RESEARCH PAPER

In - Vivo Cardioprotective Activity Of *Diptheracanthus Patulus* On Doxorubicin Induced Cardiotoxicity

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Abstract

The evaluation the efficacy of the ethanolic extract of *Diptheracanthus patulus* (DPEE) in mitigating doxorubicin-induced cardiotoxicity using a Wistar rat model, supported by biochemical, histological, and chromatographic analyses. The study was conducted using healthy adult Wistar rats, divided into the following groups: Normal control, Doxorubicin-treated (disease control), treatment group. Doxorubicin was administered intraperitoneally to induce cardiotoxicity, while DPEE was given orally. The extract was subjected to qualitative screening, and HPTLC was used to identify and quantify β-sitosterol and other phytoconstituents. The cardioprotective effect of DPEE was evaluated by using Biochemical markers: Levels of AST, ALT, LDH, CK-MB, CK-NAC, and MDA were measured to assess myocardial damage and oxidative stress. Lipid profile: HDL, LDL, VLDL, total cholesterol, and triglycerides were examined to determine the impact on lipid metabolism. Histopathological study carried by Heart tissues was examined under light microscopy using Hematoxylin & Eosin staining to assess tissue architecture and cellular damage. Doxorubicin administration resulted in elevated levels of cardiac enzymes (AST, ALT, LDH, CK-MB, and CK-NAC), increased lipid peroxidation (MDA), and dyslipidemia. Treatment with DPEE significantly attenuated these alterations, suggesting protection against oxidative stress and myocardial damage. Histopathological evaluation revealed that DPEE preserved normal cardiac tissue structure, in contrast to the degeneration observed in the doxorubicin-only group. The study concludes that ethanolic extract of *Diptheracanthus patulus* exhibits significant cardioprotective activity, likely due to its rich phytoconstituents with antioxidant potential. These findings support the therapeutic role of *Diptheracanthus patulus* in managing drug-induced cardiotoxicity.

Keywords- Diptheracanthus Patulus, HPTLC, β-Carotene, Doxorubicin, Cardiotoxicity

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Introduction-

Cancer patients now have a longer life expectancy because to new anticancer drugs and targeted therapy. However, for patients with cancer, many of these treatments might result in life-threatening side effects such heart failure, cardiomyopathy, etc. One Cardiotoxicity is the main cause of morbidity in cancer patients and is a serious issue with anticancer medications, particularly those in the anthracycline group (doxorubicin). Although their exact mechanism of cardiotoxicity is unknown, other cytotoxic medications such 5-flurouracil, cyclophosphamide, and toxoids are also linked to cardiotoxicity.2. Doxorubicin triggers the production of reactive oxygen species (ROS), which causes oxidative stress that disrupts mitochondrial function, damages cellular membranes, and leads to cytotoxic effects. 3 Numerous contemporary therapeutic agents have been explored for their ability to provide cardiac protection. However, these agents have not offered an adequate solution to the issue of cardiotoxicity resulting from medications that are crucial for treating cancers and various other conditions. *Diptheracanthus patulus* has demonstrated a protective effect on the heart against Doxorubicin-induced cardiotoxicity, which can be attributed to its antioxidant properties. Traditionally, it has been utilized to address various health issues, including pain, inflammation, fever, and intestinal diseases. The plant demonstrates a broad range of pharmacological effects, such as anti-inflammatory, anti-cancer, antioxidant, hepato-protective, and cardio-protective properties.

Dipteracanthus patulus (Jacq) Nees (DP) is a type of undershrub that belongs to the Acanthaceae family. Traditional medicine has noted that DP is utilized for treating eye ailments

by applying its extract to the eyelid. 4 Additionally, the leaves from several *Dipteracanthus* species have been employed to combat various infectious diseases.5 DP has been found to contain compounds such as lyoniresinol-9'-O-β-D-glucoside, 5,5-dimethoxy-lariciresinol-9-O-β-glucopyranoside, β-sitosterol, lupeol, α-ethyl galactose, apigenin-7-O-rutinoside, α-D-glucose, β-D-glucose, and β-D-fructose. Furthermore, various phytochemicals including ascorbic acid, phenolic compounds, tannins, lycopene, carotenoids, and α-tocopherol have also been identified in DP. 6 The assessment of phenolic levels, along with high-performance thin-layer chromatography (HPTLC) profiling and quantification of β-carotene and β-sitosterol in MEDP, was conducted to identify the phytoconstituents that contribute to this activity. 7

Method

Animals –

Prior to starting the experimentation, approval from the Institutional Animal Ethics Committee (Approval No: CPCSEA/CBPL/AH/97) was acquired for the study plan. A total of 18 male wistar, weighing between 150 and 250 g, were procured from the Cristal biological solution pone, Maharashtra, India, for carrying out experimental investigation. Throughout the study period, the mice were cared for and the experiments were carried out in accordance with the standards set by the Committee for Control and Supervision of Experimentation on Animals (CCSEA, formerly CPCSEA), India, for the use and care of laboratory animals.

Plant material

Plant Material βThe plant material was collected in the month of June 2010 from the nearby villages of Solapur district (MS), India [It is located between 17.10 to 18.32 degrees to the north latitude while it is about 74.42 to 76.15 degrees to the east longitude]. The soil is black and of fertile quality and rain fall is scanty. The collected plant material

was botanically authenticated (Certificate No. BSI/WC/Tech/2007/460 dated: 03/08/2007) by Botanical Survey of India (BSI), Pune Division, Maharashtra State, India and the voucher specimen is deposited in BSI, Pune, MS (India).

Preparation of plant extract

The required dose of *Dipteracanthus patulus* extract was prepared by suspending the weighed amount of extract in 1% carboxymethylcellulose (CMC) solution. Briefly, 1% CMC was prepared by dispersing 1 g of CMC in 100 mL of distilled water with continuous stirring until a homogenous gel-like suspension was obtained. The plant extract was triturated with a small quantity of 1% CMC and then made up to the required volume to obtain a uniform suspension. The prepared suspension was freshly made each day and administered orally to the animals using a gavage needle at a dose of 100 mg/kg body weight.

Chemicals

B-sitosterol (Sigma Aldrich, USA), ethanol, doxorubicin and other reagents of analytical grade were purchased from labware pharmaceuticals latur.

Phytochemical screening

The qualitative phytochemical analysis was performed for EEDP extracts to determine the presence/ absence of various chemical constituents viz carbohydrate, tannins, phenolics; flavonoids, steroids, carotinoids and iridoid glycoside [8 & 9].

HPTLC Analysis-

HPTLC was performed on separate precoated silica gel aluminium TLC plates 60F254S (E-Merk, Germany) for qualitative evaluation of β-sitosterol in ethanolic extract of DP. In brief, concentrated EEDP (10µL) and standard markers (5µL) were loaded on TLC plates with Camag Linomat 5 applicator with nitrogen supply. The mobile phase used for β-sitosterol was toluene: ethyl acetate (80:20 v/v). The plates were developed to a distance of 80mm in a Camag twin-trough chamber previously equilibrated with mobile phase for 20min. After development of βsitosterol plate, derivatization was carried out with 5% Anisaldehyde sulphuric acid in methanol and heated at 105oC on Camag TLC plate platform heater for 4 min. Camag TLC visualizer-2 was used for photodocumentation of at 254nm, 366nm and White R. The β Sitosterol HPTLC chromatogram was obtained using Camag TLC Scanner-3 in conjunction with Vision CATS- 3.2SP software

Group	N0. Of animals	Treatment
Group I. Normal Control	6	Distilled water daily (1 mL/kg, orally)
Group II. Disease Control	6	Distilled water daily (1 mL/kg, orally) + DOX (15 mg/kg, i.p.)
Group III.Standard	6	Diptheracanthus patulus (100 mg/kg) orally + water (1 mL/kg, orally) + DOX (15 mg/kg, i.p.)

Experimental Study Design-

Eighteen rats were randomized into three major groups of six animals each. Group I served as control group, group II was doxorubicin (DOX), group III was given DP extract. All these groups were studied for 11 days. Drugs were administered as following; Group I distilled water/Normal saline (1 mL/kg, orally). Group II Distilled water daily (1 mL/kg, orally) + DOX (15 mg/kg, i.p.) single dose on 7th day. Group III *Diptheracanthus patulus* (100 mg/kg) orally + water (1 mL/kg, orally) + DOX (15 mg/kg, i.p.) single dose on 7th day of experiment). All experiments were performed in a balanced design (six animals per group) to avoid being influenced by order and time.

Rats were divided randomly into three groups (six rats per group), and a treatment set of 10 days was selected. After 24 h of the last treatment (day 11), rats were euthanized. The sera and plasma were collected for biochemical assays. The animals were

euthanized with anesthesia (diethyl ether). The animal hearts were then removed after thorax surgery and fixed in 10% buffered formalin. Heart tissues were embedded in paraffin with 5 µm sections and stained with H&E (hematoxylin and eosin) for histology evaluation.

Biochemical parameter and lipid profile Study:

At the end of treatment rat was anesthetized with ethanol. Blood was collected from the

retro-orbital plexus without any anticoagulant. The serum was separated by centrifuging at 3000 rpm for 15 min and investigated the following parameters LDL, HDL, VLDL, Triglycerides, Total Cholesterol, AST, ALT, LDH, CK-NAC, CK-MB and oxidative stress markers by PreClinBio solution, LLP Pune, India, where they performed histopathology of heart.

Histopathology Studies:

On day 11, the rats were deeply be anesthetized and sacrificed by decapitating their heart. After decapitation, heart was carefully isolated and fixed in 10% formalin solution. The samples were then sent to PreClinBio solution, LLP Pune, India, where they performed histopathology of heart.10

Statistical Analysis:

The results were obtained using statistical analysis of means ± SEM (n = 5). Statistical analysis was performed using one-way ANOVA (analysis of variance) followed by Tukey's multiple comparison tests using GraphPad Prism (ver. 8.0). $p < 0.05$ was considered statistically significant.

Result

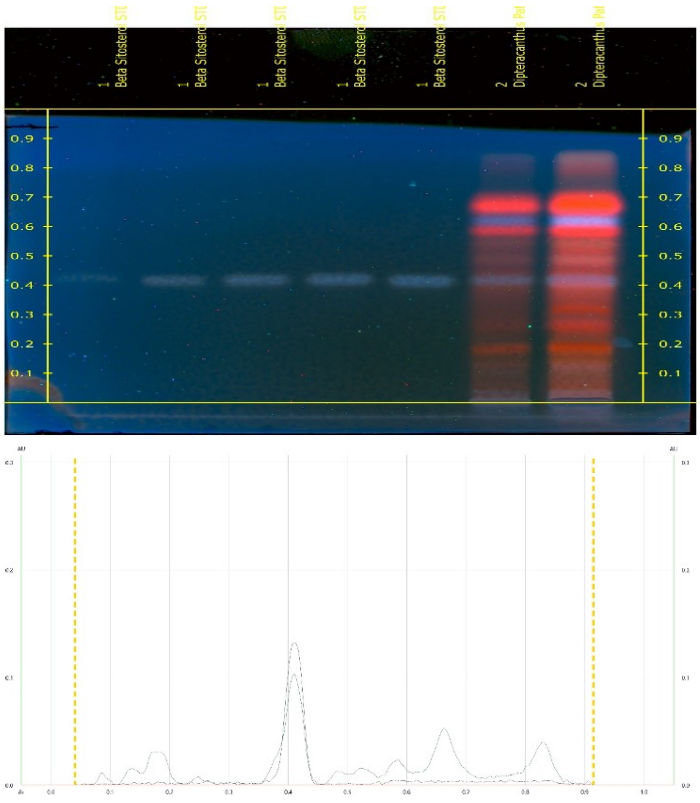
Identification Test of Phytoconstituents

Preliminary phytochemical analysis of ethanolic extract showed the presence flavonoids, phenols, alkaloid, glycosides, saponins and tannins. Observation preliminary phytochemical analysis is illustrated in Table 1

Test	*Diptheracanthus Patulus*
Tannins	+
Flavonoids	+
Saponins	+
Terpenoids	+
Alkaloids	+
Phenolic	+
*+ Indicate Presence, - Indicate Absence	

Quantitative analysis of β-sitosterol by HPTLC

HPTLC analysis of DP extract confirmed the presence of beta sitosterol at Rf value 0.41.



The HPTLC spectrum of beta sitosterol and EEDP recorded in camag TLC scanner 3 (s/n151015) at 518 nm showed complete overlapping at start, middle and end position of spectrum. The photo documentation of beta sitosterol TLC plate after derivatization anisaldehyde-sulfuric acid in methanol showed grey colour band in camag visualizer at fluorescence 366 nm. The HPTLC Chromatogram obtained after the densitometric scan of developed plates where integrated using wincats software to calculate the areas of beta- sitosterol in ethanolic extract DP and standard compound. The beta-sitosterol found was 0.19% of sample.

Biochemical Test Parameters

Biochemical parameters levels AST, ALT, CKMB, CK-NAC, LDH, MDA increase in doxorubicin treated group (Table 2-3) as compared to the control group and DP extract treated group. Lipid profile LDL, VLDL, TOTAL CHOLESTEROL, TRIGLYCERIDES also increase in doxorubicin treated group as compare to control group and DP extract treated group only HDL level decrease in doxorubicin group as compare to control and DP extract treated group. (Table 1)

Table -1 Effect of vehicle, Doxorubicin and groups treated with ethanolic extracts of *diptheracanthus patulus* for the duration of 10 days

Treatment	Dose	HDL (IU/L)	LDL(IU/L)	VLDL(IU/L)	Cholesterol (IU/L)	Triglycerides (IU/L)
Vehicle	1ml/kg orally	54.47 ±0.46	18.80 ±0.33	22.30 ±0.49	95.57± 1.27	111.50±2.44
Doxorubicin	15mg/kg i.p	41.9± 0.36***	32.93 ±0.43***	27.8± 0.43***	102.63 ±0.43***	139.0 ±2.04***
***Diptheracanthus patulus* extract**	100 mg/kg orally	52.0± 0.6###	28.0± 0.6###	11.8± 0.3###	91.08± 0.5###	59.0± 1.4###

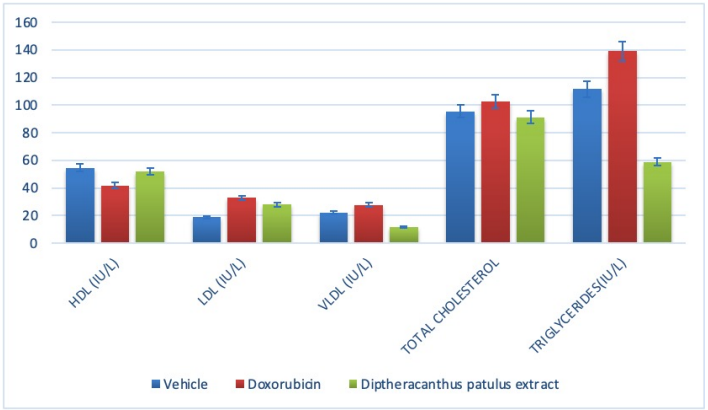


Fig 1 effect of ethanolic extract on dox induced cardio toxicity in rat with respect to serum lipid level (mg/dl) changes. *p < 0.005=significant, **p < 0.01=highly significant, **p < 0.001very highly significant p< 0.001 =***vs vehicle group; p <0.001###vs Dox group.

Table 2 Effect of vehicle, Doxorubicin and groups treated with ethanolic extracts of *diptheracanthus patulus* for the duration of 10 days

Treatment	Dose	ALT(U/L)	LDH(U/L)	CKMB(U/L)	AST(U/L)	CK-NAC(U/L)
Vehicle	1ml/kg orally	44.90±0.32	180.65±0.88	93.70±1.08	58.72±0.67	130.17±0.79
Doxorubicin	15mg/kg i.p	110.78±1.18***	390.50±1.41***	220.47±0.96***	125.45±0.76***	300.53±1.59***
Diptheracanthus patulus extract	100mg/kg orally	59.53±0.6###	252.92±6.88###	128.62±1.98###	66.80±0.62###	168.18±2.80###

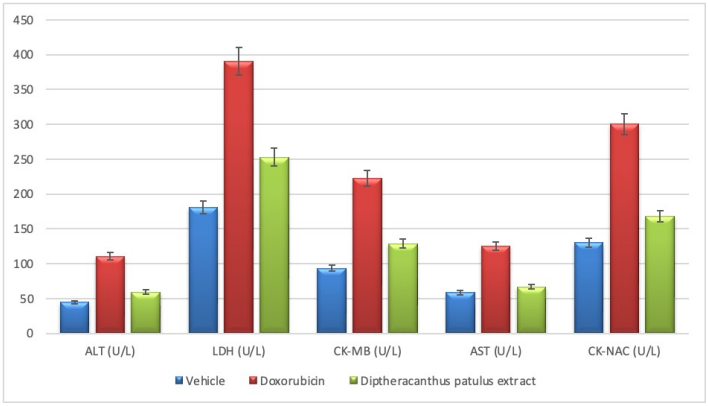


Fig 2 effect of addminitration of ethinolic extract of *Diptheracanthus patulus* extract on ALT, LDH, CK-MB, CK-NAC in DOX indusced cardio toxicity in rat. Valuve are represented as the means ± SEM (n=6); p< 0.001 =***vs vehicle group; p <0.001###vs Dox group.

Table 3 Effect of vehicle, Doxorubicin and groups treated with ethanolic extracts of *diptheracanthus patulus* for the duration of 10 days

Treatment	Dose	MDA(NMOL/ML)
Vehicle	1ml/kg orally	2.32±0.02
Doxorubicin	15mg/kg i.p	4.8±0.1***
***Diptheracanthus patulus* extract**	100mg/kg orally	1.92±0.05###

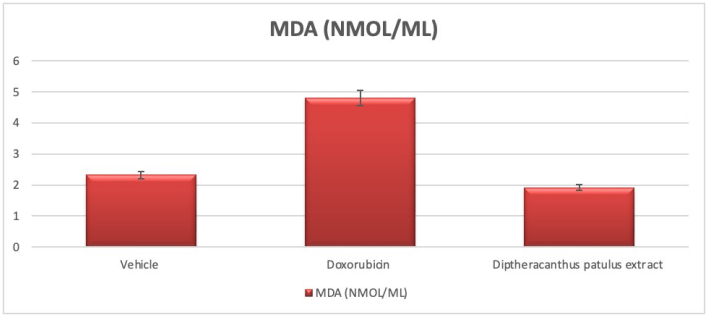
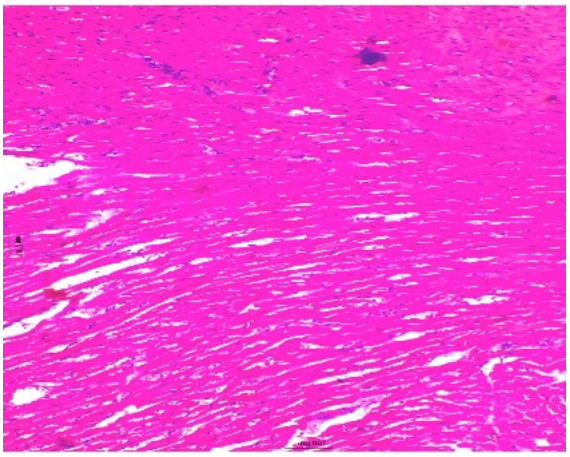


Fig 3 effect of addminitration of ethinolic extract of *diptheracanthus patulus* extract on mda in dox indusced cardio toxicity in rat. valuve are represented as the means ± sem (n=6); plant extract of *diptheracanthus patulus* was administrated orally to rats with a dose of 100mg/kg for 10 days. ***p <0.001 vs vehicle group (doxorubicin-induced cardiotoxicity), ###p <0.001vs doxorubicin. (DP extract treated).

Histopathological examination

The histology of the heart tissue from rats of control group I and group III (DP extract) showed normal morphological appearances. While in group II (DOX) disruption, loss of myofibrils and vacuolization of the cytoplasm were observed.



H1 (10X) H1(40)

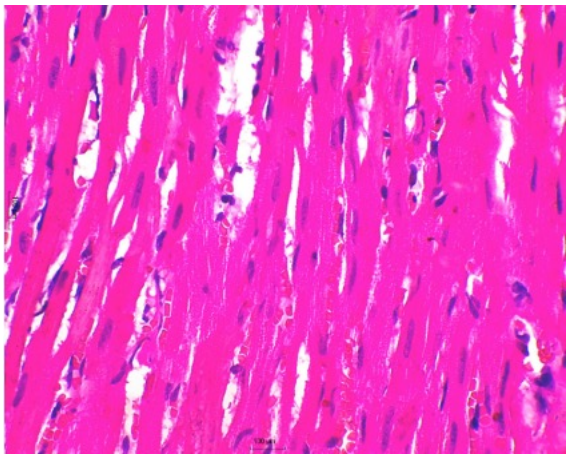


Fig-1 Microscopic features of rat heart treated with normal sal

H2(40 X) H2 (10 X)

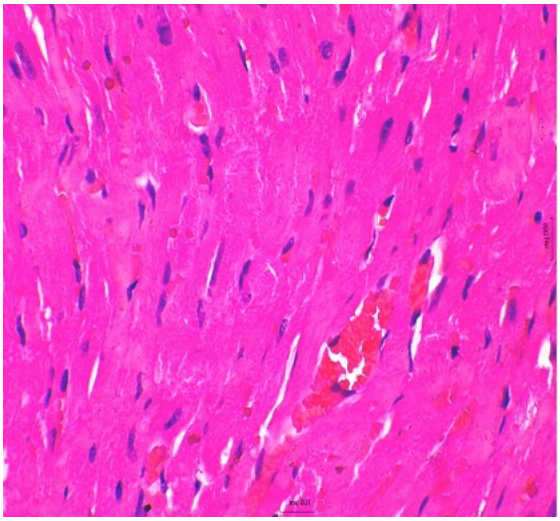
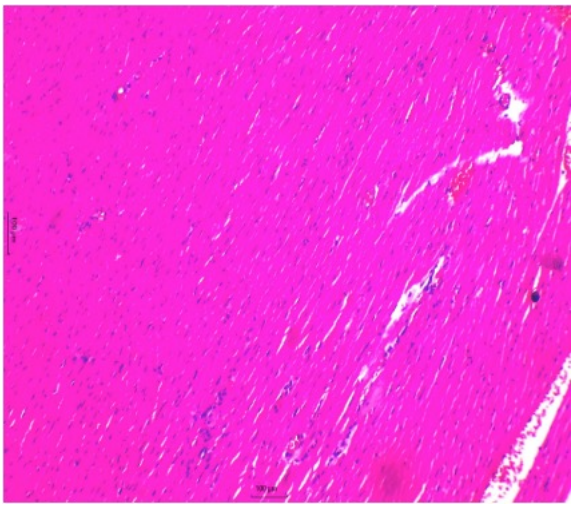


Fig-2 Microscopic features of rat heart treated with doxorubicin.



H3 (10 X) H3 (40 X)

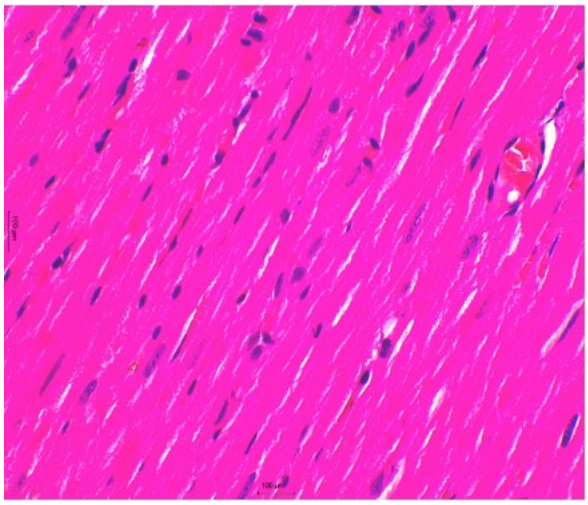
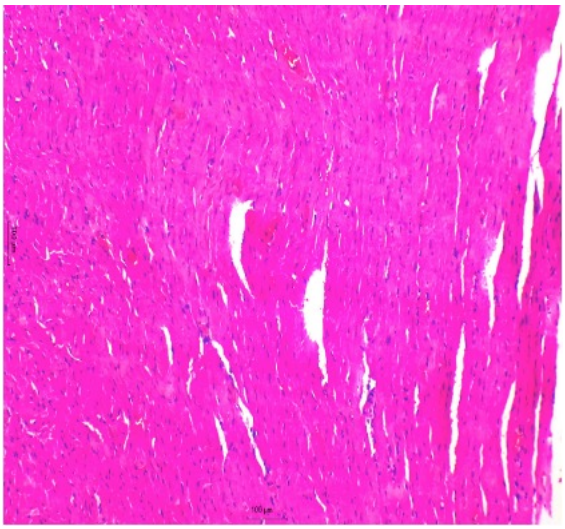


Fig-3 Microscopic features of rat heart treated with DP extract.



CONCLUSION

The present study focused on evaluating the cardioprotective potential of *Diptheracanthus patulus* ethanolic extract (DPEE) against doxorubicin-induced cardiotoxicity in rats. Doxorubicin, an anthracycline antibiotic used in cancer treatment, is well-known for inducing oxidative stress and cardiomyocyte damage through the generation of free radicals and lipid peroxidation, leading to myocardial injury and altered biochemical parameters. (107)

Preliminary phytochemical screening of DPEE revealed the presence of flavonoids, phenols, alkaloids, tannins, glycosides, saponins, and terpenoids, indicating its rich antioxidant potential. HPTLC analysis confirmed the presence of β-sitosterol (0.19%), a phytosterol known for its anti-inflammatory and cardioprotective effects. (108)

The administration of doxorubicin significantly elevated serum levels of cardiac enzymes including AST, ALT, LDH, CK-MB, CK-NAC, and MDA, indicating myocardial damage and oxidative stress. Concurrent administration of DPEE significantly reduced these elevated enzymes levels compared to the doxorubicin control group. This reduction suggests a protective role of the extract against doxorubicin-induced myocardial toxicity, likely due to the antioxidant activities of the phytoconstituents. (109)

Doxorubicin disrupted lipid metabolism, as evident from increased LDL, VLDL, total cholesterol, and triglycerides, and reduced HDL levels. In contrast, the group treated with DPEE showed a significant improvement in lipid profile, restoring these values close to normal. This lipid-lowering effect could be attributed to the presence of flavonoids and β-sitosterol, which have been reported to modulate lipid metabolism and enhance HDL levels. (110) 111)

Histological sections of cardiac tissues showed that rats in the Dox group exhibited myocardial degeneration, vacuolation, and inflammatory infiltrates, Indicative of severe cardiac damage. However, treatment with DPEE preserved myocardial architecture with only mild congestion and no significant structural abnormalities, confirming the cardioprotective potential of the extract.

The cardioprotective effect of DPEE may be primarily due to its ability to scavenge free radicals, reduce lipid peroxidation (as shown by decreased MDA), and stabilize cell membranes, thereby preventing enzyme leakage and maintaining myocardial integrity. β-sitosterol, in particular, plays a crucial role by inhibiting inflammatory cytokines and improving cardiac function. (112)

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