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Original Article

Evaluation of Fractions of *Artocarpus lakoocha* R. on Doxorubicin Induced Myocardial Toxicity in Wistar Rats

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ABSTRACT

Background: Doxorubicin, an anthracycline derivative, is a cytotoxic drug that has been shown to be effective against a variety of cancers. Its clinical utility has been limited due to dose-dependent cardiac toxicity. **Objectives:** To fractionate the ethanolic extract of bioactive *Artocarpus lakoocha* Roxb. bark and to Phytochemical investigation of fractions and pharmacological evaluation on doxorubicin induced cardiotoxicity in rats. **Materials and Methods:** *In vitro* studies conducted to determine Cardioprotective activity of the fractions of ethanolic plant extract. Cardiotoxicity induced with DOX (2.5 mg/kg body weight *i.p*) in 6 equal injections given alternatively for 2 weeks to make a total cumulative dose of 15 mg/kg body weight. Comparative studies were done between Treatment group, Dox 2.5 mg/kg on alternative day for 2 weeks followed by fractionation method-Petroleum ether fraction (11.2 mg/kg), Chloroform fraction (66 mg/kg), Ethyl acetate fraction (34 mg/kg) and aqueous fraction (44.4 mg/kg) of *Artocarpus lakoocha* R. for 2 weeks. Animals were observed for change in body weight, food and water intake ECG changes, hematology analysis such as RCB, WBC, Platelet, hematocrite level, Cardiac markers such as CK-MB, LDH and cTn I, Antioxidant enzymes such as GSH, Catalases, SOD and MDA were monitored. Heart histopathological studies carried out to evaluate cardiotoxicity. **Results:** As a result of Dox treatment, cardiomyopathy develops, which is characterized by an increase in cardiac biomarkers, changes in ECG, hematology analysis and a deficiency in antioxidant enzymes. By lowering the elevated levels of biomarker enzymes like LDH and CK-MB and the absence of CTnI, Fractions of EAAL significantly protected the myocardium from the toxic effects of Dox. **Conclusion:** Based on results of general appearance, hematology analysis, ECG studies, specific cardiac markers and antioxidant activity, it is concluded that the ethyl acetate fraction of *Artocarpus lakoocha* R. exhibited cardioprotective activity. Based on data obtained, treatment studies were found more effective when compared with preventive studies. Phytocompounds present in the plant is responsible for the cardioprotective activity.

Keywords: Doxorubicin, *Artocarpus lakoocha* R., Cardiotoxicity, Oxidative stress, Rats.

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INTRODUCTION

Cardiovascular Diseases (CVDs) are becoming increasingly common illnesses, mostly as a result of contemporary dietery habits and lifestyle choices.[1] As a result, CVDs are the world's leading cause of mortality deaths annually. A set of conditions known as CVDs impact the heart and blood vessels. These conditions include heart disease, stroke, myocardial infraction, cerebrovascular disease,[2] rheumatic heart disease,[2] angina pectoris, hypertension,[1] and other circulatory diseases.

Cancer is one of the major global causes of death.[3] Doxorubicin (DOX) is a quinine- containing chemotherapeutic drug, produced by bacteria Streptomyces peucetius,[4] used to treat cancers such as multiple myeloma, acute leukaemia, ovarian, bladder, and thyroid diseases.[5] Although an effective anticancer medication, it can cause toxicity such as nausea, vomiting, alopecia, hematopoietic suppression, and cardiotoxicity.

DOX's therapeutic use is limited primarily due to cumulative dose-related cardiotoxicity, which can result in cardiomyopathy, cardiac failure,[6] arrhythmia,[7] or left ventricular dysfunction. Doxorubicin causes cardiotoxicity by various biological processes, including oxidative stress, lipid peroxidation, DNA damage, mitochondrial injury, apoptosis, and autophagy.[8,9] Among them Oxidative stress is a critical step in DOX-induced cardiac injury.

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MATERIALS AND METHODS

In vitro experimental validation

Plant material

The barks of *Artocarpus lakoocha* L. were collected from Uttarakannada District in Karnataka, in the month of August 2023. Identified and Authenticated by S. N. Emmi H.O.D of Botany Department. of H.S. Kotambari Science Institute Vidyanagar, Hubballi. (Ref no: Klecoph/HBL//2023-24).

Preparation of Ethanolic extract

The dried barks have been ground up into small fragments and powdered. The soild extract was produced by using Soxhlet extraction (Continuous hot extraction) using 70% Ethanol. Dried, coarse powder of plant material was placed inside the porous thimble. The round-bottom flask is filled with a suitable solvent, which is heated to reflux. The solvent vapor rises, condenses in the condenser, and drips onto the solid material in the Soxhlet extractor. The solvent dissolves the target compounds from the solid, and the solution is then drained back into the flask. This extraction process continues cyclically, until the solvent flowing from extraction chamber not leave any residue behind, allowing for efficient extraction of the desired compounds. Solvent from the extract was then removed using steam distillation to have gummy concentrate of extract which were used in the present study.[10,11]

Preparation of *Artocarpus lakoocha* R. extract Fractions

Ethanolic extract were further fractioned by solvent- solvent partition with 4 different solvents of polarity i.e. petroleum ether, ethyl acetate and chloroform, water. Ten (10) g of ethanolic extract was reconstituted with 400 mL distilled water (stock solution). After that, the material is transferred into a funnel for separation, shaken, and given time to settle. The least polar solvent, petroleum ether, 120 mL, was added and mixed. In order to obtain petroleum ether fractions, ethyl acetate and chloroform, and water based on their relative polarity, the solution was divided three times in succession using petroleum ether, chloroform fraction, and ethyl acetate in a separating funnel. The aqueous layer can be removed by opening the bottom of the separating funnel once the substance has had time to settle. To obtain the petroleum ether fraction, the leftover material in the separating funnel was transferred into a sanitised container. The cycle for the ethyl acetate and chloroform fractions was similar. Since water was first used to dissolve the crude extract, the leftover amount after fractionation is known as residual aqueous fraction, or RAF for short.[12,13]

Chemicals and drugs

Doxorubicin was purchased from other analytical grade compounds, whereas enzyme assay kits were obtained from Sigma and ERBA.

Animals

Male and Female Wistar Albino rats weighing about 150-200 g was procured from KLE college of Pharmacy, Hubballi, Karnataka. Animals will be housed in group of six in polypropylene cages maintained at room temperature (25+2°C) under 12 hr light and dark cycles with free access to standard pellet diet and water *ad libitum*. All the experimental procedures were carried out in accordance with CPCSEA guidelines95). Experimental protocols were reviewed and approved by IAEC of KLEU's College of Pharmacy, Hubballi, Karnataka, India. (Proposal number: MPh/NC0222009/KLECoPH/23).

Preliminary phytochemical screening

The concentrated extract was used for preliminary screening of Phytochemicals investigations Flavonoids, Glycoside, Alkaloids, Steroids, Saponins, Tannins, Carbohydrates.[11]

Acute Oral Toxicity

The acute oral toxicity in this investigation toxicity of *Artocarpus lakoocha* R. was carried using nulliparous, non-pregnant female rats according to OECD 423 guideline. The dose of Dox was selected based on previous literature.[14]

Experimental design

- Group I-Received vehicle 5mL/kg for two weeks followed by saline *i.p* for two weeks.
- Group II-Animal will be treated with DOX (2.5 mg/kg body weight *i.p*) in 6 equal injections alternatively for 2 weeks to make a total cumulative dose of 15 mg/kg body weight.
- Group III-Animal will be treated with DOX followed by treatment with Vitamin E (100 mg/kg body weight *p.o*).
- Group IV-Animal will be treated with DOX followed by treatment with Petroleum ether fraction (11.2 mg/kg body weight *p.o*).
- Group V-Animal will be treated with DOX followed by treatment with Chloroform fraction (66 mg/kg body weight *p.o*).
- Group VI-Animal will be treated with DOX followed by treatment with Ethyl acetate fraction (34 mg/kg body weight *p.o*).

- Group VII-Animal will be treated with DOX followed by treatment with Aqueous fraction (44.4 mg/kg body weight *p.o*).

Food, water and ECG

Food and water consumption were regularly monitored, and an ECG (Biopac M35),[15,16] hematoanalysis was obtained both before and after the procedure.

Enzyme assays

After 36 hr of therapy, blood was collected from the retro-orbital plexus under light ether anaesthesia and heparinized for biomarker assessment. Lactate Dehydrogenase (LDH),[15] Creatinine Phosphokinase-MB (CK-MB)[16] and Cardiac Troponin-I (cTnI)[17] are all measured in one step using the Troponin I test. Later animals were sacrificed under anaesthesia, followed by a rapid dissection of the cardiac tissue. The cardiac tissue was washed in ice-cold saline, dried and weighed immediately. The hearts of all animals were collected for determination of endogenous antioxidants such as Glutathione (GSH), Melonldehyde (MDA), Superoxide Dismutase (SOD) and Catalase (CAT) later remaining cardiac tissue was used for histopathological analysis.

Histopathological studies

After collecting the blood for the estimation of various markers, animal was sacrificed and heart was isolated, washed with saline and weight was determined by recording relative heart weight with respect to body weight. The isolated hearts were preserved in 10% neutral formalin solution for histopathological studies. Processing of isolated heart.[18]

Statistical analysis

The experimental data were statistically analysed using one-way analysis of variance ANOVA followed by Bonferroni's Multiple Comparison Test. Results will be expressed as Mean±SEM and difference were considered significant at $p<0.05$ using GraphPad Prism® software.[14]

RESULTS

Plant material study

The Percentage yield of ethanolic extract of crudely powdered *Artocarpus lakoocha* R. obtained by Soxhlet extraction process using ethanol as solvent was found 26.66%.

Phytochemical investigation of extract

The qualitative chemical investigation of ethanolic extract of *Artocarpus lakoocha* R. was carried out to check the presence of various Phytoconstituents in extract and the result are given in Table 1.

Preparation of fractions

The Percentage yield of fractions of *Artocarpus lakoocha* R. obtained by Separating funnel fractionation process using 4 different solvents i.e. petroleum ether, chloroform, ethyl acetate, aqueous (Table 2).

Acute oral toxicity studies of *Artocarpus lakoocha* R.

The dose of plant extract was selected based on previous literature.[14] Hence, the maximum dose was set up to be 2000 mg/kg. Therefore 1/10th (200 mg/kg) was selected as high dose respectively. Further fractions were calculated as per percentage yield-pet. Ether fraction (11.2 mg/kg), ethyl acetate fraction (34 mg/kg), chloroform fraction (66 mg/kg), aqueous fraction (44.4 mg/kg). Dose of doxorubicin (2.5 mg/kg body weight *i.p*) in 6 equal injections alternatively for 2 weeks to make a total cumulative dose of 15 mg/kg body weight. and vitamin E (100 mg/kg) was used in the current study.

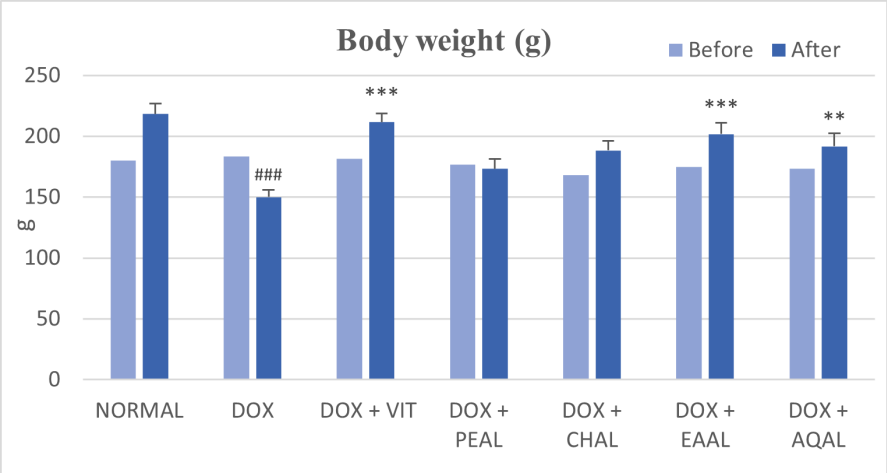

Figure 1: Effect of fractions of *Artocarpus lakoocha* R. on Body weight Values are Mean+SEM; $n=6$ in each group, ### $p<0.001$ when compared to control, *** $p<0.001$, ** $p<0.01$ when compared to DOX using one-way ANOVA (Bonferroni's Multiple Comparison Test).

Table 1: Preliminary phytochemical test.

Phytoconstituens	Results
Carbohydrates	+
Flavonoids	+
Alkaloids	+
Glycosides	+
Resins	-
Steroids	+
Tannins	+
Saponins	+

Table 2: Percentage yield of fractions.

Solvent	% Yield
Pet.ether Fraction	5.6%
Chloroform Fraction	33%
Ethyl acetate Fraction	17%
Aqueous Fraction	44.4%

General appearance

Animals were observed for their general appearance throughout the study period. Dox Induced groups developed red exudates around eye, pink tinge, fur become scruffy and soft watery faeces. These changes were less observed in Pet. ether, Chloroform and Aqueous fraction treated groups and these changes were not observed in vitamin E and Ethyl acetate fraction treated groups.

Body Weight

Figure 1, depicts that body weight decreased slowly in Dox induced group when compared with normal control (control vs DOX $p<0.001$). Vitamin E treated group and Fractions treated group shown significant increase in body weight when compared with Dox (Vitamin E, $p<0.001$; DOX+EAAL, $p<0.001$; DOX+AQAL, $p<0.01$). DOX+PEAL and DOX+CHAL treated groups failed to significantly increase body weight.

Food and Water Intake

In DOX treated group food and water consumption were significantly reduced as when compared with normal control (control vs DOX $p<0.001$). Vitamin E treated group, extract treated groups such as DOX+EAAL and DOX+AQAL shown significant changes with food and water intake when compared with Dox (Vitamin E, $p<0.001$; DOX+EAAL, $p<0.001$; DOX+AQAL). DOX+PEAL and DOX+CHAL treated groups failed to show significant changes in food and water intake compared with Dox (Figure 2).

Haematology analysis

In Dox treated group food and water WBC, RBC, PLT and hematocrite level was gradually reduced as when compared with normal control (control vs DOX $p<0.001$). Vitamin E treated group, fractions treated groups such as DOX+EAAL and DOX+AQAL shown significant changes with WBC, RBC, PLT and hematocrite level when compared with Dox (Vitamin E, $p<0.001$; DOX+EAAL, $p<0.001$; DOX+AQAL). DOX+PEAL and DOX+CHAL treated groups failed to show significant changes in WBC, RBC, PLT and hematocrite level compared with Dox (Table 3).

Serum Cardiac Biomarkers

Only Dox treated animals showed the elevation in CK-MB and LDH enzymes when compared with control group (control vs DOX $p<0.001$). Vitamin E treated group, extract treated groups such as DOX+EAAL and DOX+AQAL shown significant decrease in Cardiac biomarkers when compared with Dox treated group. Dox (Vitamin E, $p<0.001$; DOX+EAAL, $p<0.001$; DOX+AQAL, $p<0.01$). DOX+PEAL and DOX+CHAL treated groups failed to show significant changes in LDH and CK-MB enzymes when compared with Dox (Figure 3).

Presence of Cardiac troponin I was identified using one step rapid test. Only Dox treated groups showed the presence of cTn I (Figure 4) which is cardiac specific biomarker. Normal, Std+ dox and extract treated groups DOX+PEAL, DOX+CHAL, DOX+EAAL and DOX+AQAL showed the absence of Cardiac troponin I when compared to Dox treated group (Figure 3).

Cardiac troponin I

Only Dox treated animals showed the presence of Cardiac troponin I compared to all other groups (Figure 4).

Electrocardiogram (ECG) studies

Normal control, Vitamin E treated group and extract treated group i.e., DOX+EAAL showed a normal pattern of ECG. In Dox treated group there is elevation in PR interval, QT interval, decrease in amplitude of QRS complex and heart rate when compared with ECG patterns of normal (control vs DOX $p<0.001$) (Figure 5).

Whereas Vitamin E and Ethyl acetate fraction treated group showed a protective effect against Dox induced altered PR interval (Vitamin E $p<0.001$; DOX+EAAL, $p<0.001$; DOX+AQAL, $p<0.01$), QT interval (Vitamin E $p<0.001$; DOX+EAAL, $p<0.001$; DOX+AQAL, $p<0.01$), QRS complex (Vitamin E $p<0.001$; DOX+EAAL, $p<0.001$; DOX+AQAL, $p<0.01$) and heart rate (Vitamin E $p<0.001$; DOX+EAAL, $p<0.001$; DOX+AQAL, $p<0.01$). pet.ether fraction DOX+PEAL, DOX+CHAL failed to prevent Dox induced ECG changes (Figure 5).

Antioxidant activity

Doxurubicin treated rat showed significant decrease in GSH, SOD and Catalase enzyme when compared with normal control (control vs DOX $p<0.001$). Whereas Vitamin E treated and Ethyl

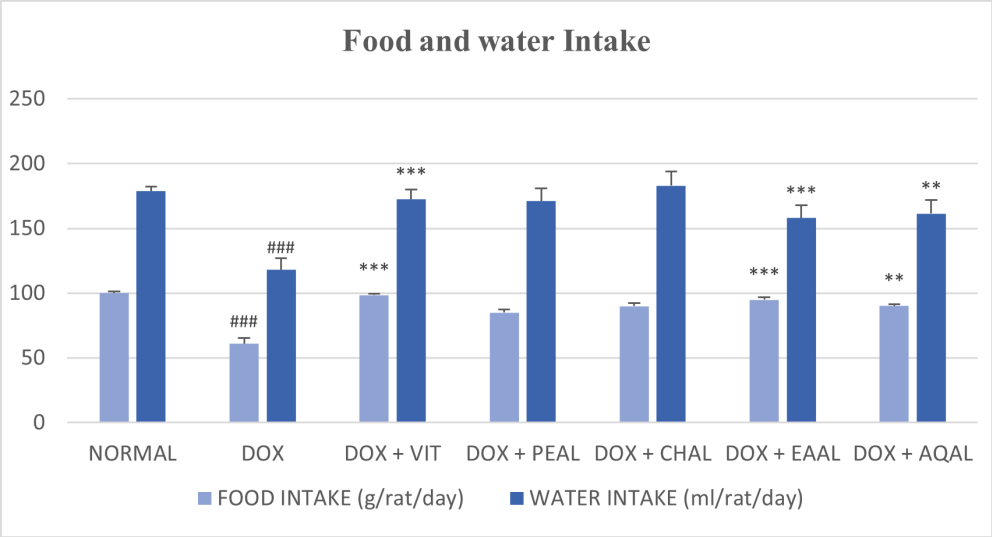

Figure 2: Effect of fractions of *Artocarpus lakoocha* R. on Food and water Intake Values are Mean+ SEM; *n*=6 in each group, ### $p<0.001$ when compared to control, *** $p<0.001$, ** $p<0.01$ when compared to DOX using one-way ANOVA (Bonferroni's Multiple Comparison Test).

Table 3: Effect of fractions of *Artocarpus lakoocha* R. on haematology analysis.

Groups	WBC (10^3/µL)	RBC (10^6/µL)	PLT (10^3/µL)	HEMATOCRITE
Normal	11.3+0.70	7.89+0.17	872.2+87.6	48.6+1.01
DOX	6.88+0.65###	5.61+0.14###	551.8+33.3###	32.9+1.31
DOX+Vit. E	10.7+0.67***	7.48+0.29***	830.3+54.5***	46.8+1.46
DOX+PEAL	7.59+0.61	5.74+0.25	658.7+56.1	35.1+1.63
DOX+CHAL	8.0+0.89	6.00+0.25**	640.7+41.1	38.4+1.63
DOX+EAAL	10.6+0.44***	7.42+0.23***	827.8+53.9***	45.1+1.43
DOX+AQAL	8.4+0.96*	6.99+0.24**	716.8+46.3**	42.5+1.64

Values are Mean+ SEM; *n*=6 in each group, ### $p<0.001$ when compared to control, *** $p<0.001$, ** $p<0.01$ when compared to DOX using one-way ANOVA (Bonferroni's Multiple Comparison Test).

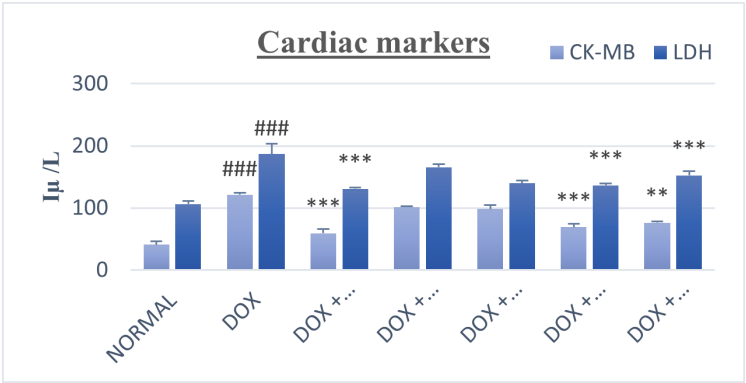

Figure 3: Effect of fractions of *Artocarpus lakoocha* R. on serum Cardiac Biomarkers Values are Mean+ SEM; *n*=6 in each group, ### $p<0.001$ when compared to control, *** $p<0.001$, ** $p<0.01$ when compared to DOX using one-way ANOVA (Bonferroni's Multiple Comparison Test).

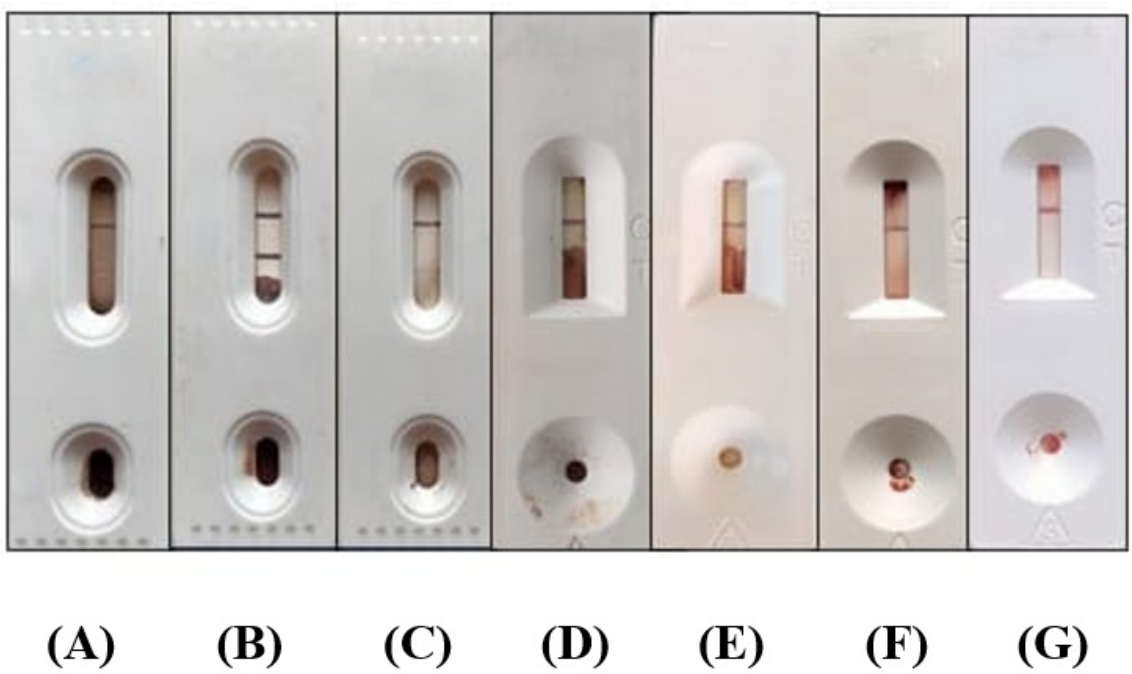

Figure 4: Effect of fractions of *Artocarpus lakoocha* R. on Cardiac troponin I.

Table 4: Effect of fractions of *Artocarpus lakoocha* R. on Histopathological study.

Groups	Cardiomyocyte degeneration	Intermuscular edema	Inflammatory cell infiltration	Vacuolization	Myofibrillar loss
Normal	-	-	-	-	-
DOX	++	+	+	++	++
DOX+Vit E	+	-	-	-	+
DOX+PEAL	++	++	+	+	+
DOX+CHAL	+	-	+	+	+
DOX+EAAL	+	-	-	-	-
DOX+AQAL	+	+	+	+	+

'+'- present '-' – absent.

acetate fraction treated group DOX+EAAL and DOX+AQAL increased GSH (Vitamin E $p<0.001$; DOX+EAAL, $p<0.001$; DOX+AQAL, $p<0.01$), SOD (Vitamin E $p<0.001$; DOX+EAAL, $p<0.001$; DOX+AQAL, $p<0.01$) and Catalase (Vitamin $p<0.001$; DOX+EAAL, $p<$; DOX+AQAL, $p<0.01$) when compared with Dox treated groups respectively.

Dox treated rats showed increase in MDA levels as compared with control group (control vs DOX $p<0.001$). Vitamin E treated and Ethyl acetate fraction treated group DOX+EAAL and DOX+AQAL decreased the MDA levels (Vitamin $p<0.001$; DOX+EAAL, $p<0.001$; DOX+AQAL, $p<0.01$) when compared with Dox treated group. Antioxidant activity is less significant with fractions DOX+PEAL, DOX+CHAL (Figure 6).

Histopathalogical studies of the heart

Doxorubicin treated rat heart tissue exhibited Cardiomyocyte degeneration, Intermuscular edema, Inflammatory cell infiltration, Vacuolization and Myofibrillar loss as compared to control rats. Whereas ethyl acetate fraction showed the minimal changes/no changes as compared to other treated groups for above parameters (Table 4 and Figure 7).

Histopathological results, Myofibrillar loss, Intermuscular edema, Inflammatory cell infiltration, Vacuolization, Congestion.

DISCUSSION

Cancer is complex disease with significant medical impact. Cardiac tissue injury is major disadvantage of anticancer treatment which includes Doxorubicin. Many theories have been proposed

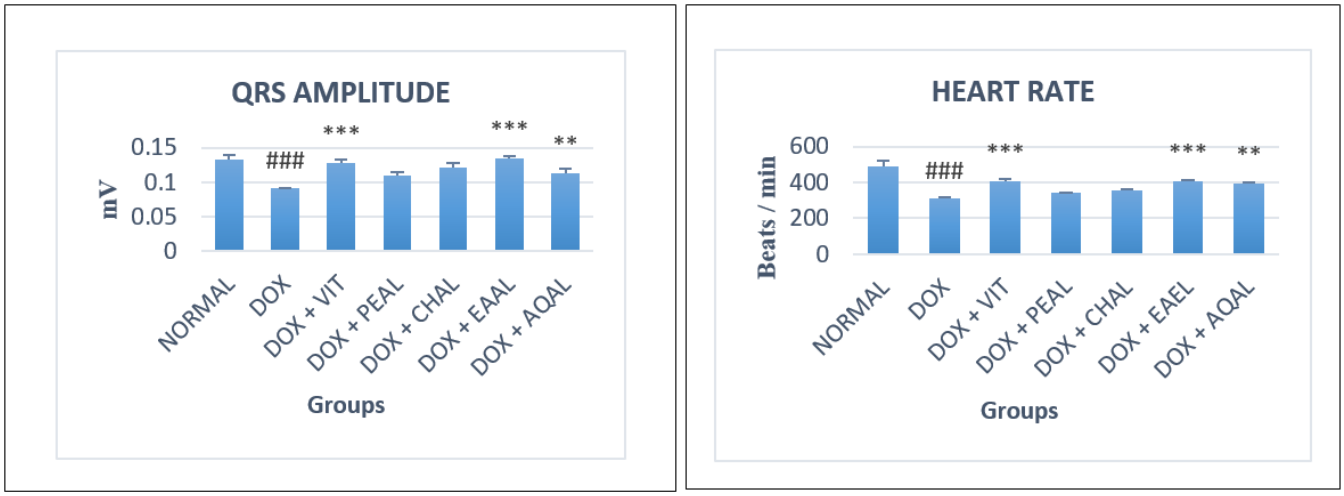

Figure 5: Effect of fractions of *Artocarpus lakoocha* R. on Electrocardiogram (ECG) Values are Mean+ SEM; *n*=6 in each group, ### $p<0.001$ when compared to control, *** $p<0.001$, ** $p<0.01$ when compared to DOX using one-way ANOVA (Bonferroni's Multiple Comparison Test).

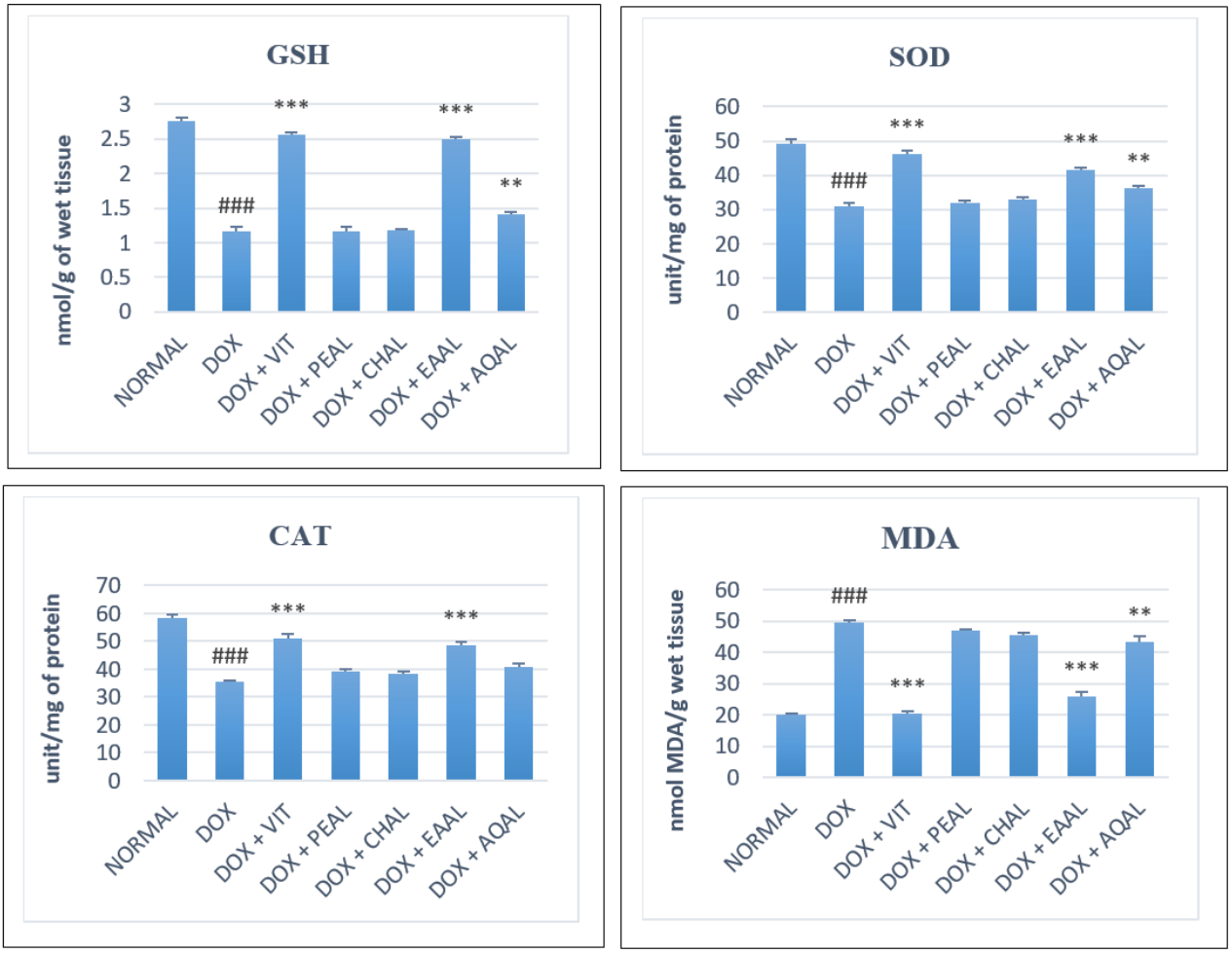

Figure 6: Effect of fractions of *Artocarpus lakoocha* R. on *In vivo* antioxidant activity. Values are Mean+ SEM; *n*=6 in each group, ### $p<0.001$ when compared to control, *** $p<0.001$, ** $p<0.01$ when compared to DOX using one-way ANOVA (Bonferroni's Multiple Comparison Test).

to determine the mechanism of action of Doxorubicin to induce cardiotoxicity. One prominent theory involves the generation of Reactive Oxygen Species (ROS) and the resulting oxidative stress within cardiac cells. Doxorubicin can induce the production of ROS, leading to oxidative damage of proteins, lipids, and DNA within the heart muscle cells. This oxidative stress can disrupt cellular signalling pathways, impair mitochondrial function, and cause cell death, ultimately leading to cardiotoxicity.

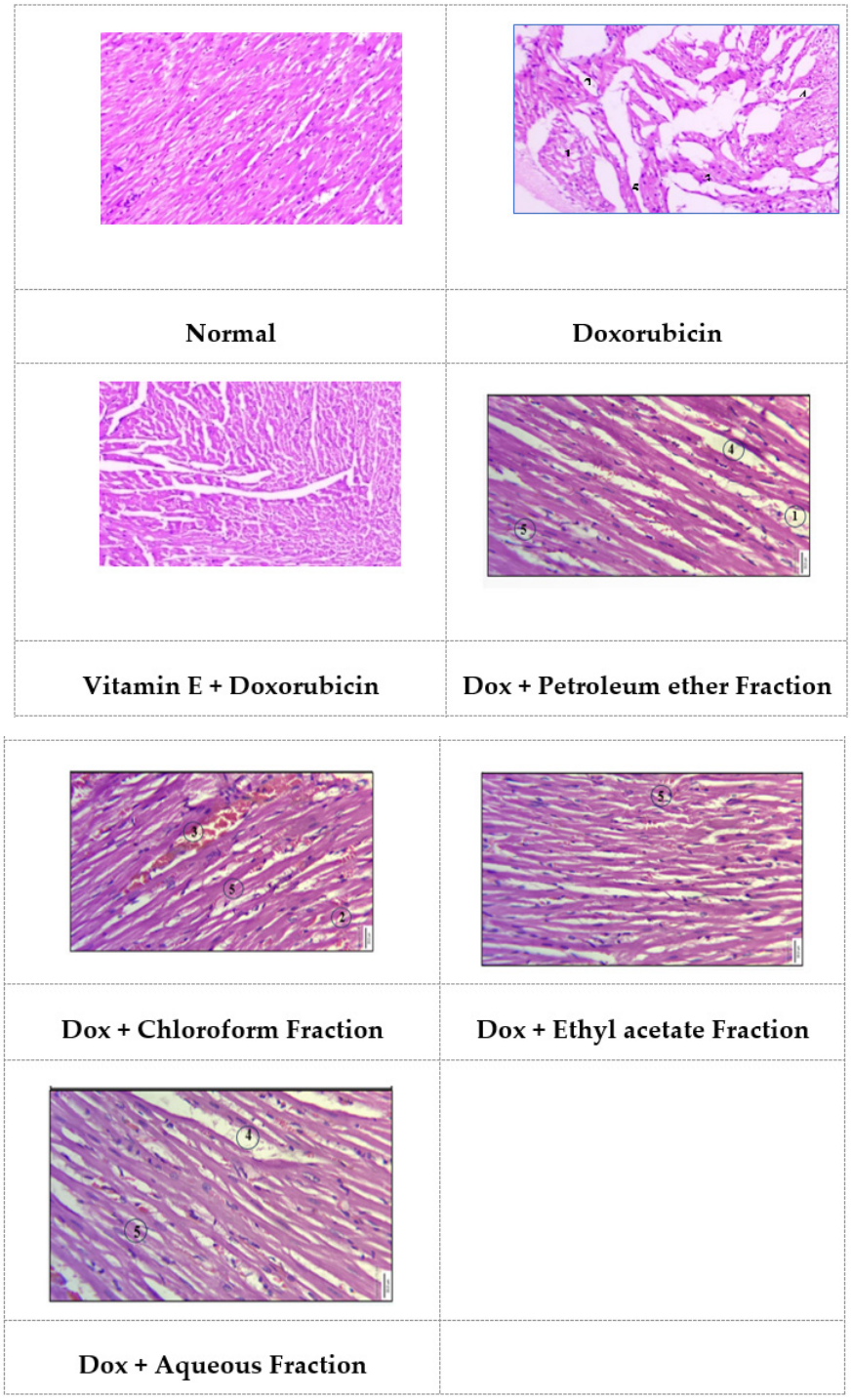

Figure 7: Histopathalogical studies of heart (All images were captured using 40X magnification).

Current study was carried out to evaluate how the Fractions of extract of *Artocarpus lakoocha* R. can affect the treatment of Doxorubicin induced Cardiotoxicity. Comparative studies were conducted to determine the effectiveness between Prophylactic and Treatment studies.

Doxorubicin is the most cytotoxic antibiotic and a key component of many chemotherapy regimens used to treat solid tumours and various types of haematological tumors.[19] Intercalation of DNA, which inhibits protein synthesis and replication, as well as topoisomerase-II inhibition, which prevents topoisomerase II-dependent relegation after double strand breaks, are the mechanisms for anti-neoplastic effects. Doxorubicin therapy has one of the most common side effects, dosage dependent cardiotoxicity, which is characterised by acute or chronic progressive cardiomyopathy, which limits its therapeutic use while being an excellent anticancer agent. Doxorubicin accumulates in cardiac tissue over a period of time dose dependently and eventually causes cardiac dysfunction.[20] Earlier

studies evidenced the generation of free radicals by chronic administration of doxorubicin in heart tissue.[21]

Generation of ROS by doxorubicin involves more than one mechanism.[22] Doxorubicin is reduced by one electron by mitochondrial reductase, which could result in anthracycline semiquinone free radicals.[23] and these are unstable in aerobic environments and quickly convert molecular oxygen to ROS such superoxide anion and hydrogen peroxide.[24] Oxidative stress is caused by increased free radical formation in cardiomyocytes and may have a number of negative effects, including perturbations in mitochondria, energetic imbalance, *p*53 accumulation, activation of *p*38 and JNK and finally cell death.[25]

Ethanolic extract of *Artocarpus lakoocha* R. bark and twigs was prepared using Soxhlet extraction using 70% ethanol and percentage yield was found to be 19.3%. Phytochemical investigations revealed the presence of carbohydrates, alkaloids, triterpenoids, Flavonoids, stilbenes and Saponin glycosides.

Effect of Fractions of *Artocarpus lakoocha* R. on body weight, food and water intake

It is observed that, there was gradual decrease in food and water intake in the rats treated with doxorubicin within the few days of treatment.[26] It is due to unfavourable side effect of doxorubicin therapy, anorexia (lack of appetite). When the energy and nutrient<!-- Inline graphics removed. Please check with MS --> demands of the cell are not met, in response cells activate autophagy as a survival mechanism to maintain cellular homeostasis and provide nutrients during times of starvation.[27,28] As induction of autophagy requires optimal production of reactive oxygen species it may cause DNA damage in response to Anorexia.[29] At molecular level, fasting alters some cell signalling pathways, which are necessary for cell survival and cell death.[27] There is stimulation of release of stress-hormones (cortisol) and activation of the RAAS due to dehydration.[30,31] As a result of decrease in water and food intake, body weight gain was gradually reduced in Dox treated group, Pet.ether and Chloroform treated group. But these changes were less observed in Aqueous, Ethyl acetate, and vitamin E treated groups.

Effect of fractions of *Artocarpus lakoocha* R. on haematology analysis

The effect of fractions of *Artocarpus lakoocha* R. on WBC, RBC and PLT in doxorubicin-induced toxicity in Wistar rats showing that doxorubicin (Dox) caused a significant decrease in the WBC, RBC and PLT when compared to the normal group. This decrease was reversed towards normal by drug treated with vitamin E and ethyl acetate fraction. As a result of decrease in WBC, RBC, PLT and hematocrite was gradually reduced in Dox treated group and also in pet ether, chloroform and aqueous fraction treated group. But these changes were less observed in EA. fraction and vitamin E treated groups.

Effect of fractions of *Artocarpus lakoocha* R. on Specific cardiac markers

Doxorubicin induced cardiac toxicity leads destruction of myocardium, due to this cardiac marker like troponins, creatine kinase-MB, lactate dehydrogenase was released into blood stream and act as the diagnostic markers of myocardial tissue damage. Serum LDH, CK- MB and cTnI are considered as specific cardiac markers to know the presence or absence of cardiac toxicity.[32] In doxorubicin treated rats these enzyme levels are significantly elevated. During a heart injury or damage, there is a disruption in the blood supply to a<!-- Inline graphics removed. Please check with MS --> portion of the heart muscle. This leads to a lack of oxygen and nutrients, causing cardiomyocyte death. When cardiomyocytes are damaged, the intracellular contents including enzymes are released into the bloodstream.[33] Animals treated with Ethyl acetate fraction and vitamin E reduce the above enzyme levels and thus responsible for maintaining common structural integrity of cardiac cells.

Effect of fractions of *Artocarpus lakoocha* R on Electrocardiography

Electrocardiographic (ECG) abnormalities are the major criteria generally used for the exact diagnosis of myocardial injury. Moreover, ECG changes are the display of the severity of doxorubicin induced-myocardial damage, which is in line with earlier reports.[15,17] The reliable changes in ECG observed with cumulative dose of doxorubicin are PR and QT interval prolongation, decrease in QRS complex amplitude and decrease in heart rate. Lipid peroxidation and increased oxidative stress might be responsible for consecutive loss of cellular membrane that leads to ECG changes.[34] The decrease in heart rate may be due to heart block.[35] Animals treated with Ethyl acetate fraction and vitamin, reduced PR and QT interval prolongation and also showed improvement in QRS amplitude and heart rate providing protective effect from cardiac damage.

Effect of fractions of *Artocarpus lakoocha* R. invo antioxidant activity

It is widely accepted that doxorubicin induced cardiotoxicity mainly involves oxidative stress and the free radical formation and also has been reported to produce DNA damage and stimulates lipid peroxidation.[36,37] It is known that doxorubicin can generate free radicals by two pathways i.e., enzymatic and non-enzymatic.[38] Doxorubicin is activated to the corresponding semiquinone by mitochondrial enzymes (e.g. NADH dehydrogenase) and this semiquinone undergoes redox-cycling and forms superoxide (O_2) and H_2O_2. Superoxide reacts with iron-sulphur centres and releases H_2O_2 which than combines to form a more potent Oxidant Hydroxyl radical (OH).[23] Though Heart is rich in mitochondria, it has less ability to protect itself against free radicals, due to less amount of catalase and inactivation of GSH cycle.[39] Cardiomyocytes have lower antioxidant capacity

than other organs, rendering them more susceptible to Dox free radical-damaging effects.[15]

Superoxide dismutase, a key defence mechanism for aerobic cells against the harmful effects of radicals, scavenges the superoxide ions produced as cellular byproducts in the body. Whereas catalase reduces H_2O_2 produced by dismutation reaction and prevents generation of free radicals (hydroxyl), thereby protecting the cellular constituents from oxidative damage.[40] The decreased levels of these enzymes in doxorubicin treated rats resulted in increase of superoxide and hydrogen peroxide respectively. Intake of doxorubicin is linked with decrease in endogenous antioxidants, which leads to an increase in free radicals, which is followed by the emergence of several alterations in the myocardium.[41,42]

Present study indicated that oxidative stress and free radicals are increased because of doxorubicin treatment responsible for myocardial GSH suppression, decrease in SOD and Catalase and increased lipid peroxidation (MDA), which are in line with previous studies. In animals given with Ethyl acetate fraction and vitamins, MDA levels was decreased, showing that lipid peroxidation was reduced, while GSH, SOD, and CAT levels increased, demonstrating that extracts had the ability to scavenge free radicals and protect myocytes from oxidative damage.

Effect of fractions of *Artocarpus lakoocha* R. on histopathology of heart

In histopathologic study, the cardiac muscle fibres of control group were uniform in size, shape and configurations with no inflammatory-cell infiltrates. Cardiac damage occurred in doxorubicin treated group, Pet. Ether and chloroform fraction treated group as indicated by cardiomyocyte degeneration, Inflammatory cell infiltration, myofibrillar loss, Intermuscular edema and vacuolization of cytoplasm which are in line with earlier reports.[15,34] In the present study rats treated with Ethyl acetate of extracts and Vitamin E showed minimal myocardial fibres loss, vacuolated cells, reduced edema, reduced inflammatory cells and cardiomyocyte degeneration. However, all of the above changes were significantly reduced in rats treated with fractions of ethanolic extract of *Artocarpus lakoocha* R and also treatment studies showed a favourable result with normal group when compared with a preventive study.

CONCLUSION

Based on results of general appearance, ECG studies, specific cardiac markers and antioxidant activity, it is concluded that the ethyl acetate fraction of *Artocarpus lakoocha* R. exhibited cardioprotective activity. Based on data obtained, treatment studies were found more effective when compared with preventive studies. Phytocompounds present in the plant might be responsible for the cardioprotective activity. Further study is required to perform isolation of phytoconstituents and combination therapy, to determine which phytoconstituents are responsible for cardioprotective property.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

CVDs: Cardiovascular Diseases; **DOX:** Doxorubicin; **ROS:** Reactive Oxygen Species; **DNA:** Deoxyribonucleic Acid; **OECD:** Organisation for Economic Co-operation and Development; **ECG:** Electrocardiogram; **LDH:** Lactate Dehydrogenase; **CK-MB:** Creatinine Phosphokinase-MB; **cTnI:** Cardiac Troponin-I; **GSH:** Glutathione; **MDA:** Melonldehyde; **SOD:** Superoxide Dismutase; **CAT:** Catalase; **IAEC:** Institutional Animal Ethics Committee; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **PEAL:** Petroleum Ether Fraction; **CHAL:** Chloroform Fraction; **EAAL:** Ethyl Acetate Fraction; **AQAL:** Aqueous Fraction; **WBC:** White Blood Cells; **RBC:** Red Blood Cells; **PLT:** Platelets; **RAAS:** Renin-Angiotensin-Aldosterone System.

REFERENCES

1. Haroon HB, Ahmed N, Sampath MK, Dinesh S, Azamthulla M, Radhakrishnan G, *et al*. *Tamarindus indica* Linn. leaves ameliorates experimental induced heart failure in Wistar rats. J Basic Clin Physiol Pharmacol. 2021;33(3):363-71. doi: 10.1515/jbcpp-2020-0338, PMID 33915613.
2. Yusuf S, Wittes J, Friedman L. Overview of results of randomized clinical trials in heart disease. I. Treatments following myocardial infarction. JAMA. 1988;260(14):2088-93. doi: 10.1001/jama.1988.03410140100032, PMID 2901501.
3. Ewer MS, Ewer SM. Cardiotoxicity of anticancer treatments. Nat Rev Cardiol. 2015;12(9):547-58. doi: 10.1038/nrcardio.2015.65, PMID 25962976.
4. Klatsky AL. Alcohol and cardiovascular diseases. Expert Rev Cardiovasc Ther. 2009;7(5):499-506. doi: 10.1586/erc.09.22, PMID 19419257.
5. Varga ZV, Ferdinandy P, Liaudet L, Pacher P. Drug-induced mitochondrial dysfunction and cardiotoxicity. Am J Physiol Heart Circ Physiol. 2015; 309(9):H1453-67. doi: 10.1152/ajpheart.00554.2015, PMID 26386112.
6. Çomez MS, Cellat M, Özkan H, Borazan Y, Aydın T, Gokçek İ, *et al.* Protective effect of oleuropein on ketamine-induced cardiotoxicity in rats. Naunyn Schmiedebergs Arch Pharmacol. 2020;393(9):1691-9. doi: 10.1007/s00210-020-01870-w, PMID 32383030.
7. Zhang S, Liu X, Bawa-Khalfe T, Lu LS, Lyu YL, Liu LF, *et al.* Identification of the molecular basis of doxorubicin-induced cardiotoxicity. Nat Med. 2012;18(11):1639-42. doi: 10.1038/nm.2919, PMID 23104132.
8. El-Awady ES, Moustafa YM, Abo-Elmatty DM, Radwan A. Cisplatin-induced cardiotoxicity: mechanisms and cardioprotective strategies. Eur J Pharmacol. 2011;650(1):335-41. doi: 10.1016/j.ejphar.2010.09.085, PMID 21034734.
9. Dugbartey GJ, Peppone LJ, de Graaf IA. An integrative view of cisplatin-induced renal and cardiac toxicities: molecular mechanisms, current treatment challenges and potential protective measures. Toxicology. 2016;371:58-66. doi: 10.1016/j.tox.2016.10.001, PMID 27717837.
10. Poojar B, Ommurugan B, Adiga S, Thomas H, Sori RK, Poojar B, *et al.* Methodology used in the Study. Asian J Pharm Clin Res. 2017;7(10):1-5.
11. Bhattacharya E, Dutta R, Chakraborty S, Mandal Biswas S. Phytochemical profiling of *Artocarpus lakoocha* Roxb. leaf methanol extract and its antioxidant, antimicrobial and antioxidative activities. Asian Pac J Trop Biomed. 2019;9(11):484. doi: 10.4103/2221-1691.270984.
12. Atawodi SE, Bulus T, Mamman M. Bioassay guided fractionation and anti-trypanosomal effect of fractions and crude aqueous and methanolic extracts of *Terminalia avicennioides* (Guill. & Perr.) Parts (Guill. and Perr.) Parts. Int J Biol. 2011;3(3):145-52. doi: 10.5539/ijb.v3n3p19.
13. Kumari P, Sahal D, Jain SK, Chauhan VS. Bioactivity Guided Fractionation of Leaves Extract of *Nyctanthes arbortristis* (Harshringar) against P falciparum. PLOS ONE. 2012;7(12):e51714. doi: 10.1371/journal.pone.0051714, PMID 23300557.
14. Saleem M, Asif A, Akhtar MF, Saleem A. Hepatoprotective potential and chemical characterization of *Artocarpus lakoocha* fruit extract. Bangladesh J Pharmacol. 2018;13(1):90-7. doi: 10.3329/bjp.v13i1.34117.

15. Ahmed AZ, Mumbrekar KD, Satyam SM, Shetty P, D'Souza MR, Singh VK. Chia seed oil ameliorates doxorubicin-induced cardiotoxicity in female Wistar rats: an electrocardiographic, biochemical and histopathological approach. Cardiovasc Toxicol. 2021;21(7):533-42. doi: 10.1007/s12012-021-09644-3, PMID 33740233.
16. Koti BC, Vishwanathswamy AH, Wagawade J, Thippeswamy AH. Cardioprotective effect of lipistat against doxorubicin induced myocardial toxicity in albino rats. Indian J Exp Biol. 2009;47(1):41-6. PMID 19317350.
17. Chester K, Zahiruddin S, Ahmad A, Khan W, Paliwal S, Ahmad S. Bioautography-based Identification of Antioxidant Metabolites of *Solanum nigrum* L. and Exploration Its hepatoprotective Potential against D-Galoctosamine induced hepatic fibrosis in rats. Pharmacogn Mag. 2019;15(62):179-88.
18. Murugesan M, Revathi R, Manju V. Cardioprotective effect of fenugreek on isoproterenol-induced myocardial infarction in rats. Indian J Pharmacol. 2011;43(5):516-9. doi: 10.4103/0253-7613.84957, PMID 22021992.
19. Marques-Aleixo I, Santos-Alves E, Oliveira PJ, Moreira PI, Magalhães J, Ascensão A. The beneficial role of exercise in mitigating doxorubicin-induced Mitochondrionopathy. Biochim Biophys Acta Rev Cancer. 2018;1869(2):189-99. doi: 10.1016/j.bbcan.2018.01.002, PMID 29408395.
20. Del Tacca M, Danesi R, Ducci M, Bernardini C, Romanini A. Might adriamycinol contribute to adriamycin-induced cardiotoxicity? Pharmacol Res Commun. 1985;17(11):1073-84. doi: 10.1016/0031-6989(85)90113-4, PMID 4089011.
21. Cardinale D, Iacopo F, Cipolla CM. Cardiotoxicity of anthracyclines. Front Cardiovasc Med. 2020;7:26. doi: 10.3389/fcvm.2020.00026, PMID 32258060.
22. Renu K, V G A, P B TP, Arunachalam S. Molecular mechanism of doxorubicin-induced cardiomyopathy – an update. Eur J Pharmacol. 2018;818:241-53. doi: 10.1016/j.ejphar.2017.10.043, PMID 29074412.
23. Davies KJ, Doroshow JH, Hochstein P. Mitochondrial NADH dehydrogenase-catalyzed oxygen radical production by adriamycin, and the relative inactivity of 5-iminodaunorubicin. FEBS Lett. 1983;153(1):227-30. doi: 10.1016/0014-5793(83)80153-7, PMID 6298008.
24. Doroshow JH, Davies KJ. Redox cycling of anthracyclines by cardiac mitochondria. II. Formation of superoxide anion, hydrogen peroxide, and hydroxyl radical. J Biol Chem. 1986;261(7):3068-74. doi: 10.1016/S0021-9258(17)35747-2, PMID 3005279.
25. Deavall DG, Martin EA, Horner JM, Roberts R. Drug-induced oxidative stress and toxicity. J Toxicol. 2012; 2012:645460. doi: 10.1155/2012/645460. PMID 22919381, PMCID PMC3420138.
26. Gilliam LA, St Clair DK. Chemotherapy-induced weakness and fatigue in skeletal muscle: the role of oxidative stress. Antioxid Redox Signal. 2011;15(9):2543-63. doi: 10.1089/ars.2011.3965, PMID 21457105.
27. Ogata T, Oishi Y, Higuchi M, Muraoka I. Fasting-related autophagic response in slow- and fast-twitch skeletal muscle. Biochem Biophys Res Commun. 2010;394(1):136-40. doi: 10.1016/j.bbrc.2010.02.130, PMID 20184860.
28. Kanamori H, Takemura G, Maruyama R, Goto K, Tsujimoto A, Ogino A, *et al.* Functional significance and morphological characterization of starvation-induced autophagy in the adult heart. Am J Pathol. 2009;174(5):1705-14. doi: 10.2353/ajpath.2009.080875, PMID 19342365.
29. Rodríguez-Vargas JM, Ruiz-Magaña MJ, Ruiz-Ruiz C, Majuelos-Melguizo J, Peralta-Leal A, Rodríguez MI, *et al.* ROS-induced DNA damage and PARP-1 are required for optimal induction of starvation-induced autophagy. Cell Res. 2012;22(7):1181-98. doi: 10.1038/cr.2012.70, PMID 22525338.
30. Judelson DA, Maresh CM, Yamamoto LM, Farrell MJ, Armstrong LE, Kraemer WJ, *et al.* Effect of hydration state on resistance exercise-induced endocrine markers of anabolism, catabolism, and metabolism. J Appl Physiol (1985). 2008;105(3):816-24. doi: 10.1152/japplphysiol.01010.2007, PMID 18617629.
31. Taylor PJ, van Rosendal SP, Coombes JS, Gordon RD, Stowasser M. Simultaneous measurement of aldosterone and cortisol by high-performance liquid chromatography-tandem mass spectrometry: application to dehydration-rehydration studies. J Chromatogr B Analyt Technol Biomed Life Sci. 2010;878(15-16):1195-8. doi: 10.1016/j.jchromb.2010.03.033, PMID 20378424.
32. Lewandrowski K, Chen A, Januzzi J. Cardiac markers for myocardial infarction. A brief review. Am J Clin Pathol. 2002; 118(1);Suppl:S93-9. doi: 10.1309/3EK7-YVV9-228C-E1XT, PMID 14569816.
33. Nimbal SK, Gadad PC, Koti BC. Effect of ethanolic extract of *Rosa centifolia* against doxorubicin induced nephrotoxicity in albino rats. J Ayurveda Integr Med. 2021;12(4):657-62. doi: 10.1016/j.jaim.2021.07.020. PMID 34801355, PMCID PMC8642703.
34. Chadchan KS, Das SN, Jargar JG, Das KK. A Comparative Study on Antidiabetic Effects of *Aqueous arietinum* extracts on alloxan Induced Diabetic Male albino Rats. J Young Pharm. 2017;9(2):230-3. doi: 10.5530/jyp.2017.9.45.
35. Kilickap S, Akgul E, Aksoy S, Aytemir K, Barista I. Doxorubicin-induced second degree and complete atrioventricular block. Europace. 2005;7(3):227-30. doi: 10.1016/j.eupc.2004.12.012, PMID 15878560.
36. Nimbal SK, Koti BC. Effect of Ethanolic Extract Fractions of *Boerhaavia diffusa* in Doxorubicin-induced myocardial toxicity in albino rats. J Young Pharm. 2017;9(4):545-9. doi: 10.5530/jyp.2017.9.104.
37. Mcquillan PJ, Morgan BA, Ramwell J. Adriamycin cardiomyopathy. Fatal outcome of general anaesthesia in a child with Adriamycin cardiomyopathy. Fatal outcome of general anaesthesia in a child with adriamycin cardiomyopathy. Anaesthesia. 1988;43(4):301-4. doi: 10.1111/j.1365-2044.1988.tb08979.x, PMID 3377152.
38. De Beer EL, Bottone AE, Voest EE. Doxorubicin and mechanical performance of cardiac trabeculae after acute and chronic treatment: a review. Eur J Pharmacol. 2001;415(1):1-11. doi: 10.1016/s0014-2999(01)00765-8, PMID 11245845.
39. Simmons TW, Jamall IS. Relative importance of intracellular glutathione peroxidase and catalase *in vivo* for prevention of peroxidation to the heart. Cardiovasc Res. 1989;23(9):774-9. doi: 10.1093/cvr/23.9.774, PMID 2611816.
40. Sivajothi V, Dey A, Jayakar B, Rajkapoor B. Antihyperglycemic, antihyperlipidemic and antioxidant effect of *Phyllanthus rheedii* on streptozotocin induced diabetic rats. Iran J Pharm Res. 2008;7(1):53-9.
41. Venkatesan N. Curcumin attenuation of acute adriamycin myocardial toxicity in rats. Br J Pharmacol. 1998;124(3):425-7. doi: 10.1038/sj.bjp.0701877, PMID 9647462.
42. Singh G, Singh AT, Abraham A, Bhat B, Mukherjee A, Verma R, *et al.* Protective effects of *Terminalia arjuna* against Doxorubicin-induced cardiotoxicity. J Ethnopharmacol. 2008;117(1):123-9. doi: 10.1016/j.jep.2008.01.022, PMID 18346858.

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