

CARDIOPROTECTIVE EFFECT OF *IXORA COCCINEA* LINN. FLOWER EXTRACT ON DOXORUBICIN INDUCED CARDIOMYOPATHY IN RATS.

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Summary

Albino Wistar rats were pretreated with 200 and 400 mg/kg of methanolic extract of *Ixora coccinea* Linn. flower (MICF) for 7 days and simultaneous treatment with doxorubicin (cumulative dose of 15 mg/kg i.p. in six divided doses for two weeks) along with the extracts for the next 14 days. On the 22nd day, parameters evaluated were ECG, Invasive blood pressure, serum markers such as creatine kinase-MB (CK-MB), lactatedehydrogenase (LDH), SGOT and SGPT, tissue antioxidant markers viz. catalase (CAT), superoxide dismutase (SOD) and extent of lipid peroxidation viz. malondialdehyde (MDA) was estimated. The extract dose dependently, significantly reduce the elevated levels of the serum enzymes (P<0.01) and restores the ECG and BP (P<0.01) close to normal, also significantly increase the tissue antioxidant levels (P<0.01), while decrease the MDA level (P<0.01) when compared with the control. The histopathological study also further confirmed the cardioprotection. In conclusion, the cardioprotection was due to the blunting action of the extract to oxidative stress induced by doxorubicin.

Keywords: *Ixora coccinea* Linn., Antioxidant, ECG, blood pressure, cardiotoxicity.

Introduction

Ixora coccinea Linn. (Fam: Rubiaceae) is a shrub with a small, obovate to oval – oblong, rounded to subcordate base leaves on branched hard heavy twigs. Height commonly goes to around 4-6 ft. (1.2 – 2 m) but capable of going upto 12 ft. (3.6 m) *Ixora coccinea* Linn. is found throughout India more common in the western peninsula in scrub jungles widely cultivated throughout the tropics. Roots are used as sedative for hic-coughs, used for nausea, loss of appetite, fever, gonorrhoea, diarrhoea, decoction is given for dysentery. Leaves are used for dermatological disorders, methanolic extract of *Ixora coccinea* Linn. leaves has shown antioxidant and reactive oxygen species scavenging property, moreover *Ixora coccinea* Linn. extract showed strong reducing power and total antioxidant capacity. Leaf and stem are used

as an ablation for infantile. Flowers and bark is used on reddened eyes and eruptions in children. Decoction of flowers is given for hemoptysis, catarrhal bronchitis and dysmenorrhoea. Flowers are used externally to sores, employed for chronic ulcer, scabies and some type of dermatitis and also used internally for cholera, diarrhoea, dysentery, leucorrhoea, antitumor and gonorrhoea, fresh juice of flower have protective action against electroconvulsions [1 - 5]. *Ixora coccinea* Linn. flowers showed chemoprotective effects on cyclophosphamide induced toxicity by increasing the life span of treated mice [6] and also on cisplatin induced toxicity [7].

Doxorubicin (Dox) is converted to semiquinone by mitochondrial, lysosomal and cystolic enzymes. Semiquinone is a charged moiety that readily donates an electron to an oxygen molecule, resulting in generation of an oxygen free radical or superoxide ion/hydroxyl radicals [8]. Due to the presence of less developed antioxidant defense mechanisms of heart, they are particularly vulnerable to apoptosis by anthracycline-induced reactive oxygen species. Therapeutic strategies, designed to augment cellular endogenous defense systems as antioxidants have been identified as a promising approach to combat against Dox toxicity [9]. As there was no previous reports available for the cardioprotective activity of the drug, the present study was undertaken to study the cardioprotective effect of methanolic extract of *Ixora coccinea* Linn. flowers on Doxorubicin induced cardiac toxicity with a view to provide a scientific evidence.

Material and Methods

Plant material:

The flowers of *Ixora coccinea* Linn. were collected in the month of August 2010 from local area of Sangli, Maharashtra and were authenticated by Dr. (Mrs.) U. S. Yadav, Dept. of Botany, Willingdon College, Sangli. The herbarium has been submitted for further reference (V. No. WC/2010/121). The plant material was shade dried at room temperature.

Preparation of extract:

The dried flowers of *Ixora coccinea* Linn. were grinded and made into a coarse powder. The coarse powder was then subjected to continuous hot extraction in soxhlet apparatus with methanol as a solvent and extracted till the solvent becomes colorless. The extract was evaporated under reduced pressure using rotary evaporator (Equitron Roteva) at a low temperature of 45°C until the extract turns syrupy and then the syrupy extract is transferred to an evaporating dish for drying at room temperature. The yield was found to be 16 % w/w.

Preliminary phytochemical investigation:

The extract was subjected to chemical tests qualitatively for the identification of different phytoconstituents like glycosides, saponins, carbohydrates, sterols, alkaloids, flavonoids, tannins, proteins and triterpenoids.

Animals:

Albino Wistar rats of 150 - 250 gm of either sex were used for the study. The inbred species of rats were obtained from animal house of Appasaheb Birnale College of Pharmacy, Sangli for experimental purpose. The animals were maintained under controlled conditions of temperature ($23 \pm 2^\circ\text{C}$), humidity ($50 \pm 5\%$) and 12 h light - dark cycles. All the animals were acclimatized for seven days before the actual study. The animals were randomized into experimental, normal and control groups, housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water ad libitum. Animals were habituated to laboratory conditions for 48 h prior to experimental protocol to minimize if any of non-specific stress. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Appasaheb Birnale College of Pharmacy, Sangli, Maharashtra (Registration No. 843/AC/04 /CPCSEA), India.

Toxicity studies:

Acute toxicity study was carried using Albino rats by up and down/staircase method as per OECD guidelines. The methanolic extract of *Ixora coccinea* Linn. flowers (MICF) was orally administered to different groups of rats at the doses of 50, 300, 1000, 2000 and 3000 mg/kg body weight respectively. The animals were observed for 48 hr to study the general behavior and for any sign of discomfort to the animals.

Chemicals:

Sodium carboxy methyl cellulose (Na-CMC), thiobarbituric acid, heparin, ketamine (Neon Labs, India), xlyazine (Indian Immunologicals, India), doxorubicin (Cipla, India), sodium hydroxide, pyridine, n – butanol, pyrogallol, anaesthetic ether etc. the enzyme kits used were creatine kinase – MB (Pathozyme, India), lactate dehydrogenase (Teco diagnostics, India), SGOT (Span diagnostics Ltd., India), SGPT (Span diagnostics Ltd., India), total protein (Pathozyme, India) etc, all the chemicals used was of analytical grade.

Pharmacological Screening:

Doxorubicin induced cardiac stress:

The rats were divided into four groups of six animals each. The groups are as follows-

Group 1: Normal (rats treated with 1% Na CMC, ~ 2 ml/kg/day, p.o)

Group 2: Control (rats treated with Dox with total cumulative dose of 15 mg/kg i.p for 2 weeks in six divided dosage).

Group 3: Rats pretreated with methanolic extract of *Ixora coccinea* Linn. flowers (MICF) 200 mg/kg p.o. along with Dox treatment.

Group 4: Rats pretreated with (MICF) 400 mg/kg p.o. along with Dox treatment.

Group 2, 3 and 4 received the treatment of Dox at alternate days for a period of 2 weeks (the days selected for Dox injection was on the 8th, 10th, 14th, 16th, 18th, 21st day after the 7 days pretreatment with the extract. On 22nd day parameters studied were general appearance, heart weight, heart/body weight ratio.

Electrocardiography:

ECG was recorded at the end of the treatment, 24h after the last dose of Dox. All rats were fasted overnight but had free access to water after the last dose administration. Biopac MP-35 (Santa Barbara, CA, USA) was used to record and monitor ECG tracings. Rats from each group were anesthetized with Ketamine – Xylazine anesthesia, a needle electrodes were inserted under the skin for the limb lead at position II. For each ECG tracing P wave, QRS complex, QT interval, RR interval and cardiac cycle were measured.

Blood pressure:

Blood pressure was determined by invasive method (carotid artery cannulation) by using BIOPAC MP-35. Carotid artery was cannulated by using PE-50 tube (cannula) which was attached to the pressure transducer loaded previously with the heparinized saline. Here, various parameters such as systolic, diastolic, mean BP and heart rate was determined.

Biochemical parameters:

Soon after the blood pressure has been recorded, the blood was collected from the retro orbital route and subjected to centrifugation to isolate serum out of it. The serum was further used for estimation of creatine kinase-MB (CK-MB) by immunoinhibition method estimated at 340 nm, lactate dehydrogenase (LDH) by kinetic method estimated at 340 nm, serum glutamate oxaloacetate transaminase (SGOT)/aspartate aminotransferase (AST) and serum glutamate pyruvate transaminase (SGPT)/alanine aminotransferase (ALT) by 2,4-DNPH method estimated at 505 nm. Heart tissue homogenate was prepared in 0.05 M phosphate buffer pH 7.4 and homogenated in tissue homogenizer at 2000 rpm for 10 min. Antioxidant enzymes estimated were catalase^[10] (CAT), superoxide dismutase^[11] (SOD) and extent of lipid peroxidation malondialdehyde^[12] (MDA).

Histopathological study:

At the end of study, the heart was isolated, washed with ice cold saline. The tissue was fixed in 10% buffered neutral formalin solution. After fixation tissues were embedded in paraffin-wax and sections were cut and stained with hematoxylin and eosin. The slides were observed under light microscope.

Statistical Analysis:

Values are expressed as Mean \pm SEM for six rats in each group, statistical analysis was performed using one way ANOVA followed by Dunnett t test (GraphPad Instat, USA). **p<0.01 was taken as the criterion of statistical significance.

Results:

The hemodynamic, electrocardiographic, biochemical and histopathological studies were assessed for methanolic extract of *Ixora coccinea* Linn. flowers on chronic administration of Dox induced cardiomyopathy.

Preliminary phytochemical investigation:

Preliminary phytochemical investigation revealed the presence of tannins, flavonoids, steroids, glycosides and alkaloids.

Toxicity studies and behavior changes:

There was no mortality found upto dose 3000 mg/kg. No behavior changes were observed during the toxicity studies.

Effect of methanolic extract of *Ixora coccinea* Linn. flowers (MICF) on doxorubicin induced cardiomyopathy:

General observation:

In Dox treated group, the animal fur became scruffy and developed a yellowish to reddish tinge. These rats also had red exudates around the eyes and nose, animals were sicker and lethargic when compared with the normal. These observations were markedly reduced in the group treated with MICF when compared with the doxorubicin control group.

Body weight and heart/body weight ratio:

Decrease in body weight, heart weight and heart/body weight ratio was seen in Dox treated rat, at the end of the study when compared with the normal. MICF 200 and 400 mg/kg, shows significant increase in body weight gain, increase in weight of heart (MICF-400 mg/kg, **P<0.01) and dose dependently demonstrate increase in heart/body weight ratio (MICF-200 and 400 mg/kg, **P<0.01), when compared with Dox control group, (Table 1).

ECG recordings:

Normal group showed a normal ECG pattern, where as animals treated with Dox alone showed significant elevation in ST segment, prolongation in P wave, QRS complex and R-R interval. In addition there was a decreased in cardiac cycles and prolongation of QT interval as compared to normal rats. Treatment with MICF 200 and 400 mg/kg for 21 days alternatively with Dox treated rats exhibited near to normal ECG pattern with a decreased elevation in ST segment. Furthermore, treatment also resulted in decrease in P wave (MICF-400 mg/kg, **P<0.01), QRS complex (MICF-400 mg/kg, *P<0.05), QT-interval (MICF-200&400 mg/kg, **P<0.01) and R-R interval (MICF-200&400 mg/kg, **P<0.01), whereas cardiac cycle was increased (MICF-200 &400 mg/kg, **P<0.01) when compared with the control. The data such as P wave, QRS complex, QT

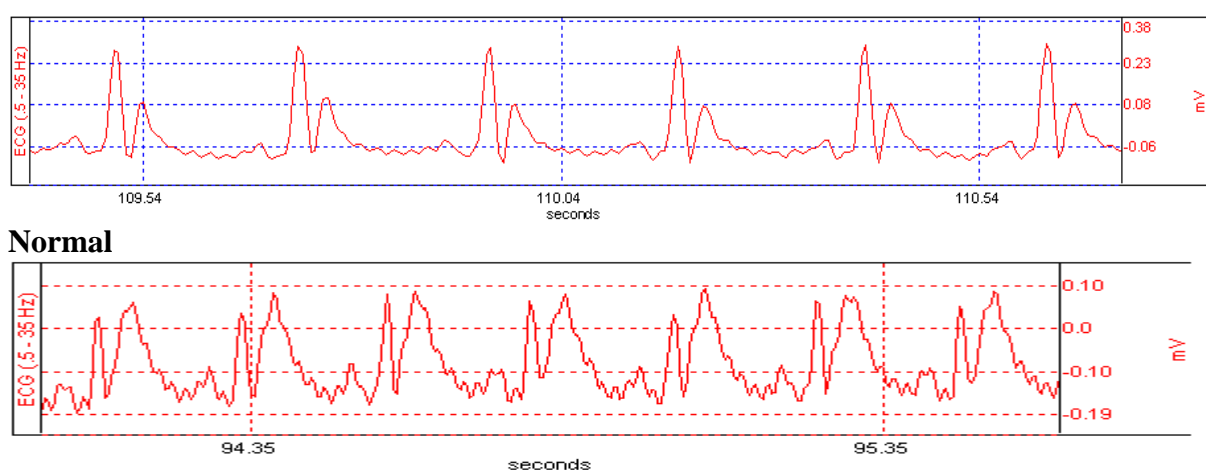
interval, R-R interval and cardiac cycle are shown in Table 2. The electrocardiographic pattern of normal, control, MICF 200 and 400 mg/kg is shown in Fig. 1.

Table 1: Effect of methanolic extract of *Ixora coccinea* Linn. flowers (MICF) on body weight, heart weight and heart/body weight ratio by doxorubicin induced cardiomyopathy.

Group No.	Groups	Initial body weight (g)	Final body weight (g)	Heart weight (g)	Heart/body weight ratio ($\times 10^{-3}$)
1	Normal	196.8711 ± 2.6093	203.5139 ± 1.9265	0.9872 ± 0.026	4.8358 ± 0.0119
2	Control Dox treated	214.6618 ± 2.9014	196.0815 ± 2.3916	0.6970 $\pm 0.0165^{##}$	3.5628 $\pm 0.0259^{##}$
3	Dox + MICF 200 mg/kg	193.6841 ± 2.7918	182.0481 ± 2.4783	0.7938 $\pm 0.0178^*$	4.1215 $\pm 0.0764^{**}$
4	Dox + MICF 400 mg/kg	197.1028 ± 2.5294	191.2358 ± 2.2019	0.8608 $\pm 0.0401^{**}$	4.5052 $\pm 0.0529^{**}$

Values are expressed as Mean \pm SEM and n = 6, *P<0.05, **P<0.01 using one way ANOVA coupled with "Dunnnett t test". **P<0.01 is considered as significant. # indicate control group compared with normal ($^{\#}P<0.05$, $^{##}P<0.01$) and * indicate other groups compared with control group. MICF - Methanolic extract of *Ixora coccinea* Linn. flowers.

Fig. 1: ECG recordings of normal, control (Dox treated), MICF 200 mg/kg, MICF 400 mg/kg.



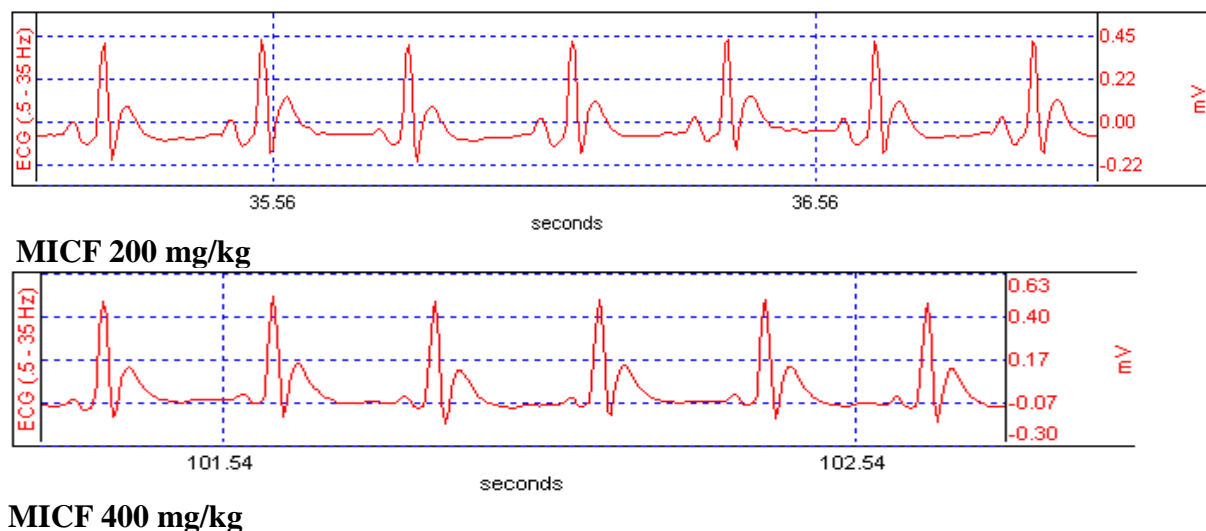


Table 2: Effect of methanolic extract of *Ixora coccinea* Linn. flowers (MICF) on ECG changes (in seconds) of rats by doxorubicin induced cardiomyopathy.

Group No.	Groups	P wave	QRS complex	QT interval	RR interval	Cardiac cycle
1	Normal	0.0267 ± 0.0011	0.0333 ± 0.0011	0.0685 ± 0.0013	0.1995 ± 0.0015	0.1658 ± 0.0059
2	Control Dox treated	0.0417 ± 0.0012 ^{##}	0.0426 ± 0.0012 ^{##}	0.1086 ± 0.0021 ^{##}	0.3205 ± 0.0082 ^{##}	0.1245 ± 0.0061 ^{##}
3	Dox + MICF 200 mg/kg	0.0356 ± 0.017*	0.0398 ± 0.0019 ^{ns}	0.0903 ± 0.0026**	0.2803 ± 0.0033**	0.1632 ± 0.0035**
4	Dox + MICF 400 mg/kg	0.0341 ± 0.0014**	0.0372 ± 0.0014*	0.0874 ± 0.0022**	0.2643 ± 0.0035**	0.1648 ± 0.0029**

The ECG parameters are expressed in seconds (sec.). Each values are expressed as Mean ± SEM and n = 6, *P<0.05, **P<0.01 using one way ANOVA coupled with “Dunnnett t test”. **P<0.01 is considered as significant. # indicate control group compared with normal (#P<0.05, ##P<0.01) and * indicate other groups compared with control group.

Blood Pressure determination:

The animals treated with Dox showed a decrease in the systolic, diastolic, mean BP and the heart rate when compared with the normal, treatment with methanolic extract of *Ixora coccinea* Linn. flowers 200 mg and 400 mg/kg showed a dose dependent, significant increase in the systolic BP (**P<0.01), diastolic BP (**P<0.01), mean BP (**P<0.01) and the heart rate (**P<0.01) respectively, when compared with the control group. Table 3 shows the data for blood pressure.

Table 3: Effect of methanolic extract of *Ixora coccinea* Linn. flowers (MICF) on blood pressure (mm Hg) of rats by doxorubicin induced cardiomyopathy.

Group No.	Groups	Systolic BP mmHg	Diastolic BP mmHg	Mean BP mmHg	Heart Rate (bpm)
1	Normal	125.7612 ± 0.7292	118.6231 ± 0.2078	113.6142 ± 0.4912	273.8215 ± 1.7391
2	Control Dox treated	78.5610 ± 0.2591 ^{##}	68.2625 ± 0.3629 ^{##}	73.8213 ± 0.2196 ^{##}	211.2621 ± 1.3017 ^{##}
3	Dox + MICF 200 mg/kg	104.1023 ± 0.3681 ^{**}	92.5415 ± 0.3996 ^{**}	99.6206 ± 0.2892 ^{**}	244.8982 ± 1.3865 ^{**}
4	Dox + MICF 400 mg/kg	110.9912 ± 0.3935 ^{**}	94.2627 ± 0.4605 ^{**}	103.6498 ± 0.3611 ^{**}	252.6137 ± 1.4578 ^{**}

Values are in mmHg and heart rate is in beats per minute (bps), expressed as Mean ± SEM and n=6, *P<0.05, **P<0.01 using one way ANOVA coupled with “Dunnett t test”. **P<0.01 is considered as significant. # indicate control group compared with normal ([#]P<0.05, ^{##}P<0.01) and * indicate other groups compared with control group.

Biochemical study:

Serum Markers: CK – MB, LDH, SGOT and SGPT:

Treatment with doxorubicin causes an elevation in level of CK-MB, LDH, SGOT and SGPT, which are considered as the selective biomarkers of myocardial damage when compared with the normal. Our study showed decrease in the elevated levels of these enzymes. Pretreatment with MICF 200 mg and 400 mg/kg showed a dose dependent, significant decrease in CK-MB (**P<0.01), LDH (**P<0.01), SGPT (**P<0.01) and SGOT (**P<0.01) when compared with the Dox control group. Table 4, shows the effect of methanolic extract of *Ixora coccinea* Linn. flowers 200 mg and 400 mg/kg on the level of creatine kinase – MB, lactate dehydrogenase, SGOT and SGPT enzymes.

Tissue antioxidant markers and lipid peroxidation of heart tissue homogenate:

Doxorubicin cause a decrease in the level of endogenous antioxidant reserves viz. SOD and CAT and shows an increase in the lipid peroxidation of the heart when compared with the normal. Pretreatment with MICF 200 and 400 mg/kg showed a significant increase in the SOD (**P<0.01) and CAT (**P<0.01). While significantly decrease the level of MDA (**P<0.01) in a dose dependent manner, when compared with the control. The antioxidant tissue markers and lipid peroxidation is shown in Table 5.

Table 4: Effect of methanolic extract of *Ixora coccinea* Linn. flowers (MICF) on CK-MB, LDH, SGOT and SGPT levels in rats by doxorubicin induced cardiomyopathy.

Group No.	Groups	CK-MB (U/L)	LDH (IU/L)	SGOT (IU/L)	SGPT (IU/L)
1	Normal	48.2411 ± 0.8212	76.6602 ± 0.5315	43.1219 ± 0.7065	24.5013 ± 0.2691
2	Control Dox treated	149.6624 ± 0.8096 ^{##}	153.9096 ± 0.3182 ^{##}	141.9981 ± 0.8635 ^{##}	45.0131 ± 0.2019 ^{##}
3	Dox + MICF 200 mg/kg	94.7138 ± 0.7661 ^{**}	98.4106 ± 0.5102 ^{**}	79.0691 ± 0.6938 ^{**}	34.6149 ± 0.2682 ^{**}
4	Dox + MICF 400 mg/kg	76.5357 ± 0.6476 ^{**}	88.3989 ± 0.5992 ^{**}	61.6120 ± 0.6259 ^{**}	27.0812 ± 0.3016 ^{**}

Values are expressed as Mean ± SEM and n = 6, *P<0.05, **P<0.01 using one way ANOVA coupled with “Dunnett t test”. **P<0.01 is considered as significant. # indicate control group compared with normal ([#]P<0.05, ^{##}P<0.01) and * indicate other groups compared with control group.

Table 5: Antioxidant enzymes and lipid peroxidation of heart tissue homogenate of rats.

Group No.	Groups	CAT (U/mg protein)	SOD (U/ mg protein)	MDA (µmole/ mg protein)
1	Normal	35.1814 ± 0.4013	2.2930 ± 0.0912	0.8231 ± 0.0015
2	Control Dox treated	16.0212 ± 0.3306 ^{##}	0.7325 ± 0.0661 ^{##}	2.7931 ± 0.0078 ^{##}
3	Dox + MICF 200 mg/kg	28.4259 ± 0.2732 ^{**}	1.9481 ± 0.0532 ^{**}	1.4193 ± 0.0036 ^{**}
4	Dox + MICF 400 mg/kg	32.3001 ± 0.2820 ^{**}	2.0024 ± 0.0556 ^{**}	1.1062 ± 0.0043 ^{**}

Values are expressed as Mean ± SEM and n = 6, *P<0.05, **P<0.01 using one way ANOVA coupled with “Dunnett t test”. **P<0.01 is considered as significant. # indicate control group compared with normal ([#]P<0.05, ^{##}P<0.01) and * indicate other groups compared with control group.

Histopathological changes on Dox-induced cardiomyopathy:

The heart sections obtained from Dox treated animals showed abundant areas of necrosis and aggregation of acute inflammatory cells and damaged vascular spaces. Animals pretreated with MICF 200 and 400 mg/kg showed improvement in the cell integrity evidenced by absence of necrosis, less vacuolization of the cytoplasm and maintenance of normal integrity of the cardiac muscles, refer Fig. 2.

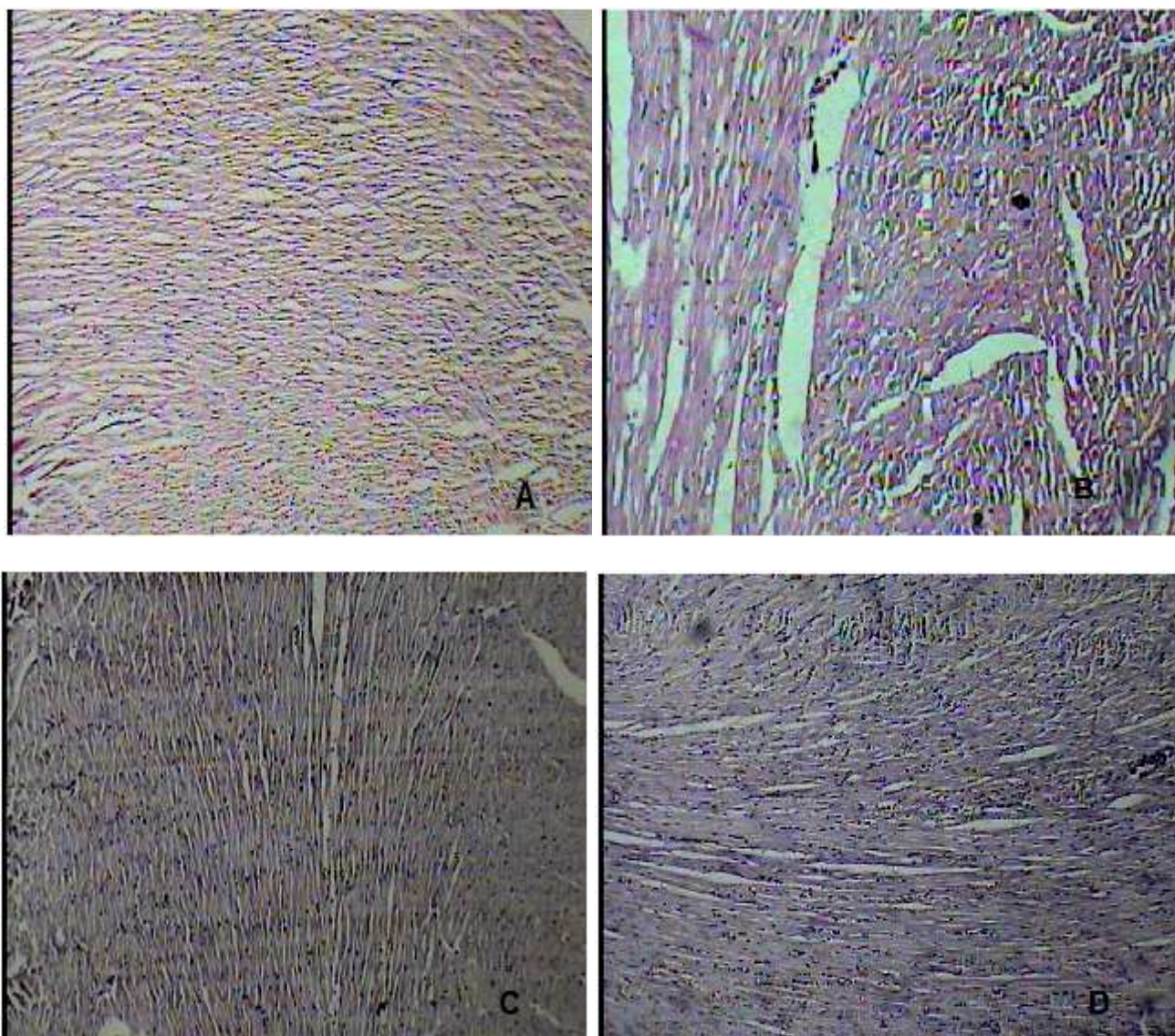


Fig. 2: Histopathological images of heart pretreated with methanolic extract of *Ixora coccinea* Linn. flowers (MICF) by doxorubicin induced cardiac toxicity. A – Normal, B – Control, C – MICF – 200 mg/kg, D - MICF – 400 mg/kg.

Discussion

The mechanism by which Dox acts is by the formation of an iron-anthracycline complex that generates free radicals, which in turn, causes severe damage to the plasma membrane, and interferes with the cytoskeletal structure [13]. Oxygen free radical formation by doxorubicin [14, 15] enhance the susceptibility of cardiac tissue to lipid peroxidation leading to a progressive dose – related irreversible loss of myofibrils, dilation of the sarcoplasmic reticulum, cytoplasmic vacuolization, swelling of mitochondria, increased number of lysosomes and myocyte necrosis [16], inhibition of nucleic acid as well as protein synthesis [17], release of vasoactive amines [18], change in adrenergic function [19], decreased activity of Na⁺ K⁺ ATPase [20], alteration in sarcoplasmic calcium transport, imbalance of myocardial electrolytes in response to the doxorubicin [21].

The present study also evidenced the formation of free radicals which brought about the hemodynamic, biochemical and the histopathological changes in rats treated with doxorubicin.

Doxorubicin treated animals have shown scruffy fur as well as significantly decreased body weight, heart weight and heart/body weight ratio. The decrease in body weight in this study is in accordance with other studies [22] and it may be attributed to reduced food intake and inhibition of protein synthesis due to Dox treatment compared to normal group. Our study demonstrated a significant increase in the bodyweight gain, heart weight and heart/body weight ratio when compared with the control, also the animal were normal and energetic against the lethargic control group. The red exudates from eyes and nose were also reduced to normal.

ECG was monitored owing to the fact that the severity of changes in ECG are parallel to the known clinical Dox cardiotoxicity [23]. Doxorubicin treatment causes prolongation of P wave, QRS complex, QT interval and RR interval while reduces cardiac cycle [24]. The ST segment elevation was also observed. The consecutive loss of cellular membrane damage due to oxidative stress might be characterized by ST elevation [25]. Treatment with MICF reflected reduction in P wave, QRS complex, QT interval and RR interval while cardiac cycle was increased, the ST segment was also near to normal. The ECG changes shown by the methanolic extract of *I. coccinea* Linn. flowers may possess protective effect or cell membrane stabilizing action on the myocardium.

Dox cause a decrease in the systolic, diastolic, mean BP and heart rate [26], this is probable due to effect of Dox on the myofibrils, causes its disruption [27] hence the systolic, diastolic, mean BP and heart rate decreases. Our study demonstrated an increase in the systolic, diastolic, mean BP and the heart rate when compared with the control. Stabilization of the myocardium due to extract, causes the decrease in the myofibrils disruption and shifts the blood pressure close to normal.

Doxorubicin causes an elevation in levels of CK-MB, LDH, SGOT and SGPT when compared with the normal. Deficiency of oxygen supply or glucose supply may cause damage to the myocardial cell membrane leading to permeable and ruptures so that the enzymes leaks out. These enzymes are also called as specific biomarkers, can be estimated to check the damage. Treatment with MICF flowers has shown a significant decrease in the level of these enzymes suggesting the protective or membrane stabilizing effect of the extract on the myocardium.

Oxidative stress and mitochondrial dysfunction are associated with disease and toxic process. It results from over production of ROS, often leading to peroxidation of membrane phospholipids and production of reactive aldehydes [28]. Treatment with doxorubicin cause a decrease in the antioxidant stores of the heart viz. catalase and superoxide dismutase while the extent of lipid peroxidation increases when compared with the normal. Our study demonstrated a significant increase in the endogenous antioxidant stores of CAT and SOD while the MDA levels were decreased when compared with the control. These results indicate the protective effect or free radical scavenging effect of the MICF in oxidative damage done by doxorubicin. The presence of antioxidant constituents such as flavonoids and tannins might be responsible for the free radical scavenging and antioxidant activity of the extract.

Histopathological examination of myocardial tissue obtained from normal animal exhibited clear integrity of myocardial membrane. The heart sections obtained from Dox treated animals showed disruption of several subcellular elements including loss of myofibrils, swelling of mitochondria, vacuolization of the cytoplasm, formation of lysosomal bodies and dilation of the sarcotubular system [29]. Treatment with the MICF flowers demonstrated less disruption of the myofibrils and less vacuolization of the cytoplasm. This further confirms the membrane stabilizing effect of the extract.

Conclusion

The present study clearly emphasize the cardioprotective effect of methanolic extract of *Ixora coccinea* Linn. flowers on doxorubicin induced cardiac myopathy. *Ixora coccinea* Linn. flowers proved effective in maintaining the hemodynamic, electrocardiographic, biochemical and histopathological parameters close to normal, by boosting the endogenous antioxidant stores and blunting the oxidative stress. Further studies focusing on the isolation, characterization and purification of the active constituent and elucidating the exact mechanism of action is to be carried out.

Acknowledgement

The author would like to thank Guide, Dr. Mrs. N. S. Naikwade and Co-Guide, Mrs. Tabassum Shikalgar. The author extends sincere thanks to the Principal, Prof. D. D. Choughule for providing excellent laboratory facilities and last but not the least Mr. Kalai Bharatesh for his help during the work.

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