Original Article

Cardioprotective Efficacy Of Eclipta Alba Methanolic Extract In Doxorubicin Induced Oxidative Cardiac Damage

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DOI: 10.47750/pnr.2023.14.03.335

Abstract

The goal of the current investigation was to assess the preclinical effectiveness of doxorubicin (DOX)induced cardiotoxicity and methanolic extract of Eclipta Alba (MEEA). Except for the control group, animals were separated into five groups at random and given either a vehicle (15 mg/kg, i.p.) or DOX (15 mg/kg, i.p.) or MEEA (200 and 400 mg/kg, p.o.) or conventional Digoxin for 14 days. On the 13th and 14th days of treatment, DOX was administered. DOXimpaired rats had a substantial (p0.05) rise in bloo d cardiac indicators (creatine kinase, lactate dehydrogenase, and cardiac troponinI), lipid peroxidation, and protein carbonyl conte nt, as well as a decrease in the levels of antioxidants (SOD, CAT, Gpx, and GSH) in the heart tissue. MEEA (200 and 400mg/kg, p.o.) treatment returned the abnormal biochemical level to normal. As a result, the study's findings indicate that MEEA demonstr atedPotential cardioprotective efficacy in DOXinduced heart injury, mediated by its membrane stabilising and antioxidant impact

Keywords: Doxorubicin, cardiotoxicity, oxidative stress, antioxidants, lipid peroxidation , Eclipta Alba

Introduction

A powerful chemotherapy drug extensively used to treat a variety of malignancies is doxorubicin (DOX). Treatment related cardiac symptoms from DOX include tachycardia, arrhythmia, hypotension, temporary reduction of left ventr icular function, and refractory lateonset cardiomyopathy [1,2]. . Meanwhile, cardiotoxicity is highly prevalent in patients receiving higher doses of DOX [3] . Further, clinical studies reports that when accumulative dose of DOX exceeds to 700mg /m^2, there is a chance of cardiac failure to nearly 50%incidence of heart failure will increase to 48% when the accumulation dose of DOX climbs to 700 [4] . Despite its efficacy, the clinical utility of DOX is limited due to its life threatening adverse effects.

A growing body of research indicates that a number of mechanisms, including oxidative stress, lipid peroxidation, ce ll death, and mitochondrial toxicity, are involved in the pathogenic mechanism of DOX-mediated cardiotoxicity. and [5,6]. Apart from the various mechanisms, oxidative stress orchestrates a cardinal role in DOX mediated myocardial injury.

In a nutshell, DOX on biotransformation results in semiquinone metabolite, which then results in superoxide anion. radicals and other highly reactive species like H_2O_2 and hydroxyl radicals and thus leads to mitochondrial damage and

cell death Thu and then induces mitochondrial dysfunction and cell injury [7,8].Therefore, the key strategy to lessen DOXinduced cardiotoxicity is to reduce oxidative stress. Due to its effectiv eness in treating numerous diseases and lack of side effects, plant nutrition for human use has recently attracted incr eased interest.

Eclipta Alba Linn. (Asteraceae) commonly referred as coat buttons is a short perennial herb having short and hairy blade like leaves usually found bushy areas and small forest. In folklore claim it has been used for the treatment for the various ailments like dysentery, malaria, diarrhea, hepatitis, hypertension and wound healing [9]. Previous studies have shown the anti diabetic and cardiovascular effects of Eclipta Alba inmurinemodels[10,11]Recent research has shown that Eclipta Alba possesses cardioprotective properties in isoprotere nolinduced myocardial infarction [12]. In light of these circumstances, the current investigation was conducted to as sess the effects of the methanolic extract of Eclipta Alba on DOX-induced oxidative cardiotoxicity.

Materials and Methods

Drugs and Chemicals

Sigma Chemical Co. in St. Louis, Missouri, USA, was where DOX was purchased.

Other necessary reagents were of the highest purity and analytical grade.

Plant material

The entire E. albaplant was procured from the numerous gardens and nurseries in Palvancha, Bhadradri district, Tela ngana, India. Dr. K. Madhava Chetty, an assistant professor at Sri Venkateswara University in Andhra Pradesh's Chi toor district, verified the authenticity of the plant that was obtained. In order to prepare the plant materials for use in the future, they were first dried in the shade, ground mechanically, and then placed in a closed container.

Preparation of extract

A simple maceration process using 1000 ml of methanol and 250 g of the powdered, shade-
dried E. albawa was used to extract the substance. Distillation was used to concentrate the extract to onefourth of its original volume. The concentrated extract was placed in a beaker and evaporated to a thick paste over a water bath t hat was kept at a temperature of about 50°C to obtain the final residue. Each yielded a final output of 12% w/w.

Phytochemical screening

The methanol extract of E. alba responded favourably to early tests for triterpenoids [13], flavonoids [14], steroids [15], tannins, saponins, and alkaloids [16], as well as the Shinoda test for flavonoids..

Animals

According to CPCSEA and the Institutional Animal Ethical Committee's requirements, all animal studies were carri ed out (IAEC). CPCSEA Regulation No. 1641/PO/E/S/14/CPCSEA.

The usual experimental methodologies and practises used in this biological evaluation are detailed below.

Acute toxicity studies

According to OECD guideline No. 425, albino mice were used in the acute toxicity experiments

.Doxorubicin induced cardiotoxicity

Male Wistar rats weighing 150 10 g were used for the investigation. Animals were fed commercially available stand ard rat pellet feed (M/s Pranav Agro Industries Ltd., India) marketed as Amrut rat/mice feed, and water was accessib le at all times. Prior to the experiment, the animals were given a 24hour fast from food but were given unrestricted a ccess to tap water. The rats were kept in climatecontrolled housing at a constant temperature of 25 degrees Celsius a nd acclimated to cycles of 12 hours of light and 12 hours of darkness.

Study Design

Five groups of six rats each were formed randomly from the experimental animals:

Group 1: For 14 days, 2% gum acacia suspension was administered to control rats.

(1 ml/kg body weight), taken orally for 14 days

Group 2: Rats were given 2% gum acacia suspension for 14 days, and on the 13th and 14th days, they were given D OX (15mg/kg b.wt; i.p).

Group 3: Rats were given digoxin for 14 days, and on the 13th and 14th days, they were given DOX (15mg/kg body weight; i.p.).

Group 4: Rats got DOX (15 mg/kg b.wt; i.p) on the 13th and 14th days, one hour after the extract was administered. Rats received 200 mg of methanolic extract of E. alba (MEEA) suspended in 2% gum acacia orally for 14 days.

Group 5: Rats got DOX (15 mg/kg b.wt; i.p) on the 13th and 14th days, one hour after the extract was administered. Rats received 400 mg of methanolic extract of E. alba (MEEA) suspended in 2% gum acacia orally for 14 days.

Following the last doses of extract and DOX, food access was restricted overnight, and on day 15, the animals under went anaesthesia with phenobarbital sodium (35 mg/kg; i.p.) before being decapitated and slaughtered. The serum w as isolated from the blood after it had been drawn from the jugular vein for the purpose of measuring cardiac marker enzymes. The cardiac tissue was removed, freed of adhering tissues, cleansed, and dried after being bathed in icecol d saline. Then, 100 mg of tissue was weighed, homogenised (10% w/v) in cold TrisHCl buffer, and utilised to exami ne various biochemical markers in DOX-induced heart damage.

Estimation of cardiac markers

Using commercial biochemicals kits from Pathozyme, India, the serum levels of creatinine kinaseMB (CKMB) and l actate dehydrogenase (LDH) were calculated. Additionally, using kits purchased from Life Technologies (India) Pvt . Ltd., the blood cardiac troponin (cTn) cTn-I was quantified by the ELISA method.

Measurement of Lipid peroxidation and protein carbonyl content

Using the Ohkawa et al. approach, malondialdehyde (MDA), a lipid peroxidation (LPO) marker, was detected spectr ophotometrically at 532 nm. Protein oxidation under DOXinduced myocardial oxidative stress was quantified by me asuring the protein carbonyl content. Using the method developed by Levine et al., the protein carbonyl content was calculated.

Measurement of antioxidants

According to the directions in the kit, which was acquired from Span Diagnostics Ltd, Gujarat, India, the cardiac lev el of the antioxidants catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione (GSH) were calculated.

Statistical analysis

The information was shown as mean standard error mean (SEM) Using SPSS version 18.0, one-way analysis of variance was used to analyse the data, and p 0.05 was regarded as statistically significant.

Results

Effect of MEEA administration on cardiac markers

Table 1 shows the impact of MEEA treatment on the levels of cardiac markers in rats that had been subjected to DO X injury. In this investigation, rats given DOX showed significantly (p0.005) increased blood levels of the cardiac m arkers CK-MB, LDH, and cardiac troponin I (cTn-I).

MEEA therapy, however, significantly (p0.05) returned the increased cardiac markers to baseline at doses of 200 an d 400mg/kg.

Effect of MEEA on cardiac lipid peroxidation and protein carbonyl levels

Rats given DOX showed a substantial (p0.05) increase in lipid peroxidation as shown by higher MDA content, a byp roduct of the LPO process, compared to the control group. In addition, when compared to rats that had consumed D OX, MEEA at doses of 200 and 400 mg/kg restored the elevated level of MDA to normal.

As a result of protein oxidation, the level of protein carbonyl in cardiac tissue was considerably (P 0.05) higher in th e DOXimpaired group than in the control group in this investigation. Intoxicated rats given 200 or 400 mg/kg of ME EA successfully reduced protein oxidation and returned the heart tissue of protein carbonyl to normal. Fig. 1 display ed the outcomes.Rats given DOX showed a substantial (p0.05) increase in lipid peroxidation as shown by higher M DA content, a byproduct of the LPO process, compared to the control group. In addition, when compared to rats that had consumed DOX, MEEA at doses of 200 and 400 mg/kg restored the elevated level of MDA to normal. As a res ult of protein oxidation, the level of protein carbonyl in cardiac tissue was considerably (P 0.05) higher in the DOXi mpaired group than in the control group in this investigation. Intoxicated rats given 200 or 400 mg/kg of MEEA suc cessfully reduced protein oxidation and returned the heart tissue of protein carbonyl to normal. Fig. 1 displayed the o utcomes.

Effect of MEEA on antioxidant status in DOX induced cardiac oxidative stress

We examined the amounts of antioxidants such SOD, CAT, GPx, and GSH in the heart tissue.

When compared to the control group, DOX inebriated were found to have significantly lower levels of SOD, CAT, GPx, and GSH (p 0.05). As DOX alone offended rats, MEEA administration at doses of 200 and 400mg/kg consider ably (p0.005) boosted the lowered level of antioxidants (Table 2).

Discussion

Even though Dox has a wide spectrum of chemotherapeutic effects, its clinical value is reduced because of dosemedi ated cumulative cardiotoxicity[17]. Clinically significant toxic consequences can include heart failure, cardiomyopat hy, and transitory electrocardiographic abnormalities [18]. The evidence is mounting that oxidative stress plays a crit ical role in the development of Dox cardiac damage [19]. The creation of an ironanthracycline complex, which relea ses free radicals and causes deformation of the plasma membrane and cytoskeleton of cardiomyocytes, is the primar y cause of Dox's cardiotoxicity mechanism [20]. Additionally, the heart is extremely vulnerable to Dox-induced oxidative injury due to a weak antioxidant defence cascade.

A recent review highlights herbal extracts displays effective cardioprotective effects against DOX-induced toxicity in preclinical models [21] Therefore, our study was designed to evaluate the cardioprotective potential of E.albaextracts against DOX induced cardiotoxicity.

Clinically, many diseases have been diagnosed by estimating the cytoplasmic enzymes which are leaked into the blood during oxidative cell membrane damage. Thus, elevated level of marker enzymes in serum is as a result of organ damage and serum marker enzymes in an indicative of tissue damage and distortion of membrane integrity. Creatine Kinase (CK-MB) and LDH are the heart specific biochemical enzymes used to measure the extent of cardiac damage during oxidative injury. During free radical attack, the membrane integrity of cardiac tissue is lost and causes the release of enzymes into the blood circulation [22] .Further, cardiac troponin, generally a protein is localized in the striated muscle of heart as thin filaments. It has three subunits such as Troponin T (cTn-T), Troponin I (cTn-I) and Troponin C. Meanwhile, cTn-I is a routinely used cardiac marker to measure the severity of myocardial damage [23] .In the present study, DOX intoxicated rats displayed significant elevation of CK-MB , LDH and cTn-T level which is in line with the previous reports [24]. Previous studies indicate that increased serum level of CK-MB and LDH elicit early and late cardiac injury [25]. Treatment with MEEA at the dose of 200 and 400mg/kg significantly decreased the serum level of cardiac markers to normal due to its cardiac membrane stabilizing action. The membrane stabilizing effect of T. procumbens has been reported in previous studies [26].

Polyunsatured fatty acids (PUFA) are the main chemical components of cytoplasmic membrane of all organs and tissues and it is prone to oxidative attack due to high amount of unsaturated bonds. Mounting reports suggests that DOX-induced cardiotoxicity involves the generation of highly reactive superoxide anions and hydroxyl radical which elicits the peroxidation lipid cell membrane [27,28]. Furthermore, DOX induced free radicals also involved in the protein oxidation, a key toxic mechanism during the oxidative tissue damage [29]. The final end product generated during this process is protein carbonyl content (PCC), an effective biochemical marker to measure the nature of protein oxidation [30]. In the present study, DOX treated rats displayed marked elevation of PCC in heart tissue as a result of oxidative stress which is in corroboration with the previous reports [31] .However, treatment with MEEA at the dose of 200 and 400mg/kg to DOX intoxicated rats significantly reduced the lipid peroxidation and protein carbonylation. The anti lipid peroxidative effect of E.albamight be due to the presence of various phytoconstituents like flavanoids, steroids and triterpenoids [32]

Mounting studies indicate that DOX mediated cardiac damage is associated with rampant generation of toxic free radicals in cardiomyocytes [33] .Cells encompass a network of antioxidants like GSH, Gpx, SOD and CAT to scavenge the free radicals during oxidative cellular damage and thus increase the cell survival. The primary antioxidant SOD, catalyses the reduction of superoxide radical (O_2^-) to hydrogen peroxide, whilst GSH , GPx and CAT are involved in the catalytic reduction of hydrogen peroxide to oxygen and water. Can catalyze the reduction of hydrogen peroxide and other peroxides, and SOD can catalytically reduce O_2^- to hydrogen peroxide [34]. In the present study, DOX intoxicated rats displayed decreased level of antioxidant like GSH, SOD, CAT and Gpx and treatment with MEEA at the dose of 200 and 400mg/kg significantly increased the antioxidants level to normal.

Previous studies reports that centaureidin and procumbenetin are the flavanoids identified in T. procumbens [35,36] .Thus, the cardioprotective potential of T. procumbens might be due to the presence of flavanoids. It has been shown that flavanoids has the capacity to scavenge the semiquinone radical and terminate the peroxidation of lipids produced by DOX which is highly attributed due to the presence of hydroxyl group [37].

Conclusion

On the basis of our findings, methanolic extract of E.alba may improve the DOX induced cardiotoxicity by regulating the cardiac marker enzymes, inhibition of lipid peroxidation, improving the status of antioxidants. Further, molecular mechanism studies are warranted to delineate the cardioprotective property of E.alba

Ethical Clearance Number: CPCSEA Reg. No: 1641/PO/E/S/14/CPCSEA

Source of Funding: None

Conflict of interest: None to declare

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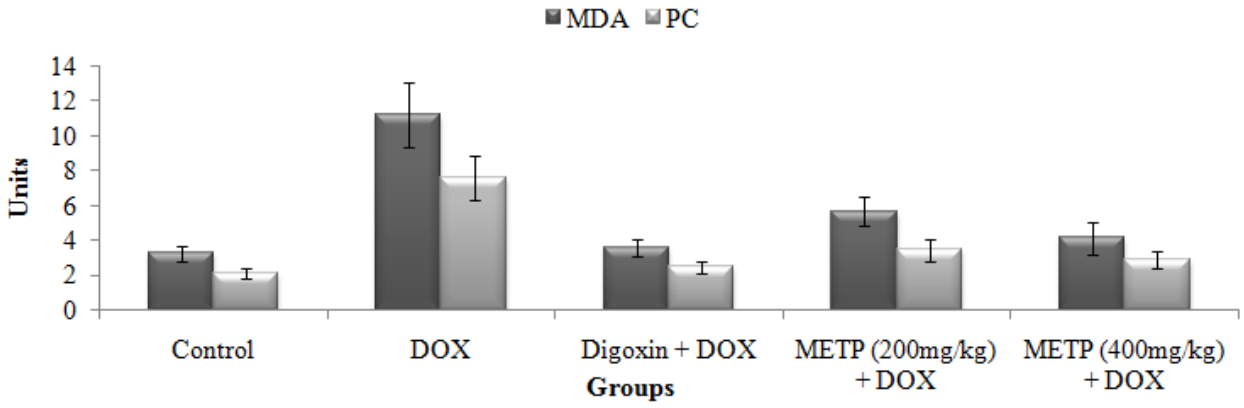
Table 1: Effect of MEEA and DOX on serum cardiac markers

Groups	LDH (IU/L)	CK-MB (IU/L)	cTnI (µg/ml)
Control	146.43 ± 6.52	106.65±5.12	1.12±0.32
DOX	556.76 ± 9.45 ^{a*}	434.28±9.85 ^{a*}	3.87±0.48^{a*}
Digoxin + DOX	150.67±4.87 ^{b*}	110.54±4.65 ^{b*}	1.32±0.32 ^{b*}
MEEA (200mg/kg) + DOX	221.65±6.76 ^{b*}	132.32±5.12 ^{b*}	1.87±0.41 ^{b*}
MEEA (400mg/kg) + DOX	186.42±4.56 ^{b*}	115.92± 6.56 ^{b*}	1.45±0.38 ^{b*}

The values were expressed as mean ± SEM (n=6). Analyses were done by one way analysis of variance (ANOVA) with Tukey's post-hoc test comparison procedure ^{a*} $p<0.05$, compared to control; ^{b*} $p<0.05$, compared to Cd. LDH: Lactate Dehydrogenase; CK-MB: Creatine kinase; cTnI: Cardiac Troponin T.



MDA –Malondialdehyde; PC- Protein carbonyl content. Units : MDA- nmole/mg tissue; PC-µmoles/mg protein.

Fig 1: Effect of MEEA on lipid peroxidation and protein carbonyl content in heart tissue. DOX induced oxidative stress caused marked increase in MDA level and protein carbonyl content in cardiac tissue. DOPET treatment effectively reduced the increased lipid peroxidation and protein carbonyl levels. The results were shown as mean ± SEM (n = 6). ^{a*} $p<0.05$, compared to Control; ^{b*} $p<0.05$, compared to DOX.

Table 2: Effect of MEEA and DOX on antioxidants levels in heart homogenate

Groups	SOD	CAT	GPx	GSH
Control	11.52±0.78	7.24±0.41	19.26±1.35	15.75±1.65
DOX	5.35±0.52 ^{a*}	3.24±0.32 ^{a*}	12.45±1.35^{a*}	6.85±0.76 ^{a*}
Digoxin + DOX	10.86±0.87 ^{b*}	7.12±0.42 ^{b*}	18.65±1.45 ^{b*}	14.58±1.34 ^{b*}
MEEA (200mg/kg) + DOX	8.24±0.76 ^{b*}	5.32±0.45 ^{b*}	16.87±1.76 ^{b*}	10.76±0.98 ^{b*}

MEEA (400mg/kg) + DOX	9.87±0.82 [b*]	6.56±0.52 [b*]	17.26±1.45 [b*]	12.12±1.34 [b*]

The values were expressed as mean ± SEM (n=6). Analyses were done by one way analysis of variance (ANOVA) with Tukey's post-hoc test comparison procedure. [a*] $p<0.05$, compared to Control; [b*] $p<0.05$, compared to DOX. Units-SOD: U/mg protein; CAT: µmoles/H_2O_2/min/mg protein; GPx: µmoles NADPH oxidized /min/mg protein; GSH: nmol/mg protein.