

## Antioxidant and Cardioprotective Activity of *Indigofera barberi* on Doxorubicin Induced Toxicity on Rats

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Worldwide, the major death causing diseases are cardiovascular diseases and today the need for herb based therapeutics is needed. Present study was undertaken the whole plant of *Indigofera Barberi* (IB) to evaluate its cardioprotective activity against cardiotoxicity on rats induced by Doxorubicin (DXR). Soxlet extraction was used to prepare extracts. Preliminary phytochemical tests and in-vitro antioxidant activity are the methods used for standardization of all the extracts. Chloroform extract of *Indigofera barberi* (CEIB) and aqueous extract of *Indigofera barberi* (AQIB) are two extracts obtained from above activity were selected against induced cardiotoxicity of DXR to determine in-vivo cardioprotective activity. Total flavonoid and phenol content was determined. Endogenous antioxidants (MDA, GSH), ECG and histopathological studies are the parameters of serum (CK, CK-MB, LDH) and non serum to evaluate the cardioprotective activity. Serum elevated levels of biomarker, decreased antioxidant activity, changes in electrocardiogram (ECG) and histopathological studies are shown by DXR alone treated rats. The toxicity produced by DXR has reversed on the rats pre-treated with CEIB and AQIB. CEIB has shown more activity when compared to AQIB. Compared to standard vitamin E the activity of CEIB was found to be significant. The protective effect of IB plant on DXR induced cardiotoxicity was revealed. To understand the mechanism of action and to reveal phytochemical responsible for the said activity the further research to be undertaken.

**Keywords:** Antioxidant; Cardioprotective; Cardiotoxicity; doxorubicin; heart failure; *Indigofera barberi*.

Heart attacks and strokes are usually acute events and are mainly caused by a blockage that prevents blood from flowing to the heart or brain. The most common reason for this is a build-up of fatty deposits on the inner walls of the blood vessels that supply the heart or brain. Strokes can be caused by bleeding from a blood vessel in the brain or from blood clots. The most important behavioral risk factors of heart disease and stroke are unhealthy diet, physical inactivity, tobacco use and harmful use of alcohol. The effects of

behavioral risk factors may show up in individuals as raised blood pressure, raised blood glucose, raised blood lipids, and overweight and obesity. These “intermediate risks factors” can be measured in primary care facilities and indicate an increased risk of heart attack, stroke, heart failure and other complications.

The antioxidant properties of medicinal plants which could be ascribed to antioxidants phytochemicals related to therapeutics actions. Numerous medicinal plants and plant products

having cardioprotective effect are documented. In the management of cardiovascular related disorders, currently available synthetic drugs serves as viable alternatives obtained from sustainable agents from natural sources<sup>6</sup>. Understanding the toxic side effects & high cost of most synthetic drugs many patients in developing countries are not reading accessible.

Doxorubicin (DOX)<sup>1</sup> which belongs to the class of anthracyclines is one among the effective antitumor antibiotics. Due to dilation and high incidence of myocardial damage its use is limited.

After cumulative DOX administration congestive heart failure, cardiomyopathy and electro cardiographic changes were demonstrated<sup>2</sup>. Oxidative myocardial damage produced by anthracyclines cause excess production of free radicals and oxidative stress at a very high level. Antioxidant effect of therapeutic interventions may offer considerable cardio protection with this regard<sup>3</sup>. The study analysis of oxidative stress in cardiac tissues and drugs therapeutic efficacy in laboratory animals which are cancerfree serves as appropriate models by DOX induced cardio toxicity. To decrease the risk of DOX induced cardio toxicity while maintaining its efficacy several approaches may be taken<sup>4</sup>.

The altered schedules of drug administration, modifications of anthracycline molecules, adjunctive treatment with  $\alpha$ -adrenergic blockers, ACE inhibitors, dexrazoxane and probucol were included. None of these have been entirely successful. To prevent or treat DOX induced cardio toxicity a new drug is needed<sup>5</sup>.

Endemic herb of high value in Tirumala hills is *Indigofera barberi* of Fabaceae family. Vernacularly name is Adavineelimanadumokka. It is an under shrub grows upto 1m tall. It has faintly angled branchlets. 3 foliated leaves, oblong ovate leaflets, pubescent, obtuse, mucronate<sup>7</sup>. Arrangement of pink color floweres is axillary congested racemes. Pods appressed, deflexed, subterete, sharply pointed and white tomentose. 2-4 number seeds. September or December is flowering and fruiting season. For controlling diabetes, leaf powder (5g) is taken orally<sup>8</sup>.

Along with buttermilk, pills of peanut size are prepared by leaves (50g), garlic (1g), pepper(1g) which are made into paste<sup>9</sup>. As practice followed by Nakkala and many tribal physicians,

for 5 days once a day 5 pills are taken to cure jaundice. It is also used as dye and colouring agent<sup>10</sup>. For the treatment of many skin diseases, peptic ulcers and to expel intestinal worms whole plant powder (5g) is taken for 10 days with rice washed water once a day. To cure wounds, cuts, burns and boils leaf juice is used as an antiseptic<sup>11</sup>.

Using biochemical makers and histopathological analysis, the study was undertaken to investigate the possible effects of *Indigofera barberi* extract against doxorubicin induces cardiotoxicity in rats was undertaken in the present study keeping these facts in view.

## MATERIALS AND METHODS

### Plant material

The plant was identified and collected in Tirumala hills (deciduous forest) in Andhra Pradesh, India. Dr. K. Madhava chetty, a Plant Taxonomist, Department of Botany, Sri Venkateswara University, Tirupathi, India authenticated the samples. Sorting, cleaning and at room temperature it was air dried for about 1 week were done to the whole plant of *Indigofera* by using the laboratory hammer mill. Until required, storage is done in water and air proof containers protected from heat and sun light.<sup>12</sup>

### Preparation of extracts

In soxhlet apparatus using petroleum ether, chloroform, ethanol and distilled water the *Indigofera barberi* powdered materials were extracted successively for 18 hrs. To get free from the solvents, the extracts were concentrated to dryness in rota evaporator<sup>13</sup>.

### Phytochemical analysis

For the presence of saponins, alkaloids, glycosides, tannins, terpenoids, flavonoids, carbohydrates, proteins, aminoacids, sterols and fixed oils phytochemical analysis of extracts were carried out by different methods<sup>14</sup>.

### Total flavonoids estimation

Aluminium chloride calorimetric assay<sup>15</sup> was used to measure the total flavonoid content. To a 10 ml volumetric flask containing 4 ml of distilled water an aliquot (1ml) of extracts and standard solution of cetechin (100mg/ml) were added. 10 % AlCl<sub>3</sub> about 0.3ml was added after 5 min. Then after 1 min, the volume was made up to 10ml with distilled water by addition of 2 ml of 1M NaOH.

The content of the solution were mixed well and an absorbance was measured against prepared blank reagent at 510 nm. Fresh weight expressed as mg catechin equivalents (CE)/100G for total flavonoid contents of roots. Analysis of samples are analysed in triplicates.

#### Estimation of total phenols

Based on Folin-Ciocalteu reagent assay<sup>16</sup> total phenolics are determined. To 25 ml of volumetric flask, containing 9 ml distilled water an aliquot (1ml) extracts and standard solution of Gallic acid (100mg/ml) were added. The distilled water was used as blank. To the mixture 1 ml of Folin-Ciocalteu reagent was added and shaken. 10ml of 7% Na<sub>2</sub>CO<sub>3</sub> solution was added to the mixture after 5 min. The solution was diluted with water and mixed to a volume of 25 ml. An absorbance of 750nm with UV-vis spectrophotometer was determined against prepared blank reagent after incubation for 90 min at room temperature. Fresh weight of root extracts expressed as mg gallic acid equivalents (GAE)/100G gives the total phenolic content. Samples are all analyzed in triplicates.

#### Acute toxicity studies

According to OECD guidelines<sup>17</sup>, for chloroform extract acute toxicity studies were conducted on albino mice (20-25g). Healthy adult mice, were divided into groups (n=5), at the doses of 5, 50, 300 and 200 mg/kg body weight, chloroform extract was administered orally. For mortality and physical/behavioural changes animals were observed for over 14 days.

#### In-vitro antioxidant studies

##### DPPH radical scavenging activity assay

The free radical scavenging activity of the *Indigofera barberi* (IB) extracts, in-vitro, was measured by using 2,2-diphenyl 1-1-picrylhydrazyl (DPPH). 24 mg of DPPH was dissolved in 100 ml methanol and stored at 20°C which is used as stock solution. To succeed an absorbance approximately 0.95 ± 0.02 by using the spectrophotometer at an absorbance of 517 nm, DPPH solution was impoverished which serves as working solution. 100 µl of extracts at various concentrations (10-100 µg/ml) and aliquant of the solution (3-4ml) were mixed thoroughly<sup>18</sup>. At room temperature in a dark room for about 15 min the reaction mixture which is shaken well is then incubated. The absorbance was taken at 517 nm. As the procedure mentioned above, the control

was prepared without any extract. Based on the following equation, the percentage scavenging activity of DPPH was estimated.

$$\text{Scavenging effect (\%)} = \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} \times 100$$

##### Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) scavenging activity

In a 50 mM phosphate buffer freshly prepared H<sub>2</sub>O<sub>2</sub> (2mM) was added<sup>19</sup>. Different extracts of aliquants (0.01ml) were transferred into test tubes by adding 50mM phosphate buffer (pH 7.4), and made volumes up to 0.4ml. Tubes are vortexed after addition of 0.6ml H<sub>2</sub>O<sub>2</sub> solution and an absorbance of H<sub>2</sub>O<sub>2</sub> was determined at 230 nm which is performed against blank after 10 min. The abilities to scavenge H<sub>2</sub>O<sub>2</sub> was calculated by using the specified equation:

$$\text{H}_2\text{O}_2 \text{ scavenging action} = \left[ \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} \right] \times 100$$

##### Nitric oxide scavenging activity

By taking 2ml of 10mM sodium nitroprusside and a saline phosphate buffer (pH 7.4) of 0.5ml which is mixed with various concentrations of 0.5 ml of extract prepared in ethanol and mixture was incubated for about 30 min at 25°C. To each test tube, 1.5 ml of Griess reagent (contains phosphoric acid 3%, sulphanilamide 1% and naphthylethylenediamine dichloride 0.1%) is added correctly<sup>20</sup>. The absorbance was measured at 546 nm and with reference to ascorbic acid as standard; the percentage scavenging activity was compared. Nitric oxide radicals scavenging activity was calculated by comparing the absorbance values of control and absorbance values of test samples (extracts) using the following equation, the % inhibition of nitric oxide liberated was determined:

$$\% \text{ inhibition of nitric oxide} = \left[ \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} \right] \times 100$$

#### In-vivo cardioprotective studies

##### Animals and experimental protocol

In accordance with the guidelines of CPCSEA, male wistar rats of about 250-300 g of weight used in the study. In a temperature controlled room at 22 ± 3°C with 12h light-dark cycle, well ventilated room animals were housed. Water was provided *ad libitum*<sup>21</sup> and food consisted

of normal rat chow. To avoid stressful conditions care taken between 8 and 10 m experimental procedures were performed. Rats were divided randomly into four groups (12 rats each).

Group I: Received normal saline (1 ml/200 g body weight/day) orally for 10 consecutive days

Group II: Received normal saline orally for 10 consecutive days and a single dose of DOX (15 mg/kg, i.p.) on day 7

Group III: Received Vitamin E combined with DOX

Group IV: Received the CEIB (250 mg/kg) combined with DOX

Group V: Received the CEIB (500 mg/kg) combined with DOX

Group VI: Received the AQIB (250 mg/kg) combined with DOX

Group VII: Received the AQIB (500 mg/kg) combined with DOX

For 10 consecutive days the plant was extracted was administered and on day 7 DOX was administered. ECG was recorded using Niviqure Inco polygraph system and changes in heart rate and QT and ST changes were computed from the ECG after 48 hours of DXR challenge.

With thiopentone (35 mg/kg, i.p.) rats were anesthetized. In serum separating tubes by orbital puncture blood samples were collected. To separate the sera the blood was centrifuged at 3000g for 15 min and for the biochemical analysis they were kept at 70°C. The hearts were rapidly dissected out, by opening the abdomen of each rat, in ice-cold isotonic saline they were washed and between two filter papers they were blotted. In 10% formalin four hearts from each group were fixed for histopathological examination and for subsequent analysis the remaining hearts from each group were homogenized in ice-cold 0.1 M potassium phosphate buffer (pH 7.4) and stored at -70°C.

#### **Cardiac biochemical assay**

According to method of Adams<sup>22</sup>, cardiac GSH was determined. According to the method of Hissin and Hilf<sup>23</sup> GSH level was assessed and values were expressed as nmol/mg protein. According to the method of Mihara and Uchiyama<sup>24</sup> by measuring malondialdehyde (MDA) content in tissue homogenates liquid peroxidation products were determined. At 532 nm by spectrophotometrically the MDA content

was measured. Based on a standard curve using 1,1,3,3-tetra ethoxypropane as a standard the MDA content was calculated. As nmol/g protein values are expressed.

#### **Serum biochemical assay**

Using diagnostic kits the activities of creatine kinase (CK), creatine kinase isoenzyme-MB (CK-MB) and lactate dehydrogenase<sup>25</sup> (LDH) were determined according to standard methods. By measuring the rate of NADPH formation at 340 nm the CK-MB activity was assayed by measuring the rate of nicotinamide adenine dinucleotide reduced from (NADH) formation at 340 nm, the LDH activity was determined<sup>26</sup>.

#### **Histopathological study**

Under a light microscope, examination of hearts which were cut at 0.5 im are mounted on slides, stained with hematoxylin and eosin were examined.

#### **Statistical analysis**

As mean  $\pm$  SEM results are presented by use of one-way ANOVA followed by Dunnetts multiple comparison, compares data between treatment groups were done. At  $p < 0.05$ ,  $< 0.01$  and  $< 0.001$  values were considered statistically significant.

## **RESULTS**

#### **Phytochemical analysis**

The results were carried out and displayed for the phytochemical screening of various extracts i.e., petroleum ether extract of IB (PEIB), chloroform extract of IB (CEIB), ethanol extract of IB (EEIB), and aqueous extract of IB (AQIB) in Table 1.

#### **Total flavonoids and phenolic content of IB extracts**

Presence of total flavonoids and phenols in different extracts of IB are given in table 2. The maximum amount of flavonoids and phenolic compounds are present in CEIB followed by AQIB, PEIB, MEIB shows the result.

#### **Acute toxicity studies**

For chloroform extract acute toxicity studies were conducted on albino mice (20-25g). the extract was found to be safe at 2000 mg/kg.

#### **In-vitro antioxidant studies**

By DPPH medical scavenging activity assay, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) scavenging

activity and nitric oxide scavenging activity determines the antioxidant activity of various extracts of IB in table 3,4,5 respectively. The % inhibition at various concentrations was more for (10-100 ig/ml) CEIB when compared to others was the result revealed.

### In-vivo cardioprotective studies

#### Non-serum parameters

When compared to normal group on DXR administration there is a significant increase in ST and QT intervals whereas the heart rate was decreased. The changes caused by DXR

**Table 1.** Phytochemical screening of successive solvent extraction of IB

Phytoconstituents	Method	Aqueous extract	Ethanolic extract	Chloroform extract	Pet. Ether extract
Flavonoids	Shinoda Test	+	+	+	-
	Zn. Hydrocholride Test	+	+	+	-
	Lead acetate Test	+	+	+	-
Volatile Oil	Stain Test	-	+	-	-
	Wagner Test	-	-	-	-
Alkaloids	Hager's Test	-	-	-	-
	FecI3 Test	+	+	+	-
Tannins & Phenols	Pot.Dichromate Test	+	+	+	-
	Foaming Test	+	+	-	-
Saponins	Salkowski Test	+	+	-	+
Steroids	Molish Test	-	-	-	-
Carbohydrates	Litmus Test	-	-	-	-
Acid Compounds	Keller-Killani Test	+	+	-	-
Glycoside	Ninhydrin Test	-	-	-	-
Amino Acids	Biuret	-	-	-	-
Proteins					

"+": Present; "-": Absent

**Table 2.** Total flavonoids and phenols of different extracts of IB

Extract	Total Flavonoids (% w/w) Mean $\pm$ SEM	Total Phenols (% w/w) Mean $\pm$ SEM
PEIB	5.61 $\pm$ 0.12	3.38 $\pm$ 0.19
CEIB	7.12 $\pm$ 0.61	5.76 $\pm$ 0.88
EEIB	5.51 $\pm$ 0.45	3.12 $\pm$ 0.21
AQIB	6.23 $\pm$ 0.59	5.4 $\pm$ 0.52

**Table 3.** DPPH radical scavenging activity of *Indigofera barberi* extracts

Extract	Concentration ( $\mu$ g/ml) and % inhibition					
	10	20	40	60	80	100
Ascorbic acid	58.51 $\pm$ 0.11	65.59 $\pm$ 0.51	72.36 $\pm$ 0.14	78.21 $\pm$ 0.28	81.36 $\pm$ 0.14	91.68 $\pm$ 0.19
PEIB	11.89 $\pm$ 0.19	18.45 $\pm$ 0.46	20.76 $\pm$ 0.05	23.18 $\pm$ 0.52	25.51 $\pm$ 0.33	27.33 $\pm$ 0.10 <sup>+</sup>
CEIB	30.47 $\pm$ 0.22	33.52 $\pm$ 0.64	45.09 $\pm$ 0.14	51.17 $\pm$ 0.64	54.71 $\pm$ 0.46*	63.15 $\pm$ 0.01*
EEIB	14.15 $\pm$ 0.31	17.19 $\pm$ 0.59	22.09 $\pm$ 0.28	24.15 $\pm$ 0.33 <sup>+</sup>	26.61 $\pm$ 0.28	29.16 $\pm$ 0.08
AQIB	16.28 $\pm$ 0.45	23.28 $\pm$ 0.87	27.98 $\pm$ 0.36	33.65 $\pm$ 0.41 <sup>+</sup>	39.71 $\pm$ 0.91*	43.31 $\pm$ 0.11

Each value represents the Mean $\pm$  SEM. \*P<0.01, <sup>+</sup>P<0.05 equated to postive control

administration were reversed by pretreatment with chloroform extract of *Indigofera barberi* (CEIB) as well as vitamin E. When compared to normal as well as test treated animals, a significant decrease in body weight, heart weight have shown on rats treated with DXR.

#### Serum parameters

In DXR treated rats, the serum CKMB levels of IB extracts shown protective effects which is shown in table 7. DXR treated rats had serum CKMB level of  $235.9 \pm 1.06$  UI/L which shows induced cardio toxicity on rats on DXR treatment. Serum CKMB level decreased to  $156.32$

$\pm 1.4$  UI/L on rats treated with vitamin E, which was significantly lower when compared to DXR control group. A serum CKMB level of  $196.6 \pm 0.36$  and  $174.11 \pm 0.23$  respectively was observed on treatment with CEIB at 250 mg/kg and 500 mg/kg. A serum CKMB level of  $201.32 \pm 1.56$  and  $193.6 \pm 1.18$  respectively was observed on treatment with AQIB at 250mg/kg and 500 mg/kg when compared to DXR control group, both values are significantly lower.

#### Histopathological study

On doxorubicin intoxicated rats the cardioprotective activity of CEIB was given in

**Table 4.** Hydrogen peroxide scavenging activity of *Indigofera barberi* extracts

Extract	Concentration ( $\mu\text{g/ml}$ ) and % inhibition					
	10	20	40	60	80	100
Ascorbic acid	54.95 $\pm$ 0.11	62.48 $\pm$ 0.95	73.56 $\pm$ 0.28	79.79 $\pm$ 0.11	83.57 $\pm$ 0.23	92.58 $\pm$ 0.51
PEIB	10.15 $\pm$ 0.28	12.74 $\pm$ 0.62	15.01 $\pm$ 0.32	19.08 $\pm$ 0.08	21.73 $\pm$ 0.28	23.85 $\pm$ 0.33
CEIB	31.76 $\pm$ 0.59	34.73 $\pm$ 0.23	46.71 $\pm$ 0.46	53.75 $\pm$ 0.17	59.95 $\pm$ 0.29 <sup>+</sup>	63.09 $\pm$ 0.12 <sup>*</sup>
EEIB	10.75 $\pm$ 0.76	14.86 $\pm$ 0.58	18.31 $\pm$ 0.89	22.17 $\pm$ 0.42	26.55 $\pm$ 0.25 <sup>+</sup>	30.91 $\pm$ 0.28
AQIB	17.34 $\pm$ 0.97	23.85 $\pm$ 0.31	29.51 $\pm$ 0.91 <sup>*</sup>	37.84 $\pm$ 0.25	39.76 $\pm$ 0.16 <sup>*</sup>	43.78 $\pm$ 0.21

Each value represents the Mean $\pm$  SEM. <sup>\*</sup>P<0.01, <sup>+</sup>P<0.05 equated to postive control

**Table 5.** Nitric oxide scavenging activity of *Indigofera barberi* extracts

Extract	Concentration ( $\mu\text{g/ml}$ ) and % inhibition					
	10	20	40	60	80	100
Ascorbic acid	57.41 $\pm$ 0.11	66.34 $\pm$ 0.25	71.63 $\pm$ 0.26	76.91 $\pm$ 0.78	83.08 $\pm$ 0.91	91.67 $\pm$ 0.48
PEIB	11.14 $\pm$ 0.23	15.38 $\pm$ 0.41	19.11 $\pm$ 0.61	21.76 $\pm$ 0.72	23.51 $\pm$ 0.08	27.65 $\pm$ 0.55
CEIB	23.16 $\pm$ 0.56	27.85 $\pm$ 0.85	32.16 $\pm$ 0.58	42.74 $\pm$ 0.41 <sup>*</sup>	57.99 $\pm$ 0.15	67.56 $\pm$ 0.66
EEIB	14.56 $\pm$ 0.78	20.45 $\pm$ 0.96	27.61 $\pm$ 0.27 <sup>+</sup>	34.11 $\pm$ 0.26	38.67 $\pm$ 0.61 <sup>*</sup>	42.56 $\pm$ 0.72
AQIB	12.14 $\pm$ 0.09	20.43 $\pm$ 0.23	26.91 $\pm$ 0.71	38.51 $\pm$ 0.29 <sup>+</sup>	52.13 $\pm$ 0.35	59.61 $\pm$ 0.77 <sup>+</sup>

Each value represents the Mean $\pm$  SEM. <sup>\*</sup>P<0.01, <sup>+</sup>P<0.05 equated to postive control

**Table 6.** ECG changes of IB extracts on DXR intoxicated rats

Group	Treatment	ST interval (msec)	QT interval (msec)	Heart rate (msec)
I	Normal	36.12	66.88	353.8
II	Control (DXR)	48.14	81.86	301.3 <sup>*</sup>
III	Vitamin E + DOX	37.43 <sup>*</sup>	65.89	402.5
IV	CEIB (250 mg/kg) + DOX	38.52	69.23	369.2
V	CEIB (500 mg/kg) + DOX	40.12	71.45 <sup>**</sup>	375.1
VI	AQIB (250 mg/kg) + DOX	43.21	70.39	381.2 <sup>*</sup>
VII	AQIB (500 mg/kg) + DOX	46.69 <sup>*</sup>	75.21	389.2

Each value represents the Mean  $\pm$  SEM. <sup>\*</sup>P<0.01, <sup>\*\*</sup>P<0.05 equated to postive control

fig 1. I. On treatment with vehicle to normal heart tissue exhibited histology with normal myocardial cells with well defined myoplasm, prominent nuclei and nucleolus. II histology of heart section shows fatty changes, inflammation and damage of myocardial architecture with myocardial necrosis when treated with doxorubicin. III. Histology of heart tissue showed potential recovery of normal myocyte when compared to doxorubicin treated group upon treatment with standard vitamin E. IV. Histology of heart showed less necrosis when treated with CEIB (250 mg/kg). V. Histology of heart tissue returned to quite normal from injured heart when treated with CEIB (500 mg/kg) VI. Histology of heart tissue showed less vacuolization of the cytoplasm when treated with AQIB (250 mg/kg). VII histology of heart tissue showed less necrosis when compared to doxorubicin treated group when treated with AQIB (500 mg/kg).

## DISCUSSION

Preparation of chloroform and ethanol extracts of IB was done and CBIE has highest yield. Many biological activities were exhibited by flavonoids and phenols through many studies. Thus, from CEIB and AQIB flavonoids and phenols are isolated<sup>27</sup>.

For the investigation of IB and DOX induced myocardial toxicity the present study was done<sup>28</sup>. The result suggests that DOX induced cardiomyopathy in rats is prevented by IB. For evidence the following lines are emphasized from the present study.

In the study, a significant elevation in the level of the diagnostic markers CK, CK-MB, LDH by DOX treated rats moreover, the severity

of DOX induced myocardial damage is indicated by the elevated levels of these enzymes, which is in line with an earlier report<sup>29</sup>. DOX induced elevation of serum marker was significantly reduced which is shown by prior administration of CEIB 250mg/kg +DOX and 500mg/kg+ DOX. For maintaining architectural integrity and normal structural of cardiac myocytes can be done by restriction of leakage of these enzymes, CEIB is responsible which is confirmed by the reduction in the enzyme level through which membrane stabilizing property of CEIB can be accounted<sup>30</sup>.

For the definite diagnosis of myocardial injury, electrocardiograph abnormalities are the main criteria generally used. As compared to normal rats DOX induced rats shows significant alteration of ECG patterns. Decrease in the heart rate and prolongation of ST interval were the characteristic finding<sup>31</sup>. Prolongation of QT interval was also found in addition to this. The severity of DOX induced myocardial damage is indicated by the ECG changes, which is in line with earlier report. By ST prolongation, oxidative stress caused due to consecutive loss of cellular damage might be characterized. A protective effect against DOX induced altered ECG patterns were shown of pretreatment of CEIB 250 mg/kg + DOX and 500mg/kg +DOX.

From the present study, the mechanism of cardiotoxicity induced by a DOX is not clearly known, although large body of evidence shows a decrease in endogenous antioxidants, increased oxidative stress by increase in oxygen free radicals, which is followed by development of a variety of subcellular changes in the myocardium, typical of DOX induced cardiac injury. We found a significant increase in heart tissue in rat treated with DOX<sup>32</sup>.

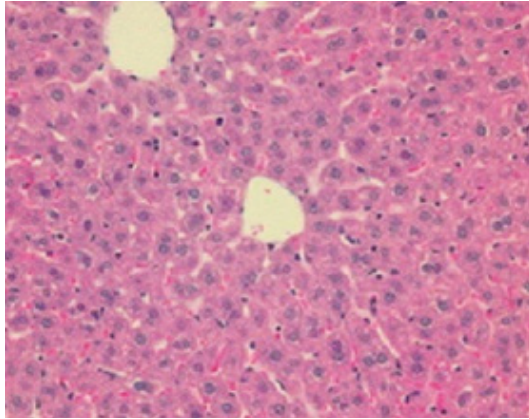
**Table 7.** Effect of IB extracts on CK, LDH, CK-MB, MDA and GSH

Group	CK (UI/L)	LDH (UI/L)	CK-MB (UI/L)	MDA (nmol/g heart tissue)	GSH (nmol/g heart tissue)
I Normal	155.4 ± 1.57	239.5 ± 0.91	134.71 ± 1.5	16.95 ± 0.96	2.98 ± 0.05
II Control (DXR)	328.2 ± 0.9	418.7 ± 1.18	235.9 ± 1.06**	48.91 ± 0.39	1.53 ± 0.03
III Vitamin E + DOX	226.3 ± 1.12*	321.5 ± 1.32	156.32 ± 1.4	24.94 ± 0.35	2.46 ± 0.11*
IV CEIB (250 mg/kg) + DOX	281.51 ± 0.89	385.3 ± 1.16	196.6 ± 0.36	39.56 ± 0.51	1.92 ± 0.09
V CEIB (500 mg/kg) + DOX	232.45 ± 0.89	336.5 ± 0.98**	174.11 ± 0.23	29.12 ± 0.62	2.12 ± 0.06
VI AQIB (250 mg/kg) + DOX	294.34 ± 1.32	393.61 ± 1.11	201.32 ± 1.56	40.26 ± 1.3**	1.78 ± 0.21
VII AQIB (500 mg/kg) + DOX	275.3 ± 0.36	356.21 ± 1.33	193.6 ± 1.18*	38.21 ± 1.25	2.09 ± 0.06

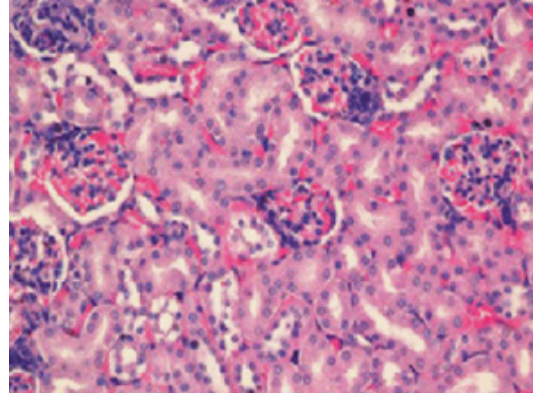
Each value represents the Mean ± SEM. \*P<0.01, \*\*P<0.05 equated to positive control

Decrease in levels of GSH and increased lipid per oxidation are suggested by MDA levels. Depletion antioxidants and increased oxidative stress are responsible for the damage of the cardiac

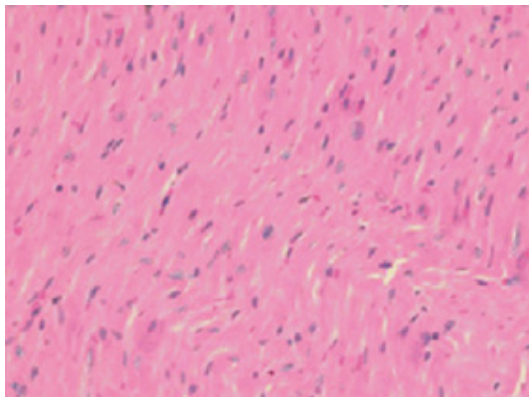
tissue as reported earlier. The oxidative stress and cardiac damage are confirmed by an increase in heart tissue MDA levels with an decrease in levels of GSH on DOX treated rats. The changes in MDA



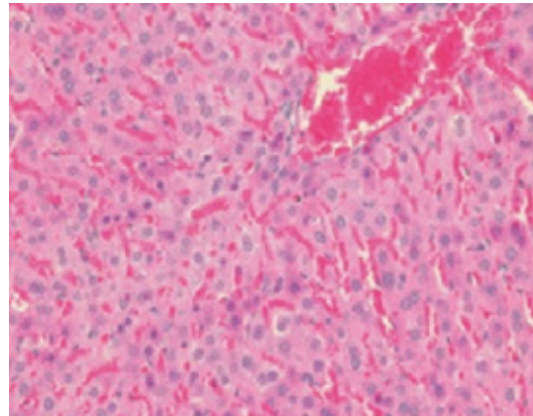
Group I: Normal group



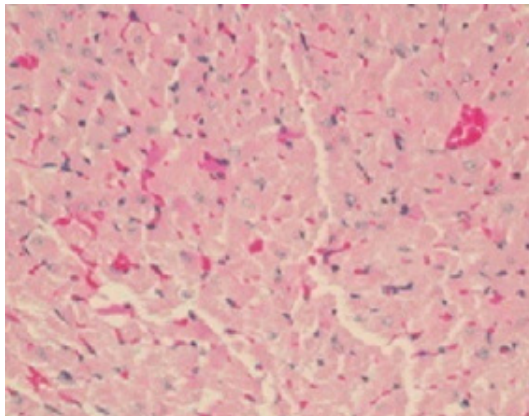
Group II: Disease control group



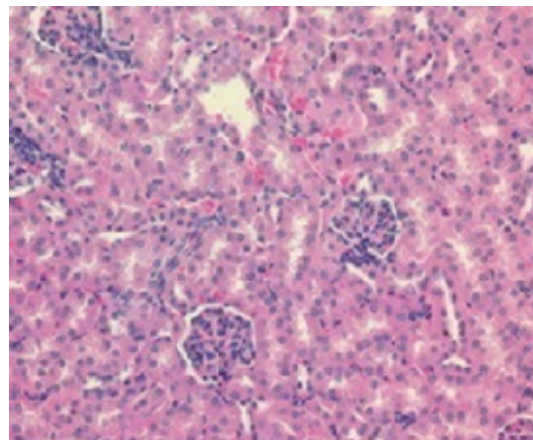
Group III: Vitamin E + DOX treated group



Group IV: CEIB (250 mg/kg) + DOX treated group

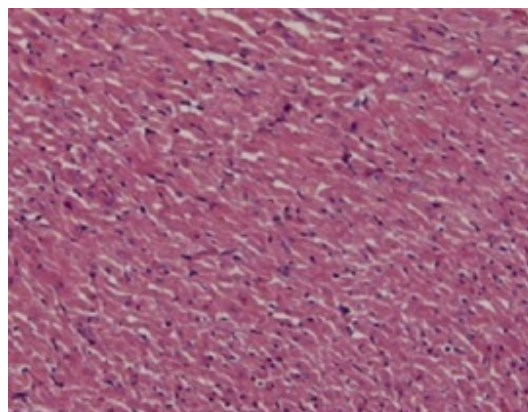


Group V: CEIB (500 mg/kg) + DOX treated group



Group VI: AQIB (250 mg/kg) + DOX treated group





Group VII: AQIB (500 mg/kg) + DOX treated group

**Fig. 1.** Cardioprotective activity of different extracts of IB

and enzyme levels by DOX induced changes are prevented by CEIB. In heart tissue of CEIB 250mg/kg + DOX, CEIB 500 mg/kg +DOX and vitamin E treated groups shown significant increase in the GSH activity and decrease in lipid peroxidation was found.

In the pathogenesis of DOX induced myocardial toxicity reactive oxygen species (ROS) are involved. In mitigating free radical-induced cell injury, the antioxidant enzyme GSH plays an important role. Because of its ability to use and remove organic and inorganic peroxide, in the heart, the GSH is extremely important. As observed in the present study, increased GSH consumption caused by enhanced lipid peroxidation by depletion of GSH in heart of rats is known to result<sup>33</sup>. The susceptibility to free radicals can be decreased by the prior administration of CEIB which protects the myocytes against DOX induced myocardial toxicity. The formation of hydroxyl radicals (OH), initiation, propagation of lipid peroxidation are promoted by declined GSH contents by DOX<sup>34</sup>.

The data of the present study clearly shows the beneficial action of CEIB as a cardioprotective agent is suggested by electrophysiological, biochemical and histological parameters which were maintained to near normal status in DOX treated rats, which are modulated by CEIB.

### CONCLUSION

We this conclude that it is evident that CEIB whole plant possess promising antioxidant

and cardioprotective activity in in-vivo model, through the research findings based on the observations and results obtained. As stated earlier, the presence of free-radical scavenging activity activity, which may be due to presence of flavonoids and phenolic compounds in the extracts may attribute to its cardioprotective activity. To elucidate detailed cardioprotective mechanism and to support the present assumption further studies are required.

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