Research Article

Evaluation of cardioprotective effect of ethanolic extract of *Abelmoshcus esculentus* on Doxorubicin induced cardiotoxicity in rats

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Abstract

Objective: The present study was taken up to evaluate the possible protective effect of ethanolic extract of *Abelmoschus esculentus*. L. against doxorubicin (DOX) induced cardiotoxicity in rats. **Material and methods:** In this experiment, 30 Albino Wistar rats (250 g) were divided into five groups (n = 6). Control group received distilled water for 10 days. Dox treated group received vehicle for 10 days. The remaining three groups received vitamin C and ethanolic extract of *Abelmoschus esculentus* (100 and 200 mg/kg, *p.o.*) for 10 days. Cardiotoxicity was induced by administration of single dose of DOX (10mg/kg *i.p.*) on 7th day of study. Various biochemical parameters are estimated in serum and heart tissue which includes Creatinine kinase (CK-MB), lactate dehydrogenase (LDH), reduced glutathione (GSH), Super oxide dismutase (SOD) and catalase (CAT) and along with histopathological studies. **Results and conclusion:** DOX treated rats showed a significant increase in myocardial tissue damage markers such as Creatinine kinase (CK-MB), Lactate dehydrogenase (LDH) and significant declines in the levels of reduced glutathione (GSH), Super oxide dismutase (SOD) and catalase (CAT). All biochemical changes which are brought to normal after oral administration of ethanolic extract of *Abelmoschus esculentus* at doses 100 and 200mg/kg, *p.o* for 10 days. Moreover, in this study, we have found that oral administration of *Abelmoschus esculentus* prevented DOX-induced cardiotoxicity by accelerating heart antioxidant defence mechanisms and membrane stabilizing effect.

Keywords: *Abelmoschus esculentus* extract, Antioxidants, Cardiotoxicity, Doxorubicin, Histopathology, Myocardial injury markers

Introduction

Doxorubicin is a potent broad-spectrum chemotherapeutic agent that is highly effective in treating patients with acute lymphoblastic leukaemia, Hodgkin lymphoma, aggressive non-Hodgkin lymphomas, breast carcinoma, ovarian carcinoma and many solid tumours (Wang et al., 2014). However, the clinical use of this drug has been seriously limited by undesirable side effects especially dose-dependent myocardial injury, leading to potentially lethal congestive heart failure (Mustafa et al., 2015). Due to the great importance of DOX in chemotherapy for the treatment of many types of cancer, researchers have exerted great efforts to attenuate the side effects of DOX. In view of this several strategies have been followed for dosage optimization and use of analogues or combined therapy but no promising results have been found (Smith et al., 2010; Das et al., 2011). The use of several DOX analogues available clinically did not show stronger antitumor efficacy as compared to DOX (Yang et al., 2014). Antitumor action of DOX is mediated by a wide number of mechanisms but one of the activities, i.e., generation of the free radicals, is among the main causes of cardiotoxicity. This fact allows the researchers to develop strategies to reduce the toxic effects of DOX without interfering with its antitumor properties.

Herbal extracts have many properties like antioxidant, anti-allergic, anti-inflammatory, antiviral, anti- proliferative and anti-carcinogenicity (Wallace, 2003). Natural antioxidants, which are capable of protecting the cells from oxidative injury, should be included in the potential antioxidant therapy. Therefore, there is a need for identifying alternative, natural and safer sources of antioxidants (Yeh et al., 2004).

Abelmoschus esculentus contain roots, leaves, fruits and seeds

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contain mucilage. Leaves, flower petals and fruit husk contain β-sitosterol and its glycosides. Flowers contain flavonoids, myricetin, its glucoside and cannabis sand petals contain myricetin and its glucoside. Seeds contain phospholipids and essential oil. Seed oil contains 18.90% of linoleic acid.

Materials and methods

Chemicals and drugs

Doxorubicin was procured from Dabur Pharmaceuticals Ltd., New Delhi, India. Vitamin E was procured from Merck Ltd., India. LDH, CK and AST assay kit were purchased from Span Diagnostics Ltd., Surat, India. All other chemicals used during the study were of analytical grade.

Plant material

The fresh fruits of *Abelmoschus esculentus* were collected from the local areas of Anantapur, Andhra Pradesh, India. The plant was identified and authenticated by Dr. J. Ravindra Reddy, Professor, Department of Pharmacognosy and Phytochemistry, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur, where the voucher specimen was deposited for further reference.

Preparation of plant extract

The fruits of *Abelmoschus esculentus* were washed thoroughly in water to remove foreign matter and allowed to shade dry with a relative humidity of 40-45%. Then, the leaves were powdered in roller grinder and passed through a sieve (No.40). About 300 g of dried fruit powder was mixed with 900 ml of ethanol as drug to the solvent ratio is 1:3 and the maceration process was continued for 72 hrs for the extraction. The ethanolic extract was filtered and concentrated to dry mass. A greenish- brown extract was found to be 24.37%w/w.

Phytochemical Screening

The preliminary phytochemical screening was performed with the ethanolic extract of *Abelmoschus esculentus* for the detection of various phytochemicals (Khandelwal et al., 2000).

Experimental animals

Albino Wistar rats (200-250 g body weight) were used for this study. They were housed at ambient temperature (22±10°C), relative humidity (55± 5%) and 12 h/12 h light dark cycle animals had free access to Amrut brand rat pellet diet supplied by (VRK Nutrition solution Maharashtra) and water given ad libitum. The Institutional Animal Ethical Committee (878/ac/05/CPCSEA/003/2017) as approved the experimental protocol at Post Graduate Department of pharmacology, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur., Andhra Pradesh, India.

Acute toxicity studies

Healthy adult male *albino* rats were fasted overnight prior to the experiment. Different doses (50-2000 mg/kg, P.O) of the ethanolic extract of *Abelmoschus esculentus* were administered to each group of rats (Each group carries 8 rats) and they were observed continuously for 1 hour and then at half-hourly intervals for 4 hours, for any gross behavioral changes and further up to 72 hours, followed 14 days for any mortality as per the OECD (Organization for Economic Co-operation and Development) Guideline 425. The fruit extract of *Abelmoschus esculentus* was found to be non-toxic up to the maximum dose of 2000 mg/kg body weight. Dose selected for cardioprotective activity was 100, 200 and 600 mg/kg respectively (OECD et al., 2001).

Experimental protocol

A total of 30 animals are allocated in to five groups and each group consists of six animals. The following groups received the respective samples for the period of 10 days.

Group I: Rats in this group were given distilled water 1 ml/200 g body weight /*p.o.*/day

Group II: Rats in this group were given 10mg/kg of *Doxorubicin* by *i.p.* on the seventh day of the experiment (Gustav Holmgren et al., 2015).

Group III: Rats in this group were treated with 140mg/kg of standard i.e.-Vitamin-C for 10 days through oral administration (Vucskits et al., 2010). On 7th day rats injected with *doxorubicin* at the dose of 10 mg/kg *i.p.*

Group IV: Rats in this group were treated with ethanolic extract of *Abelmoschus esculentus* at dose of 100 mg/kg/day given orally. On 7th day rats injected with doxorubicin at the dose of 10mg/kg *i.p*.

Group V: Rats in this group were treated with ethanolic extract of *Abelmoschus esculentus* at a dose of 200 mg/kg orally and on the 7th day rats were injected with *doxorubicin* at the dose of 10mg/kg *i.p.*

On day 11, blood was withdrawn under light ether anesthesia by retro orbital puncture in Eppendorf tubes and allowed to clot. The clotted blood was centrifuged at 3000 rpm for 15 min. Carefully taken out the serum and kept at -80°C up to further usage for biochemical analyses. After phlebotomy, animals were sacrificed and hearts were immediately isolated, washed with ice-cold phosphate-buffered saline (pH = 7.4), blotted, and subjected to freezing at -80°C until further use. A small section of the myocardial tissue from each group was fixed in 10% neutral buffered formalin for histopathological examination.

Biochemical determinations

Serum parameters

The serum samples were analysed for determination of

levels of CK-MB, LDH using standard kit according to manufactures instructions using semi auto analyser (Mispa Plus).

Antioxidant Parameters

Animals were euthanized and hearts tissue were quickly dissected out and washed in ice cold phosphate buffer, dried on filter paper and quickly weighed. A 10% w/v tissue homogenate is prepared in ice cold 0.05 M phosphate buffer using tissue homogenizer. The chilled tissue homogenate was used for estimation of level of MDA, SOD, CAT, Glutathione, and total protein.

Measurement of Superoxide Dismutase

SOD activity was measured by determining the ability of sample to inhibit autooxidation of pyrogallol by Improved Pyrogallol Autoxidation Method. One unit of SOD activity is defined as amount of enzyme required to inhibit the rate of pyrogallol autooxidation by 50%. At pH 7.4, 50 µL of sample solution was mixed with 2950 µL of Tris-HCl buffer (0.05 M, pH 7.4, 37 °C) containing 1 mM Na_2EDTA and 50 µL of pyrogallol (60 mM in 1 mM HCl, 37°C) and then rapidly shaken by hand at 37 °C. The absorbance was measured against the Tris-HCl buffer every 30 s for 5 min at 325 nm using UV visible double beam spectrophotometer (Jasco V550). The oxygen radical scavenging ability was calculated as {ΔA (control)- ΔA (test)} ΔA (control) × 100 T Here, ΔA (control) is the increase in Absorbance at 325 nm of the mixture without the sample and ΔA (sample) is that for the mixture with the sample; T = 5 min (Misra et al., 1972).

Measurement of catalase activity

Catalase enzyme degrades hydrogen peroxide (H2O2) into oxygen and water. Ultraviolet absorption of H2O2 can be measured at 240 nm. In the presence of catalase, absorption decreases due to degradation of H2O2. 0.1 ml of tissue homogenate is mixed with 1.0 ml freshly prepared hydrogen peroxide and 1.9 ml phosphate buffer into cuvette. Blank were similarly prepared without tissue homogenate. Absorption of test was measured at 240 nm against blank, by using UV-visible double beam spectrophotometer (Jasco V- 550). For at least 3 min at 240 nm. Activity of CAT was expressed in unit/mg of protein and calculated using molar extinction coefficient 43.6 M^{-1} cm^{-1} (Xican, 2012).

Measurement of glutathione activity

GSH reacts with Ellman's reagent (5, 5-dithio bis Nitrobenzoic acid or DTNB) to produce a chromophore Thio nitrobenzoic acid (TNB) that give maximal absorbance at 412 nm. Absorbance value can give the estimation of enzyme value. 1 ml of 10% tissue homogenate is mixed with 1 ml 20% trichloroacetic acid (TCA) containing 1mM EDTA and mixture is centrifuged for 10 min at speed 1000 rpm. In 1 ml supernatant 0.5 ml DTNB solution and 3 ml Phosphate Buffer were added. In 0.2 ml of supernatant is added to new set of test tubes containing 1.8 ml of Elman's reagent (0.1 mM DTNB (5, 5'-dithio bis-2-nitrobenzoic acid) prepared in 0.3 M Phosphate buffer containing sodium citrate). Mixed well and absorbance was measured the at 412 nm using UV visible double beam spectrophotometer (Jasco V- 550) against blank the amount of glutathione is Calculate using the molar extinction coefficient 13600 M^{-1} cm^{-1} (Aebi, 1974).

Histopathological examination of heart

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

Statistical analysis

Data were expressed as the Standard Error Mean (SEM). For a statistical analysis of the data, group means were compared by one-way analysis of (ANOVA) with *post-hoc* analysis. The Turkey-Kramer *post-hoc* test was applied to identify significance among groups; $p < 0.05$ was considered to be statistically significant.

Results

Preliminary phytochemical screening

The *Abelmoschus esculentus* showed the presence of flavonoids, alkaloids, terpenoids, tannins, saponins, carbohydrates and amino acids.

Effect of ethanolic extract of *Abelmoschus esculentus* on myocardial marker enzymes

The effect of AE on serum levels of CK-MB and LDH are summarized in figures 1 and 2 respectively. A significant increase in serum levels of CK-MB and LDH are observed in doxorubicin treated groups as compared to normal group ($p < 0.0001$). AE treated groups at both doses (100 and 200 mg/kg) showed a significant decrease in the level of serum CK-MB ($p<0.05$ and $p<0.001$) and LDH ($p<0.01$ and $p<0.001$) are observed as compared with DOX alone treated rats.

Effect of ethanolic extract of *Abelmoschus esculentus* on superoxide dismutase, catalase and reduced glutathione activities

Cardio toxicity induced by doxorubicin shows (Figure 3, 4 & 5) evidence of a significant decrease of reduced glutathione ($p<0.001$), superoxide dismutase ($p<0.0001$) and catalase ($p<0.01$) content as compared to the normal rats. AE counteracted the deleterious effect of doxorubicin by significant increasing the levels of SOD ($p<0.05$ and $p<0.01$), GSH ($p<0.05$ and $p<0.01$) and catalase (NS and

p<0.01). AE (200 mg/kg) showed maximum protection against the antioxidant parameters.

Effect of ethanolic extract of *Abelmoschus esculentus* on histopathological changes in the rat myocardium

Histopathological observations are shown in figure 6. Hematoxylin and eosin-stained sections of rat heart, which were examined under high power (400x) of light microscope. Figure 6A; represents normal control rat showing normal myocardial fibers, no vacuolation, necrosis or inflammation. 6B; represents DOX alone treated rats, showing a large and irregularly shaped hypertrophic myocardial fiber with other fibers in the vicinity showing small and large vacuoles. 6C; represents vitamin E (100 mg/kg *p.o.*) + DOX treated rats, showing cardiac muscle fibers of normal shape, size and configuration. A single vacuole is seen in one of the myocardial fibers. (6D) represents *Abelmoschus esculentus* (100 mg/kg *p.o.*) + DOX treated rats, showing scattered vacuoles in the myocardial fibers. (6E) represents *Abelmoschus esculentus* (200 mg/kg *p.o.*) + DOX treated rats, showing cardiac muscle fibers of normal shape, size and configuration. A single myocardial fiber with an intracytoplasmic vacuole is seen in the photograph.

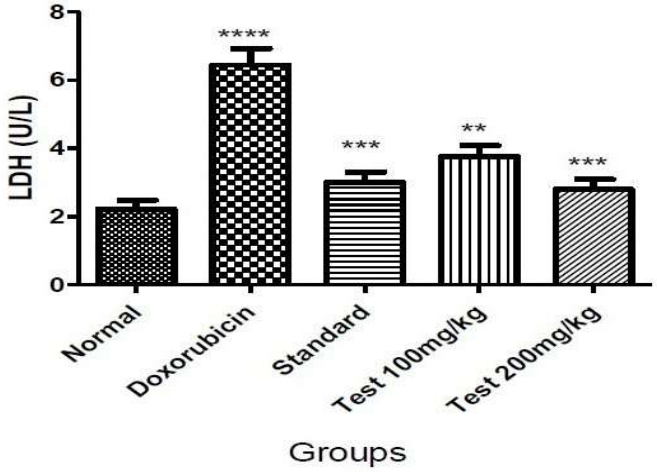

Figure 1. Effect of AE (100 and 200 mg/kg) on serum LDH level of DOX induced cardio toxicity in rats. The data are expressed as the mean± SEM (n = 6). P < 0.0001 is indicated by ****, p < 0.001 is indicated by ***, p < 0.01 indicated by **, p < 0.05 is indicated by *.

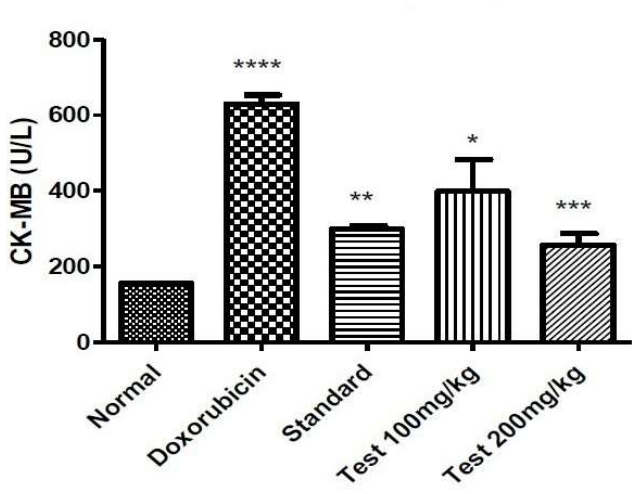

Figure 2. Effect of AE (100 and 200 mg/kg) on serum CK-MB level of DOX induced cardio toxicity in rats. The data are expressed as the mean± SEM (n = 6). P < 0.0001 is indicated by ****, p < 0.001 is indicated by ***, p < 0.01 indicated by **, p < 0.05 is indicated by *.

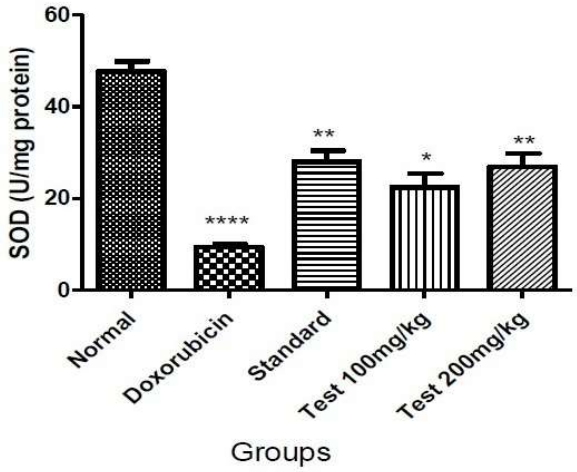

Figure 3. Effect of AE (100 and 200 mg/kg) on tissue levels of superoxide dismutase of DOX induced cardio toxicity in rats. The data are expressed as the mean± SEM (n = 6). P < 0.0001 is indicated by ****, p < 0.001 is indicated by ***, p < 0.01 indicated by **, p < 0.05 is indicated by *.

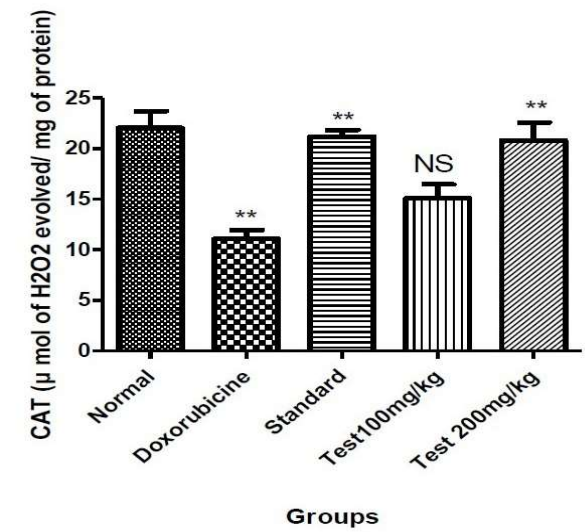

Figure 4. Effect of AE (100 and 200 mg/kg) on tissue levels of Catalase of DOX induced cardio toxicity in rats. The data are expressed as the mean± SEM (n = 6). P < 0.0001 is indicated by ****, p < 0.001 is indicated by ***, p < 0.01 indicated by **, p < 0.05 is indicated by *.

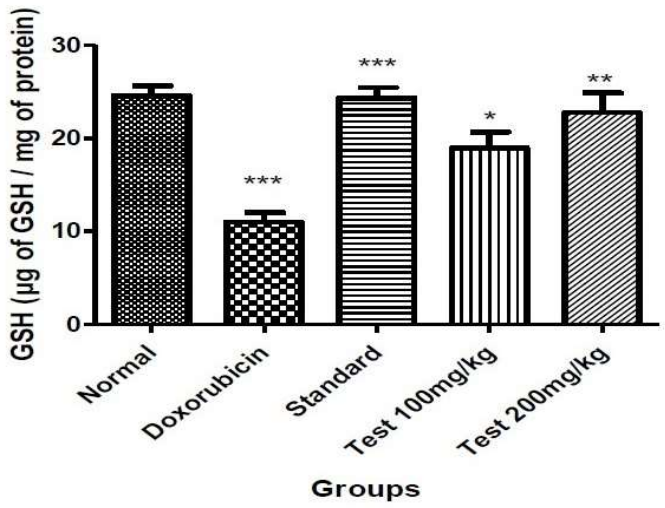

Figure 5. Effect of AE (100 and 200 mg/kg) on tissue levels of Reduced glutathione of DOX induced cardio toxicity in rats. The data are expressed as the mean± SEM (n = 6). P < 0.0001 is indicated by ****, p < 0.001 is indicated by ***, p < 0.01 indicated by **, p < 0.05 is indicated by *.

Discussion

The present study was undertaken to evaluate of cardio protective activity of ethanolic extract of *Abelmoschus esculentus* fruits against doxorubicin induced cardiotoxicity. Doxorubicin generates free radicals through semiquinone intermediates in an iron-dependent reaction. These reactive oxygen species (ROS) interact with proteins, lipids of cell membrane and nucleic acids of heart muscle. All these events cause cellular apoptosis (Billingham et al., 1978; Davies et al., 1986). Cardio specific enzymes are generally present in very low levels in serum. When the membrane disrupts theses enzymes like CK-MB and LDH leak out in to the plasma and rise in appreciable quantities, which gives information about the extent of myocardial damage due to lipid peroxidation. Results of the present study significantly demonstrated rise in the serum levels of LDH and CK-MB after doxorubicin administration (Kumar et al., 2000). Treatment with *Abelmoschus esculentus* significantly reduced the serum levels of LDH and CK-MB. It is hypothesized that decreased permeability of membrane as a result of protection from

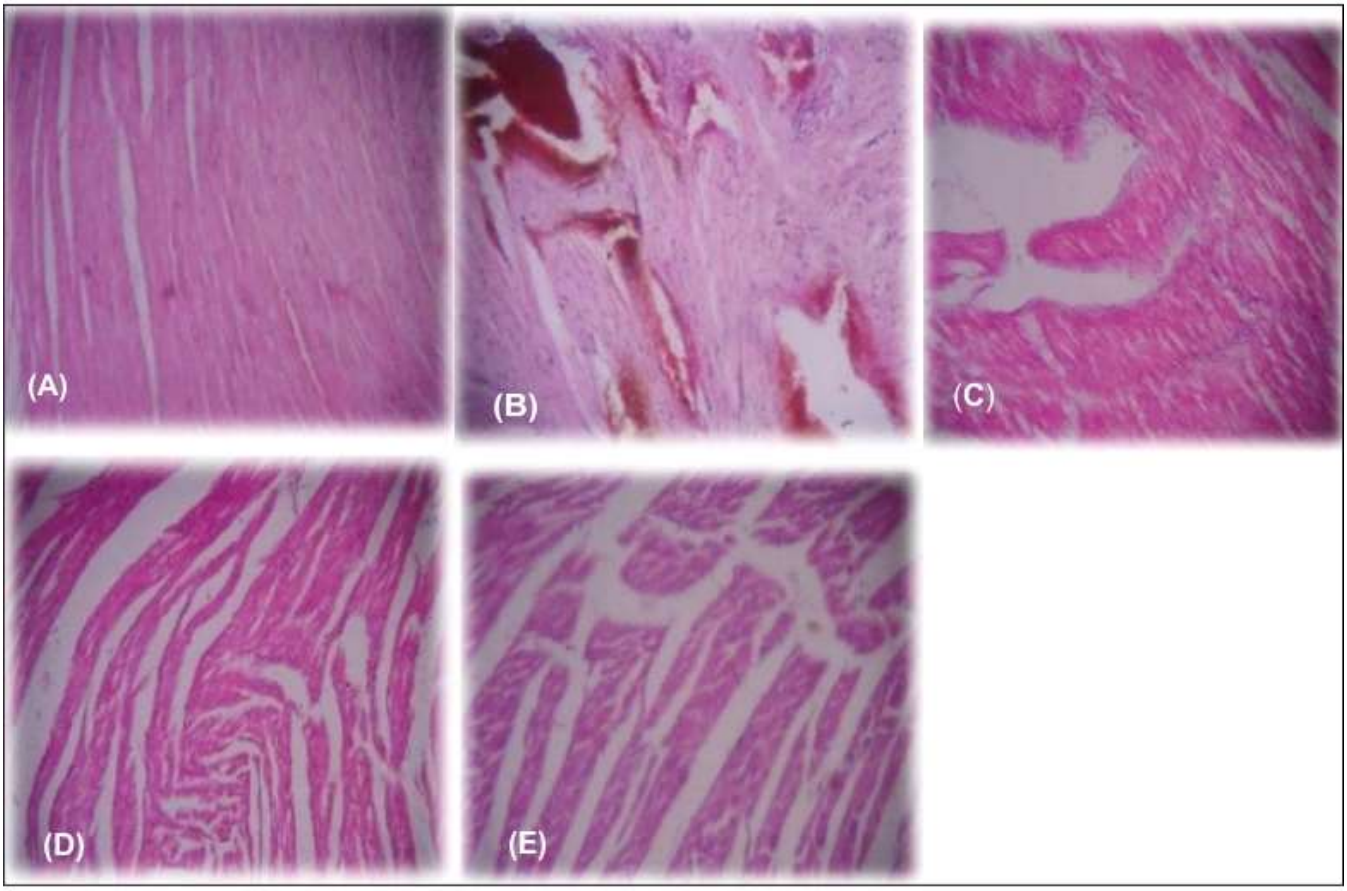

Figure 6. Histopathological changes in the rat myocardium: (A) Normal control rat; (B) DOX alone treated rats; (C) Vitamin E (100 mg/kg *p.o.*) + DOX treated rats; (D) *Abelmoschus esculentus* (100 mg/kg *p.o.*) + DOX treated rats; (E) *Abelmoschus esculentus* (200 mg/kg *p.o.*) + DOX treated rats

oxidative damage was responsible for this effect. Endogenous antioxidant system efficiently neutralizes reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical, hydrogen peroxide, and nitric oxide. When balance between ROS and antioxidant is disrupted, ROS accumulate and produce various pathological changes in body tissues which ultimately contribute as causative factors for many diseases as in the case of cardiovascular diseases (Preus et al., 1988; Thompson et al., 1986).

Doxorubicin enhances free radical formation and decrease antioxidant enzymes due to overwhelming consumption of antioxidants which can lead to cardiac failure (Ayaz et al., 2005). Similar findings were observed in the present study where decreased levels of SOD, CAT, GSH were observed in the cardiac tissue following doxorubicin administration. After the treatment with the ethanolic extract of *Abelmoschus esculentus* (100 and 200 mg /kg b.w), the cellular antioxidant levels were normalized significantly when compared to doxorubicin administered rats. These findings suggest that the ethanolic extract of *Abelmoschus esculentus* protects the myocardium due to its antioxidant activity. As seen in the present study, DOX treatment caused significant histological changes including marked infiltration of inflammatory cells, myofibril loss and cytoplasmic vacuolization (Sivakumar et al., 2012). In rat treated with AE, these DOX-induced histological changes were minimal, suggesting protection from cellular damage.

Conclusion

In the present study, we concluded that the ethanolic extract of *Abelmoschus esculentus* (200mg/kg) is proven to have a cardioprotective effect in doxorubicin induced cardiotoxicity. This study suggests a possible usefulness of *Abelmoschus esculentus* as a cardioprotective agent contributing to a safer use of doxorubicin patient subjected to chemotherapy. However, cardioprotective effect was observed more in the higher dose of prove the efficacy of *Abelmoschus esculentus*. Further dose response and bioavailability studies are needed to *Abelmoschus esculentus* as a cardioprotective drug.

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Conflicts of interest: Not declared.

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