



# Phytochemical And Pharmacological Evaluation Of *Prunus Domestica* L. Seed Extracts In Doxorubicin-Induced Cardiotoxicity In Wistar Rats

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## Abstract

*Prunus domestica* L., a member of the Rosaceae family, has a wide range of biological properties, including hepatoprotective, antibacterial, antioxidant, and antihaemolytic effects. In the present investigation, we assessed the nutritional value, conducted phytochemical screening, determined the total phenolic content, and examined the protective impact against doxorubicin-induced cardiotoxicity. We have concentrated on the biochemical changes caused by the induction of cardiotoxicity and the potential therapies using our experimental medicine, as well as comparing them to the conventional drug, Aspirin. The presence of heightened biochemical markers, including triglycerides, total cholesterol, LDL, CPK-NAC, SGPT, and SGOT, serves as a strong indicator of cardiotoxicity produced by Doxorubicin. The findings indicate that it serves as a viable energy source. Through phytochemical screening, a wide range of secondary metabolites were identified, including alkaloids, carbohydrates, glycosides, proteins, steroids, terpenoids, fixed oils and fats, as well as phenolic compounds. The findings from animal research indicate that the use of plant seed extracts derived from *Prunus domestica* L leads to a decrease in increased biochemical markers during the therapy of cardiotoxicity.

Keywords- *Prunus domestica*, Cardiotoxicity, Doxorubicin, Total Phenolic Content

## Introduction

Diverse fruits are a significant component of the human diet. There is a global consensus that consuming a diet rich in fruits and vegetables reduces the risk of chronic illnesses such as cardiovascular diseases and cancer, and promotes longevity and good health (1). Nutraceuticals or functional foods have been often associated with these food items. Foods that possess high levels of antioxidants, such as phenolic acids and flavonoids, are in great demand within the contemporary market as nutraceuticals. Fruits are considered to be

a low-fat and low-calorie source, although dried fruits are known for their high protein and mineral content (2).

*Prunus domestica* L., a member of the Rosaceae family, is often known as plum (2, 3). The Rosaceae family is the 19th biggest family of plants, consisting of over 3000 species. In contrast, the *Prunus* genus has around 400-430 species, although only 89 of these are officially recorded in the Genetic Resources Information System. In the Indian context, a total of 36 *Prunus* species have been documented, out of which 18 species have been identified as suitable for various agricultural applications (5-9).

In conjunction with other medications, it is used for the management of leucorrhoea, irregular menstrual cycles, and miscarriage resulting in debility (10). Plums and prunes have a laxative action on the stomach. The use of the bark of *P. domestica* as a febrifuge and the roots as an astringent has been documented (11). *P. domestica* serves as a major provider of essential nutrients such as calcium, magnesium, vitamin A, potassium, and fibre (12). This particular species has a distinct position within the Indian pharmaceutical system owing to its multitude of health advantages (2). The composition of this substance is rich in many bioactive phytochemicals, including anthocyanins, pectins, carotenoids, lignans, abscisic acid, glucoside, flavonoids, flavonoids glycosides, bipyrrole, dihydroflavonols, and carbohydrates (12-19). The content of these bioactive chemicals varies based on several pre-harvesting parameters (20). Plums are a very nutritious food that greatly contributes to human nutrition. This fruit has substantial antioxidant activity due to its abundance of significant phytochemicals (21). Moreover, many extracts derived from *P. domestica* have significant effects, including antibacterial, anticancer, antihyperlipidemic, blood pressure-lowering, anxiolytic, and antidiabetic properties (2, 22, 23).

The *P. domestica* fruit is succulent and succulent, often devoured, although the seed is frequently disposed away. Our work seeks to assess the phytopharmacological efficacy of the seed (or kernel) of *P. domestica* against doxorubicin-induced cardiotoxicity in wistar rats, acknowledging the potential of this ignored component.

## Materials and Methods

### Reagents and Chemicals

Petroleum ether (SD fine), methanol (Merck), diethyl ether (SD fine), ethyl acetate (Merck), double distilled water, folin's reagent (Merck), sodium hydroxide (Fisher), sodium acetate trihydrate (Merck), nitric acid (Fisher), gallic acid (Merck), sodium carbonate monohydrate (Merck), sulphuric acid (Fisher), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Alpha acer), ascorbic acid (Merck) and 2,4,6-tris-(2-pyridyl)-s-triazine (TPTZ) (SRL chem). Other than that, all of the chemicals that were used in this investigation were of an analytical grade.

## Sample collection

In order to gather the seeds, plums were taken from a garden located in the Almora area of Uttarakhand. Under the shade, the seeds were allowed to dry. It was determined by the CSIR-NISCAIR in New Delhi that the plant was legitimate and authentic. In order to verify the authenticity of the plant material, specimens were made and then placed in the Raw material herbarium and museum in New Delhi before being submitted for certification.

## Analysis of Physical Parameters and Estimates of Probability

Evaluation of the physical parameters and proximate analysis for the purpose of calculating the nutritive value were carried out in accordance with the Indian pharmacopoeia and a previous work on *Bombax ceiba* (24, 25).

**Getting Ready to Prepare Extracts To extract the substance, a Soxhlet extractor was used.** For the purpose of this brief explanation, the thimble of the soxhlet extractor was filled with 200 grammes of the powdered plant material. Petroleum ether, diethyl ether, ethyl acetate, methanol, and double-distilled water were the solvents that were used in the extraction process. The sequence of the solvents was determined by the increasing polarity of the solvents. For each solvent, about 72 cycles of syphoning were carried out, or the extraction process was prolonged until the syphoning tube seemed to be colourless. Following the extraction process, the obtained extracts from the various solvents were concentrated using a rotary evaporator under decreased pressure. The resulting concentrates were then placed in a refrigerator for further analysis.

## Phytochemical Analysis and Review

Using established qualitative procedures (26, 27), phytochemical screening was performed on the various extracts to determine whether or not they contained the various phytoconstituents being sought. Each concentration was examined to see whether or not it included natural active mixtures such as alkaloids, sugars, glycosides, amino acids, proteins, triterpenoids and steroids, flavonoids, phenolics, and gums. Also included are mucilages, naphthoquinones, and other similar substances.

## Quantity of Phenolics in total

The folin-ciocalteau technique was chosen for the purpose of determining the total phenolic content of each extract, with certain adjustments being made to the existing procedure. In a nutshell, a 100  $\mu$ l dilution of the extract, with a concentration of 1000  $\mu$ g/ml, or gallic acid, with a concentration ranging from 10 to 400  $\mu$ g/ml, was placed in a test tube that contained 7.9 ml of pure water. Additionally, distilled water may be used as a blank. The volumetric flask was then filled with 500 microliters of the folin's reagent, which was then shaken well before being incubated for eight minutes at room temperature. After adding 1.5 ml of 20%  $\text{Na}_2\text{CO}_3$  to the combination described above, the total volume was determined to be 10 ml, and the mixture was then incubated at room temperature for two hours. At a wavelength of 765 nm, an absorbance measurement was taken using a UV-Vis double beam spectrophotometer (Systronics 2205). The calibration curve of gallic acid was used with the purpose of carrying out the calculations. The amount of total phenolic content in each extract was expressed as milligrammes of gallic acid equivalents (GAE) per gramme of dry weight (mg GAE/g dw) (28).

## Experimental animals

This study was conducted in Wistar albino rats (200-250 g), which were kept in the animal house of Siddhartha Institute of Pharmacy, Dehradun, India, for one week before starting the experimental protocol for proper acclimatization under the controlled condition of illumination (12 h light/12 h darkness) and temperature  $25^{\circ}\text{C} \pm 5^{\circ}\text{C}$ . They were housed under ideal laboratory conditions and maintained on a standard pellet diet and water *ad libitum* throughout the experimental period. The Institutional Animal Ethics Committee (IAEC) approved the study, SIP, Dehradun, India, with protocol number SIP/IAEC/PCOL/10/2020.

## Cardiotoxicity Induction

The animals' cardiotoxicity was induced by the intraperitoneal administration of Doxorubicin 15mg/kg on the 7<sup>th</sup> day of experimentation. This induction administration was done to all group animals except the normal group.

## Experimental Design

In this experiment, a total of 30 Wistar rats were used. The rats were randomly divided into eight groups comprising six animals in the group as follows:

Group 1: Normal control, received normal saline (1 ml/kg p.o.) for 18 days

Group 2: Disease control, received doxorubicin (15 mg/kg i.p) from the 7<sup>th</sup> day up to the 18<sup>th</sup> day

Group 3: Standard, received Aspirin (1 mg/kg) for 18 days and received doxorubicin (15 mg/kg i.p ) from the 7<sup>th</sup> day up to the 18<sup>th</sup> day

Group 4: Test group PDE1 received (100 mg/kg, oral) for 18 days and received doxorubicin (15 mg/kg i.p ) from the 7<sup>th</sup> day up to the 18 day

Group 5: Test group PDE2, received (200 mg/kg, oral) for 18 days and received doxorubicin (15 mg/kg i.p ) from the 7<sup>th</sup> day up to the 18 day

On the 31<sup>st</sup> day, blood samples were collected from the rat tail veins for biochemical determinations. Later, the rats were sacrificed under the influence of anesthesia. The hearts were excised immediately, rinsed in ice-cold normal saline, and used for the following assays.

## Biochemical determinations

Parameters such as Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Creatinine Kinase (CK), Total Cholesterol (TC), Triglycerides (TG), Low-Density Lipoprotein (LDL), High-Density Lipoprotein (HDL) and serum inflammatory markers CK-NAC (U/L), TNF- $\alpha$ , IL-6, IL-1, NF- $\kappa$  levels were estimated at Hi-Tech Pathology Lab Dehradun, Uttarakhand.

## Histopathological examination of the heart

The heart was isolated immediately after the animal was sacrificed, washed with ice-cold normal saline, and fixed in a 10% buffered neutral formalin solution. After fixation, the heart tissue was processed by embedding it in paraffin. Then, the heart tissue was sectioned and stained with hematoxylin and eosin (H.E.) for histopathological examination.

### Statistical Parameters

Results were expressed as mean  $\pm$  SEM (n=6). Statistical analysis was performed with a one-way analysis of variance (ANOVA) followed by a student t-test. P value less than  $<0.05$  was considered significant. \* $P<0.05$ , \*\* $<0.01$  and \*\*\* $<0.001$ .

## RESULTS

### Phytochemical Screening

Phytochemical screening shows the presence of carbohydrates, glycosides, protein, steroids and terpenoids, fixed oil and fats, flavonoids and phenolic compounds. Bioactivity and the therapeutic value of the plant extract are attributed to the phytoconstituents of the plant extract.

### Total Phenolic Content

The total phenolic content of the different fractions was found to be in the order methanol > ethyl acetate > diethyl ether > water > petroleum ether in an amount of  $1.72 \pm 0.177 > 1.02 \pm 0.056 > 0.95 \pm 0.023 > 0.53 \pm 1.732 > 0.11 \pm 0.02$ . All the total phenolic contents are expressed in terms of mgGAE.gm<sup>-1</sup>dw. Calculating the total phenolic content calibration curve tannic acid with equation  $y = 0.0088x$  and  $R^2 = 0.9994$ .

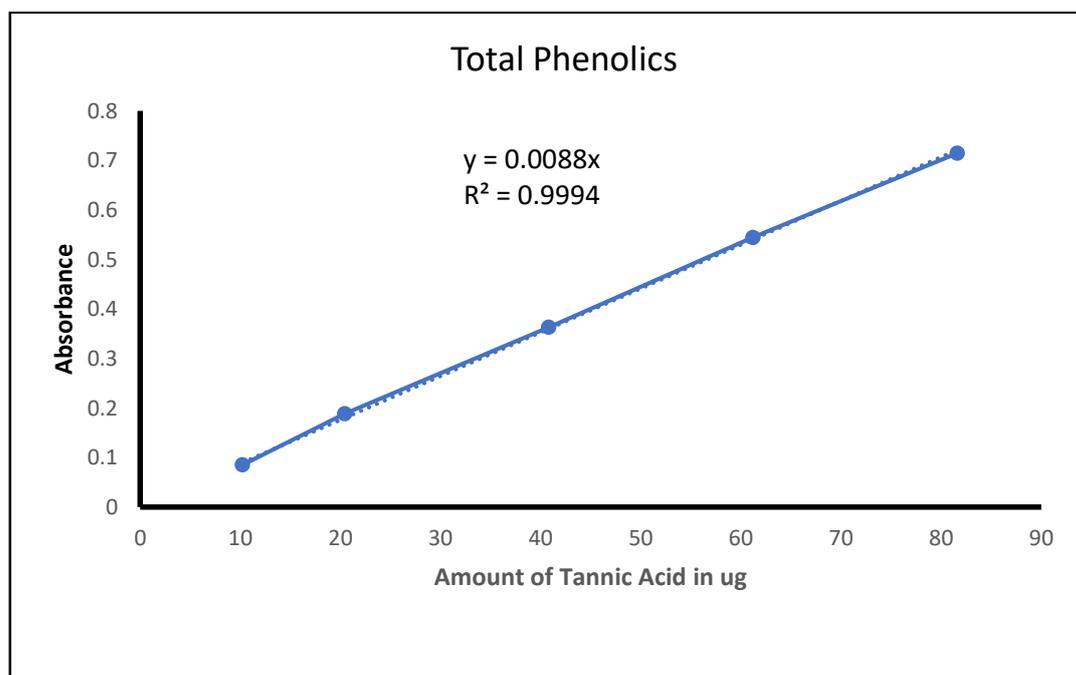


Fig.1 Graph of Total Phenolic Content of *Prunus domestica* L seed extract (PDE)

**Effect of PDE1 and PDE2 on SGPT, SGOT, Total Cholesterol, Triglycerides, HDL and LDL****Table 1 Effect of various pharmacological interventions on level of SGPT (IU/L)**

S.No.	Group	SGPT Level
1.	Normal Control	20.66±2.16
2.	Disease Control	43.16±1.94****
3.	Standard Control	24.56±1.05***
4.	PDE 1	32.96±1.24****
5.	PDE 2	28.7±1.01****

**Table 2. Effect of various pharmacological interventions on the level of SGOT (IU/L)**

S.No.	Group	SGOT Level
1.	Normal Control	55.83±1.47
2.	Disease Control	97.5±1.87****
3.	Standard Control	60.81±1.48****
4.	PDE 1	82.93±1.44****
5.	PDE 2	75.05±1.39****

**Table 3. Effect of various pharmacological interventions on the level of Total Cholesterol (mg/dL)**

S.No.	Group	Total Cholesterol Level
1.	Normal Control	78.53±0.25
2.	Disease Control	146.5±1.87****
3.	Standard Control	95.83±1.47****
4.	PDE 1	128.43±1.00ns
5.	PDE 2	121.5±1.00****

**Table 4. Effect of various pharmacological interventions on the level of Triglycerides (mg/dL)**

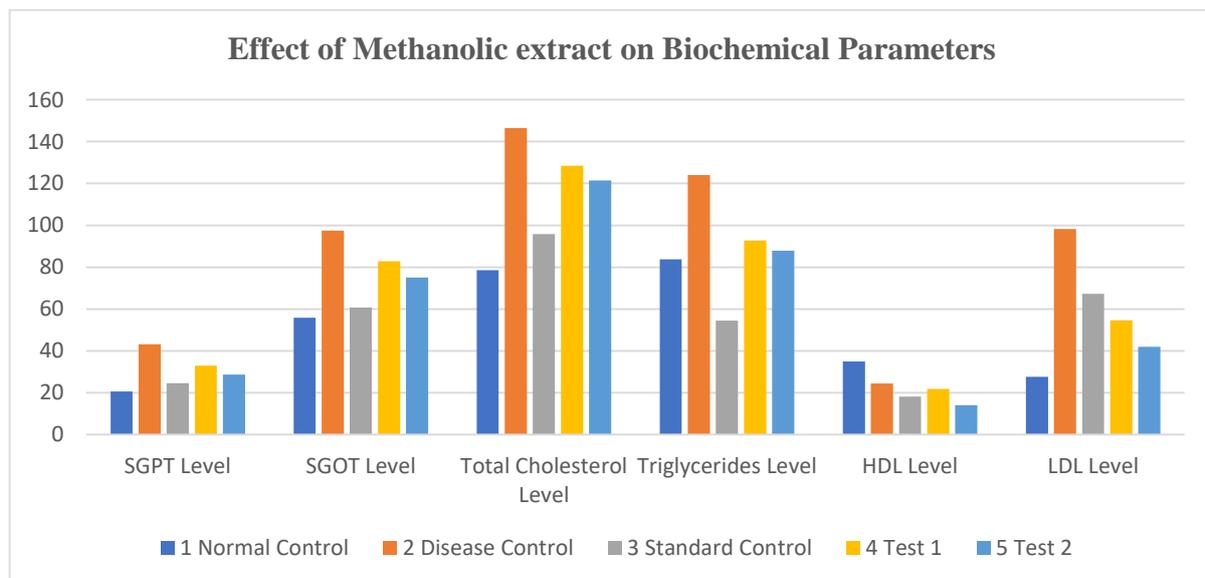
S.No.	Group	Triglycerides Level
1.	Normal Control	83.78±1.79
2.	Disease Control	124±2.09****
3.	Standard Control	54.5±1.87****
4.	PDE 1	92.7±1.31 ns
5.	PDE 2	87.83±1.12****

**Table 5. Effect of various pharmacological interventions on the level of HDL (mg/dL)**

S.No.	Group	HDL Level
1.	Normal Control	35.03±0.37
2.	Disease Control	24.5±1.87****
3.	Standard Control	18.23±1.35**
4.	PDE 1	21.86±1.45 ns
5.	PDE 2	14.08±1.42****

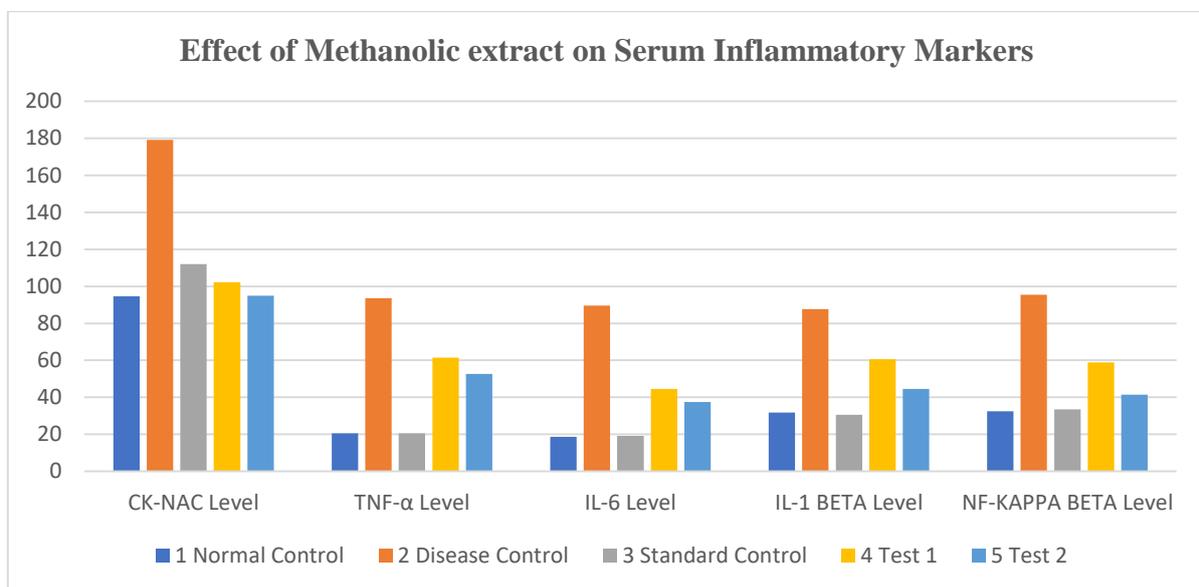
**Table 6. Effect of various pharmacological interventions on the level of LDL (mg/dL)**

S.No.	Group	LDL Level
1.	Normal Control	27.68±1.26
2.	Disease Control	98.2±1.49****
3.	Standard Control	67.3±0.78****
4.	PDE 1	54.7±0.91 ns
5.	PDE 2	42.06±0.70****

**Figure 2 shows the effect of Methanolic extract on Biochemical Parameters**

**Table 7. Effect of extract on serum inflammatory markers CK-NAC (U/L), TNF- $\alpha$  Level, IL-6 Level, IL-1 Level, NF-KAPPA Level**

S.No	Group	CK-NAC Level	TNF- $\alpha$ Level	IL-6 Level	IL-1 Level	NF-KAPPA Level
1.	Normal Control	94.5 $\pm$ 1.87	20.5 $\pm$ 1.87	18.75 $\pm$ 1.54	31.83 $\pm$ 1.47	32.5 $\pm$ 1.87
2.	Disease Control	179.16 $\pm$ 2.31****	93.5 $\pm$ 1.87****	89.5 $\pm$ 1.87****	87.7 $\pm$ 1.59****	95.5 $\pm$ 1.87****
3.	Standard Control	112.03 $\pm$ 1.41****	20.5 $\pm$ 1.87 ns	19.16 $\pm$ 1.72 ns	30.5 $\pm$ 1.87 ns	33.5 $\pm$ 1.87 ns
4.	PDE1	102.1 $\pm$ 1.05****	61.5 $\pm$ 1.87****	44.5 $\pm$ 1.87****	60.6 $\pm$ 1.87****	58.9 $\pm$ 1.43****
5.	PDE 2	94.91 $\pm$ 1.42 ns	52.7 $\pm$ 1.59****	37.5 $\pm$ 1.87****	44.5 $\pm$ 1.87****	41.5 $\pm$ 1.87****

**Figure 3 shows the effect of Methanolic extract on Serum Inflammatory Markers**

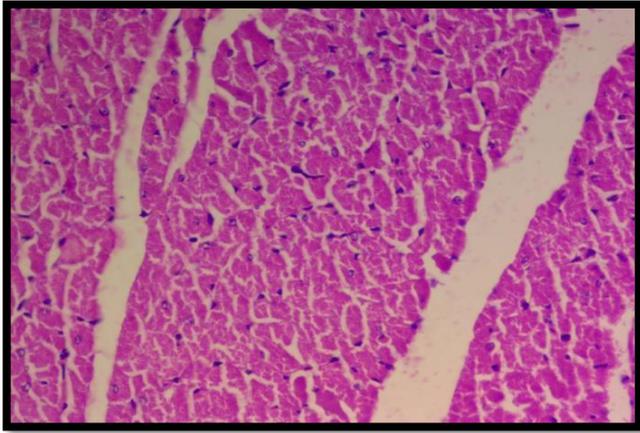
The above results show that triglycerides, total cholesterol, LDL, CPK-NAC, SGPT, and SGOT levels markedly increase during disease induction. This thing suggests that the heart is majorly affected after the administration of Doxorubicin at 15mg/kg dose. SGPT, SGOT, total cholesterol, and CPK-NAC levels are decreased in the standard control and test groups when compared with disease control groups, which indicates that the standard drug Aspirin and plant extract *Prunus domestica* L shows its therapeutic effect on the heart. In the normal control group, biochemical parameters SGPT, SGOT, triglycerides, total cholesterol, HDL, LDL, and CPK-NAC were normal, while inflammatory parameters TNF-alpha, interleukin-6, interleukin-1B, and nuclear factor- kappa B were also within the normal range.

In the disease control group (Doxorubicin 15mg/kg), biochemical parameters SGPT, SGOT, triglycerides, total cholesterol, LDL, and CPK-NAC were increased, while HDL was within the normal range.

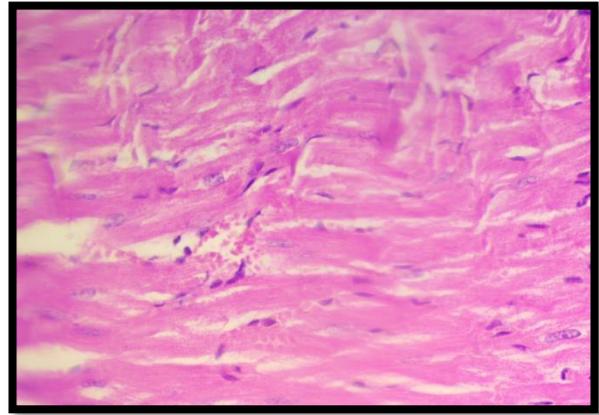
In the standard control, biochemical parameters such as SGPT, SGOT, triglycerides, total cholesterol, HDL, LDL, and CPK-NAC were normalized by Aspirin (1mg/kg) administration.

In test 1 and test 2 (*Prunus domestica* L 100mg/kg and 200mg/kg), SGPT, SGOT, triglycerides, total cholesterol, HDL, LDL, and CPK-NAC were normalized after *Prunus domestica* L administration.

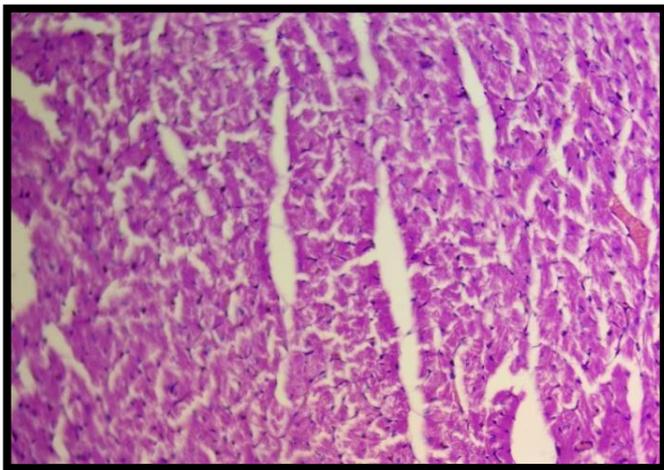
**Histopathology of Heart Tissue**



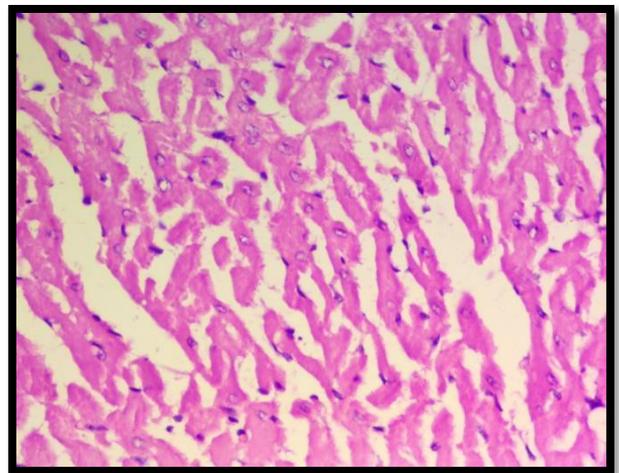
**Fig 4 Normal Control**



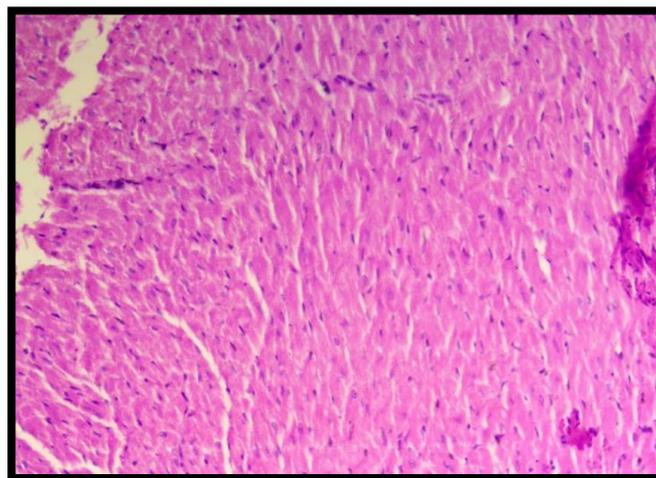
**Fig 5 Disease Control**



**Fig 6 Standard Control**



**Fig 7 Test ME (100mg/kg)**



**Fig 8 Test ME (200 mg/kg)**

### Interpretation of Histopathology:

**Fig 4: Normal Control Group** showed heart parenchyma with cardiac muscles and leaflets. Cardiac muscles were normal. There was no evidence of ischemic changes. Leaflets were normal.

**Fig 5: Disease Control Group** showed degenerative changes, focal necrosis with vascular congestion, lymphoplasmatic infiltration, and edema. A degenerative change was noted in the leaflets. Sections also showed hyalinization and lymphomononuclear infiltration.

**Fig 6: Standard Group showed that cardiac muscles were regenerating. Minimal** lymphoplasmacytic infiltration and mild edema were noted. Leaflets showed regenerative changes. This section showed the usual architecture of a rat heart.

**Fig 7: PDE 1 (100mg/kg) Group** showed cardiac muscles with degenerative and focal regenerative changes. Vascular congestion, enlarged cardiac muscle nuclei, and necrosis were also noted.

**Fig 8: The PDE 2 (200mg/kg) Group** showed heart parenchyma with cardiac muscles and leaflets. Cardiac muscles normalized from an ischemic condition and showed degenerative and focal regenerative changes.

### Conclusion

The potential of PDE to restore normality in tissue that is experiencing oxidative stress is likely attributable to the fact that it is an antioxidant, which means that it is able to neutralise the effects of free radicals which are causing the overall protective effect. However, the particular chemical mechanism by which PDE protects against oxidative damage has not yet been determined. This is something that has to be done. It is possible that PDE might have considerable therapeutic importance as an adjuvant treatment associated with doxorubicin if this protective activity is shown to be present in cancer patients.

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