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Attenuation of Doxorubicin Induced Cardiotoxicity in Wistar Rats by Extracts of Fruits of *Ziziphus jujuba* Lam

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Abstract

The objective of the study was to evaluate the cardioprotective activity of fruits of *Ziziphus jujuba* Lam. in doxorubicin induced cardiotoxicity in Wistar rats. The ethanolic and aqueous extract of fruits of *Z. jujuba* has been prepared. Male Wistar rats were divided into seven groups. Vehicle control was Group 1. Doxorubicin 5 mg/kg i.p. was administered to Group 2 animals on the 7th and 14th day. Animals in groups 3 and 4 were dosed with ethanolic extracts of 200 and 400 mg/kg p.o. while groups 5 and 6 received aqueous extracts of 200 and 400mg/kg i.p. Animals from group 7 were given nebivolol 5 mg/kg p.o. All the animals were treated for 28 days. Doxorubicin (2.5 mg/kg) was injected intraperitoneally to group 2 to group 7 on day 7th and day 14th. Body weight, ECG, blood pressure, CK-MB, Cardiac troponin-I and LDH were the study parameters. The histology of the heart was performed at the end of the study. Doxorubicin showed cardiotoxicity manifested by changes in serum marker enzymes, ECG and hemodynamic parameters which were further confirmed by histology of the heart. These changes induced by doxorubicin were attenuated by treatment with extracts of the fruit of *Ziziphus jujuba* Lam. It is concluded that aqueous extract of the fruit of *Ziziphus jujuba* Lam. (400 mg/kg p.o.) treatment for 28 days protects the heart of rats in doxorubicin induced cardiotoxicity.

Keywords: Cardioprotective, Doxorubicin, ECG Parameters, Hemodynamic Parameters, Nebivolol, *Ziziphus jujuba* Lam

1. Introduction

Doxorubicin is an anthracycline antibiotic anticancer medicine that is commonly used to treat a variety of haematological and solid tumour malignancies, such as breast cancer, leukaemia, and sarcomas[1,2]. Its dose-dependent adverse effect of cardiotoxicity, which may cause permanent cardiomyopathy and ultimately heart failure, restricts its therapeutic use[3]. Above a cumulative dosage of 550 mg/m^2 of body surface area, the risk of doxoru-

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bicin-related cardiotoxicity significantly rises. So, 500 or 450 mg/m^2 was proposed as the maximum cumulative dosage of doxorubicin[4]. Cardiotoxicity caused by doxorubicin is mediated by several distinct pathways, including the generation of free radicals, calcium excess, mitochondrial malfunction, and the development of peroxynitrite. Enzymatic reduction of doxorubicin to its semiquinone radical occurs. This doxorubicin semiquinone is radical transfers its electron to molecular oxygen immediately, producing hydrogen peroxide and the superoxide radical[1–3]. Role of Reactive Oxygen Species (ROS) in doxorubicin-induced toxicity as possible medicinal and/or protective agents, several substances having antioxidant characteristics have been studied. The following fruits and phytoconstituents were included: Cranberry[5], p-coumaric acid[6], *Withania somnifera* L.[7], *Lycium barbarum* L.[8], Catechin[9], Carvedilol[10], Nebivolol[11].

The ability of polyphenols and flavonoids to shield molecules or cells against oxidative cardiac harm has garnered a lot of interest in recent years. *Ziziphus jujuba* Lam. (ZJ) is a polyphenolic phytonutrient plant that is widespread in the berry family and has drawn attention because of the vast range of biological features it has. Previous research suggested that *Ziziphus jujuba* Lam. had free radical scavenging action[12,13] anti-apoptotic[14], anti-inflammatory[15], anti-cataract[16], cardioprotective[17], gastroprotective and ulcer healing properties[18], as well as antifibrotic[19], antidiabetic[20], hypolipidemic[21], anti-atherosclerotic[22], and estrogenic/antiestrogenic properties[23]. Numerous medicinal plants' polyphenols and flavonoids have remarkable cardioprotective properties.

The objective of the current investigation was to determine the different *Ziziphus jujuba* Lam. extracts had cardioprotective effects on doxorubicin induced cardiotoxicity in Wistar rats. By examining the ECG parameters, hemodynamic parameters, and biochemical changes in doxorubicin induced cardiotoxicity, this research also tried to elucidate the potential mechanism of the *Ziziphus jujuba* Lam.

2. Materials And Methods

2.1 Materials

Active pharmaceutical ingredient form of doxorubicin was purchased from Naprod Life Sciences Pvt. Ltd., Urethane (HIMEDIA lab chemicals), Isoflurane (Baxter Healthcare Corporation Deerfield, IL 60015 USA), formaldehyde solution (Loba Chemie, India), Heparin (Bhavya Industries, Gujarat) and water for injection (Tara Bioscience Ltd., Haryana) was purchased from local vendors. Assay kits for LDH (Lactate Dehydrogenase), cardiac troponin-I, creatine kinase-MB (CK-MB), and Electrolyte concentration level (Na^+, K^+, Cl^-), were obtained from Accurex Biomedical Pvt. Ltd., Mumbai (India). Reagents for histopathological analysis were procured from Sigma-Aldrich-Merck Ltd., Bangalore (India). All reagents and chemicals were of analytical grade. Reagents were equilibrated at room temperature for 30 minutes before the biochemical estimations.

2.1.1 Authentication of Plant

Ziziphus jujuba Lam. fruit was purchased from local vendors in Bhilai, Chhattisgarh. Identification and authentication were carried out at the Department of Dravyaguna, Bharati Vidyapeeth (Deemed to be) University, College of Ayurved, Pune, India and voucher specimen number has been submitted to the college.

2.1.2 Preparation of Extracts

Whole fruit was cut into small flakes and dried on a tray dryer at a temperature of 30-40° C. The dried flakes were pulverized to make a fine powder.

2.1.2.1 Ethanolic Extract

Coarsely powdered plant material (50 g) was macerated in ethanol (250 ml) for 48 h, with occasional shaking. Macerate was decanted and filtered through cloth and then through filter paper to obtain a clear extract. This process was repeated with the same volume of ethanol. Macerates were pooled, transferred to a previously weighed petri dish and evaporated to dryness at room temperature to obtain dried extracts. After drying, the petri dish was weighed again. The yield was represented as per cent yield.

2.1.2.2 Aqueous Extracts

The plant material that remained after ethanolic extraction, was dried in air. The dried plant material was macerated following the same procedure as described above by using distilled water (1:1 v/v) as solvent, to obtain dried aqueous extract.

2.1.3 Experimental Animals

The Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy in Pune, which was established by the regulations and standards of the Committee for Control and Supervision of Experimental Animals (CPCSEA), India, authorized the experimental protocol (IAEC approval no. PCP/IAEC/2021-22/1-19). Male Wistar rats weighing 175-250 g, aged 3 to 4 months, and obtained from Global Bioresearch Solution Pvt. Ltd., Nhavi, Pune (India), an authorized breeder of laboratory animals, were used in all the tests. We were kept in polypropylene cages (47-34-20 cm) lined with a husk that was changed out every 24 hours and kept at a temperature of 22° C and 3° C with 50% humidity. Food and water were freely available to the rats. Rats were given a typical pellet diet (VRK Nutritional Solutions, Pune, Maharashtra, India).

2.1.4 Experimental Design and Protocol

Forty-two male Wistar rats were divided into seven groups, each group contained six animals. Animal grouping is as follows:

Group 1 (vehicle control): normal saline
Group 2 (DOX control): DOX 2.5 mg/kg (single dose); i.p. on the 7th and 14th day
Group 3 (ethanolic extract 200 mg/kg/day orally-EtOH200) for 28 days + DOX 2.5 mg/kg (single dose); i.p. on the 7th and 14th day.
Group 4 (ethanolic extract 400 mg/kg/day orally-EtOH400) for 28 days + DOX 2.5 mg/kg (single dose); i.p. on the 7th and 14th day.
Group 5 (aqueous extract 200 mg/kg/day orally-AQ200) for 28 days + DOX 2.5 mg/kg (single dose); i.p. on the 7th and 14th day.
Group 6 (aqueous extract 400 mg/kg/day orally-AQ400) for 28 days + DOX 2.5 mg/kg (single dose); i.p. on the 7th and 14th day.
Group 7 (Nebivolol (Std.) 5mg/kg/day orally-N5) for 28 days + DOX 2.5 mg/kg (single dose); i.p. on the 7th and 14th day.

2.2 Methods

2.2.1 Body Weight Parameters

The body weight of each rat was recorded till the completion of the 28-day study.

2.2.2 Serum Parameters

On the last day, blood was collected from the retro-orbital plexus of each rat under light anaesthesia. Serum was separated by using cryo-centrifuge (Eppendorf, India) at 4^0 C at 7000 rpm for 15 minutes and kits were used to assay LDH, cardiac troponin-I and CK-MB (Accurex Pvt. Ltd., India) using an autoanalyzer (Nihon Kohden, Japan).

2.2.3 ECG and Hemodynamic Parameters

All rats were fasted overnight and allowed unrestricted access to water. On the 28th day of the study, animals were anaesthetized with urethane (1.25 g/kg/5 ml, i.p.). The ECG was recorded using a power lab instrument (AD Instruments) and the labchart-7 pro software. For each ECG trace, needle electrodes were implanted beneath the skin for the leg lead at position II in rats from each group. QRS complexes, QT interval, PR interval, and Heart Rate (HR) were evaluated.

Each rat's left carotid artery was cannulated and the pressure transducer was connected to the cannula, which was filled with heparinized saline. The hemodynamic parameters were measured by a power lab device after 30 minutes of stabilization. Systolic Blood Pressure (SBP), Diastolic Blood Pressure (SBP), and Mean Arterial Blood Pressure (MABP), the hemodynamic parameters were studied.

Animals were euthanized by cervical dislocation after all parameters were recorded. Each rat's heart was isolated and heart weight and body weight ratio were carried out by using the formula:

$$Ratio = \frac{weight\ of\ heart}{final\ weight\ of\ body}$$

The heart was deposited in a 10% formalin solution. The organ specimens were dehydrated with xylene (1 hour each) for 2 hours each. The infiltration and impregnation were performed twice, each for 1 hour, using paraffin to make paraffin L moulds, paraffin wax was utilized. Specimens were cut into 3-5mm thick sections and stained with haematoxylin and eosin (H and E stain).

Histopathology parameters such as necrosis and cellular infiltration were examined in the heart section. The parameters' grading method was (00: No changes, +: Less than 25% changes, ++: Less than 50% changes, +++: Less than 75% changes, and ++++: More than 75% changes).

3. Statistical Analysis

Data were expressed as the mean ± SEM. Statistical analysis was carried out by a One-way ANOVA Dunnett test followed by a Two-way ANOVA Bonferroni multiple comparison tests using GraphPad Prism 9.0 for Windows (GraphPad Software Inc., La Jolla, CA, USA). The P value was considered significant when $^*p < 0.05$.

4. Results

4.1 Organoleptic Evaluation of Fruits of *Ziziphus jujuba* Lam

The dried powder of fruits of *Ziziphus jujuba* Lam. was red-brown with a pungent odour and had a faintly bitter taste.

4.2 Preparation and Preliminary Phytochemical Investigation of Extracts

The dried powder of the plant was extracted by successive cold maceration method and yielded a high percentage of extract i.e., 18.9% and 12.2% w/v using 99.9% v/v ethanol and distilled water. All the plant extracts were tested for the presence of metabolites based on preliminary phytochemical screening met by the standard methods[24] (Table 1).

4.3 Survival

All the animals receiving ethanolic, aqueous extracts and Nebivolol (NEB) survived throughout the study

4.4 Body Weight

Body weight in DOX treated animals was significantly decreased as compared to the control group. Animals receiving ethanolic extract (200 and 400 mg/kg p.o.), aqueous extract (200 and 400 mg/kg p.o.) and NEB showed a significant increase in body weight as compared to the DOX group (Table 2).

4.5 Heart Weight to Body Weight Ratio

DOX group animals showed a non-significant decrease in heart weight when compared to the control group. In the heart weight to body weight ratio of animals of groups 3, 4, 5, 6 and NEB, group 7 increased non-significantly (Table 3).

4.6 Evaluation of ECG Parameters

A significant decrease in HR was observed in DOX treated animals when compared with the vehicle-treated animals. HR in the group EtOH 200, 400, AQ200 and NEB treated animals showed a non-significantly increase. While animals from the group AQ400 showed a significant increase in HR ($^*p < 0.05$). DOX treated animals showed a non-significant increase in PR, QT, QTc and QRS complex compared to the control group. Animals from EtOH 200, 400, AQ200, 400 and NEB showed a non-significant decrease (Table 4).

4.7 Evaluation of Hemodynamic Parameters

DOX treated animals showed a significant reduction in SYSBP, DIABP, max dp/dt and min dp/dt as compared to the control group. Animals treated with EtOH 200,

Table 1. Preliminary phytochemical identification tests for ethanolic and aqueous extracts of *Ziziphus jujuba* Lam

Sample Name	Extracts	Reducing Sugar	Iodine	Glycoside	Saponin	Tannins	Phenols	Alkaloids	Flavonoids	Steroids	Ninhydrin	Xanthoproteic	Quinine
ZJ	Aqueous	-	-	-	+	+	-	-	+	+	-	+	-
	Ethanolic	-	-	+	+	-	-	-	+	+	-	+	+

The ethanolic extract contains glycosides, saponins, flavonoids, steroids, xanthoproteic, and quinine while saponins, tannins, flavonoids, steroids, and xanthoproteic were present in aqueous extract.

Table 2. Effect of ethanolic and aqueous extract (200 mg/kg p.o. and 400 mg/kg p.o.) of fruits of *Ziziphus jujuba* Lam. on body weight (in gm) in Wistar rats

DAYS	VEHICLE CONTROL	DISEASE CONTROL (DOX 5 mg/kg i.p.)	DOX+ ETOH200	DOX+ ETOH400	DOX+AQ200	DOX+ AQ400	DOX+NEB (5 mg/kg p.o.)
0	161.00 ± 3.91	165.17 ± 7.77	230.33 ± 6.20	218.33 ± 7.76	153.33 ± 8.96	157.67 ± 9.30	158.00 ± 1.98
3	161.33 ± 3.94	166.00 ± 7.79	230.33 ± 6.20	218.33 ± 7.76	153.33 ± 8.96	157.67 ± 9.30	158.00 ± 1.98
6	161.83 ± 3.70	165.17 ± 7.77	230.67 ± 6.73	222.17 ± 5.83	153.33 ± 8.96	159.33 ± 9.01	159.17 ± 1.74
9	163.00 ± 3.70	138.33 ± 3.87	222.83 ± 6.93	218.00 ± 5.65	167.67 ± 5.04	162.50 ± 7.35	161.67 ± 9.84
12	164.00 ± 3.56	123.17 ± 7.92	226.00 ± 6.92	228.00 ± 5.04	187.17 ± 6.82	161.50 ± 4.14	180.50 ± 8.22
15	165.17 ± 3.03	122.67 ± 7.79	227.17 ± 5.17	234.67 ±3.67	196.50 ± 6.96	164.17 ± 4.24	170.00 ± 13.23
18	170.50 ± 14.10	125.50 ± 8.71**	231.33 ± 5.10***	244.67 ± 6.54***	234.67 ± 10.05***	206.33 ± 6.52*	209.17 ± 19.75*
21	180.00 ± 15.54	124.00 ± 8.50***	238.67 ±2.81***	251.33 ± 8.82***	282.83 ± 11.55***	226.17 ± 15.68**	257.33 ± 1.98***
28	180.00 ± 15.54	121.25 ± 7.65***	243.83 ± 1.78***	253.33 ± 8.81***	284.33 ± 11.62***	227.50 ± 15.45**	275.83 ± 1.98***

The values are expressed as mean ± SEM (n = 6); *p < 0.05, **p < 0.01. ***p < 0.0001, compared to Doxorubicin group, **p < 0.001 compared to the control group (Two-way ANOVA followed by Bonferroni test).

Table 3. Effect of ethanolic and aqueous extract (200 mg/kg p.o. and 400 mg/kg p.o.) of fruits of *Ziziphus jujuba* Lam. on the heart to body weight ratio in Wistar rats

VEHICLE CONTROL	DISEASE CONTROL (DOX 5 mg/kg i.p.)	DOX+ETOH200	DOX+ETOH400	DOX+AQ200	DOX+ AQ400	DOX+NEB (5 mg/kg p.o.)
0.005 ± 0.0005	0.003 ± 0.0002	0.003 ± 0.0002	0.003 ± 0.0001	0.004 ± 0.0004	0.004 ± 0.0005	0.005 ± 0.0006

The values are expressed as mean ± SEM (n = 6); *p < 0.05, **p < 0.01. ***p < 0.0001, compared to Doxorubicin group, **p < 0.001 compared to the control group (One-way ANOVA followed by Dunnett test).

400, AQ200, and 400 showed a significant increase in the SYSBP, DIABP, max dp/dt and min dp/dt but NEB showed a significant reduction in SYSBP, DIABP, and max dp/dt and min dp/dt respectively. (***p < 0.0001) (Table 5, 6).

4.8 Evaluation of Serum Parameters

LDH, cardiac troponin-I and CK-MB levels in the DOX group were significantly higher than in the control group. Rats treated with EtOH 200 and 400 mg/kg showed non-significant changes in LDH, cardiac troponin-I and CK-MB levels. On the other hand, animals dosed with AQ200 and 400 mg/kg showed a significant reduction in the levels of serum concentration of LDH, cardiac troponin-I and CK-MB. The serum concentration of CK-MB was not altered significantly in NEB treated animals. The level of LDH and cardiac troponin-I was significantly attenuated by NEB as compared to DOX treated group (Table 7).

4.9 Histopathology

The histology of the heart in the control group showed no significant alterations. DOX administration, on the other hand, caused necrosis and cellular infiltration. The heart of EtOH200, 400, AQ200, 400 and NEB showed mild necrosis and cellular infiltration. (Figure 1-7) (Table 8).

Figure 1-7 Photomicrographs of histological changes of rat heart. The red arrow shows necrosis, and the blue arrow shows cellular infiltration. H and E stain x400.

Table 4. Effect of ethanolic and aqueous extract (200 mg/kg p.o. and 400 mg/kg p.o.) of fruits of *Ziziphus jujuba* Lam. on ECG parameters in Wistar rats

PARAM-ETERS	VEHICLE CONTROL	DISEASE CONTROL (DOX 5 mg/kg i.p.)	DOX+ ETOH200	DOX+ ETOH400	DOX+ AQ200	DOX+ AQ400	DOX+ NEB (5 mg/kg p.o.)
HR	368.6 ±12.85	172.2 ±1.26***	343.83 ± 19.47	348.68 ± 13.12	355.95 ± 0.26	413.85 ± 19.05*	339.15 ± 8.86
PR	0.045 ± 0.0058	0.04 ± 0.0003	0.049 ± 0.00028	0.051 ± 0.0004	0.046 ± 0.0004	0.0054 ± 0.0004	0.0041± 0.0004
QRS	0.017 ± 0.004	0.030 ± 0.01	0.016± 0.002	0.015 ± 0.0009	0.019± 0.001	0.013 ± 0.001	0.015 ± 0.001
QT	0.050 ± 0.011	0.051 ± 0.006	0.0506 ± 0.003	0.052 ± 0.006	0.0464± 0.001	0.052 ± 0.006	0.038 ± 0.003
QTc	0.116± 0.01	0.121 ± 0.01	0.121 ± 0.010***	0.125 ± 0.016	0.114 ± 0.006	0.135 ± 0.01	0.089 ± 0.007

The values are expressed as mean ± SEM (n = 6); *p < 0.05, **p < 0.01. ***p < 0.0001, compared to Doxorubicin group, **p<0.001 compared to the control group (Two-way ANOVA followed by Bonferroni test).

Table 5. Effect of ethanolic and aqueous extract (200 mg/kg p.o. and 400 mg/kg p.o.) of fruits of *Ziziphus jujuba* Lam. on blood pressure in Wistar rats

PARAM-ETERS	VEHICLE CONTROL	DISEASE CONTROL (DOX 5 mg/kg i.p.)	DOX+ ETOH200	DOX+ ETOH400	DOX+ AQ200	DOX+ AQ400	DOX+NEB (5 mg/kg p.o.)
SYSBP	110.96± 1.97	106.88 ± 1.66	99.37 ± 0.83***	119.43 ± 0.10***	84.79 ± 0.14***	102.93 ± 0.97***	70.21± 0.16***
DIABP	79.75 ± 3.64	68.11 ± 2.09***	84.55± 0.46	107.78 ± 0.23***	79.15± 0.18	83.78 ± 0.72	55.91± 0.67***

The values are expressed as mean ± SEM (n = 6); *p < 0.05, **p < 0.01. ***p < 0.0001, compared to Doxorubicin group, **p < 0.001 compared to the control group (One-way ANOVA followed by Dunnett test).

Table 6. Effect of ethanolic and aqueous extract (200 mg/kg p.o. and 400 mg/kg p.o.) of fruits of *Ziziphus jujuba* Lam. on left ventricular function in Wistar rats

PARAM-ETERS	VEHICLE CONTROL	DISEASE CONTROL (DOX 5 mg/kg i.p.)	DOX+ ETOH200	DOX+ ETOH400	DOX+ AQ200	DOX+ AQ400	DOX+NEB (5 mg/kg p.o.)
Max dp/dt	1968.36 ± 130.40	1246.24 ± 94.34***	265.43 ± 3.40***	724.83 ± 69.20***	410.86 ± 3.97***	1188.90 ± 39.68***	478.42 ± 15.46***
Min dp/dt	747.68 ± 36.89	426.29 ± 25.19***	118.40 ± 5.42***	181.19 ± 10.15***	167.68 ± 2.27***	337.75 ± 5.42***	93.77 ± 2.35***

The values are expressed as mean ± SEM (n = 6); *p < 0.05, **p < 0.01. ***p < 0.0001, compared to Doxorubicin group, **p < 0.001 compared to the control group (One-way ANOVA followed by Dunnett test).

Table 7. Effect of ethanolic and aqueous extract (200 mg/kg p.o. and 400 mg/kg p.o.) of fruits of *Ziziphus jujuba* Lam. on serum parameters in Wistar rats

PARAM-ETERS	VEHICLE CONTROL	DISEASE CONTROL (DOX 5mg/kg i.p.)	DOX+ ETOH200	DOX+ ETOH400	DOX+ AQ200	DOX+ AQ400	DOX+NEB (5mg/kg p.o.)
CK-MB	0.32 ± 0.01	0.48 ± 0.06	0.74 ± 0.22**	1.41 ± 0.003***	0.31 ± 0.004	0.32 ± 0.008	0.32 ± 0.006
Troponin-I	4.08 ± 0.04	1.46 ± 0.01***	0.02 ± 0.005***	1.03 ± 0.03***	0.01 ± 0.0004***	0.51 ± 0.01***	11.83 ± 0.47***
LDH	1004.7 ± 236.42	2465.8 ± 125.83*	1273.1 ± 238.60**	1346.4 ± 176.74*	1478.0 ± 109.8**	1336.5 ± 37.43	549.2 ± 100.86*

The values are expressed as mean ± SEM (n = 6); *p < 0.05, **p < 0.01. ***p < 0.0001, compared to the Doxorubicin group, **p < 0.001 compared to the control group (One-way ANOVA followed by Dunnett test).

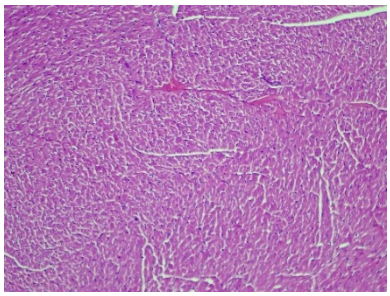


Figure 1. Control group.

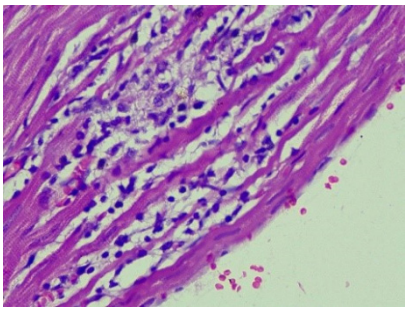


Figure 2. DOX group.

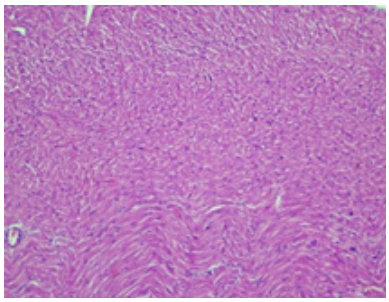


Figure 3. EtOH200 + DOX group.

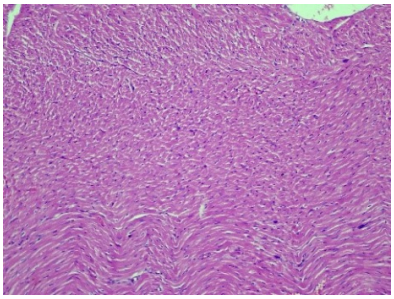


Figure 4. EtOH400 + DOX group.

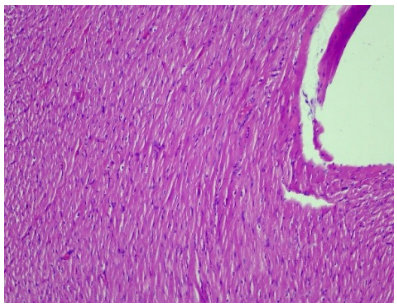


Figure 5. AQ200 + DOX group.

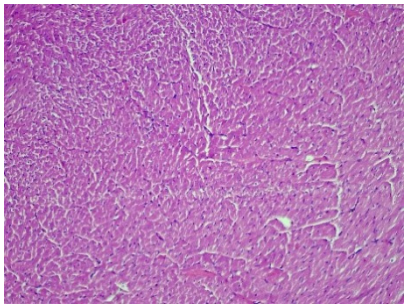


Figure 6. AQ400 + DOX group.

Table 8. Assessment of toxicity in the heart of various groups of rats

Parameters	Control	DOX	EtOH200 + DOX	EtOH400 + DOX	AQ200 + DOX	AQ400 + DOX	Std. + DOX
Necrosis	0	++	0	0	0	0	0
Cellular infiltration	0	++	0	0	0	0	0

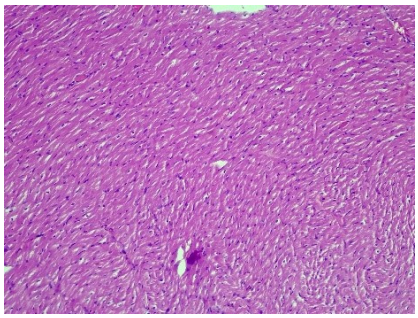


Figure 7. Std. + DOX group.

5. Discussion

Doxorubicin (DOX; Adriamycin) is an effective anticancer agent used to treat several forms of solid tumours, leukaemia, and lymphomas. The main limiting consequence is acute and chronic toxicity. Acute cardiotoxicity manifested as symptoms such as arrhythmias, however, chronic toxicity can progress to irreparable cardiomyopathy, which affects around 30-40 % of individuals who receive a total dosage of 500 mg/mm^2 [25]. DOX is a toxin to all biological components, including genetic material, kidney, heart, and liver, as well as myocardial tissue genetic material. DOX induces acute cardiac failure, which limits its effectiveness. As a result, innovative treatment agents with proven efficacy appear to be important for clinical approaches[26].

From cardiac hypertrophy to heart failure, compensatory systems such as the sympathetic nervous system are activated. These reactions have a large impact on biochemical, physiological, and molecular alterations. As a result, we chose doxorubicin-induced cardiac damage. However, the majority of scientists who have addressed this issue believe that cardiomyopathy is the most substantial contributor to the death of experimental animals following short-term DOX administration. Excessive fluid build-up was seen in rats treated with DOX in the pleural, pericardial, and peritoneal cavities. DOX administered intraperitoneally at a dosage of 20 mg/kg caused cardiac toxicity characterised by oxidant injury[25], as did doses of 25 mg/kg and 18 mg/kg induced heart toxicity as well. Doxorubicin was administered two times in equal dosages in the current study, for a total cumulative dose of 5 mg/kg i.p. Our findings demonstrated that a cumulative dosage of 5 mg/kg i.p. DOX caused cardiac damage in rats. The administration of DOX relies on the production of free radicals which is the primary concern regarding cardiac cell injuries. The mechanism regarding the generation of ROS and concern with damage to heart tissue involves many enzymes like NOS (Nitrous oxide), NADPH Oxidase (NOX) etc, which allow generating of oxidative stress[27]. Induction of Dox semiquinone radical formation due to binding of DOX to eNOS (endothelial Nitric Oxide Synthase). This formed radical reduces the free oxygen to superoxide free radical by an enzymatic one-electron reduction reaction, where the conversion shows cardiotoxic effects. The presence of flavoenzymes (NADPH-cytochrome P450 reductase and NADH dehydrogenase) helps in completing the reaction. As the Dox binds to eNOS (the reductase enzyme), there is an alteration in free radicals which leads to cardiotoxicity[27,28]. Antisense eNOS mRNA, interestingly, inhibits the caspase-3 activity stimulation which indicates the cardioprotective impact against DOX-induced cardiotoxicity[29]. This bi-nature drug is very potential to bind over the plasma protein as well as the cell membrane. The entry of DOX leads to the blocking of nucleotide replication which causes the inhibition of DNA and RNA polymerase activity. This further causes the interaction of DOX with topoisomerase-II which forms a complex concerning DNA rupturable, which is the key mechanism of cell-killing action by DOX[30]. Drug-membrane interactions cause changes in the physical characteristics of the cell membrane as a result of the drug's behaviour within the membrane. The physical characteristics of the membrane are affected by how the drug orients in the membrane, which components of the lipid membrane it interacts with, and whose relationships within the membrane it disrupts. This, in turn, can alter cell signalling and the function of membrane proteins such as transporters and

ion channels. This cell membrane causes the leakage of enzymes from cells into the blood. DOX has a high capacity for activity and affinity towards the cardiolipin protein, which causes a change in the function of the cardiolipin and the generation of superoxide radicals[31,32]. DOX lowers the heart's long fatty acid chain and increases glucose metabolism in the absence of oxygen, a process known as anaerobic metabolism. The increase in the levels of various enzymes caused by doxorubicin therapy is most likely due to the medication causing cardiac toxicity, although LDH, troponin-I and CK-MB are more specific for myocardial damage. DOX increased the activity of all of these enzymes in serum in this investigation. Individually, they are not specific for myocardial damage; however, evaluating these enzymes combined may be a sign of myocardial injury[25]. These findings corroborated prior doxorubicin-induced heart damage investigations conducted by other researchers[33,34]. In our study, the considerable rise in LDH, troponin-I and CK-MB produced by DOX was reduced in the EtOH400 and AQ400 treatment groups. Arrhythmia is another kind of doxorubicin cardiotoxicity that can develop at any time and after any dosage. Only the DOX group showed a substantial increase in PR, QTc, QT, and QRS intervals. ECG alterations are associated with increased action potential length, although it is thought that DOX has the greatest impact on the recovery phase of the transmembrane action potential, altering Ca^{2+} migrations across the cellular membrane preferentially[35]. Pre-treatment of EtOH and AQ groups of ZJ inhibits the DOX-induced alterations in PR, QT, QTc and QRS complex. Doxorubicin is a systolic and diastolic heart function inhibitor. Furthermore, the metabolite, Doxorubicinol is a more powerful inhibitor of three-ion pumps than doxorubicin. In addition, Doxorubicinol inhibited the calcium-loading activity of cardiac sarcoplasmic reticulum vesicles. Doxorubicin is known to impair cardiac function in both humans and experimental animals[36]. In the current study, a drop in systolic and diastolic pressure, max dp/dt, min dp/dt were found in the DOX group, which was reversed in the AQ400 treatment groups. Xue *et al.*, 2021 reported that rutin[37], epicatechin[38], kaempferol[39] are the flavonoids which are present in ZJ[40] and exhibit antioxidant action. Antioxidants present in ZJ, prevent necrosis and cellular infiltration by inhibiting the generation of free radicals[41]. Cancer and cardiovascular disease may arise as a result of oxidative damage[42]. ZJ has several antioxidant components that defend against damaging free radicals and are significantly linked to a lower incidence of cardiovascular disease.

6. Conclusion

In the present study, an aqueous extract of ZJF (400 mg/kg) showed a cardioprotective effect in doxorubicin induced cardiotoxicity. This study suggested a possible usefulness of ZJF as a cardioprotective agent contributing to a safer use of doxorubicin in patients subjected to chemotherapy. However, a cardioprotective effect was observed only in the higher dose of ZJF. Further dose response and bioavailability studies are needed to prove the efficacy of ZJF as a cardioprotective drug.

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