

CARDIOPROTECTIVE EFFECTS OF ETHANOLIC LEAF EXTRACT OF *IPOMOEA BATATAS* ON DOXORUBICIN INDUCED CARDIOTOXICITY IN RATS

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Received: 02 January 2015, Revised and Accepted: 31 January 2015

ABSTRACT

Objective: This study was aimed to investigate the protective effects of ethanolic leaf extract of *Ipomoea batatas* (EEIB) on doxorubicin (DOX) induced cardiotoxicity using Albino Wistar rats by monitoring the enzymatic, non-enzymatic antioxidant levels, serum enzyme levels, microscopical examinations and the study in electrocardiography (ECG) alterations.

Methods: The rats were treated with EEIB (300, 600, 900 mg/kg body weight, n=6) and vitamin C (50 mg/kg) orally for 10 days and the rats were also treated with DOX 15 mg/kg on 7th day. On the 11th day, the rats were treated with anaesthetized with anesthetic ether, ECG and the levels of biochemical and histological observations of the heart tissues were performed.

Results: The levels of cardiac markers like creatinine kinase (CK-MB), CK, lactate dehydrogenase and lipid peroxides were significantly decreased in the extract (600, 900 mg/kg) and ascorbic acid pretreated rats when compared to DOX with significance $p < 0.001$. In heart superoxide dismutase, catalase, tissue protein and glutathione levels were significantly increased in the extract (600, 900 mg/kg) and ascorbic acid pretreated rats when compared to DOX with significance of $p < 0.001$. The heart weight and electrographic alterations were gradually recovered by the extract in a dose-dependent manner. There was no significant recovery of the above-mentioned parameters with an extract dose of 300 mg/kg.

Conclusion: The study revealed that the extract and vitamin C have a dose-dependent potential to prevent oxidative damage/free radical damage induced by DOX in heart.

Keywords: Doxorubicin, Vitamin C, *Ipomoea batatas*, Serum enzymes, Antioxidant enzymes.

INTRODUCTION

Cardiovascular disease (CVD) is a group of problems that occur when the heart and blood vessels are not working the way they should. CVD include coronary heart disease (heart attacks), rheumatic heart disease, peripheral artery diseases, congenital heart disease and heart failure. The major causes of CVD are tobacco use, alcohol consumption, physical inactivity and an unhealthy diet [1]. Diabetes mellitus and hypercholesterolemia are well-established risk factors for coronary artery disease [2]. Vascular disease includes the retinopathy, nephropathy, peripheral vascular disease, stroke and coronary artery disease [3].

Coronary artery disease and its consequence, myocardial infarction (MI) continue to be a prime cause of mortality and morbidity in the western world [4]. MI is a complex phenomenon affecting the mechanical, electrical, structural, and biochemical properties of the heart. The heart failure is one of the most common causes of death in industrialized nations. United States of America is alone having over 4.6 million patients who have been diagnosed by this disease and it is the cause of death in several hundred thousand patients each year [5,6].

CVD remains the principle cause of death in the both developed and developing countries, accounting for roughly 20% of all worldwide deaths per year. CVD in India cause 3 million deaths per year, accounting for 25% of all mortality. The World Health Organization predicts that deaths due to circulatory system disease are projected to double between 1985 and 2015 [7].

Doxorubicin (DOX) is an anthracycline antibiotic used effectively in tumor therapy besides various anti-neoplastic agents, also used in solid and hemopoietic malignancies. Anthracycline structures are related to anthraquinone or naphthaquinone core linked with a glycoside bond at a ring atom 7 to an amino sugar, quinones, and phenolic group's and their derivatives.

The principle mechanisms of cardiotoxicity are increased oxidative stress, as an evident from increased levels of reactive oxygen species (ROS) like superoxide anion and H_2O_2 and lipid peroxidation (LPO) by DOX leads to causing impairment of cell functioning and cytolysis.

Decreased activity of Na^+ and K^+ adenosine triphosphatase (ATPase), vasoactive amine release, decreased levels of antioxidants and sulfhydryl group's results in inhibition of nucleic acids and protein synthesis, altered adrenergic function and decreased expression of cardiac-specific genes are the proposed mechanism.

Ipomoea batatas is the sixth most important food crop in the world. It is a dicotyledonous plant, belongs to the family Convolvulaceae. The leaf extracts of *I. batatas* (sweet potato) have shown the presence of total flavonoids, total phenolics. *I. batatas* contained highest level of total phenolics and scavenging activity with the IC_{50} value of 372.4 $\mu g/ml$ and thus a potential source for antioxidants. Besides, all the different varieties of *I. batatas* leaves are found to be stronger scavenger compared to vitamin C. The reducing power of *I. batatas* leaf extract increased with concentration with higher level of reducing power. Despite differences in their storage roots, all the *I. batatas* leaves varieties contained antioxidants that are beneficial to the human body. The action of flavonoids can be divided into two different mechanisms, scavenging process and chelating process [8].

I. batatas leaves are an excellent source flavonoids with anti-oxidative poly-phenols, with 6 polyphenolics compounds, 15 anthocyanins and phenolic acids such as caffeic, mono, di and tri caffeoylquinic acids and are superior with other vegetables.

The major constituent of flavonoids is pro-anthocyanins and two or more flavan-3-ol such as catechin, epicatechin or gallo-catechin. Catechin contains two benzene rings to be the powerful scavenger. It

also contains vitamin A, C, E, B1, B2 and nutrients like Ca, Fe, S, Cu, Zn, P, Mg, Na, K, Mn and Al.

I. batatas leaves are used as a vegetable, in tea, breads, confectioneries and as a nutritional supplement and for industrial alcohol production. Research suggests that *I. batatas* may be beneficial in diabetes, anti-ulcer, anti-inflammatory, dengue, anti-proliferative and wound healing.

METHODS

Drugs and chemicals

All the drugs and chemicals used were of analytical grade. DOX was procured from Parenteral Drugs Ltd. and vitamin C was purchased from S. D. Fine Chemicals Ltd., Mumbai were used in this study.

Plant material and preparation of extracts

The fresh leaves of *I. batatas* was collected from the fields near Kolar, district in the month of October and the plant material was identified and authenticated by a qualified botanist at National Ayurveda Dietetics Research Institute (Govt. Central Pharmacy Annexe), Jayanagar Bengaluru.

The ethanolic leaf extract of *I. batatas* (EEIB) was collected from an authentic supplier, M/S Green Chem Pvt. Ltd., Bengaluru as a gift sample.

Preliminary phytochemical screening

Ethanolic extract of *I. batatas* was subjected to preliminary phytochemical screening for the detection of various plants constituents like carbohydrates, saponins, flavonoids, glycosides, proteins, alkaloids and phenols [9,10].

Animals

Inbred Wistar albino rats weighing between 150 and 200 g were housed in a group of 5-6. All rats were feed with a pelleted diet (Pranav Agro Industries Ltd., Sangli, India) and water *ad libitum*. Institutional Animals Ethics Committee approved the experimental protocol and care of animals was taken as per guidelines of CPCSEA, Department of Animal Welfare and Government of India.

Acute toxicity studies

The acute oral toxicity studies were carried out by using Albino Wistar rats weighing between 150 and 200 g of either sex as per OECD 425 guidelines by employing the up and down method prior to evaluation of cardioprotective activity.

Evaluation of cardio-protective effects of EEIB on DOX induced cardiotoxicity [11,12]

The animals were randomly divided into six groups consists of six animals each.

Wistar rats weighing (180-200 g) either sex were selected under healthy conditions for experimental purpose.

Group 1: Normal control received distilled water (vehicle) for 10 days.

Group 2: Toxic control received DOX 15 mg/kg i.p at a single dose on 7th day, other days it received only vehicle (distilled water), orally.

Group 3: Standard control received 50 mg/kg of vitamin C for 10 days and received DOX on 7th day at a dose of 15 mg/kg, i.p route.

Group 4: Served as pre-treatment group received 300 mg/kg of ethanolic extract of *I. batatas* orally for 10 days and DOX on 7th day at a dose of 15 mg/kg, i.p route.

Group 5: Served as pre-treatment group received 600 mg/kg of ethanolic extract of *I. batatas* orally for 10 days and DOX on 7th day at a dose of 15 mg/kg, i.p route.

Group 6: Served as pre-treatment group received 900 mg/kg of ethanolic extract of *I. batatas* orally for 10 days and DOX on 7th day at a dose of 15 mg/kg, i.p route.

After 24 hrs (11th day) rats were anaesthetized and electrocardiograph (ECG) was recorded using power lab system. Blood samples were collected by retro-orbital, intracardiac method, and the serum was separated and used for the estimation of serum biomarkers i.e. creatine phosphokinase, lactic dehydrogenase (LDH), and creatinine kinase (CK-MB). Following, the animal was sacrificed by cervical dislocation and the heart specimen was collected and used for histopathology and for tissue biochemical estimations tissue proteins, LPO, superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT) [13].

Statistical analysis

Values were expressed as mean±standard error mean from six animals. Statistical differences were evaluated using a one-way analysis of variance, followed by Dunnet's t-test. Results were considered to be statistically significant at p<0.05.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

The phytochemical screening of the ethanolic extract of *I. batatas* revealed the presence of carbohydrates, saponins, flavonoids, glycosides, alkaloids, phenols, proteins and amino acids (Table 1).

Effect of EEIB on various ECG parameters

The ECG of different groups was compared. Normal rats showed a standard ECG pattern, whereas animals treated with DOX alone showed significant elevation in QRS complex, ST segment, and R-R interval. The heart rate was decreased due to widening of QRS, prolongation of QT interval, and increased T-wave was observed when compared to normal control rats. Rats pretreated with EEIB at dose of 600, 900 mg/kg and vitamin C (50 mg/kg) resorted the elevated levels of P-wave, RR interval, QT interval, ST segment and heart rate near to the normal with a significance of p<0.001. Extract treated with 300 mg/kg showed no significant value for P-wave, RR interval and ST segment, but showed significance for heart rate and QRS complex with p<0.001 and p<0.05 respectively. The data of the experimental animals such as P-wave, QRS complex, QT interval, R-R interval, ST height and heart rate are shown in the Table 2.

Effect of EEIB on CK -MB, CK, LDH and SOD

Rats treated with DOX (15 mg/kg) alone shows a significantly increase in the levels of CK-MB, LDH, CK and SOD when compared to normal control rats. Rats pretreated with different doses of extract (300, 600, 900 mg/kg) and vitamin C (50 mg/kg) to shows significant decrease when compared to DOX treated rats. (p<0.001) in the level of CK-MB, LDH, serum glutamic pyruvic transaminase and serum glutamic oxaloacetic transaminase as compared to DOX alone treated rats.

Table 1: Phytochemical screening of ethanolic extracts of the leaf of *I. batatas*

S. no.	Phytoconstituents	Ethanolic extracts of whole plant of <i>C. ternatea</i>	Aqueous extracts of whole plant of <i>C. ternatea</i>
1	Carbohydrates	+	+
2	Saponins	+	+
3	Flavonoids	+	+
4	Glycosides	+	+
5	Proteins and amino acids	+	+
6	Alkaloids	+	+
7	Phenols	+	+

C. ternatea: *Clitoria ternatea*, +: Presence of phytoconstituents in the extract, -: Absence of phytoconstituents in the extract, *I. batatas*: *Ipomoea batatas*

CK-MB

The level of CK-MB in the DOX alone treated animals with a dose of 15 mg/kg showed a significant raise in the CK-MB levels with mean±standard deviation (SD) (228.3±7.87) and significance of $p<0.001$ when compared to normal (69.99±7.48). The pretreated animals with vitamin C 50 mg/kg (96.78±7.30, $p<0.001$) and extract at a dose of 600, 900 mg/kg (170.6±9.16), (126±6.14) with $p<0.001$ showed a significant decrease in the levels of CK-MB. The extract 300 mg/kg showed significance with (210±12.89, $p<0.01$) when compared to DOX treated group. The extract has shown a dose dependent effect.

CK

The level of CK in the DOX alone treated rats showed a significant raise in the CK levels with mean±SD (438.1±15.80) and significance of $p<0.001$ when compared to normal (207.6±14.55). Pretreated animals with vitamin C 50 mg/kg and extracts at a dose of 600, 900 mg/kg showed a significant decrease in the levels of CK (287.4±10.91) and (365.7±21.36), (233.8±21.12) with $p<0.001$, extract 300 mg/kg showed significance with (407.2±19.15, $p<0.05$) when compared to DOX treated group.

LDH

The level of LDH in the DOX alone treated rats showed an elevated levels of LDH with mean±SD (511.0±18.18) and significance of $p<0.001$ when compared to normal (244.4±8.80). Pretreated animals with vitamin C 50 mg/kg and extracts at a dose of 600, 900 mg/kg showed a significant decrease in the levels of LDH (297.9±22.13) and (420.9±8.15), (318.6±9.76) with $p<0.001$ respectively when compared to DOX treated group. Despite the LDH level in the extract dose 300 mg/kg showed no significance as compared to toxic group.

SOD

The level of SOD in the DOX alone treated rats with a dose of 15 mg/kg showed a significant decrease in the SOD levels (7.55±0.99) with mean±SD and significance of $p<0.001$ when compared to normal (21.55±1.32). Pretreated animals with vitamin C 50 mg/kg and extracts at a dose of 300, 900 mg/kg showed a raise in the levels of SOD (20.60±1.44) and (3.75±0.93), (15.63±0.57) with $p<0.001$ and the extract 600 mg showed raise in significance (9.37±0.77) with $p<0.05$ respectively when compared to DOX group (Table 3).

EEIB on CAT, GSH, LPO and tissue protein**CAT**

The CAT levels in the DOX alone treated rats with a dose of 15 mg/kg showed a significant decrease in the CAT levels (68.56±8.01) with mean±SD and significance of $p<0.001$ when compared to normal (206.70±10.14). The CAT level in the extract dose; 300 mg/kg was not significant as compared to toxic group. The rats pretreated with vitamin C 50 mg/kg and extracts at a dose of 600, 900 mg/kg showed a raise in the levels of CAT (117.30±10.68) and (113.2±12.36), (161.40±9.69) with $p<0.001$ respectively when compared to DOX treated group.

GSH

The GSH levels in the DOX alone treated rats showed a significant decrease in the GSH levels (1.43±0.34) with mean±SD and significance of $p<0.001$ when compared to normal (5.15±0.29). The GSH level in the extract dose 300 mg/kg was not significant as compared to toxic group. The rats pretreated with vitamin C 50 mg/kg and extracts at a dose of 900 mg/kg showed a raise in the levels of CAT (5.00±0.15) and (3.89±0.34) with $p<0.001$ respectively, but dose of 300 mg has significance (2.39±0.41), with $p<0.01$ when compared to DOX treated group.

LPO

There was a dose dependent inhibition in *in-vivo* LPO in 600, 900 mg/kg of extract (18.84±0.31), (15.89±0.72) and in vitamin C (13.12±0.46) with a significance of $p<0.001$ when compared to toxic group. DOX alone treated rats showed a significant increase in the LPO levels (23.26±0.55) with mean±SD and significance of $p<0.001$ when compared to normal (11.78±0.73). The rats pretreated with extract of 300 mg/kg reduced the levels of LPO (22.23±0.61) with mean±SD and significance of <0.05 when compared to toxic group.

Tissue protein

The protein content in the DOX alone treated rats with a dose of 15 mg/kg showed a significant decrease in the protein levels (3.22±0.21) with mean±SD and significance of $p<0.001$ when compared to normal (8.08±0.24). Pretreated animals with vitamin C 50 mg/kg and extracts at a dose of 300, 600, 900 mg/kg showed a raise in the levels of protein (7.91±0.22) and (4.82±0.26), (6.45±0.40), (7.22±0.56) respectively when compared to DOX treated group. The extract has shown a dose dependent effect (Table 4).

Table 2: Effect of EEIB on ECG in doxorubicin induced cardio toxicity

Group	P-wave (S)	QRS complex (S)	RR interval (S)	QT interval (S)	ST height (mV)	Heart rate (bpm)
G1	0.0212±0.0004	0.0277±0.0012	0.3135±0.0076	0.0538±0.0015	0.0589±0.0008	278.50±6.50
G2	0.0293±0.0008###	0.0562±0.0021###	0.5842±0.0099###	0.0823±0.0006###	0.0801±0.0009###	170.45±4.40###
G3	0.0189±0.0010***	0.0264±0.0012***	0.3355±0.0104***	0.0568±0.0013***	0.0643±0.0004***	298.16±10.65***
G4	0.0296±0.0010 ^{NS}	0.0418±0.0005*	0.5386±0.0053 ^{NS}	0.0684±0.0041***	0.0810±0.0033 ^{NS}	186.75±4.69***
G5	0.0157±0.0005***	0.0478±0.0202 ^{NS}	0.5023±0.0111***	0.0461±0.0021***	0.0482±0.0010***	227.28±2.92***
G6	0.0188±0.0005***	0.0220±0.0010***	0.3148±0.016***	0.0544±0.0029***	0.0538±0.0029***	285.75±4.92***

Each value is expressed as mean±standard deviation for groups of six animals in each group. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ will be considered as significant when compared to toxic group. # $p<0.05$, ## $p<0.01$, ### $p<0.001$, will be considered as significant when compared to the normal group. One-way ANOVA followed by Dunnett's multiple comparison tests. EEIB: Ethanolic leaf extract of *I. batatas*, ECG: Electrocardiography

Table 3: Effect of EEIB on CK-MB, CK, LDH and SOD

Group	Drug treatment	CK-MB (IU/L)	CK (IU/L)	LDH (IU/L)	SOD units/mg of protein
G1	Normal control distilled water	69.99±7.48	207.6±14.55	244.4±8.80	21.55±1.32
G2	Doxorubicin (15 mg/kg/7 th day)	228.3±7.87###	438.1±15.80###	511.0±18.18###	7.55±0.99###
G3	Vitamin C (50 mg/kg/day) DOX	96.78±7.30***	287.4±10.91***	297.9±22.13***	20.60±1.44***
G4	EEIB (300 mg/kg/day) DOX	210±12.89**	407.2±19.15*	515.9±26.16 ^{NS}	3.75±0.93***
G5	EEIB (600 mg/kg/day) DOX	170.6±9.16***	365.7±21.36***	420.9±8.15***	9.37±0.77*
G6	EEIB (900 mg/kg/day) DOX	126±6.14***	233.8±21.12***	318.6±9.76***	15.63±0.57***

Each value is expressed as mean±standard deviation for groups of six animals in each group. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ will be considered as significant when compared to toxic group. # $p<0.05$, ## $p<0.01$, ### $p<0.001$, will be considered as significant when compared to the normal group. One-way ANOVA followed by Dunnett's multiple comparison tests. CK: Creatine kinase, LDH: Lactic dehydrogenase, SOD: Superoxide dismutase, DOX: Doxorubicin, EEIB: Ethanolic leaf extract of *I. batatas*, *I. batatas*: *Ipomoea batatas*

Table 4: Effect of EEIB on catalase, GSH, LPO and tissue protein

Drug treatment	Catalase micro mole of H ₂ O ₂ consumed/minutes	GSH units/mg of protein	LPO μ moles/MDA/minutes/mg of protein	Protein g/dL tissue
Normal control distilled water	206.70 \pm 10.14	5.15 \pm 0.29	11.78 \pm 0.73	8.08 \pm 0.24
DOX (15 mg/kg/7 th day)	68.56 \pm 8.01 ^{###}	1.43 \pm 0.34 ^{###}	23.26 \pm 0.55 ^{###}	3.22 \pm 0.21 ^{###}
Vitamin - C (50 mg/kg/day) DOX	117.30 \pm 10.68 ^{***}	5.00 \pm 0.15 ^{***}	13.12 \pm 0.46 ^{***}	7.91 \pm 0.22 ^{***}
EEIB (300 mg/kg/day) DOX	81.22 \pm 6.03 ^{NS}	1.45 \pm 0.26 ^{NS}	22.23 \pm 0.61 [*]	4.82 \pm 0.26 ^{***}
EEIB (600 mg/kg/day) DOX	113.2 \pm 12.36 ^{***}	2.39 \pm 0.41 ^{**}	18.84 \pm 0.31 ^{***}	6.45 \pm 0.40 ^{***}
EEIB (900 mg/kg/day) DOX	161.40 \pm 9.69 ^{***}	3.89 \pm 0.34 ^{***}	15.89 \pm 0.72 ^{***}	7.22 \pm 0.56 ^{***}

Each value is expressed as mean \pm standard deviation for groups of six animals in each group. *p<0.05, **p<0.01, ***p<0.001 will be considered as significant when compared to toxic group. #p<0.05, ##p<0.01, ###p<0.001, will be considered as significant when compared to the normal group. One-way ANOVA followed by Dunnett's multiple comparison tests. GSH: Glutathione, LPO: Lipid peroxidation, DOX: Doxorubicin, EEIB: Ethanolic leaf extract of *I. batatas*, *I. batatas*: *Ipomoea batatas*

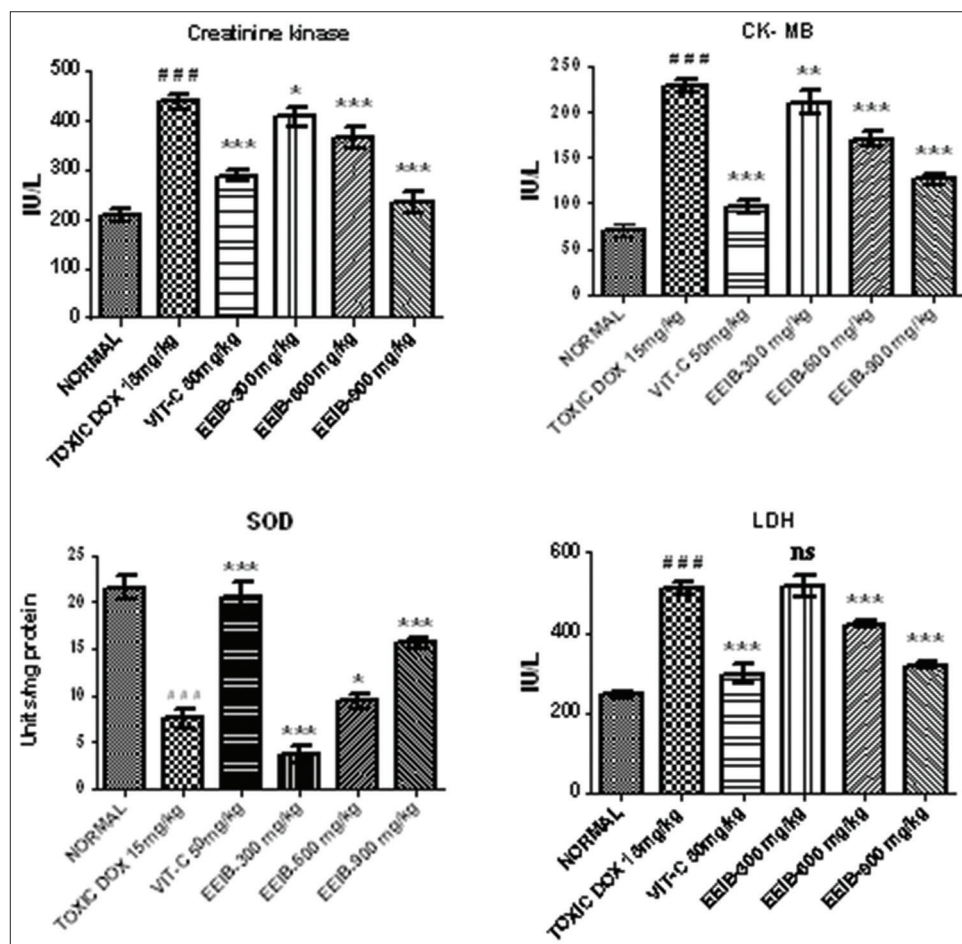


Fig. 1: Effect leaves extra of *Ipomoea batatas* on creatine kinase (CK)-MB, CK, lipid peroxidation and superoxide dismutase

Histopathological studies of rat's heart

In the present study, screening of EEIB on DOX induced cardio toxicity was investigated.

DOX an anti-cancer drug causes damage to heart muscles and to its chambers which leads to reduced cardiac function and leads to complicated diseases like blood pressure alterations, stroke. DOX generates ROS, which reported to play a vital role in the development of cardio toxicity and gives an insight of pathology. In the present study it is to be found that EEIB showed a protective effect on DOX induced cardio toxicity.

Heart tissue is particularly susceptible to free radical damage, because it contains low levels of free-radical detoxifying enzymes such as SOD, CAT and GSH and also due to the high affinity of DOX toward phospholipids component of the mitochondrial membrane in the

myocardial cells, thus leads to the accumulation of DOX in the heart tissue (Fig. 1).

Accounting the above point the decrease in the activities or concentrations of these enzymes in the heart may cause damage to myocardial cells. Thus, the extracellular fluid contains elevated levels of CK, CK-MB and LDH, which are analyzed in serum [14].

In the present study, DOX treated groups showed significant increased levels of CK, CK-MB and LDH when compared to normal. This is due to the myocardial cells, containing CK, LDH, are damaged due to incomplete oxygen supply or glucose, the cell membrane becomes permeable or may rupture, which results in the leakage of these enzymes. Pre-treatment with extract at a dose of 600 mg/kg and 900 mg/kg, significantly lowered the DOX - induced elevation of serum levels of these diagnostic marker enzymes. This shows that the EEIB

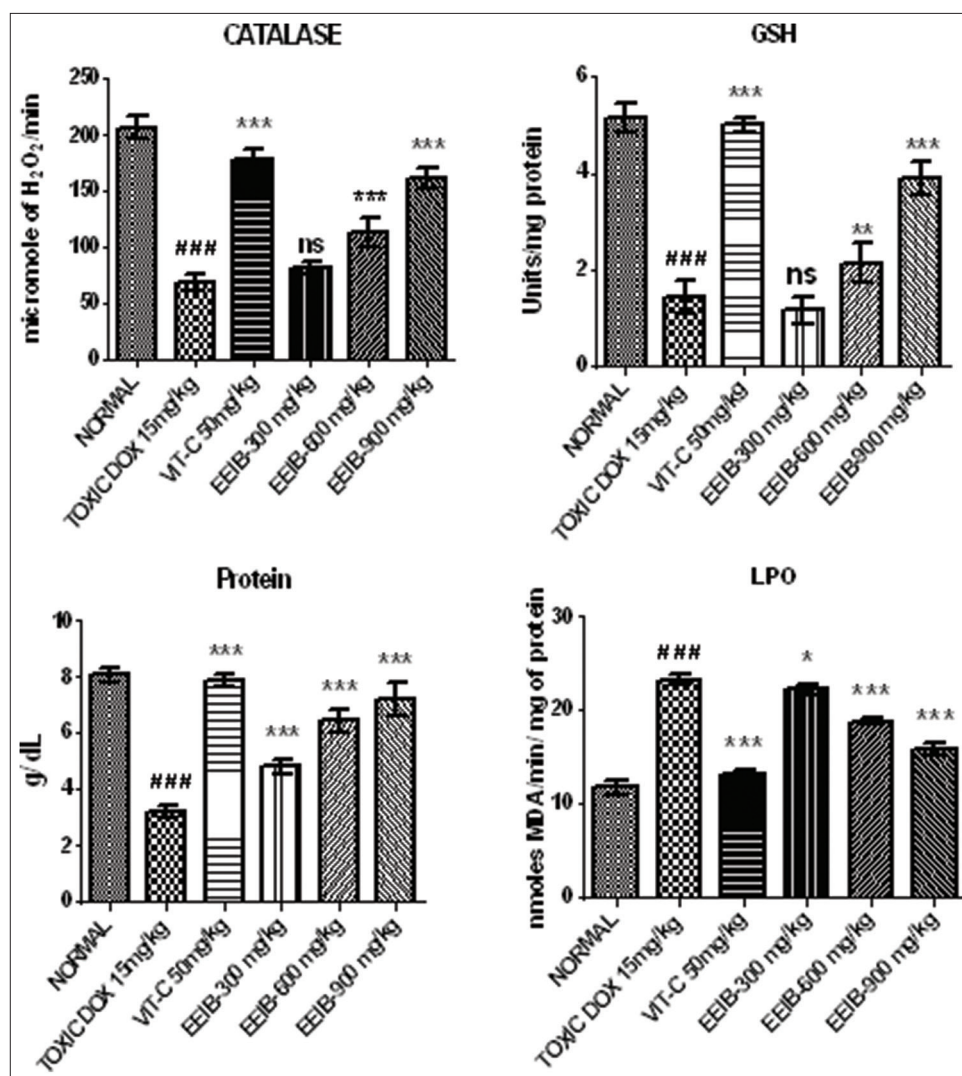


Fig. 2: Effect of ethanolic leaves extract of *Ipomoea batatas* on catalase, glutathione, lipid peroxidation and tissue protein

can prevent the leakage of these enzymes into extracellular fluid by maintaining the membrane integrity of myocardium [15].

A significant increase in the cardiac enzymes was found which was parallel to the extent of myocardial injury. One of the best marker is LPO in serum is malonaldehyde, which indicates the amount of membranes damaged by ROS. LPO is enhanced during ischemia i.e. due to decreased antioxidant defense mechanisms and decreased ATP production [16]. LPO involves the formation and proliferation of lipid radicals, uptake of oxygen and rearrangement of double bonds in unsaturated lipids which results in the destruction of membrane lipids [17]. Pre-treatment with different doses of extract prior to DOX administration showed reduction in LPO compared to toxic group. This may be due to the electron and proton donating capacity of flavonoids present in the leaf extract of *I. batatas* and showed a cardio protective effect when compared with vitamin C 50 mg/kg.

LDH is an intracellular enzyme, which CAT the readily reversible reaction involving the oxidation of lactate to pyruvate with nicotinamide adenine dinucleotide serving as coenzyme. This enzyme level rises within 24-48 hrs after a heart attack and peaks in 2-3 days in the serum. DOX induced cardio toxicity results in elevation of LDH levels in serum and extracellular fluid is due to leakage of this enzyme from mitochondria. Pre-treatment with extract at a dose of 600 mg/kg and 900 mg/kg resulted in decrease levels of LDH in serum when compared to DOX alone treated rats. This protective mechanism of extract is by

protecting the cell membrane from destructive effect of free radicals and also by inhibiting the oxidative modification of LDL as well by balancing lipid profile [18].

Dox generates superoxide free radicals due to conversion of quinone to semi quinone moiety, whereas SOD enzyme dismutates this free radical to hydrogen peroxide. By this process any increase in SOD activity of the organ appears to be beneficial in the event of increased free-radical generation.

Three antioxidant enzymes SOD, GSH and CAT plays important role in prevention of cell injury induced by free radical. These also promote the formation of OH radicals, initiation and proliferation of LPO. GSH is involved in removing organic and inorganic peroxide. SOD levels were significantly decreased in DOX treated animals when compared to the normal groups. The rats pre-treatment with extract (EEIB 600 and 900 mg) reversed the SOD activity in dose-dependent manner which may be due to the cardio-protective activity of EEIB. The antioxidant activity of EEIB may be responsible for this protective action. The activity of vitamin C also exhibited significant increase in the levels of SOD (Fig. 2).

Studies reported that a rise in SOD activity, without a concomitant rise in the activity of CAT /GSH might be disadvantageous. This is due to the fact that SOD generates H₂O₂ as a metabolite, which is cytotoxic and needs to be scavenged by CAT/GSH. Thus a simultaneous increase in CAT/GSH activity is essential for an overall beneficial effect of increase in SOD activity.

Inhibition of DOX induced oxidative stress and tissue injury might be due to an increase in levels GSH, myocardial SOD and CAT activities gained by treatment with extracts. The observed increase in CAT activity in DOX induced animals supports the above hypothesis that this increase is possibly required to overcome excessive oxidative stress. GSH levels were also lowered significantly in DOX induced animals, while pre-treatment with vitamin C, 600 and 900 mg of extract showed significant increase in GSH levels in DOX induced animals.

CAT is an antioxidant enzyme which catalyzes the breakdown of H_2O_2 into oxygen and water. CAT activity was decreased in DOX treatment groups when compared to normal, which may be due to the decrease activities/concentrations or due to mitochondrial damage in the heart causes damage to myocardial cells. The DOX treated rats showed significant decrease in the levels of CAT when compared to the normal. The rats pretreated with vitamin C 50 mg/kg and extract further increased its activity significantly at 600 mg/kg and 900 mg/kg dose levels [19].

A single dose of DOX 15 mg/kg lowered the protein content ($p < 0.001$) in cardiac tissue when compared to normal rats. Rats pretreated with vitamin C and extract showed a significant raise in protein levels ($p < 0.001$) when compared to the DOX treated groups.

ECG parameters

This parameter is generally used for the diagnosis of myocardial injury. In the present there was a significant change in ECG patterns in DOX treated groups as compared to the normal group. DOX treated animals showed a significant alteration in the ECG parameters. The most interesting findings consisted of a dose-dependent reversible prolongation of the QRS complex and a progressive lengthening of the QT interval; changes in ST wave voltages and prolongation of P duration were more variable followed by decreased in heart rate. These changes may be due to the damage of myocardium (120). The oral pre-treatment EEIB showed a dose-dependent action and vitamin C also prevented the pathological alterations in ECG induced by DOX at a significance $p < 0.001$ (Figs. 3-8).



Fig. 3: Effect of vehicle on rats electrocardiography



Fig. 4: Effect of doxorubicin (15 mg/kg) on rats electrocardiography



Fig. 5: Effect of vitamin C (50 mg/kg) on rats electrocardiography

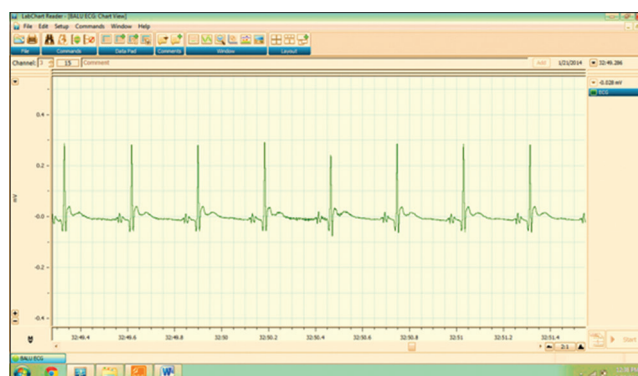


Fig. 6: Effect of ethanolic leaf extract of *Ipomoea batatas* (300 mg/kg) on rats electrocardiography



Fig. 7: Effect of ethanolic leaf extract of *Ipomoea batatas* (600 mg/kg) on rats electrocardiography



Fig. 8: Effect of ethanolic leaf extract of *Ipomoea batatas* (900 mg/kg) on rats electrocardiography

Microscopically studies

indicated that heart damage caused by DOX showed morphological changes in loss of integrity of myocardial membrane, myofibrillar structures with loss of striations and increased interstitial space finally lead to cardiomyopathy illustrated by enlarged, swollen mitochondria. The pretreated rats with EEIB (600 and 900 mg/kg) and vitamin C reduced significantly these changes when compared to toxic group. The 300 mg/kg extract did not reduce alterations induced by DOX.

Thus the EEIB have shown a significant antioxidant property. The free radical scavenging property may be the possible mechanism by the extract, protected the heart with comparison to the standard drug vitamin C against DOX and carbon tetrachloride induced cardio toxicity in rats (Fig. 9).

ACKNOWLEDGMENTS

We thank Dr. S. Mohan, Director and Principal, PES College of Pharmacy, Bengaluru, Karnataka, India, for providing this opportunity to carry out this research work.

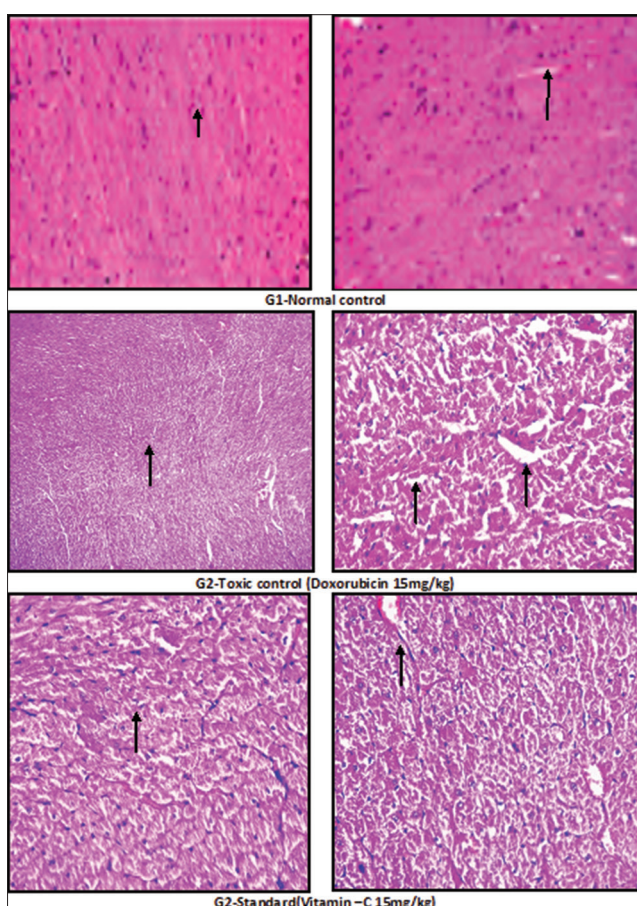


Fig. 9: Histopathological studies of rat's heart

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