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Protective Effect of Aqueous Extract of *Bauhinia Purpurea* Flower in Doxorubicin Induced Cardiomyopathy in Albino Wister Rats

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A B S T R A C T

The present study was undertaken to evaluate the cardio protective activity of Bauhinia purpurea family Fabaceae. Aqueous extract of flower of Bauhinia purpurea Linn. were prepared successively. Preliminary Phytochemical studies revealed the presence of chemical constituents such as Saponins, flavonoids, Tannins and Proteins in aqueous extract. The experiment was started with the treatment of Aqueous extract of Bauhinia purpurea flower by oral route in a dose of 10mg/kg, 20mg/kg, 30mg/kg, 40mg/kg and 50mg/kg of body weight to all groups for 7 days. On 8th day animals were treated with doxorubicin 10mg/kg was administrated to all groups. After 72hrs of Doxorubicin induction the rats was fasted for 18hrs and anaesthetized with Thiopentone sodium 30mg/kg. The blood was collected by cardiac puncture. The heart was dissected out, washed immediately in Ice-chilled physiological saline, blotted and weighed. Serum was separated from blood by centrifugation at 10,000 rpm for 20min. Serum samples were used for various biochemical parameters. Cardio protective activity produced in aqueous extract of Bauhinia purpurea flower decreases the level of Infarct size, SGOT, SGPT and CK-MB.

Keywords: Cardioprotectivity, Flavonoids, Bauhinia purpurea, Doxorubicin.

A R T I C L E I N F O

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1. Introduction

Doxorubicin is a member of the Anthracycline group and one of the most widely used drug to treat many forms of cancer such as leukemia, lymphoma and solid tumors [1]. However, its clinical uses are limited by seriously high

incidence of cardiotoxicity. An initial acute effect includes hypotension and transient electrocardiographic abnormalities. Cardiomyophathy is dose dependent which accounts for high mortality [2]. However, the mechanisms by which DOX induces cardiac injury and dysfunction are incompletely understood. Some of these include cellular toxicity from metabolites of DOX [3], generation of myocytes. A number of DOX-induced biochemical changes have been identified that can damage cardiac reactive oxygen species [4], production of reactive nitrogen species [5]; selective inhibition of cardiac muscle gene expression [6], disturbance of myocardial adrenergic signaling and induction of cardiac cell apoptosis [7]. The heart is particularly vulnerable to the free radicals produced by DOX administration, as it contains less free radical detoxifying substances such as superoxide dismutase, glutathione and catalase than do other metabolic organs such as liver or kidney and its highly oxidative metabolism.

Bauhinia purpurea (Orchid tree, Camel's foot tree) is a plant belongs to a family fabaceae.It is a small to medium-sized deciduous fast-growing shrub or tree. The stem & bark of *bauhinia purpurea* contains chemical constituents like carbohydrates, glycosides, Saponins, sterols and triterpenoids were present in methanolic extract [9]. Literature revealed that the plant having anti tumour activity, anti diabetic activity, anti inflammatory activities, analgesics, anti ulcer activity, antioxidant, anti hyperlipidemic activity, anti eosinophilic anthelminthic activity, nephroprotective activity immunomodulatroy activity and hepatoprotective activity [10]. The purpose of the present study was to elucidate whether aqueous extract of *Bauhinia purpurea* Flower could contribute to controlling important parameters that have roles in inducing and aggrevating DOX cardiomyopathy.

2. Materials and Methods

Materials

Collection of plant material

The flowers were collected during the month September to November and the plant was authenticated by a botanist. Then it was shade dried, powdered, weighed and stored in a clean, dry and air tight container.

Chemicals

Doxorubicin and TTC were purchased from Sigma-Aldrich chemicals Ltd, Bangalore. All other chemicals and reagents used were of analytical grade.

Selection of experimental animals

Adult male Wister rats, weighing 200 to 250g were selected for the study. The protocol of study was reviewed and approved by the institutional animal ethical committee and conforms to the Indian National Science Academy guidelines for the use and care of experimental animals in research. Rats were housed in Poly acrylic cages (38X23X10cm) with not more than 3 animals per cage. They were housed in an air conditioned room and were kept in standard laboratory conditions under natural light and dark cycle (Approximately 14h light/10h dark) and maintained humidity 60±5% and an ambient temperature of 25±2°C. All experiments were performed between 9:00am and 4:00pm. The animals were free access to standard diet and tap water ad libitum and allowed to acclimatize for one week before the experiments.

Methods

Extraction

Powder was packed in a condenser and extracted with distilled water at 100°C by Soxhlet extractor. After extraction the residue was dried on water bath at 100°C to get a solid mass. The percentage yield of the aqueous extract of *Bauhinia purpurea* flower (AEBPF) was calculated [11].

Preliminary phytochemical investigation:

The aqueous extract of *Bauhinia purpurea* flower was subjected for the qualitative Preliminary Phytochemical identification by the standard methods described in practical Pharmacognosy by Dr.C.K.Kokate[12] and Khandelwal K.R [13].

Cardioprotective activity

A. Experimental design

The rats were divided into 6 groups. Group I maintain as diseased control group. Group II animals were treated with 10mg/kg of AEBPF orally for 7 days. Group III animals were treated with 20mg/kg of AEBPF orally for 7 days. Group IV animals were treated with 30mg/kg of AEBPF orally for 7 days. Group V animals were treated with 40mg/kg of AEBPF orally for 7 days. Group VI animals were treated with 50mg/kg of AEBPF orally for 7 days. On 8th day animals were treated with doxorubicin 10mg/kg was administrated to all groups. After 72 hrs of doxorubicin induction the rats was fasted for 18 hrs and anaesthetized with Thiopentone sodium 30mg/kg. The blood was collected by cardiac puncture. The heart was dissected out, washed immediately in ice-chilled physiological saline, blotted and weighed and used for histopathology study. Serum was separated from heart by centrifugation at 10,000 rpm for 20min. Serum samples were used for various biochemical estimations like CK-MB[14], Aspartate Transaminase (AST) and Alanine Transaminase in (ALT) [15].

B. Quantification of infarct size

At the end of table work, animals were sacrificed with excess dose of anesthesia. Heart was excised from thorax, greater vessel above atrio-ventricular groove were removed. The heart was washed with normal saline, blotted dry and weighed. The left ventricle was separated from the heart and was weighted. It was sliced from the atrio-ventricular groove to 0.1 cm thick section and slices were incubated in 1% TTC Solution prepared in PH 7.4 phosphate buffer for 30min. at 37°C in viable myocardium. TTC is covered by dehydrogenase enzyme to a red formazan pigment that stains tissue to dark red. The infarcted myocardium that doesn't take TTC stain where the dehydrogenase enzyme or drained off remains as pale in color. The pale necrotic myocardial tissue was separated from the stained portion and weighed on an electronic balance. Myocardial infarct size was expressed quantitatively in terms of Percentage Left Ventricle necrosis (PLVN) [16].

Statistical analysis: The results were expressed as Mean ± Standard Deviation (SD) differences in groups for PLVN and Serum Biochemical Estimations. Statistical analysis

was determined by one way analysis of variance (Anova), individual groups were compared using Tukey's test. *P* value <0.05 has been considered as statistical significance level.

3. Results and Discussion

Percentage yield of the extract

The aqueous extract of *Bauhinia purpurea* flower is found to be sticky with dark brown appearance and the percentage yield of extract was found to be 20gm.

Preliminary phytochemical evaluation

The various chemical tests were carried out for the detection of Tannins, Proteins, Saponins and Flavonoids in Aqueous extract of *Bauhinia purpurea* Flower.

Table 1: Details of Qualitative Phytochemical Tests

S.No	Tests	Aqueous Extract
1	Sterols	+
2	Glycosides	-
3	Saponins	+
4	Carbohydrates	-
5	Alkaloids	-
6	Flavonoids	+
7	Tannins	+
8	Proteins	+
9	Triterpenes	-

"+" Indicates Positive (present),
"-" Indicates Negative (absent).

Cardio protective activity

Biochemical estimations

A. Apartate transaminase

With Normal control, serum Aspartate Transaminase (AST) was found to be 341.35±1.64 IU/L. Hence all the values of treatment groups were compared with Normal control group. When AEBPF was administrated orally at the dose of 10,20,30,40 and 50 mg/ bwt AST was significantly reduced to 336.13±2.93IU/L (P<0.001), 281.58±1.08IU/L (P<0.001) , 226.4± 2.23IU/L (P<0.001), 171.93±2.01IU/L (P<0.001) and 133.76±2.55IU/L (P<0.001) respectively.

B. Alanine transaminase (ALT)

Serum Alanine Transaminase (ALT) values of treatment groups were compared with the normal control group with Normal control (190.412.59IU/L). AEBPF administered rats with different doses 10,20,30,40 and 50 mg/kg bwt showed significant reduction in their ALP levels to 186.65±2.90IU/L (P<0.001), 166.75±2.07IU/L (P<0.001), 159.56±1.88IU/L (P<0.001), 98.3±1.36IU/L (P<0.001) and 78.71±2.31 IU/L (P<0.001) respectively.

C. CK-MB

Isoenzyme CK-MB was measured in test groups and compared with normal group(705.54.56 IU/L). AEBPF at doses 10,20,30,40 and 50 mg/kg bwt showed significant reduction in 683.41±4.16IU/L (P<0.001), 497.63±4.66IU/L (P<0.001), 448.16±3.71IU/L (P<0.001), 289.08±2.51IU/L (P<0.001) and 289.08±2.51IU/L (P<0.001) respectively. In the three biochemical parameters there was a dose dependent decrease in AST, ALT and CK-MB with AEBPF of 40mg/kg & 50mg/kg as depicted in table 2.

Table2. Effect of AEBPF on serum biochemical parameters

Group	Biochemical parameters(IU/ml) [Mean±SD]		
	AST	ALT	CK-MB
Disease control	341.35±1.64	190.41±2.59	705.5±4.56
AEBPF 10mg	336.13±2.93	186.65±2.90	683.41±4.16
AEBPF 20mg	281.58±1.08	166.75±2.07	497.63±4.66
AEBPF 30mg	226.4±2.23	159.56±1.88	448.16±3.71
AEBPF 40mg	171.93±2.0***	98.3±1.36***	289.08±2.51***
AEBPF 50mg	133.76±2.55***	78.7±1.23***	159.9±1.77***

Significant at ***P<0.001, **P<0.01, *P<0.05, n=6.Compared with Normal control group.

Percentage of infarct

With Normal control, Percentage Left Ventricular Necrosis (PLVN) was found to be 38.85±1.58. AEBPF of 10,20,30,40 and 50 mg/kg bwt shows significant reduction in their infarct size ie., 38.56 ± 1.21 (P<0.001), 31.71±1.43 (P<0.001), 24.±2.06 (P<0.001), 17.5±0.57. (P<0.001) and 13.65±0.59 (P<0.001) respectively. There was a dose dependent decrease in PLVN with AEBPF of 40mg/kg and 50mg/kg as illustrated in figure 1.

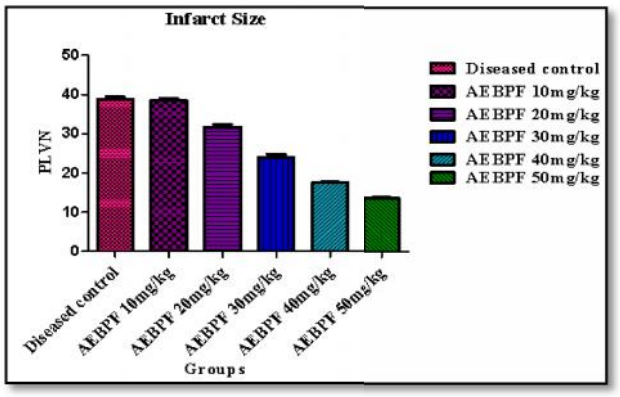


Figure 1: Effect of AEBPF on infarct size

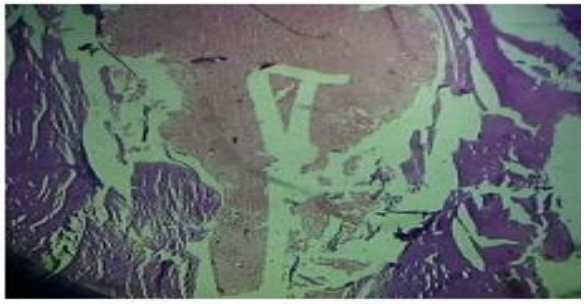
Histopathology

Teatment with AEBPF with doses 40 nad 50 mg shows muscle bundles with dilatations with congested blood vessels compared to disease control in which focal areas of hemorrhage, necrosis and milkd inflammatory cells found.

Discussion

The current study estimates the cardioprotective potential of the Aqueous Extract of Bauhinia purpurea Flower against DOX induced cardiotoxicity. Doxorubicin induced a myocardial damage have been well established in patients and in experimental animal models. In the present study, the development of oxidative cardiac injury mainly due to administration of single dose (10mg/kg) of DOX was confirmed by the significant increase in serum cardiac biomarker enzymes such as ALT, AST and CK-MB. When there is a deficient supply in oxygen or glucose the myocardial cells are damaged or destroyed and the

membrane of the cardiac cells becomes permeable or may rupture which results in leakage these enzymes. These enzymes are entering into the blood stream thus increasing their concentration in the serum. Activities of these enzymes in serum decreased in the AEBPF treated rats which would have reduced the extent of myocardial damage induced by DOX and thereby restricted the leakage of these enzymes from myocardium. It is widely accepted that oxygen-free radicalsare generated during Doxorubicin Oxidation-Reduction Cycling are responsible for the damage to the heart. Oxygen radical generation affects the heart because of doxorubicin and its toxic metabolite like doxorubicinol accumulation in cardiac tissue that has low antioxidant levels. Therefore, the present study was designed to determine the Cardioprotective effect of BP is supported by increased myocardial antioxidant enzyme activity and results were shown that administration of DOX intraperitonially, produced signs of Cardiomyopathy was prevented by oral administration of aqueous extract of *Bauhinia purpurea* flower was produced significant decrease in the cardiac biomarker enzymes such as ALT, AST and CK-MB and also observed in Histopathological studies.



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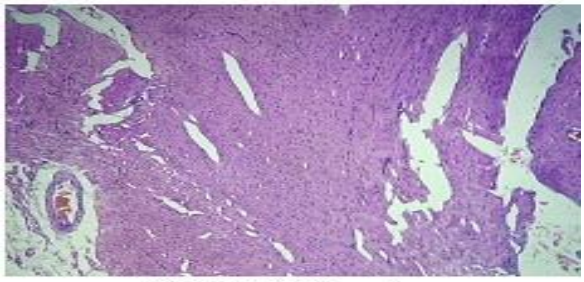
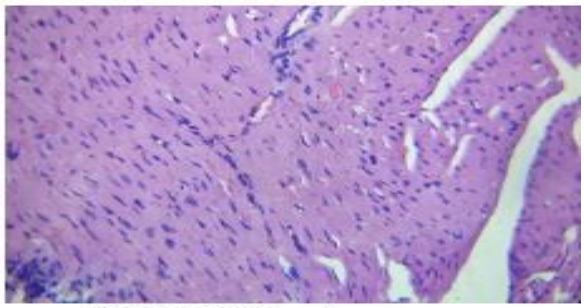
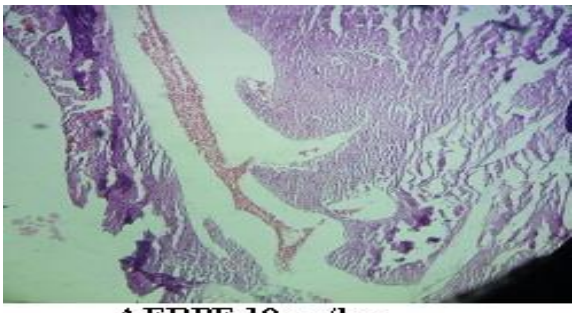


AEBPF 20mg/kg

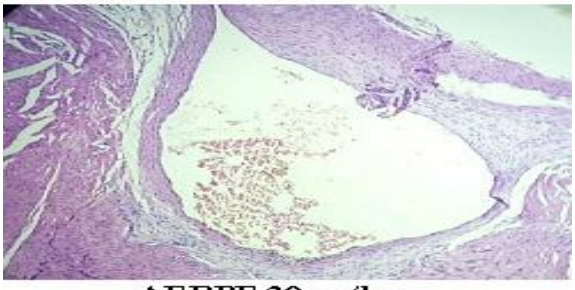


AEBPF 40mg/kg

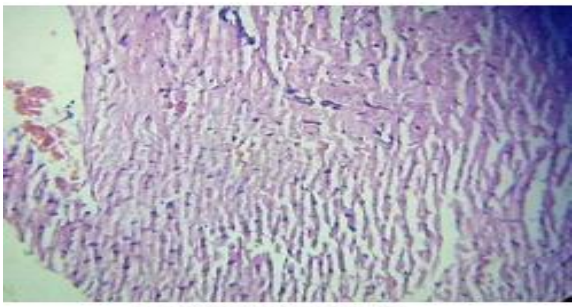
From the above information, it can be concluded that administration of aqueous extract of *Bauhinia purpurea* flower protect against acute DOX cardiotoxicity via improving cardiac enzymes and modulating the pathways that activates cardiac apoptosis by decreased the levels of Percentage Left Ventricular Necrosis, ALT, AST and CK-MB. Therefore, we demonstrate that supplementation of *Bauhinia purpurea* could be used in doxorubicin combinations which protect against cardiomyopathy without disturbing the clinical efficacy of DOX, by avoiding the need to take other medications and also enhance the patient's quality of life.



AEBPF 10mg/kg



AEBPF 30mg/kg



AEBPF 50mg/kg

4. Conclusion

In the present investigation it was observed that cardiotoxicity induced by 10mg of Doxorubicin was prevented by treatment of groups with aqueous extract *Bauhinia purpurea* flower decreases the levels of Percentage Left Ventricular Necrosis, ALT, AST and CK-MB. This protective effect of Aqueous Extract may be due to the presence of Saponins, Tritepenoids, Flavonoids and Tannins. Further studies are needed to elucidate the particular Saponins, Tritepenoids, Flavonoids and Tannins which are responsible for Cardioprotective effect.

5. References

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