mean ± standard error and significance was observed at 5% level.

## RESULTS

### Extractability and phytochemical analysis

The percent extractability of hydroethanolic extract of leaves of plant FR powder was found to be 9.13%. The extract was found to be black-brown in color, bitter in taste and pleasant in odor. In phytochemical analysis of FR leaves substantiated the existence of tannins, phenols, alkaloids, terpenoids, flavonoids and glycosides.

### General preclinical observations

ISO treated animals of group II, showed symptoms like dullness, dyspnoea, reduced feed and water intake. Group IV and V rats pretreated with FR showed mild preclinical toxic symptoms like dullness and dyspnoea. Group III rats treated with only FR showed normal behavioural pattern. Any mortality was not observed across the groups.

### HW/BW Ratio and percent infarcted area

HW/BW ratio was significantly increased in ISO treated animals. FR treated animals showed significantly ( $P < 0.05$ ) improved HW/BW ratio as compared with ISO treated animals. Myocardial infarcted area was significantly developed in ISO treated animals as compared to normal control. Percentage of infarcted area in FR treated animals (group V) significantly ( $P < 0.05$ ) decreased as compared with ISO treated animals (Table 2).

### Haematological findings

In ISO administered rats, significant ( $P < 0.05$ ) decrease was observed in Hb, PCV, TEC and lymphocytes count, whereas significant ( $P < 0.05$ ) increase was observed in TLC and granulocyte count as compared to control group rats. In FR administered rats (group V), hemoglobin, total leucocytes count and granulocytes values showed significant ( $P < 0.05$ ) lesser alterations as compared to ISO treated group II rats. These hematological values in the FR group indicating restoration towards normalcy (Table 3).

### Serum lipid profile and biochemical parameters

Total cholesterol, LDL and TG levels were significantly ( $p < 0.05$ ) increased, whereas HDL level was significantly ( $p < 0.05$ ) decreased in positive control group as compared to normal control. In FR treatment group V, TC, LDL, TG and HDL levels were significant ( $P < 0.05$ ) ameliorated as compared with ISO treated rats (Table 4). The ALT

and AST values were significantly ( $P < 0.05$ ) increased in ISO treated group. Whereas, FR treated group rats, showed significant ( $P < 0.05$ ) retrieval in ALT and AST as compared to ISO treated rats (Table 4).

### Myocardial enzymes

LDH and CK-MB in blood serum increased significantly ( $P < 0.05$ ) in ISO treated group II. In group IV and V FR significantly ( $P < 0.05$ ) protected the alterations in serum LDH and CK-MB levels. LDH and CK-MB in heart tissue homogenate significantly ( $P < 0.05$ ) decreased in ISO treated rats but these enzymes showed significant ( $P < 0.05$ ) improvement in FR treated group V rats as compared to ISO group indicating protective effect of *F. religiosa* (Table 5).

### Lipid peroxidation assay

The cardiac lipid peroxidation in ISO treated group II was significantly ( $P < 0.05$ ) increased. Animals pretreated with FR in group IV and V showed significant ( $P < 0.05$ ) improvement in lipid peroxidation level as compared to group II. Thus, the restorative values in lipid peroxidation assay in group IV and V showed the protective role of FR in myocardial infarction (Table 5).

### Electrocardiography

The heart rate, QT interval and ST segment in ISO treated group II rats were significantly ( $P < 0.05$ ) increased as compared to the normal control group. Whereas, FR treated group V showed significant ( $P < 0.05$ ) lesser alterations in these cardiac parameters when compared to ISO treated rats. The QRS complex in ISO treated group II was significantly ( $P < 0.05$ ) decreased but in group V animals significant ( $P < 0.05$ ) improvement was observed (Table 6; Figure 1).

### Histopathological Examination

In histopathological examination, in the control and FR alone group, the normal histoarchitecture of cardiac muscle fibers and heart parenchyma was observed. Whereas, group II rats given ISO alone showed myocardial infarction, mononuclear cell infiltration, myocardial degeneration and necrosis of cardiac muscle fibers. The FR treated rats with 100mg/kg BW showed mild to moderate degeneration and necrosis of myocardial fibers along with mononuclear cell infiltration, mild separation of muscle cell fiber and vacuolation. FR treated with 200mg/kg BW showed mild degenerative changes along with focal necrosis of muscle fibers and comparatively normal heart parenchyma (Figure 2).

**Table 2:** Heart weight to body weight ratio and percent infarcted area in different groups

| Group | Treatment           | HW/BW ratio               | Infarcted area (%)        |
|-------|---------------------|---------------------------|---------------------------|
| I     | Control             | 3.34 ± 0.18 <sup>c</sup>  | 0.16 ± 0.01 <sup>d</sup>  |
| II    | ISO                 | 4.56 ± 0.23 <sup>a</sup>  | 50.53 ± 0.25 <sup>a</sup> |
| III   | <i>F. religiosa</i> | 3.20 ± 0.07 <sup>c</sup>  | 0.16 ± 0.01 <sup>d</sup>  |
| IV    | FR - 100 + ISO      | 4.19 ± 0.22 <sup>ab</sup> | 25.25 ± 0.24 <sup>b</sup> |
| V     | FR - 200 + ISO      | 3.87 ± 0.11 <sup>b</sup>  | 20.57 ± 0.31 <sup>c</sup> |

Values indicated as mean ± SEM. Mean values of heart weight to body weight were expressed as heart weight per kg of body weight. Data were analysed using one way ANOVA followed by Turkey's HSD test. Values with different alphabet as superscript are differ significantly ( $P < 0.05$ ,  $n = 6$ ).

**Table 3:** Heamatological parameters in different groups

| Groups | Treatment           | Hemoglobin (g/dl)         | PCV (%)                   | TEC (10 <sup>6</sup> /cumm) | TLC (10 <sup>3</sup> /cumm) | Lymphocytes (%)           | Monocytes (%)             | Granulocytes (%)           |
|--------|---------------------|---------------------------|---------------------------|-----------------------------|-----------------------------|---------------------------|---------------------------|----------------------------|
| I      | Control             | 13.22 ± 0.39 <sup>a</sup> | 40.17 ± 1.20 <sup>a</sup> | 7.86 ± 0.20 <sup>a</sup>    | 11.78 ± 0.27 <sup>c</sup>   | 71.32 ± 0.60 <sup>a</sup> | 4.05 ± 0.1 <sup>a</sup>   | 30.15 ± 0.86 <sup>c</sup>  |
| II     | ISO                 | 10.95 ± 0.30 <sup>c</sup> | 33.33 ± 0.96 <sup>b</sup> | 6.46 ± 0.19 <sup>c</sup>    | 16.28 ± 0.30 <sup>a</sup>   | 58.15 ± 0.78 <sup>c</sup> | 3.775 ± 0.25 <sup>b</sup> | 40.23 ± 0.55 <sup>a</sup>  |
| III    | <i>F. religiosa</i> | 13.20 ± 0.19 <sup>a</sup> | 39.33 ± 0.80 <sup>a</sup> | 7.60 ± 0.13 <sup>a</sup>    | 11.043 ± 0.27 <sup>d</sup>  | 70.35 ± 0.24 <sup>a</sup> | 4.58 ± 0.17 <sup>b</sup>  | 30.97 ± 0.70 <sup>c</sup>  |
| IV     | FR -100+ ISO        | 11.22 ± 0.19 <sup>c</sup> | 33.83 ± 0.95 <sup>b</sup> | 6.87 ± 0.15 <sup>bc</sup>   | 14.87 ± 0.12 <sup>b</sup>   | 60.11 ± 0.35 <sup>b</sup> | 3.88 ± 0.09 <sup>b</sup>  | 39.15 ± 0.33 <sup>ab</sup> |
| V      | FR - 200 + ISO      | 12.18 ± 0.22 <sup>b</sup> | 34.50 ± 0.76 <sup>b</sup> | 7.07 ± 0.10 <sup>b</sup>    | 15.16 ± 0.19 <sup>b</sup>   | 61.46 ± 0.55 <sup>b</sup> | 3.99 ± 0.05 <sup>b</sup>  | 37.55 ± 0.58 <sup>b</sup>  |

Values indicate mean ± SEM. Data were analysed using one way ANOVA followed by Turkey's HSD test. Values with different alphabet as superscript are differ significantly (P< 0.05, n=6).

**Table 4:** Serum lipid profile and biochemical parameters in different groups

| Group | Treatment              | Total cholesterol (mg/dl)  | HDL (mg/dl)                | LDL (mg/dl)               | Triglyceride (mg/dl)       | ALT (IU/L)                 | AST (IU/L)                 |
|-------|------------------------|----------------------------|----------------------------|---------------------------|----------------------------|----------------------------|----------------------------|
| I     | Control                | 70.97 ± 0.41 <sup>d</sup>  | 20.84 ± 0.44 <sup>a</sup>  | 40.64 ± 5.63 <sup>c</sup> | 40.80 ± 0.62 <sup>c</sup>  | 31.08 ± 0.73 <sup>d</sup>  | 56.19 ± 0.72 <sup>c</sup>  |
| II    | ISO                    | 110.25 ± 2.73 <sup>a</sup> | 14.84 ± 0.66 <sup>c</sup>  | 61.10 ± 7.14 <sup>a</sup> | 80.46 ± 0.57 <sup>a</sup>  | 82.09 ± 0.73 <sup>a</sup>  | 102.44 ± 1.61 <sup>a</sup> |
| III   | <i>Ficus religiosa</i> | 65.29 ± 1.49 <sup>e</sup>  | 21.15 ± 0.47 <sup>a</sup>  | 39.40 ± 5.20 <sup>c</sup> | 41.048 ± 0.74 <sup>c</sup> | 33.033 ± 0.84 <sup>d</sup> | 58.70 ± 0.69 <sup>c</sup>  |
| IV    | FR - 100 + ISO         | 100.72 ± 1.88 <sup>b</sup> | 16.23 ± 0.45 <sup>bc</sup> | 59.81 ± 4.75 <sup>a</sup> | 78.15 ± 0.71 <sup>b</sup>  | 76.39 ± 0.64 <sup>b</sup>  | 96.33 ± 0.63 <sup>b</sup>  |
| V     | FR - 200 + ISO         | 91.61 ± 1.43 <sup>c</sup>  | 17.64 ± 0.59 <sup>b</sup>  | 57.41 ± 5.46 <sup>b</sup> | 76.14 ± 0.82 <sup>b</sup>  | 72.07 ± 0.78 <sup>c</sup>  | 87.03 ± 0.73 <sup>d</sup>  |

Values indicate mean ± SEM. Data were analysed using one way ANOVA followed by Turkey's HSD test. Values with different alphabet as superscript are differ significantly (P< 0.05, n=6).

**Table 5:** Myocardial enzymes in different groups

| Group | Treatment              | CK-MB (IU/L)                 |                           | LDH (IU/L)                   |                             | LPO (nM MDA/mg protein)  |
|-------|------------------------|------------------------------|---------------------------|------------------------------|-----------------------------|--------------------------|
|       |                        | Serum                        | Heart homogenate          | Serum                        | Heart homogenate            |                          |
| I     | Control                | 292.36 ± 8.79 <sup>d</sup>   | 80.77 ± 0.93 <sup>a</sup> | 797.95 ± 5.39 <sup>d</sup>   | 405.68 ± 10.66 <sup>a</sup> | 0.81 ± 0.02 <sup>d</sup> |
| II    | ISO                    | 601.11 ± 18.278 <sup>a</sup> | 50.19 ± 0.64 <sup>d</sup> | 1889.19 ± 70.26 <sup>a</sup> | 248.49 ± 9.97 <sup>d</sup>  | 2.06 ± 0.06 <sup>a</sup> |
| III   | <i>Ficus religiosa</i> | 287.53 ± 8.18 <sup>d</sup>   | 81.13 ± 0.99 <sup>a</sup> | 784.36 ± 14.04 <sup>d</sup>  | 402.97 ± 6.05 <sup>a</sup>  | 0.97 ± 0.07 <sup>d</sup> |
| IV    | FR - 100 + ISO         | 499.54 ± 6.08 <sup>b</sup>   | 59.38 ± 1.15 <sup>c</sup> | 1588.94 ± 30.15 <sup>b</sup> | 292.68 ± 9.09 <sup>c</sup>  | 0.97 ± 0.07 <sup>b</sup> |
| V     | FR - 200 + ISO         | 404.94 ± 22.57 <sup>c</sup>  | 71.11 ± 1.01 <sup>b</sup> | 1234.18 ± 61944 <sup>c</sup> | 337.75 ± 13.26 <sup>b</sup> | 1.40 ± 0.08 <sup>c</sup> |

Values indicate mean ± SEM. Data were analysed using one way ANOVA followed by Turkey's HSD test. Values with different alphabet as superscript are differ significantly (P< 0.05, n=6).

**Table 6:** Electrocardiography in different groups

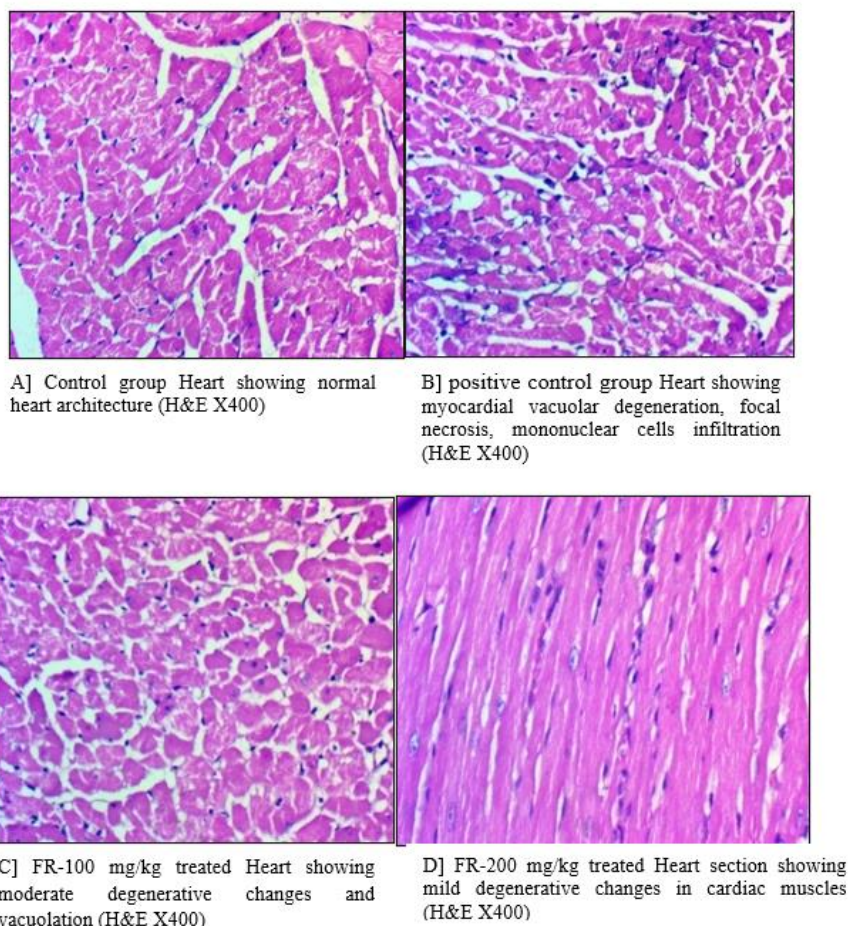
| Group | Treatment              | Heart rate (bpm)            | RR interval (sec)        | QRS complex (mV)          | QT interval (sec)          | ST segment (mv)            |
|-------|------------------------|-----------------------------|--------------------------|---------------------------|----------------------------|----------------------------|
| I     | Control                | 450.83 ± 17.44 <sup>c</sup> | 6.02 ± 0.16 <sup>a</sup> | 0.45 ± 0.009 <sup>a</sup> | 0.023 ± 0.002 <sup>c</sup> | 0.067 ± 0.004 <sup>c</sup> |
| II    | ISO                    | 589.17 ± 8.05 <sup>a</sup>  | 5.51 ± 0.10 <sup>b</sup> | 0.31 ± 0.008 <sup>d</sup> | 0.050 ± 0.001 <sup>a</sup> | 0.181 ± 0.005 <sup>a</sup> |
| III   | <i>Ficus religiosa</i> | 415.83 ± 19.29 <sup>c</sup> | 6.03 ± 0.23 <sup>a</sup> | 0.46 ± 0.015 <sup>a</sup> | 0.023 ± 0.002 <sup>c</sup> | 0.074 ± 0.004 <sup>c</sup> |
| IV    | FR - 100 + ISO         | 540.67 ± 16.39 <sup>b</sup> | 5.65 ± 0.12 <sup>a</sup> | 0.36 ± 0.007 <sup>c</sup> | 0.042 ± 0.039 <sup>b</sup> | 0.164 ± 0.003 <sup>b</sup> |
| VI    | FR - 200 + ISO         | 516.67 ± 16.39 <sup>b</sup> | 5.90 ± 0.14 <sup>a</sup> | 0.40 ± 0.014 <sup>b</sup> | 0.039 ± 0.002 <sup>b</sup> | 0.157 ± 0.005 <sup>b</sup> |

Values indicate mean ± SEM. Data were analysed using one way ANOVA followed by Turkey's HSD test. Values with different alphabet as superscript are differ significantly (P< 0.05, n=6).



**Figure 1:** Effect of *Ficus religiosa* on ECG examination

(A) Control showing normal electrocardiograph, (B) ISO showing increased QT and ST segment, (C) FR (100 mg/kg) showing moderate increased QT and ST segment in electrocardiograph. (D) FR (200 mg/kg) showing mild increased QT and ST segment in electrocardiograph



**Figure 2:** Effect of *Ficus religiosa* on histopathological examination

## DISCUSSION

The FR leaf extract was found to be safe upto 4000mg/kg doses and no toxicity or mortality was reported up to the dose of 4000mg/kg, when administered orally in rats [19]. Therefore, considering the toxicity study, the doses of FR selected as 100 and 200mg/kg in present study are highly safe in rats. In ISO treated rats, toxic symptoms like dullness, depression, dyspnoea, nervousness, reduced feed and water intake were observed. However, FR treated animals showed mild symptoms in comparison with the group II. Similar symptoms reported by Wexler<sup>[20]</sup> in spontaneously hypersensitive rats treated with ISO @50mg/100g revealed that within minutes of receiving isoprenaline, rats exhibited tachycardia, dyspnea, nervousness, reduced feed and water intake. The pre-treatment of FR in the present study showed minimal clinical symptoms in group IV and group V animals.

Increase in heart to body weight ratio in the present study might be due to infiltration of inflammatory cells in the damaged myocardial tissues, increased edematous intermuscular space and accumulation of water in intramuscular spaces. Increased HW/BW ratio is indicative of cardiac hypertrophy which allows ventricular remodeling process, causes infarct expansion as well as dilation of the non-infarcted left ventricle [21]. ISO @100mg/kg s/c at an interval of 24 h for two days caused cardiac hypertrophy due to excessive positive inotropic effect [22]. In the present study, oral pretreatment with FR @ 200mg/kg elucidate least alteration in heart weight to body weight ratio.

2-3 Triphenyl tetrazolium chloride (TTC) dye staining to the heart tissue slices is a highly accepted method to identify the necrosis of myocardial tissue<sup>[23]</sup>. TTC dye forms red formazan precipitate with available intact lactate dehydrogenase in the viable myocardial tissue, but the infarcted myocardial area fails to stain with TTC dye<sup>[24]</sup>. The area of necrosis is related to leakage of LDH enzymes and loss of

membrane integrity<sup>[22]</sup>. The findings of the present study showed white patches in the heart transverse section of staining with TTC dye due to necrosis of heart tissue indicating myocardial infarction. The findings of the present study align with earlier report of induced myocardial infarction in rats given ISO @100mg/kg s/c at an interval of 24 h for two days<sup>[22]</sup>. However, myocardial injury was significantly lesser in the group treated with FR @200mg/kg body weight.

ISO activates adenylyl cyclase, which mediates the lipolytic action by stimulating cAMP-dependent protein kinase (PKA) which phosphorylates hormone sensitive lipase, resulting in hydrolysis of the stored triacylglycerol and contribute to marked hyperlipidemia<sup>[25]</sup>. The reduction in the high-density lipoprotein and elevated values of total cholesterol, serum low density lipoprotein and triglycerides in ISO induced myocardial injury in the present study were found to be similar with the study conducted by Prince and Rajadurai<sup>[20]</sup> in ISO (@200mg/kg s/c) induced myocardial infarction in rats. The similar findings of serum lipid profile coincides with previous report of isoproterenol (100mg/kg) induced myocardial injury in rats<sup>[27]</sup>. An increase in total cholesterol, triglycerides and serum LDL results due to increase in lipid biosynthesis by cardiac cyclic adenosine monophosphate<sup>[28]</sup>. The increased levels of LDL, total cholesterol and low levels of HDL show a positive correlation with myocardial infarction. In ISO treated animals, the triglycerides levels increased significantly, which was due to an increase in synthesis or accumulation of acetyl CoA<sup>[25]</sup>. The moderate restoration of total cholesterol, high density lipoprotein, low density lipoprotein and triglycerides in treatment groups of FR revealed its protective effect in cardiac disease.

The increase in activity of ALT and AST enzymes in the serum in the present study could be due to the leakage of these enzymes from the heart as a result of ISO induced necrosis of heart tissue<sup>[29]</sup>. The elevated values of AST and ALT in ISO treated animals cause

necrotic damage to myocardial membrane. The elevated levels of these enzymes in plasma are a presumptive marker of the occurrence of necrotic lesions in the myocardial membrane<sup>[26,30]</sup>. In the findings of the present study, FR showed a protective effect against AST and ALT values. In a related study, Yadav et al. reported a protective role of FR@200 mg/kg b. wt. in doxorubin induced myocardial toxicity in rats<sup>[31]</sup>.

The leakage of CK-MB isoenzyme and LDH enzyme from the heart is the diagnostic marker of myocardial infarction. Therefore, the determination of CK-MB isoenzyme and LDH is a useful parameter for assessing myocardial damage<sup>[32]</sup>. The myocardial cells contain CK-MB isoenzyme and LDH which are damaged due to low oxygen supply or glucose, which leads to Ca<sup>+2</sup> overloading and free radicals production, which causes, loss of integrity of cell membranes and membrane becomes more porous and permeable resulting into leakage of these enzymes which act as markers of ischemic heart disease<sup>[33,34]</sup>.

The altered CK-MB isoenzyme and LDH in the present study are in agreement with the past study in ISO induced myocardial infarction<sup>[32]</sup>. Thus, FR offers a protective effect in myocardial damage and the similar effect of the protective role of FR has been reported in related previous study on alterations of these enzymes in doxorubin induced toxicity<sup>[31]</sup>.

The lipid peroxidation is an important pathological mechanism in myocardial necrosis and accumulation of lipid hydroperoxides causes cardiac damage<sup>[35]</sup>. The increased lipid peroxides in isoprenaline-induced myocardial necrosis occur due to the presence of free radicals mediated membrane damage<sup>[32]</sup>. Isoprenaline-generated free radicals initiate the peroxidation of membrane-bound polyunsaturated fatty acid, which leads to loss of structural and functional integrity of myocardium by changing membrane permeability<sup>[36]</sup>. The high vulnerability of myocardium to peroxidative damage caused due to free radicals and consequent reduction in the level of free radical scavengers<sup>[37]</sup>. *F. religiosa* leaves contain kaempferol, quercetin, myricetin, phenolics, flavonoides and other phytoconstituents. Kaempferol is an important phytoconstituent present in various extracts of *F. religiosa*, which is responsible for the scavenging activity of DPPH, hydroxyl, and oxygen free radicals and due to these actions *F. religiosa* considered as a potent candidate in treating free-radical-induced diseases particularly cardiovascular and inflammatory diseases<sup>[38,39]</sup>. The earlier report suggested that ethanolic extracts of FR possess highest antioxidant properties. It is also a well known fact that numerous flavonoids generated as secondary metabolites from various plants have demonstrated to scavenge free radicals. Thus, the data of this study and the related earlier reports indicated that FR alcoholic extract has remarkable protective effect in free radicals induced cardiac disease.

ISO caused alteration in ECG, leads to several physiological and functional changes in the heart<sup>[40]</sup>. ISO is a  $\beta$ -adrenergic agonist, it increases heart rate and further leads to cardiac dysfunction<sup>[41]</sup>. This is due to suppression of Ca<sup>2+</sup> transport, leads to intracellular Ca<sup>2+</sup> overload and disturbs sympathetic and parasympathetic input to the heart<sup>[42]</sup>. ISO induced myocardial injury in rat ECG is characterized by elevation of ST segment, prolongation of QT segment and attenuation of QRS complex. These changes in ECG may be due to damage to the integrity of myocardial cells. The ST segment elevation represents the ischemic and non-ischemic zones potential difference followed by loss of cell membrane function<sup>[42]</sup>. The QRS complex on an electrocardiogram delineates the cumulative duration of ventricular depolarization. Deviations in the QRS morphology signify potential abnormalities in myocardial function. The QT interval represents ventricular repolarization with inward sodium and calcium current and outward potassium and chloride currents<sup>[43]</sup>. The QT interval also represents the period of electric systole, which determines the functional integrity of the myocardium<sup>[40]</sup>. The similar findings in alteration of ECG were supported by the study conducted by Khanam et al.<sup>[42]</sup> reported alterations in ECG in isoproterenol @ 85mg/kg b. wt. s/c induced toxicity. Pretreatment with FR demonstrates a

protective effect against ISO-induced ST-segment elevation, indicative of its cell membrane-protecting properties. It also exhibits cardio protection by significantly restoring the QRS complex and QT interval.

The oxidative stress resulting from myocardial injury culminates in myocardial infarction. ISO causes decline in oxygen supply with increase in myocardial stress<sup>[32]</sup> which might be the reason for significant MI in group II. The similar histopathological changes in the heart like marked inflammatory signs like membrane damage and cellular infiltration along with focal myonecrosis were also reported by Pullaiah et al in the rats given ISO @ 100mg/kg<sup>[44]</sup>. Vacuolar degenerations and necrosis were also reported by Ganesan et al.<sup>[45]</sup> in rats given ISO @ 11mg/100g BW intraperitoneally. Mild to moderate histopathological alterations in FR treated animals in comparison to ISO treated animals were indicating the protective role of FR @200 mg/kg in myocardial injury.

## CONCLUSION

Pretreatment with *F. religiosa* elucidate significant protection of myocardial infarction in isoprenaline treated rats. The potential cardioprotective action of FR might be due to free radical scavenging properties of its phytoconstituents like Kaempferol, and other phytoconstituents that determined antioxidant and anti-hypolipidemic properties. Thus, the present study through this investigation has given the insight on potent effect of *F. religiosa* in treating free-radical-induced cardiovascular diseases. Further detailed studies on *F. religiosa* are required before its potential application in the treatment of cardiovascular diseases.

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## Conflict of interest

The authors declared no conflict of interest.

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## Ethics Approval

All the experimental studies were undertaken after approval (Approval No. 312/4/01/2000/20) of the Institutional Animal Ethics Committee (IAEC 312/GO/ReBi/S/2000 CPCSEA), PGIVAS, Akola as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

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