

## Cardioprotective Effect of *Lagenaria siceraria* (Molina) Standley (Cucurbitaceae) Fruit Juice on Doxorubicin Induced Cardiotoxicity in Rats

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**Abstract: Problem statement:** The cardio toxicity of Doxorubicin (Dox) has limited its use as an anticancer drug. The aim of present study was to investigate the cardio protective effect of *Lagenaria Siceraria* (Cucurbitaceous) Fruit Juice (LSFJ) against the cardio toxicity of Dox in rats. **Approach:** Male wistar rats (250-300 g) were randomly divided into four groups. Group I- was control, Group II- Dox (10 mg kg<sup>-1</sup>), groups III and IV- Dox + LSFJ (5 and 10 mL kg<sup>-1</sup> for 18 days). Dox (10 mg kg<sup>-1</sup> iv) was administered in group II, III and IV on day 16. After anesthetizing the animals on the 18th day, Electrocardiogram (ECG) was recorded and blood was investigated for Creatine Kinase-MB is enzyme (CK-MB), Lactate Dehydrogenase (LDH) and Aspartate Aminotransferase (AST) while determination of Superoxide Dismutase (SOD), Reduced Glutathione (GSH), Lipid Per Oxidation (LPO) and histopathology was carried out for heart. **Results:** Group 3 and 4 of animals showed decreased QT interval (p<0.05) and was non significant in ST interval and heart rate compared to Group 2. Significant decrease in serum CK-MB and AST of group 3 (p<0.05) and group 4(p<0.01) of animals was observed as compared to group II. In group 4 significant increase in the level of GSH (p<0.01) and decrease in MDA content (p<0.001) was observed as compared to group 2, whereas SOD was non-significant. Histopathological study of LSFJ10 mL kg<sup>-1</sup> treated group showed protection against myocardial toxicity induced by doxorubicin. **Conclusion:** It is concluded that *Lagenaria siceraria* fruit juice possessed cardio protective effect at dose of 10 mL kg<sup>-1</sup> against doxorubicin induced cardio toxicity in rats.

**Key words:** Antioxidant, electrocardiogram, histopathology, tissue parameters

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### INTRODUCTION

*Lagenaria siceraria* (Molina) Standley (Cucurbitaceae) fruit (bottle gourd), a commonly used vegetable in India is described as cardiogenic (Deshpande *et al.*, 2008). Doxorubicin, an anthracycline antibiotic, has a broad antitumor spectrum and has been used against a wide variety of hematopoietic malignancies and solid tumors (Ogura, 2001). Unfortunately, the cardio toxicity of Dox, resulting in a cardiomyopathy with irreversible congestive heart failure and high mortality, is one of the main factors that limit its use. The molecular mechanisms explaining the cardio toxicity of anthracyclines are complex, but it

appears that the induction of an oxidative stress within myocardial tissue constitutes a common denominator (Vergely *et al.*, 2007). It is hypothesized that antioxidants and iron-chelators may protect the heart against Dox toxicity. Dexrazoxane is an iron chelator and possesses potent antioxidant properties and has been reported to prevent Dox induced cardio toxicity. However, due to the high incidence of dexrazoxane-induced myelosuppression, its use has been limited to some advanced stages of malignant disorders (Seifert *et al.*, 1994). On the other hand, many plants have been reported to possess antioxidant activity. There is paucity of reports on the cardio protective activity of *Lagenaria siceraria* in Dox-induced cardio toxicity.

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The objective of the present study was to investigate the protective effects of LS fruit juice against Dox-induced cardio toxicity in rats.

## MATERIALS AND METHODS

**Animals:** Male Wistar albino rats (250-300 g) obtained from Lupin Research Park, Pune, were housed under standard housing conditions of 25°C, relative humidity 60% and photo period of 12 h dark/12 h light. Pellet diet (Chakan Oil Mills, Pune, India) and water were provided *ad libitum*. The protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Poona College of pharmacy, Bharati Vidyapeeth University, Pune, India.

**Chemicals and sample:** The fruit of LS was purchased locally and authenticated by Dr. A.M. Mujumdar of Agharkar Research Institute, Pune, India. Doxorubicin powder was gifted by Serum Institute of India Ltd., Pune. CK-MB kit was purchased from Randox Laboratories Ltd., Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom, BT29 4QY and LDH as well as AST from Ecoline, Merck Ltd, Worli, Mumbai.

All solvents/chemicals used were of analytical grade and chemicals required for sensitive biochemical assays such as 5, 5-Dithiobis (2-Nitro Benzoic acid) (DTNB) and Thiobarbituric Acid (TBA) were purchased from Sigma Chemical Co., USA.

**Experimental procedure:** The animals were randomly divided into four groups (Group 1-control, Group 2-Dox, Group 3-LSFJ5 mL kg<sup>-1</sup> and Group 4-LSFJ 10 mL kg<sup>-1</sup>) containing eight rats in each group. The body weights were recorded daily. LSFJ was administered (5 and 10 mL kg<sup>-1</sup>) orally to rats of group 3 and 4 daily for 18 days whereas Group 1 and 2 received distilled water. The control group received a single parenteral dose of sterile water for injection (1 mL kg<sup>-1</sup>) through femoral vein on the 16th day, while the groups 2, 3 and 4 received Dox (10 mg kg<sup>-1</sup>) dissolved in sterile water and injected within 1 h. After 48 h of the injection, the rats were anaesthetized by anesthetic ether and ECG was recorded using 8 channels Power Lab System (AD Instruments Pty. Ltd., Unit 13, 18-22 Lexington Drive, Bella Vista NSW 2153, Australia) and the changes in QT and ST intervals and heart rate were computed from the ECG. Blood was withdrawn from Retro Orbital Plexus (ROP) for measuring serum parameters. The animals were allowed to recover and finally euthanized on the 18th day. The hearts of

animals were removed and weighed. Four hearts were randomly selected for histopathology. The heart tissues from remaining four animals were processed and homogenized in 10% chilled tris hydrochloride buffer (10 mM, pH 7.4) by tissue homogenizer (Remi Motors, Mumbai, India) and centrifuged at 7500 rpm for 15 min at 4°C using Eppendorf 5810-R high speed cooling centrifuge. The clear supernatant was used for the estimation of SOD, GSH, MDA content and total protein.

**Serum parameters:** Serum levels of CK-MB, LDH and AST enzymes were measured by automated chemistry analyzer, Micro lab 300, Merck, using reagent kits.

**Tissue parameters: Lipid peroxidation assay (MDA content):** This assay method was described by Slater and Sawyer (1971). In this method freshly prepared 0.67% w/v thiobarbituric acid reagent was used and the absorbance was measured by UV/VIS spectrophotometer (JASCO-V-530, Japan) at 532 nm. Calibration curve was prepared by using 1, 1, 3, 3-tetraethoxypropane as a standard.

**Estimation of GSH:** The assay of GSH was carried out using DTNB reagent as per the method described by Moron *et al.* (1979). The color developed was read at 412 nm against reagent blank. Standard glutathione was in the range of (10-50 µg) and the content of reduced glutathione was expressed as µg of GSH g<sup>-1</sup> of protein.

**Estimation of SOD activity:** The SOD activity was assayed by the change in the optical density min<sup>-1</sup> at 480 nm of the reaction initiated by epinephrine bitartrate (3 mM) as described by Misra and Fridovich (1972). SOD activity was expressed as units mg<sup>-1</sup> protein. Change in optical density per min at 50% inhibition of epinephrine to adrenochrome transition by the enzyme was taken as the enzyme unit. Calibration curve was prepared by using 10-125 units of SOD.

**Determination of total protein:** Protein concentrations were determined using the method of Lowry *et al.* (1951). Diluted membrane fraction aliquots were taken and color developed by adding of 1N Folin's phenol reagent was measured at 640 nm against reagent blank. Different concentrations (40-200 µg) of standard protein Bovine Serum Albumin (BSA) were used for standard curve. The values were expressed as mg of protein g<sup>-1</sup> of wet tissue (mg g<sup>-1</sup>).

**Histopathological studies:** Heart preserved in 10% formalin, processed and embedded in paraffin. Four  $\mu\text{m}$  thick paraffin sections were cut on glass slides and stained with Hematoxylin and Eosin (H and E) reagents and observed under light microscope to evaluate myocardium injury.

**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  $\pm$  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 with correction for multiple comparisons by the Tukey's test. P-value less than 0.05 was considered statistically significant. The entire statistical analysis was performed using statistical package called Graph pad prism version 4.03 (Graph pad software, Inc. USA).

## RESULTS

**ECG changes:** Doxorubicin administration significantly increased QT ( $p < 0.01$ ) and ST ( $p < 0.05$ ) intervals compared to control group. LSFJ 10 mL  $\text{kg}^{-1}$  administration significantly decreased QT interval ( $p < 0.05$ ) while heart rate and ST interval were non significant compared to Dox group (Table 1).

**Serum parameters:** Administration of Dox (10 mg  $\text{kg}^{-1}$ ) increased serum CK-MB, LDH and AST levels compared to that of control, whereas LSFJ (5 and 10 mL  $\text{kg}^{-1}$ ) administration decreased the levels of CK-MB and AST significantly compared to that of Dox alone. The level of LDH decreased non significantly (Table 2).

**Tissue parameters:** There was a significant increase ( $p < 0.01$ ) in MDA contents in the Dox group compared to the control group. Significant decrease in the level of MDA contents was observed in group IV ( $p < 0.001$ ) in comparison to group II. Significant ( $p < 0.001$ ) decrease in myocardial GSH was observed in Dox group compared to control group. In group IV there was significant increase ( $p < 0.01$ ) of myocardial GSH compared to Dox group. In groups 3 and 4 increase in

SOD was not statistically significant compared to Dox group, whereas SOD in Dox group decreased significantly ( $p < 0.05$ ) compared to control group (Table 3).

**Histopathological changes:** The incidence of marked tissue injury with myocardial atrophy, nuclear pyknosis, cytoplasmic vacuoles and cytoplasmic eosinophilia were observed in doxorubicin treated group. Control group showed normal myocardial structure. Treatment of LS FJ 5 mL  $\text{kg}^{-1}$  for 18 days showed week protection against doxorubicin induced myocardial damage, whereas LSFJ 10 mL  $\text{kg}^{-1}$  for 18 days showed moderate protection. There was no change in the incidence of inflammation and vascular congestion in the four study groups (Fig. 1).

Table 1: Effect of Dox (10 mg  $\text{kg}^{-1}$ ) and LSFJ (5 and 10 mL  $\text{kg}^{-1}$ ) + Dox on ECG changes

Groups/ treatment $\text{kg}^{-1}$	ST interval (m sec)	QT interval (m sec)	Heart rate (bpm)
Control	36.00 $\pm$ 1.95	66.88 $\pm$ 1.56	353.8 $\pm$ 16.17
Dox 10 mg	43.14 $\pm$ 1.86 <sup>*</sup>	76.86 $\pm$ 1.99 <sup>**</sup>	308.3 $\pm$ 12.47 <sup>NS</sup>
LSFJ 5 mL + Dox 10 mg	38.25 $\pm$ 1.25 <sup>NS</sup>	69.38 $\pm$ 1.56 <sup>+</sup>	300.5 $\pm$ 14.13 <sup>NS</sup>
LSFJ 10 mL + Dox 10 mg	37.75 $\pm$ 2.21 <sup>NS</sup>	67.50 $\pm$ 1.85 <sup>+</sup>	335.7 $\pm$ 24.50 <sup>NS</sup>

**Note:** Values are mean  $\pm$  SEM; <sup>\*</sup>: p-value compares Dox with control group while <sup>+</sup>: p-value compares Dox with LSFJ + Dox groups. <sup>\*</sup>:  $p < 0.05$ , <sup>\*\*</sup>:  $p < 0.01$ , <sup>+</sup>:  $p < 0.05$ , NS: Non Significant

Table 2: Changes in the levels of CK-MB, LDH and AST enzymes in the four experimental groups

Groups/ treatment $\text{kg}^{-1}$	CK-MB (IU L <sup>-1</sup> )	LDH (IU L <sup>-1</sup> )	AST (IU L <sup>-1</sup> )
Control	82.8 $\pm$ 15.71	179.8 $\pm$ 19.97	201.2 $\pm$ 24.59
Dox 10 mg	220.8 $\pm$ 18.83 <sup>***</sup>	393.4 $\pm$ 38.54 <sup>***</sup>	377.4 $\pm$ 15.53 <sup>***</sup>
LSFJ 5 mL + Dox 10 mg	153.0 $\pm$ 4.74 <sup>+</sup>	308.6 $\pm$ 10.07 <sup>NS</sup>	297.8 $\pm$ 20.2 <sup>+</sup>
LSFJ 10 mL + Dox 10 mg	126.0 $\pm$ 20.01 <sup>++</sup>	293.0 $\pm$ 11.16 <sup>NS</sup>	270.3 $\pm$ 11.91 <sup>++</sup>

**Note:** Values are expressed as mean  $\pm$  SEM; <sup>\*</sup>: p value compares Dox with control group while <sup>+</sup>: p value compares Dox with LSFJ + Dox groups. <sup>\*\*\*</sup>:  $p < 0.001$ , <sup>+</sup>:  $p < 0.05$ , <sup>++</sup>:  $p < 0.01$ , NS: Non Significant

Table 3: The levels of MDA, GSH and SOD in the heart tissue of four experimental groups in mg of protein

Groups/ treatment $\text{kg}^{-1}$	Lipid peroxidation (nM of MDA)	GSH ( $\mu\text{g}$ of GSH)	SOD (U)
Control	2.69 $\pm$ 0.21	27.51 $\pm$ 0.83	9.24 $\pm$ 0.91
Dox 10 mg	4.09 $\pm$ 0.21 <sup>**</sup>	17.79 $\pm$ 1.16 <sup>***</sup>	4.76 $\pm$ 0.79 <sup>**</sup>
LSFJ 5 mL + Dox 10 mg	3.83 $\pm$ 0.15 <sup>NS</sup>	8.26 $\pm$ 0.41 <sup>NS</sup>	5.99 $\pm$ 0.53 <sup>NS</sup>
LSFJ 10 mL +Dox 10 mg	2.59 $\pm$ 0.21 <sup>+++</sup>	23.46 $\pm$ 0.78 <sup>++</sup>	6.58 $\pm$ 0.77 <sup>NS</sup>

**Note:** Values are expressed as mean  $\pm$  SEM; <sup>\*</sup>: p-value compares Dox with control group while <sup>+</sup>: p-value compares Dox with LSFJ + Dox groups; <sup>\*\*</sup>:  $p < 0.01$ ; <sup>\*\*\*</sup>:  $p < 0.001$ ; <sup>++</sup>:  $p < 0.01$ ; <sup>+++</sup>:  $p < 0.001$ , NS: Non Significant

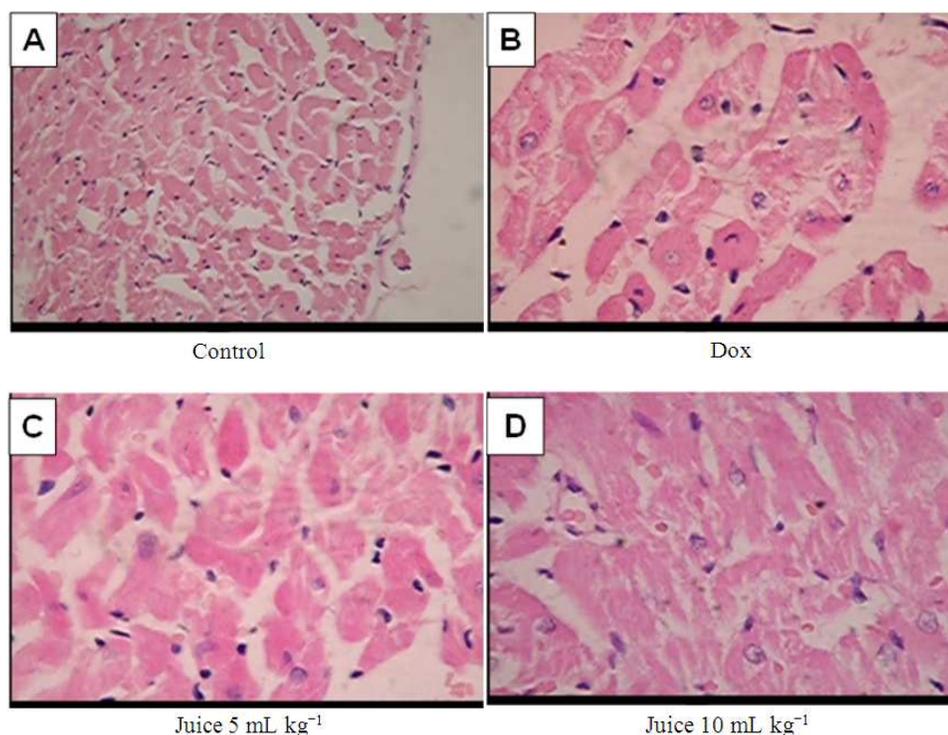


Fig. 1: Histopathological changes of rat heart (A) Control: Normal histological structure of rat heart; (B) Treated with Dox ( $10 \text{ mg kg}^{-1}$  on day 16), showing severe nuclear pyknosis and cytoplasmic vacuoles in the rat heart; (C) Treated with LSFJ ( $5 \text{ mL kg}^{-1}$  for 18 day) + Dox showing moderate to severe type of nuclear pyknosis and cytoplasmic vacuoles than Dox treated animals. (D) Treated with LSFJ ( $10 \text{ mL kg}^{-1}$  for 18 day) + Dox showing mild type of nuclear pyknosis and cytoplasmic vacuoles than Dox treated animals. (Magnification 40X and 100X)

## DISCUSSION

Doxorubicin, which possesses potential for generating free radicals, causes an unusual and often irreversible cardiomyopathy. It has quinone and hydroquinone moieties that can form semiquinone radical intermediates, which in turn can react with oxygen to produce superoxide anion radicals. These can generate both hydrogen peroxide and hydroxyl radicals ( $\cdot\text{OH}$ ), which attack DNA and oxidize DNA bases (Serrano *et al.*, 1999). The production of free radicals is significantly stimulated by the interaction of Dox with iron (Chabner *et al.*, 2006). Doxorubicin treatment changes ECG and causes prolongation of QT interval in rats (Cirillo *et al.*, 2000).

The results of present study clearly demonstrated that there was increase in myocardial injury as indicated by increase in QT and ST intervals of ECG pattern in Dox treated group. Administration of LSFJ along with Dox ( $10 \text{ mg kg}^{-1}$ ) restored QT and ST intervals close to control level.

The results also demonstrated that acute administration of doxorubicin induced cardio toxicity manifested by a significant increase in serum CK-MB, AST and LDH. These results are consistent with previous studies (Mostafa *et al.*, 1999; Sayed-Ahmed *et al.*, 2001; Saad *et al.*, 2001). In the present study the serum levels of CK-MB and AST in LSFJ treated groups decreased significantly.

The most abundant Reactive Oxygen Species (ROS) generated in living cells are superoxide anion and its derivatives, particularly highly reactive and damaging hydroxyl radical, which induces peroxidation of cell membrane lipid (Hemnani and Parihar, 1998). In this respect, any increase in SOD activity of the organ appears to be beneficial in the event of increased free radical generation. A single dose administration of Dox ( $10 \text{ mg kg}^{-1}$ ) caused a significant increase in MDA content (an index of lipid peroxidation) in cardiac tissues and decrease in myocardial GSH compared to control group indicating an increase in oxidative stress.

Table 4: The average of heart weight to body weight ratio in four study groups

Groups/treatment/kg	Heart weight to body weight ratio
Control	2.72±0.02×10 <sup>-3</sup>
Dox 10 mg	2.67±0.11×10 <sup>-3</sup>
LSFJ 5 mL + Dox 10 mg	2.69±0.04×10 <sup>-3</sup>
LSFJ 10 mL + Dox 10 mg	2.71±0.07×10 <sup>-3</sup>

**Note:** Values are expressed as mean ±SEM. There was no statistical significant difference between the averages of ratio across the groups

These results correlate with previous studies which have demonstrated the involvement of oxidative stress and lipid peroxidation in doxorubicin induced cardiomyopathy (Fadillioglu *et al.*, 2004; Patil and Balaraman, 2005). Administration of LSFJ improved the biochemical marker levels indicating decrease in oxidative stress as evident by increased level of GSH and SOD with decreased lipid peroxidation. LSFJ contains polyphenolic compounds (6.8 mg/10 mL of juice, obtained by our group). The antioxidant activity of polyphenolic compounds from others plants have been reported earlier (Karou *et al.*, 2005; Maisuthisakul *et al.*, 2007).

Histopathological studies revealed myocardial atrophy, nuclear pyknosis, cytoplasmic vacuoles and cytoplasmic eosinophilia in Dox treated rat heart. Similar observations have also been made in earlier studies on acute doxorubicin induced cardiotoxicity (Saad *et al.*, 2001; Morishima *et al.*, 1998). Histopathological observations showed less damage in LSFJ 10mL/kg + Dox group than Dox group.

Doxorubicin also causes decrease in heart weight to body weight ratio, which indicates loss of myofibrils and cytoplasmic vacuolization in myocytes (Mukherjee *et al.*, 2003). In this study the average of heart weight to body weight ratio in Dox group was not statistically significant compared to control group as well as LSFJ + Dox treated groups Table 4.

### CONCLUSION

It is thus concluded that LSFJ administration prevented Dox-induced cardiotoxicity and decreased myocardial injury by preservation of endogenous antioxidants and reduction of lipid peroxidation in rat heart.

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