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Research Article

5-FU Induced Cardiotoxicity Amelioration by Aqueous Leaf Extract of *Ficus religiosa*

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Abstract

The present study was aimed to evaluate phytochemical analysis, *In-vitro* antioxidant potential, and the protective effect of *Ficus religiosa* leaf extract (FRLE) on 5-Fluororuacil (5-FU) induced cardiotoxicity in rats. A total of 48 animals were divided into experimental and control groups and were divided into 8 groups of 6 animals each in group throughout the course of study of 14 days. FRLE control received FRLE alone (400 mg/kg b.wt.) once daily by oral intubation for 14 days. Captopril control received Captopril alone @ 20 mg/kg b.wt/day, once daily by oral intubation for 14 days. Group 4 received 5-FU (20mg/kg b. wt) from 9th day onward for 5 days by intraperitoneal injection. Groups 5 and 6 were administered 5-FU + FRLE, and 5-FU + Captopril, respectively as per the above schedule and dosages. Myocardial antioxidant parameters, body weight, heart weight, relative heart weigh to body weight, thickness of left ventricle wall and lipid profile were determined and also for both Light and Electron Microscopical examination. 5-Fluororuacil significantly ($P<0.05$) altered all the parameters in study in group 4, while the groups 5 and 6 that received FRLE, TALE and Captopril along with 5-FU revealed significant improvement in all the parameters and the values were comparable to the control groups 1 to 3.

Pre-treatment with *Ficus religiosa* extract and its concomitant administration with 5-FU for 14 days attenuated 5-FU induced myocardial damage and effectively reverted the abnormal structural changes near to normalcy. In conclusion, these results suggest that *Ficus religiosa* extract has a protective potential in ameliorating 5-FU induced cardiotoxicity and the results were comparable to those of Captopril.

Keywords: *Ficus religiosa*; 5-Fluororuacil; Myocardial Antioxidants; HP; TEM

Introduction

Medicinal drugs, manufactured from the plants. Medicines prepared from plants were investigated for their pharmacological actions in treatment of variety of diseases. The cardioprotective effect of medicinal plants is due to the presence of various phytochemicals such as flavonoids, phenolic acid, diterpenes and alkaloids [1,2]. The plant *Ficus religiosa* Linn belongs to the family Moraceae. It is popularly known as the Peepal tree in Hindi and figure in English. *Ficus religiosa* contains flavonoids, glycosides, alkaloids, phenolic acids, steroids, saponins, coumarins, tannins, triterpenoids, oleanolic acid, rusolic acid α-hydroxy ursolic acid, protocatechuic acid and maslinic acid. The non-enzymatic constituents include phenolic compounds, flavonoids and vitamin C [3]. It is traditionally used to treat gonorrhoea, diarrhea, dysentery, leukorrhea, menorrhagia, for vaginal and other urogenital disorders, haemorrhoids, ulcers, and gastrohelcosis [4].

Material and Methods

Chemicals

5-FU was purchased from Neon Laboratories Limited, Andheri (E), Mumbai and was given intraperitoneally at a dose of 20 mg/kg. This dose was guided by previous studies (5, 6). Captopril was purchased from Sigma-Aldrich Chemical Co., St. Louis, MO, USA and was administered by oral gavage at a dose of 20 mg/kg according to [7,8].

Experimental animals

The animals were acclimatized to experimental conditions for seven days before the start of the experiment. Rats were selected based on the body weight and randomly distributed to different groups such that the mean body weight variations were not > 20%. A total of 48 animals were randomized into experimental and control groups. They were divided into 8 groups with 6 animals in each group.

Experimental protocols

- **Group 1 (Control):** The animals were given normal saline (2 ml/kg b.wt./day orally), parallel to the drug-treated groups, throughout the study of 14 days.
- **Group 2 (FRLE alone):** The animals received leaves extract of FRLE alone (250 mg in 2 ml normal saline per kg b.wt.) once daily by oral intubation for 14 days.
- **Group 3 (Captopril alone):** The animals received Captopril alone (20 mg in 2 ml normal saline per kg b.wt.) once daily by oral intubation for 14 days.
- **Group 4 (Toxic control):** The animals first receive normal saline (2 ml/kg b.wt./day) by oral intubation for 9 days, and subsequently received 5-FU (20 mg in 2ml normal saline per kg b.wt.) once daily by intraperitoneal injection for additional 5 days.
- **Group 5 (5FU+ FRLE):** The animals first received leaves extract of Ficus religiosa alone (250 mg in 2 ml normal saline per kg b.wt.) once daily by oral intubation for 9 days and subsequently received 5-Fluorouracil once daily by intraperitoneal injection in association with FRLE for additional 5 days.
- **Group 6 (5-FU + Captopril):** The animals first received Captopril alone at a dose of (20 mg/kg b.wt./day) by oral intubation for 9 days, and subsequently received 5-FU (20 mg/kg b.wt./day) by intraperitoneal injection along with Captopril for additional 5 days.

At the end of the experimental period, all the rats were anesthetized with light anesthetic ether and 2ml of blood was collected from the retro-orbital plexus, the serum was separated and used for the determination of diagnostic marker enzymes like LDH, CK-MB, cTn, CRP, TG, TC, LDL, HDL and VLDL. It was also planned to estimate MDA, GSH, CAT, GPx and SOD. The histopathology of heart was also studied.

The thickness of the left ventricle wall

Each rat was anesthetized with ketamine-Xylazine and then thoracotomies. The beating heart was excised from the chest cavity and immersed briefly in three changes of Tyrode's solution at room temperature to wash out blood from the chambers. The heart was then immersed in ice-cold glutaraldehyde (2%)-paraformaldehyde (2%) fixative and fixed for at least 24h. Later on, each heart was removed from the fixative and excessive fat trimmed off. The atria were separated from the ventricles. The right and left ventricles were then separated such that the left ventricle was composed of the left ventricular free wall plus the septum. The weight and height of the left ventricle were taken. The left ventricle was then serially cut into two halves. The thickness of the left ventricular free wall was measured using a Vernier calipers. To measure the thickness, the heart was cut horizontally, and the thickness of the left ventricle wall was measured using a Vernier calipers with a sensitivity of 0.01 [8].

Antioxidant profile in heart

Immediately after the sacrifice of animals, the heart tissue samples were collected and washed with ice-cold normal saline, blotted dry and stored at -20 ^{0}C for further analysis. From each sample, a piece of weighing about 500mg was taken and homogenized by tissue homogenizer (Heidolph, Germany) at 4^{0}C and 10% w/v homogenate was prepared in cold PBS (pH 7.4).

Light microscopic (LM) study

The heart tissues were collected from the rats that were sacrificed at the end and fixed in 10% neutral buffered formalin (NBF) for histopathological studies. The fixed tissues were processed and stained with Haematoxylin and Eosin (H&E) stain as described by [9].

Transmission electron microscopy (TEM) of heart

For electron microscopic studies, small heart samples were transferred to vials and fixed in 3% glutaraldehyde in 0.05 M phos-

phate buffer (pH 7.2) for 24 h at 40 C and post fixed in 2% aqueous osmium tetroxide in the same buffer for 1 h. After the post-fixation, samples were dehydrated in a series of graded alcohol and infiltrated and embedded in Araldite 6005 resin. Ultra-thin sections (50-70 nm thickness) were cut with a glass knife on a Leica Ultra cut UCT-GA-D/E-1/00 ultramicrotome and mounted on grids. Then the sections were stained with saturated aqueous uranyl acetate and counterstained with 4% lead citrate and were observed at various magnifications under a transmission electron microscope (Model Hitachi, H-7500) at RUSKA Lab, College of Veterinary Science, Rajendranagar, Hyderabad, India.

Results and Discussions

The biological activities of medicinal plants are due to the presence of various biologically active compounds like flavonoids, phenolic compounds, tannins, alkaloids, saponins, glycosides and steroids (1, 2),and have been researched extensively for their therapeutic benefits in the healing of number of disorders.

Administration of 5-FU to rats was found to affect the thickness of the ventricle wall in this study. The increased thickness of ventricle wall by 5-FU was successfully reduced and brought back to normal with FRLE, and Captopril treatment and presented in table 1. Antioxidant balance in the heart has a very important role in protecting the heart and in allowing normal cardiac contractile performance. The results of myocardial antioxidants parameters in different groups of rats are given in table 2. In the present study, the activity of SOD, CAT and GPx, and the concentration of GSH were decreased significantly in toxic control group 5FU. The decreased removal of superoxide anions which can be harmful to the myocardium is because of decrease SOD activity. The activities of H_2O_2 scavenger's viz., GSH, GPx and CAT were also decreased significantly after 5-FU treatment in the present study. Similar findings were reported by [10] and Selmi., *et al.* (2018), who recorded significant reduction of SOD, CAT, GSH and GPx in guinea pigs treated with 5FU. Pre-treatment with FRLE, and Captopril prior to 5-FU effectively prevented increase in MDA and decrease in SOD, CAT, GSH and GPx, which may be correlated directly to the scavenging of radicals.

Group	Thickness of left ventricle wall (mm)
1. Normal control	2.43 ± 0.13
2. FRLE control	2.71^{b} ± 0.11
3. Captopril control	2.65 ± 0.84
4. 5- FU toxic control	4.46^{a} ± 0.15
5. 5-FU ± FRLE	2.60^{c} ± 0.08
6. 5-FU ± Captopril	2.81 ± 0.04

Table 1: Thickness of left ventricular wall in different groups of rats.

Data are presented as Mean ± SE; n = 6; One way ANOVA; (a= $p < 0.05$, b= $p < 0.001$) all group compared to the control group;

c= $p < 0.05$, d=$p < 0.01$) 5-FU + FRLE, 5-FU + CA group compared to the 5-FU group. 5-FU= 5- Fluorouracil: FRLE = *Ficus religiosa* leaf (aqueous) extract; (aqueous) extract.

Group	TBARs (n mol/g wet tissue)	SOD (mol/g wet tissue)	CAT (U/g tissue)	GSH (mg/g wet tissue)	GPx (U/g tissue)
1.Normal control	80.99 ± 0.94	121.18 ± 0.38	22.01 ± 0.94	126.42 ± 0.31	34.12 ± 0.52
2. FRLE control	80.83 ± 1.52	105.44^{b} ± 2.02	20.99^{b} ± 0.73	119.15^{b} ± 0.52	30.66^{b} ± 0.73
4. Captopril control	62.65^{b} ± 1.85	104.27 ± 2.20	20.62 ± 0.31	119.64 ± 0.94	30.56 ± 1.78
5. 5- FU toxic control	145.17^{a} ± 1.18	77.89^{a} ± 2.20	13.40^{a} ± 0.93	65.07^{a} ± 0.31	19.08^{a} ± 1.36
6. 5-FU ± FRLE	76.34^{b} ± 2.36	122.35^{b} ± 1.35	20.56^{b} ± 2.20	129.34^{b} ± 1.15	21.39^{b} ± 0.31
8. 5-FU ± Captopril	75.85^{b} ± 2.36	119.38^{c} ± 1.52	20.69 ± 2.20	120.36 ± 0.94	30.66^{c} ± 1.36

Table 2: Myocardial antioxidant profile in different groups of rats.

Data are presented as Mean ± SE; n = 6; One way ANOVA; (a= $p < 0.05$, b= $p < 0.001$) all group compared to the control group; c= $p < 0.05$, d= $p < 0.01$) 5-FU + FRLE, 5-FU + CA group compared to the 5-FU group. 5-FU= 5- Fluorouracil: FRLE = *Ficus religiosa* leaf (aqueous) extract.

Similar observations were recorded by [12,13] found that the administration of aqueous *Ficus* extract improves SOD activity and lowered Catalase activity The histopathology of left ventricle wall after the experimental period of 14 days is illustrated in figure (A to f). Cardiac muscle fibers of the left ventricle sections showed normal architecture in the normal control group. Sections of cardiac muscle fiber of left ventricle from 5-FU control group showed dilatation of cardiac muscle fiber, focal atrophy of cardiomyocyte with severe interstitial edema, and dilated blood vessels. Sections of cardiac muscle fiber of the left ventricle of all the treatment control groups showed no notable abnormal histological changes compared to 5-FU toxic control group. 5-FU + FRLE exhibited reduced damage to cardiac architecture with fewer areas of congestion along with vacuolization. However, there was congestion of blood capillaries, swelling around the nuclei and focal haemorrhage in between the myocardial bundle, partial preservation of myofibrils and decreased breaks in muscle fibers, myofibrillar vacuolization and marked cellular infiltration.

The Ultrastructural findings on left ventricle wall after the experimental period of 14 days are illustrated in figure (A to F). The normal structure of cardiac muscle fibers, which are connected side to side and end to end to one another by intercalated disc, dilated myofibrils with normal nucleus and fibrin were observed in heart tissue of the control group after Ultrastructural examination. Ultrastructural examination of the left ventricular myocardium of FRLE, and Captopril treated groups confirmed the light microscopic findings and demonstrated dilated myofibrils with normal nucleus mild sarcoplasmic vacuolation and little myofibrillar damage. Ultrastructural examination of left ventricular myocardium of 5-FU toxic control group 5 confirmed the light microscopic findings and demonstrated irregular nuclear envelope, dilated myofibrils with degenerating nucleus, dilatation of nucleus, dilatation of sarcoplasmic reticulum, disorganized, electron-dense and bizarre shaped mitochondria and congestion of blood capillaries. Ultrastructural examination of the left ventricle of groups treated with 5-FU + FRLE, and 5-FU + Captopril revealed myofilaments and nuclei appear somewhat normal. Degeneration of endothelial cells and undifferentiated myofibril looks like degenerated mass. The normal orientation of myofibril and architecture was found. Eman and Ghada (2016) confirmed myofibrillar breaks and detachment of muscle strands accompanied by degeneration of muscle fibers due to 5-FU. Captopril is considered a standard drug for chemotherapy-induced cardiotoxicity. Hala [6] reported that pre-treatment with Captopril and its concomitant administration with 5-FU for 14days attenuated 5-FU induced myocardial damage and effectively reverted the abnormal structural changes near to normal.

Figure 1A: Histological examination of section of heart of normal group showing normal.

Figure 1B: Heart section of 5-FU group showing inflammation and fibrosis of myocardial fibres (H&E; X 400).

Figure 1C: Figure B: Heart section of 5-FU group showing loss of striation necrosis and fragmentation (H&E; X 400).

Figure 1D: Heart section of 5-FU group showing myocardial fibres atrophy (H&E; X 400).

Figure 1E: Figure B: Heart section of FRLE treated group showing ameliorated myocardial damage (H&E; X 400).

Figure 1F: Heart section of Captopril treated group showing normal appearance of cardiac muscle fiber (H&E X400).

Figure 2A: Transmission electron microscopy of section of heart of normal group showing vesicular large nucleus parallel arrays of myofilaments, striation of myofibrils, intercalated disc between the two adjacent cardiac muscle fibre (X4000)showing normal heart architecture (H&E; X400).

Figure 2B: Transmission electron microscopy of section of heart of normal group showing vesicular large nucleus parallel arrays of myofilaments, striation of myofibrils, intercalated disc between the two adjacent cardiac muscle fibre (X4000)showing normal heart architecture (H&E; X400).

Figure 2C: Heart section of 5-FU group showing irregular nuclear envelop, swelling of perinuclear area myofibrillar damage and dilatation of sarcoplasmic reticulum (X 4000).

Figure 2D: Figure B: Heart section of 5-FU group showing loss of striation of myofibrils (X 4000).

Figure 2E: Figure C: Heart section of FRLE treated group showing ameliorated myocardial damage (X 4000).

Figure 2F: Heart section of Captopril treated group showing normal appearance of cardiac muscle fiber (X 4000).

Conclusion

In conclusion, the current study suggests that *Ficus religiosa* may has Therapeutic value in lowering cardio toxicity which, induced by 5-Fluorouracil due to reducing serum lipid profile.

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