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Cardioprotective effect of aqueous leaf extract of *Ficus religiosa* Linn on 5-Fluorouracil induced toxicity in rats

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Abstract
Various parts of Peepal have been used for various medicinal purposes. The present study was planned to investigate the cardioprotective effect of aqueous leaf extract of Ficus religiosa against 5-Fluoroacil induced toxicity in rats. Male wistar albino rats were divided randomly into 6 groups of six rats each: control (Normal saline), 5-FU (20mg/kg IP), FRLE alone (400mg/kg oral), FRLE + 5-FU. After anaesthetized the animals on the last day, electrocardiograph was recorded and blood was analyzed for LDH, CK-MB, CRP and c-Troponin. The FRLE group showed decrease in QT duration and ST segment and increase in heart rate, QRS complex compared to the 5-FU group. Significant decrease in LDH, CK-MB, CRP and c-Troponin were observed as compared to the 5-FU. Result indicates that FRLE has cardioprotective effect against dose induced cardiotoxicity in rats.

Keywords: cardiotoxicity, 5-fluorouacil, electrocardiograph, cardiac markers

Introduction
Various parts of Peepal, *Ficus religiosa* Linn, have been used for various medicinal purposes. Many studies have shown that *Ficus religiosa* extract possesses antioxidant activity (Alipour and Wankhede 2014, Yadav 2015) [1, 2], antimicrobial (Pal *et al.*, 2018) [3], anticancer (Ibrahim and Khulood 2013) [4], antiulcer (Gregory *et al*., 2013) [5], antifungal (Hemaiswaraya *et al*., 2008) [6], anti-inflammatory (Jung *et al*., 2008) [7], hepatoprotective (Parameswari *et al.*, 2012) [8], nephroprotective (Yadav and Srivastava 2013) [2], antihyperlipidemic activities (Pandit *et al*., 2010) [10]. 5-Fluoroacil is an antimetabolite Fluropyrimides based antineoplastic agent which is used in the treatment of breast, gastrointestinal, pancreatic, oesophagus, colon, cervical and skin cancers (Sorrentino *et al*., 2012) [11]. Unfortunally, the cardiac toxicity of 5-FU resulting in a cardiomyopathy with irreversible congestive heart failure is one of the main factors that limits its use. The molecular mechanism explaining the cardiotoxicity of 5-FU are complex but it appears that the induction of an oxidative stress within myocardial tissue constituents a common denominator (Tao Xu *et al*., 2019 [12], Lamberti *et al*., 2012) [13]. Considerable efforts have been made on using antioxidants to protect heart against 5-FU toxicity. Captopril prevents 5-FU induced cardiotoxicity which is an ACE inhibitor and possesses potent antioxidant properties. Cardioprotective effects of various plants have been reported to be due to antioxidant activity (El-Sayyad *et al.*, 2012). Therefore we have hypothesized that leaf extract of *Ficus religiosa* Linn (FRLE) may exert considerable cardioprotective effects.

Material and Methods
Drugs and Chemicals
5-Fluorouracil was purchased from Neon Laboratories Limited Andheri (E) Mumbai). Captopril was purchased from Sigma-Aldrich Chemical Co., St. Louis, MO, USA. Aqueous extract of *Ficus religiosa* Linn was prepared in department and administered as suspensions in freshly prepared 0.5% w/v carboxymethyl cellulose sodium salt. Serum CK-MB, CRP, cardiac Troponin and LDH were determined using a commercial kit purchased from Krishgen Biosystems, Mumbai, India. All other chemicals used were of fine analytical grade.

Animals
All experimental protocols of the animal studies were approved by the Institutional Animal Ethic committee of C.V.Sc., Rajendranagar, Hyderabad. The animals were purchased from GenTox Hyderabad. Male wistar rats (250-300g) were housed under standard conditions of 27 ^{o}C, relative humidity 70%, photoperiod of 12h day/12hr night, pellet (NIN, Hyderabad) and

water were provided ad libitum.

Experimental protocol

Effects of 5-FU, *Ficus religiosa,* Captopril and their combination on the ECG of anesthetized rats

Rats were divided into six groups of 6 animals each. Group 1, 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 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 received Captopril alone (20 mg in 2 ml normal saline per kg b.wt.) once daily by oral intubation for 14 days. Group 4 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 first received leaves extract of *Ficus religiosa* (FRLE) 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 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.

On the 15th day, the rats were anesthetized with ketamine + xylazine and electrocardiographic recording was carried out according to the method of Pradeep Kumar *et al*. (2017). Lead II ECG was recorded in the anesthetized animals using needle electrodes. Electrodes were inserted under the skin in the right upper limb, right lower limb and the left lower limb. Three needle electrodes were placed under the skin of the rats in limb lead II position (depicted in Figure 3): the negative electrode was placed to the skin of the front paws and the positive electrode to the left hind leg paws. ECG recordings were taken 1 min by computerized MP-150 multi-channel physiological analysis system (BioPac Systems Inc.; USA). Changes in ECG pattern (heartbeat (bpm) duration of the P wave, QRS complex, QT interval and amplitude of ST-segment) were assayed (Kumar *et al*., 2017).

Blood sampling and tissue preparation

Blood samples were collected 24 hours after the last dose, and all rats were sacrificed by cervical decapitation. The obtained sera were monitored for LDH, CRP cTn and CKMB activity. Heart tissues were excised after dissection of the animals and designated for biochemical analysis. The excised heart tissue (0.5g) was homogenized in ten volumes of ice cold phosphate buffer (pH: 7) until a uniform suspension was obtained. The homogenate was then centrifuged at 20,000×g for 10 min at 4 °C using high speed cooling centrifuge. The clear supernatant was used for the assay of LDH, CRP cTn and CKMB and IL-1β and IL-10.

Measurement of ventricle wall thickness: 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 24 h. 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 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 (Charlers and Oyunde, 2005) [15].

Heart weight to body weight ratio

In each group, heart weight to body weight ratio was calculated. Body weight was the weight of animal on the day of sacrifice. Heart weight was measured after placing the heart in cold saline and squeezing out the blood.

Statistical analysis

The data was expressed as mean ± standard error of mean (SEM). One way analysis of variance (ANOVA) was applied to test the significance of difference between average biochemical and ECG parameters of different groups, and multiple comparisons were determined by Tukey's test. P value less than 0.05 was considered statistically significant. The statistical analysis was performed using the program Graph Pad Prism version 4.03.

Results

ECG changes

5-FU administration significantly increased QT (p<0.05) and ST (p<0.05) intervals and significantly decreased heart rate (p<0.05) compared to the control group. FRLE administration (400 mg/kg) significantly decreased QT interval and increased heart rate (p<0.05) whereas there was no significant effect on ST interval as compared to the 5-FU group (Table 1 and Figure 1).

Table 1: Electrocardiographic (ECG) parameters in different groups of rats

Group	Heart rate (bpm)	P Wave Duration (sec)	QRS complex (sec)	PR duration (sec)	QT interval Duration (sec)	ST segment amplitude (Sec)
1. Normal control	287.33 ± 5.06*	0.032 ± 0.0007*	0.06 ± 0.005*	0.084 ± 0.001*	0.052 ± 0.008*	0.059 ± 0.001*
2. FRLE control	281.16 ± 6.74*	0.031 ± 0.0006*	0.052 ± 0.005*	0.089 ± 0.008*	0.055 ± 0.009*	0.053 ± 0.001*
3. Captopril control	284.5 ± 8.80*	0.032 ± 0.001*	0.056 ± 0.002*	0.084 ± 0.002*	0.055 ± 0.002*	0.056 ± 0.001*
4. FU toxic control	203.16 ± 0.66	0.012 ± 0.0006	0.035 ± 0.002	1.120 ± 0.003	0.087 ± 0.002	0.166 ± 0.009
5. FU + FRLE	262 ± 4.41*	0.026 ± 0.0005*	0.057 ± 0.001*	0.087 ± 0.005*	0.060 ± 0.002*	0.055 ± 0.001*
6. FU + Captopril	257.83 ± 2.07*	0.030 ± 0.0008*	0.058 ± 0.004*	0.080 ± 0.003*	0.058 ± 0.001*	0.059 ± 0.002*

Table 2: Body weight, heart weight and relative heart weight to body weight in different groups of rats

Group	Body weight (g)	Heart weight (g)	Heart weight to body weight ratio (*1000)
1. Normal control	201.00 ± 4.09*	1.03± 0.07*	5.31± 0.06*
2. FRLE control	227.33 ± 8.19*	1.17± 0.05*	5.16± 0.03*
3. Captopril control	224.16 ± 5.32*	1.02± 0.16*	4.75± 0.05*
4. FU toxic control	186.66 ± 11.73	1.82± 0.08	9.96± 0.73
5. FU + FRLE	219.00 ± 5.45*	1.42± 0.10*	6.49± 0.03*
6. FU + Captopril	222.33 ± 4.91*	1.35± 0.09*	6.04± 0.05*

Table 3: Thickness of left ventricular wall in different groups of rats

Group	Thickness of left ventricle wall (mm)
1. Normal control	2.43 ± 0.13*
2. FRLE control	2.71 ± 0.11*
3. Captopril control	2.65 ± 0.84*
4. FU toxic control	4.46 ± 0.15
5. FU + FRLE	2.60 ± 0.08*
6. FU + Captopril	2.81 ± 0.04*

Serum parameters

Administration of 5-FU (20 mg/kg) increased serum CK-MB, LDH, CRP and cTn levels compared to that of control, whereas FRLE (400 mg/kg) administration decreased the levels of CK-MB and LDH significantly compared to that of the 5-FU group alone (Figure 4).

Table 4: Cardiac biomarkers in different groups of rats

Group	LDH (U/L)	CK-MB (IU/L)	CRP (mg/dl)	cTn (ng/ml)
1. Normal control	351.33 ± 25.48*	161.52 ± 4.28*	66.39 ± 1.19*	11.43 ± 0.85*
2. FRLE control	321.30 ± 36.69*	160.07 ± 6.92*	69.47 ± 1.31*	11.20 ± 1.46*
3. Captopril control	239.74 ± 44.59*	151.50 ± 6.09*	67.07 ± 5.29*	10.80 ± 1.18*
4. FU toxic control	502.12 ± 10.14	305.29 ± 6.95	104.11 ± 9.29	23.53 ± 1.24
5. FU + FRLE	376.20 ± 27.11*	138.27 ± 7.14*	72.80 ± 3.35*	11.81 ± 1.48*
6. FU + Captopril	305.81 ± 55.19*	135.43 ± 2.57*	69.00 ± 1.15*	9.60 ± 0.82*

Administration of 5-FU (20mg/kg) caused an increased IL-1β and decreased IL-10, whereas FRLE administration decreased IL-1β and increased IL-10 levels significantly compared to that of the 5-FU group alone (Fig 5).

Table 5: Interleukin-1β and Interleukin-10 concentration in different groups of rats

Group	IL-1β (pg/mg)	IL-10 (pg/mg)
1. Normal control	47.80 ± 3.26*	20.59 ± 1.68*
2. FRLE control	40.34 ± 1.53*	20.22 ± 1.52*
4. Captopril control	38.39 ± 2.05*	17.67 ± 0.54*
5. FU toxic control	75.37 ± 6.71	11.41 ± 0.47
6. FU + FRLE	37.60 ± 0.91*	19.07 ± 1.60*
8. FU + Captopril	47.78 ± 6.61*	18.30 ± 1.69*

Discussion

The antimetabolite Fluropyrimides 5-FU is one of the most effective chemotherapeutic agents against a wide variety of cancers. It possesses potential for generating free radicals and causes an unusual and often irreversible cardiomyopathy. It increases Reactive oxygen species (ROS) and reactive oxygen nitrogen (RON), which is responsible for oxidative stress in different pathophysiological conditions. Treatment with 5-FU increases reactive oxygen species such as superoxide anions in rat cardiomyocyte and diminished activity of antioxidant agents such as SOD, GPx and increased free radical species leading to oxidation of protein, lipid and other macromolecule (Jaskanwal *et al*., 2018) [16]. In the present study, 5-FU toxic control group 5 showed decrease in the body weight of rats, which might be due to loss of skeletal muscles and adipose tissue coupled with diarrhoea due to 5-FU. This finding was also reported by Song *et al.* (2013) [17], Safa Mustafa *et al*., (2015) [18] and Saif Mohammed *et al.* (2001). However, 5-FU treated groups that were additionally treated with FRLE, and Captopril revealed significant increase in the body weight. Parameswari *et al.* (2012) [8], Gregory *et al.* (2013) [5], Ibrahim *et al.* (2013) [4] and Pochii and Muddeshwar (2017) [20] observed significant restoration in body weight in rats treated with *Ficus religiosa* leaf extracts. Similarly, an increase in weight gain with Captopril was reported by Hall (2012) and Batta and Azim (2015). There was a significant increase in the heart weight and relative heart weight to body weight (HW/BW) in 5FU group that was kept as 5-FUcontrol throughout the study. This increase of heart weight and relative heart weight to body weight by 5-FU was successfully reduced and brought back to normal in FRLE and Captopril group.

5-FU treatment changes ECG and causes prolongation of QT interval and ST segment (Dogan *et al*., 2011, Spencker *et al*., 2007) [23]. The present study has demonstrated increased myocardial injury as indicated by increase in QT interval and ST segment of ECG pattern and decrease in QRS complex and heart rate in 5-FU treated groups. Administration of FRLE along with 5-FU restored QT interval and ST segment to control level and increased heart rate, QRS complex near to normal.

The result also showed that administration of 5-FU induced cardiotoxicity manifested by a significant increase in serum CK-MB, LDH, CRP and cTn levels. These results are supported by earlier studies which have reported similarly (Khudair and Intesar 2014, Eman and Ghada 2016) [24, 25]. In this study the significant decrease in levels of CK-MB, LDH, CRP and cTn levels confirm the protective effect of FRLE against 5-Fu induced cardiotoxicity.

Many pro-inflammatory cytokines such as IL-1β, IL-6 and TNA-α are known to be involved in the pathophysiology of

heart failure and organ damage that is induced by chemotherapy agents such as 5-FU. Several reports demonstrate enhanced expression and release of inflammatory cytokineIL-1β, which is responsible for an increase in cardiac dysfunction (Daman *et al*., 2000) [26].

The present study demonstrated significant increased of IL-1β and decrease of IL-10 in 5FU treated groups. In this study, the significant decrease in levels of IL-1β and increase in IL-10 confirms the protective effect of FRLE against 5-FU induced cardiotoxicity. 5-Fluorouracil is a potent inducer of several types of cytokines including IL-1β and the administration of 5-FU showed significant increase in IL-1β concentration in cardiac homogenate, indicating the inflammatory effect of 5-Fluorouracil (Mohammed *et al.*, 2016). Methanolic leaf extract of *Ficus religiosa* inhibited lipopolysaccharide-induced production of Nitric oxide and pro-inflammatory cytokines viz., TNF-*alpha*, Interleukin-1 *beta* and IL-6 in a dose-dependent manner (Jung *et al*., 2008) [7].

In conclusion, our study showed that *Ficus religiosa* extract exerts good protection against toxic effects of 5-FU. Therefore, *Ficus religiosa* Linn may be considered as a potentially useful candidate in the combination chemotherapy with 5-FU to limit its cardiotoxicity.

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Declaration of interest

The authors report no declarations of interest

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