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Research Article
Aqueous Extract of *Andrographis paniculata* Ameliorates Cardiotoxicity Induced by Doxorubicin *in vivo*

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Abstract

Background and Objective: Cardiomyopathy is one of the life-threatening complications of doxorubicin chemotherapy. Doxorubicin is one of the chemotherapeutic agents used to treat cancer. The drug is effective against different types of cancers. However, the clinical application of the drug is limited due to its side effects on almost all tissues and organs. Cardiotoxicity is one of the life-threatening side-effects of doxorubicin. This study aimed to reduce the toxicity of the drug by supplementing the aqueous extract of *Andrographis paniculata*. **Materials and Methods:** In the present study, we used zebrafish (*Danio rerio)* as a model organism. Fishes were administered with Doxorubicin and supplemented with the aqueous extract to study the effect of the supplement. **Results:** The supplement successfully reduced Reactive Oxygen Species (ROS) by improving the activity of antioxidant enzymes such as Superoxide Dismutases (SOD), glutathione and catalase. Cellular damage induced by the drug was also reduced to a greater extent as indicated by lactate dehydrogenase assay and creatinine kinase activity. Histological sections of the heart treated with doxorubicin and *A. paniculata* also revealed the improvement in the micro-architecture and reduced inflammation as indicated by impaired neutrophil infiltration. **Conclusion:** Overall, it is apparent that *A. paniculata* is a cardioprotective agent against doxorubicin-induced cardiomyopathy.

Key words: Zebrafish, doxorubicin, cardiomyopathy *Andrographis paniculata*, ROS, SOD, GSH

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Doxorubicin belongs to the anthracycline group of antibiotics which is widely known for its anti-neoplastic potential. It is effective against a wide range of cancers from solid tumours to leukaemias. The drug strongly binds to intercalate with DNA and causes DNA strand breaks. Doxorubicin also inhibits topoisomerase II and thereby induces DNA damage which culminates in apoptosis. Despite its beneficial effects, the drug's clinical application is limited due to its adverse side effects. The side effects caused by the chemotherapeutic agent include mild symptoms like nausea vomiting to life-threatening cardiomyopathy[1]. However, the mechanism of doxorubicin-induced cardiomyopathy remains elusive.

The toxicity induced by doxorubicin may be accounted for or several mechanisms[2]. Impaired calcium signaling[3], altered iron signaling[4], reduced cardiac repair[5], induction of apoptosis[6], oxidative stress[7] are some of the mechanisms of Doxorubicin-induced cardiomyopathy. The drug has been shown to induced type 2 diabetes-like condition[8]. Many types of extracts have been attempted to reduce the toxicity induced by the chemotherapeutic agent.

Andrographis paniculata is one of the plants having rich bioactive compounds[9]. The plant has several phytoconstituents including flavonoids terpenoids phenolic acids etc. The plant extract has been shown to process antidiabetic, anti-inflammatory, analgesic, anti-viral and cardioprotective activities. Therefore, this study aimed to evaluate the protective effect of *A. paniculata* on doxorubicin-induced cardiotoxicity. Zebrafish used as a model system to evaluate the effect of the extract on audio toxicity induced by doxorubicin.

MATERIALS AND METHODS

Study Area: The study was carried out at the Department of Cardiology, Qingdao Municipal Hospital, China from December, 2020-April, 2021.

Collection and maintenance of animals: A total of 72 fishes were used for the research. Zebrafish (*Danio rerio*), were purchased from the aquarium. They were acclimatized under laboratory conditions in 35 L glass aquaria containing dechlorinated water, aerated continuously through stone diffusers connected to a mechanical air compressor. The zebrafish is a hardy fish and can withstand a pH ranging between 7.2 and 7.5. The temperature of the water was maintained at 26±1°C throughout the study. During the acclimation period, the adult fish was fed twice per day with readily available commercial fish food. The care and husbandry of the zebrafish used in this study conformed with the guidelines that regulate the care and use of laboratory animals by humans for research purposes. Fishes were treated with the extract of dried leaves of *Andrographis paniculata* in 0.01% ethanol (Final concentration).

Preparation of plant extract: A total of 10 g of the plant powder (*A. paniculata*) was dissolved in 10 mL of Sterile Milli Q water, boiled in the water bath at 100°C for 1hr. The boiled extract was cooled and centrifuged at 5000 rpm for 10 min, the pellet was discarded and the supernatant was stored at 4°C for further assays.

Determination of LC50c: Zebra fishes of interest were weighed and separated in various tanks for the study. To optimize the concentration of extract of *A. paniculata* 2-10 µg mL^{-1} and then 10-50 µg mL^{-1} was given. Finally, the doses tried as 20, 40, 60, 80 and 100 µg mL^{-1}. Finally, the dosages calculated for 2 L to analyse the acute effects. Also, control fishes maintained in a separate tank. The fishes were observed under treatment for 7 days to check the dose effect.

Treatment with doxorubicin: After fixing the safe doses of the *A. paniculata*, the fishes are injected with doxorubicin and again treated with the fixed doses of the *A. paniculata*.

Dissection of fishes for tissues: Each fish was slowly anaesthetized by adding ice chips into the fish water until the temperature reaches 12°C. When the fish remains still to any external stimuli then those were taken for dissection. The heart removed carefully and stored in 10% formalin for histopathology and the remaining tissues used for the other enzyme assay.

Catalase assay: Catalase activity was assayed by the method of Moustafa *et al.*[10]. The tissues were ground using liquid nitrogen. The stable complex of hydrogen peroxide and ammonium moly date was formed resulting in a yellow compound that has an absorption maxima at 405 nm. The calculated catalase activity is expressed in terms of KUL^{-1}.

Estimation of reduced glutathione (GSH): Estimation of reduced glutathione was done according to Aldubayan *et al.*[11]. Tissues were homogenized using 0.5 M potassium phosphate buffer and were deproteinated using

4% sulfosalicylic acid. The assay is based on the reaction between reduced glutathione and Dithiobis 2-Nitrobenzoic acid which produces a yellow colour with absorption maxima of 412 nm. The concentration of reduced glutathione is expressed in terms of mM mL^{-1}.

Estimation of lactate dehydrogenase(LDH): The fish tissue was homogenized using liquid nitrogen. The reaction mixture contains 100 mM Potassium Phosphate (pH 7.5),100 mM sodium pyruvate and 13.1 mM NADH disodium trihydrate salt in the ratio of 28:1.2:0.8. A coloured compound is formed when the tissue homogenate is added. A spectrophotometer (Changsasamy instruments and Equipment Co. Ltd, Changsha city, Hunan, China) reading at 340 nm at a different time of incubation gives the increase or decrease in the activity of LDH. The activity is expressed in terms of KUL^{-1}.

Superoxide radical scavenging assay (SOD): Superoxide dismutase is known to be one of the prominent superoxide scavengers. The superoxide scavenging ability of the fish tissues is assayed by its ability to reduce Nitro Blue Tetrazolium (NBT) to give a blue colour compound with absorption maxima of 560 nm as specified by Saggu *et al.*[12]. The superoxide scavenging ability is given in terms of the percentage of product formation.

Estimation of creatinine kinase (CK): Creatinine kinase estimation was performed according to Valvassori *et al.*[13]. Creatinine phosphate and ADP in the presence of Creatinine kinase could give rise to creatinine and Adenosine Triphosphate (ATP). The ATP formed in this reaction was used in another reaction with Glucose 6 Phosphate dehydrogenase to give a coloured compound that can be measured using a spectrophotometer (Changsasamy instruments and Equipment Co. Ltd, Changsha city, Hunan, China) at 340 nm. This absorbance is directly proportional to the amount of CK present in the tissues. The concentration is expressed in terms of UL^{-1}.

Estimation of nitric oxide (NO): The amount of Nitric oxide in the fish tissues are estimated using the method of Tarpey *et al.*[14].

Determination of nitric oxide is done by the colorimetric analysis of its stable decomposition products such as NO_3^- and NO_2^-. NO_3^- has to be reduced to NO_2^- and then can be colorimetrically estimated using Griess reaction. The Griess reagents are 0.2% (w/v) Naphthalene ethylenediamine and 2% Sulfonamide in 5% (v/v) Phosphoric acid. The reaction between NO_2^- and the Griess reagents in the dark gives a purple/magenta colour which has an absorption maxima between 520 and 550 nm. The absorbance is compared with the standard values and nitric oxide concentration is expressed in terms of mg L^{-1}.

Measurement of total protein: Fish tissues were homogenized using liquid nitrogen and were extracted using 1XPBS. The total protein content of the tissues was analyzed by the Bradford method.

Histopathological analysis: Heart tissues were taken from treated and untreated fish groups and were fixed in 10% formalin. The samples were dehydrated and embedded in paraffin. Tissues were then sectioned at 4 µm, stained with haematoxylin and eosin (H and E) and examined for histopathological evidence under Olympus BX40 Photomicroscope (Olympus Corporation, Tokyo, Japan).

RESULTS

Catalase activity: Doxorubicin is known to cause high amounts of oxidative stress in cardiac tissue. Therefore, the activity of catalase enzyme activity after treatment with *A. paniculata* was evaluated. Treatment with doxorubicin reduced the activity of the enzyme. Surprisingly we observed a dose-dependent increase in the activity of the catalase enzyme. In doxorubicin treated tissues, *A. paniculata* at a dose of 5 µg mL^{-1} increased the activity of the enzyme to 0.0250 ± 0.00816 KUL^{-1}. Similarly, *A. paniculata* at doses 10 and 15 µg mL^{-1} caused elevation in the activity of catalase to 0.0277 ± 0.00094 and 0.0313 ± 0.00104, respectively (Fig. 1). Overall, it is apparent that the extracts of *A. paniculata* caused a statistically significant elevation in the activity of the enzyme catalase.

Level of reduced glutathione: Similar to catalase activity, GSH levels were also significantly elevated by the extracts of *A. paniculata*. Doxorubicin suppressed the reduced GSH levels (0.1021667 ± 0.0016472 µ Mm L^{-1}) compared to control (0.1021667 ± 0.0016472) which is an indication of elevated oxidative stress. However, the extract of *A. paniculata* caused an elevation in the reduced GSH. A dose of 5, 10 and 15 µg mL^{-1} caused an elevation in the reduced GSH to 0.1326667 ± 0.0024944, 0.1503333 ± 0.0032998 and 0.1723333 ± 0.0017997 µM m L^{-1}, respectively (Fig. 2). There was a dose-dependent increase in the levels of reduced GSH.

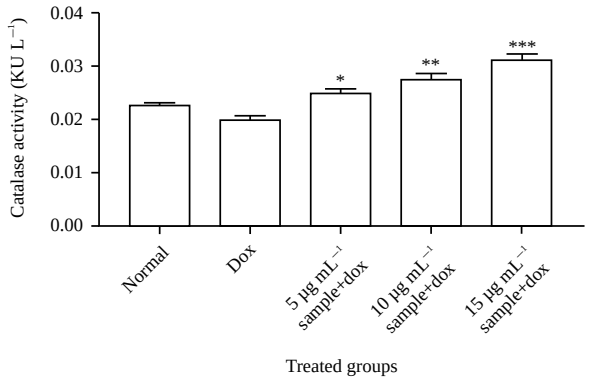

Fig. 1: Effect of *A. paniculata* extract on catalase in the presence of doxorubicin

Impact of doxorubicin in the activity of stress-reducing enzyme catalase and the role of *A. paniculatain* neutralizing the effect. *A. paniculata* extract at the lowest dose helps the tissues to recover and enhances the activity of catalase to a normal level. *,**,***Statistically significant increase in groups

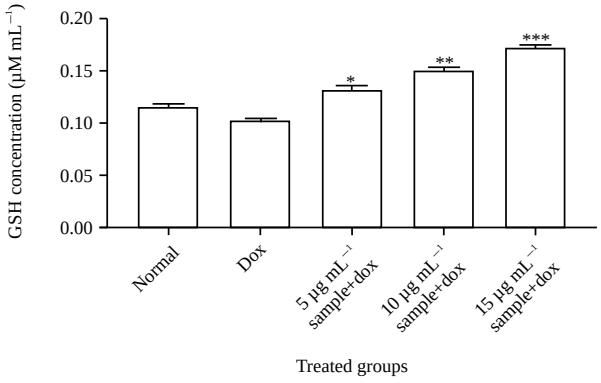

Fig. 2: Extract of *A. paniculata* restores the activity of GSH in the presence of doxorubicin

Impact of doxorubicin in anti-oxidant properties of the tissues by measuring the levels of GSH is shown in and the role of *A. paniculata* in the recovery of the tissues. *A. paniculata* extract at the lowest dose helps the tissues to recover and enhances the production of GSH to a normal level. *,**,***Statistically significant increase in groups

Nitric oxide level: Doxorubicin being a potent inducer of ROS, elevated nitric oxide levels in the tissue (from 1.1567±0.044672-1.6586±0.051651 mg L^{-1}. However, in treatment with the extract of *A. paniculata,* there was a significant decrease in the nitric oxide levels. Doses of 5, 10 and 15 µg mL^{-1} resulted in suppression of nitric oxide levels to 1.5039±0.048262, 1.3070±0.013201 and 1.0350±0.041899 mg L^{-1}, respectively (Fig. 3).

Superoxide dismutase activity: The activity of superoxide dismutase was estimated, an enzyme involved in neutralizing oxidative stress. Doxorubicin suppressed the

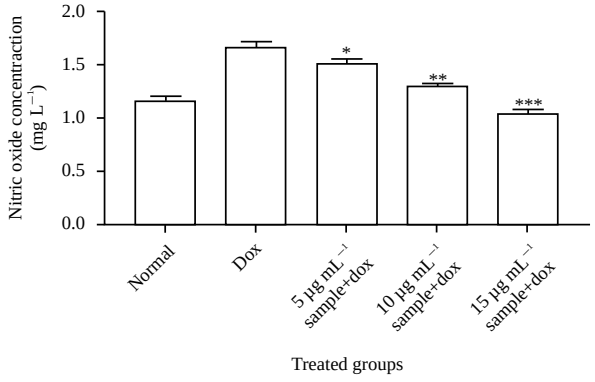

Fig. 3: Extract of *A. paniculata* reduces the nitric oxide concentration induced by doxorubicin

Impact of doxorubicin in inducing the release of reactive oxygen species such as Nitric oxide and the role of *A. paniculata* in the recovery of the tissues by reducing the stress levels. *A. paniculata* extract helps the tissues to recover from a minimal dose and lowers the concentration of nitric oxide that is equal to normal at 10 µg mL^{-1} dose in the tissues. *,**,***Statistically significant decrease in groups

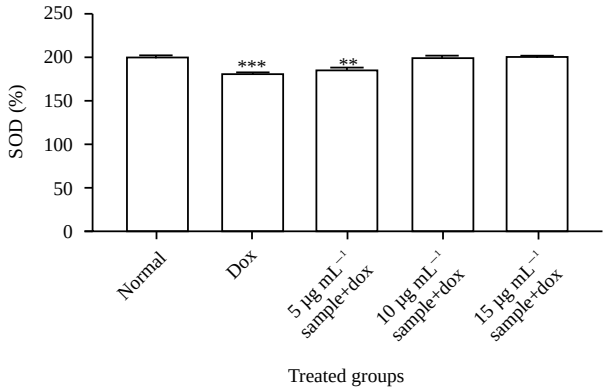

Fig. 4: Restoring effect of *A. paniculata* extract on SOD activity in the presence of doxorubicin

Impact of doxorubicin in inducing the production of stress enzymes such as superoxide dismutase that gives rise to toxic peroxide products and the role of *A. paniculata* in the recovery of tissues by reducing the stress levels. *A. paniculata* extract helps the tissues to recover from a minimal dose and lowers the concentration of SOD that is equal to normal at 10 µg mL^{-1} dose in the tissues. **,***Statistically significant decrease in groups

activity of the enzyme. (Control: 200.52±1.909226%, Doxorubicin treated: 180.54±1.411528%) Interestingly, the extract of *A. paniculata* caused an elevation in the activity of SOD in a dose-dependent manner. A dose of 5 µg mL^{-1} caused a mild increase in SOD activity (185.99±1.645256%) while 10 µg mL^{-1} showed a moderate increase in SOD activity (200.52±1.061822%). Stronger SOD activity was observed with 15 µg mL^{-1} of *A. paniculata* extract (215.68±1.447834%) (Fig. 4).

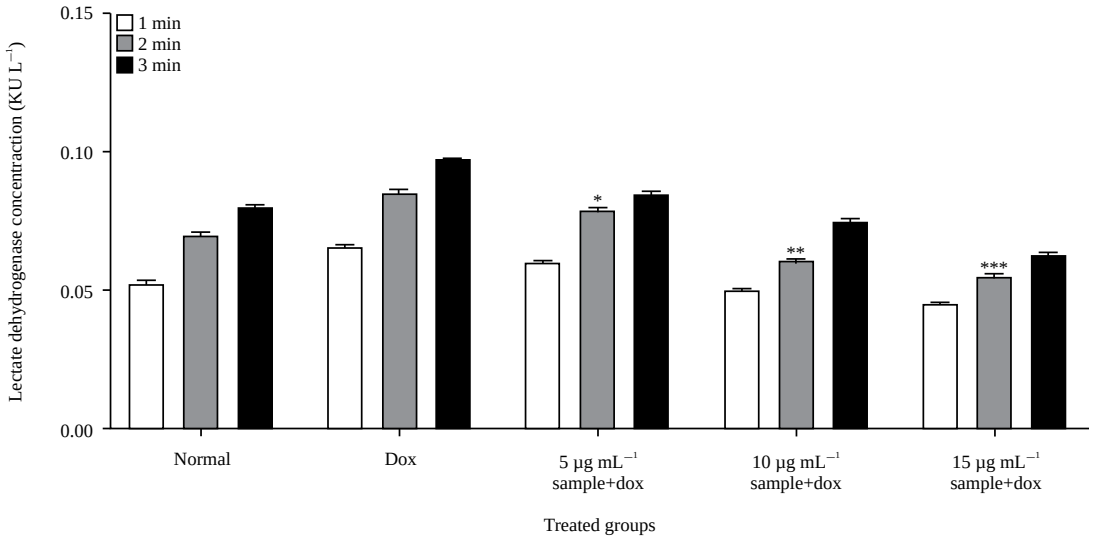

Fig. 5: *A. paniculata* extract ameliorates the cellular damage induced by doxorubicin

Impact of doxorubicin in inducing the release of Lactate dehydrogenase, an indication of cell damage and the role of *A. paniculata* in the recovery of the tissues by curing the cell damage. Cell damage increases with time after administered with doxorubicin. However, treatment with the lowest dose of *A. paniculata* extract decreases the LDH level to normal indicating cell recovery. *,**,***Statistically significant decrease in groups

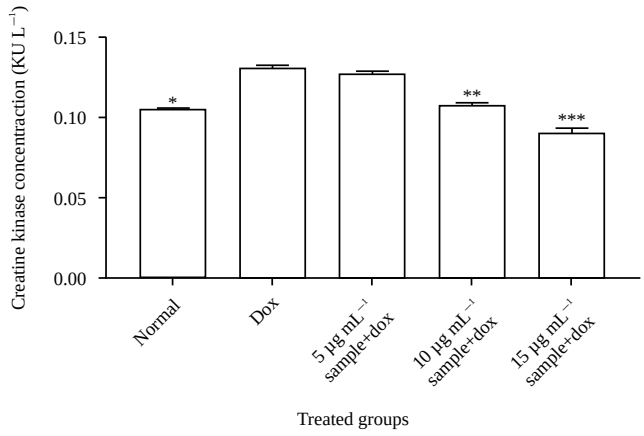

Fig. 6: Neutralizing effect of *A. paniculata* extract on creatinine kinase levels altered by doxorubicin

Impact of doxorubicin in inducing the release of creatinine kinase which is a clinical marker for Dox mediated cell toxicity and the role of *A. paniculata* in the recovery of the tissues indicated by the decreasing creatinine kinase levels. Treatment with the lowest of *A. paniculata* recovered the levels of creatinine kinase to normal. *,**,***Statistically significant decrease in groups

Lactate dehydrogenase activity: Lactate dehydrogenase is an enzyme present in all the cells across species. It is used as a biomarker for tissue injury as it is let out from tissues upon damage. We assessed the extent of tissue damage in the presence of *A. paniculata* at three-time points (1, 2 and 3 min). Control tissue released 0.0523333±0.001247, 0.0700000±0.000816 and 0.0800000±0.000826 KU L^{-1} in 1, 2 and 3 min, respectively. Doxorubicin treatment caused a drastic rise in LDH activity which are 0.0653333±0.001247, 0.0850000±0.001633 and 0.0973333±0.000471 KU L^{-1} in three-time points, respectively. 5 µg mL^{-1} of *A. paniculata* extract caused 0.0600000±0.000816, 0.0786667±0.001247 and 0.0850000±0.000716 KU L^{-1} in the first three minutes respectively. 10 µg mL^{-1} of *A. paniculata* extract suppressed the LDH activity with 0.0500000±0.000816, 0.0603333±0.000943 and 0.0750000±0.000814 KU L^{-1} with 1, 2, 3 min, respectively whereas 10 µg mL^{-1} of the extract strongly suppressed the LDH activity. (0.0450000±0.000813, 0.0550000±0.000812 and 0.0630000±0.000856 KU L^{-1} in 1, 2, 3 min, respectively) (Fig. 5).

Creatinine kinase activity: Creatinine kinase is another biomarker of tissue damage. We evaluated creatinine kinase activity to determine the protective activity of the extract of *A. paniculata.* Dox elevated creatinine kinase activity from 0.105000±0.000816-0.131000±0.000816 KU L^{-1}. The extract suppressed creatinine activity in a dose-dependent manner. A total of 5 µg mL^{-1} of the extract reduced the activity of creatinine kinase activity to a milder extent (0.127333±0.001247 KU L^{-1}). A total of 10 and 15 µg mL^{-1} of the extract suppressed the activity of creatinine kinase to 0.107666±0.001247 and 0.089666±0.003643 KU L^{-1}, respectively (Fig. 6).

Total protein content: Total protein from each of the groups was estimated. Total protein content was suppressed by doxorubicin (0.014000±0.000616 mg mL^{-1}) whereas the

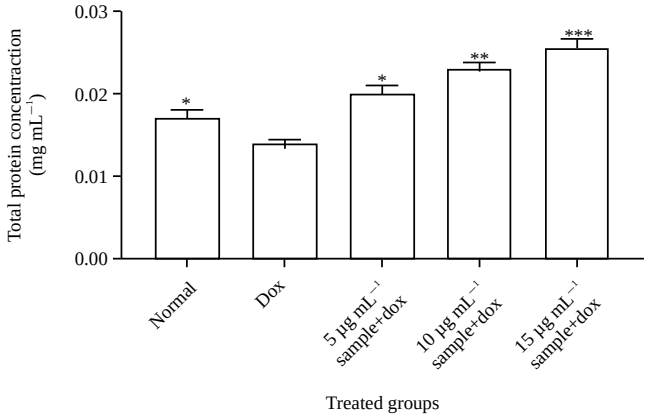

Fig. 7: Effect of *A. paniculata* extract on total protein concentration which is reduced by doxorubicin

Graph depicts the pathological conditions induced by treatment with doxorubicin as the total protein content drops lesser than normal. Treatment with the lowest dose of *A. paniculata* helps recovers the total protein content. *,**,***Statistically significant increase in groups

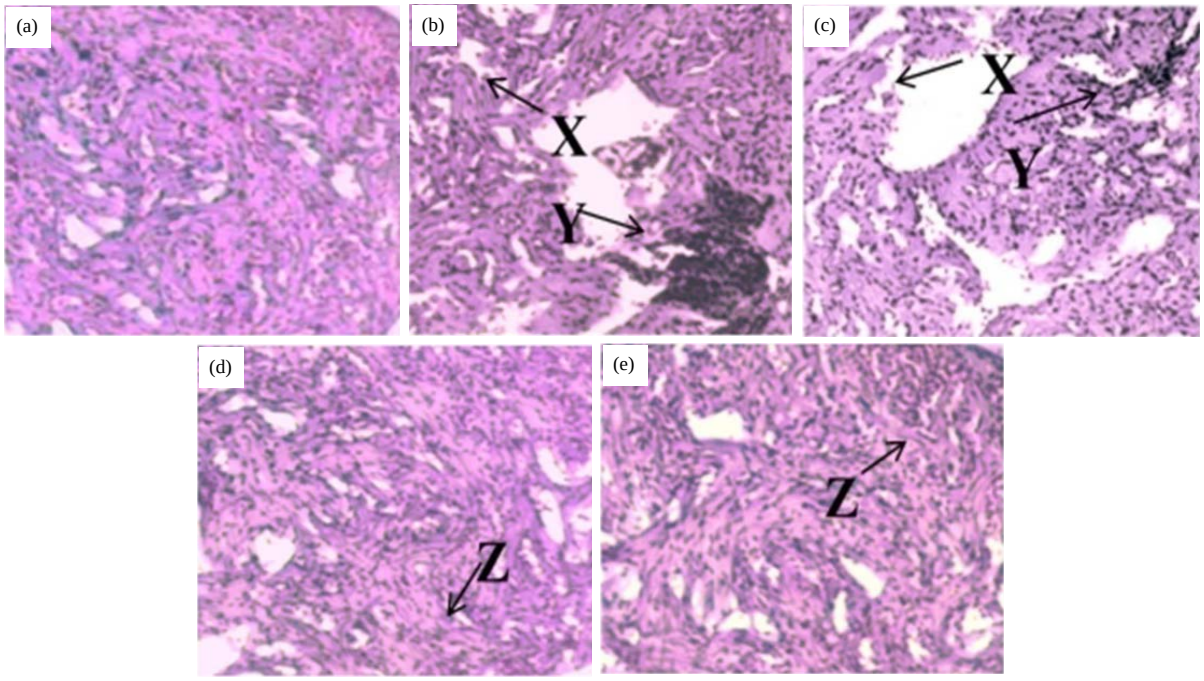

Fig. 8(a-e): Histopathological analysis of zebrafish heart tissues from different treatment groups

(a) Control, (b) Doxorubicin-induced, (c) Doxorubicin-induced and treated with µg mL^{-1} *A. paniculata*; (d) Doxorubicin-induced and treated with 10 µg mL^{-1} *A. paniculata* and (e) Doxorubicin-induced and treated with 15 µg mL^{-1} *A. paniculata*. The marking shown on the images depicts, (X)Tissue damage, (Y) Neutrophil accumulation, (Z) Recovered tissue. Doxorubicin has induced serious tissue damage and has also induced mononuclear response in the tissues. *A. paniculata* at 5 µg mL^{-1} concentration reduces the neutrophil accumulation to a considerable extent. At 10 µg mL^{-1}, the neutrophils get scattered back to normal and the tissues start to recover. At 15 µg mL^{-1}, the tissue has almost recovered from damage and the normal tissue architecture could be seen

control group showed 0.017033±0.001047 mg mL^{-1}. Surprisingly, there was an increasing trend in the total protein content upon treatment with the extract of *A. paniculata*. 0.019993±0.001047, 0.023000±0.000816 and 0.025667±0.001047 mg mL^{-1} from 5, 10 and 15 µg mL^{-1} of the extract, respectively (Fig. 7).

Histopathology: The heart tissue of the control group showed normal architecture without necrosis, oedema and inflammation (Fig. 8a). The Doxorubicin-induced group showed ruptured cardiac myofibers with necrosis and mononuclear infiltration (Neutrophil accumulation) as indicated by arrow marks A and B (Fig. 8b). Zebrafish treated

with 5 µg mL^{-1} showed very mild mononuclear infiltration with necrosis and ruptured cardiac myofibers, thus the immune response in being partially neutralized at 5 µg mL^{-1} (Fig. 8c). On treatment with 10 µg mL^{-1}, the tissues start to regenerate with completely reduced mononuclear infiltration (Fig. 8d). And complete tissue regeneration was observed on treatment with 15 µg mL^{-1} showing almost normal architecture of heart tissue showing protection from cardiomyopathy (Fig. 8e).

DISCUSSION

Doxorubicin despite being an effective anticancer drug causes a wide range of adverse drug reactions. The most devastating effect of the drug is cardiotoxicity. *A. paniculata* is a strong cardioprotective agent. In the current work, we evaluated the effect of extract of *A. paniculata* to reduces the cardiotoxicity caused by the drug. Zebrafish as the model organism in this study. One of the major contributors of doxorubicin-induced cardiotoxicity is reactive oxygen species[15-17]. Doxorubicin-mediated accumulation of reactive oxygen species could be the reason behind a wide range of toxicities in other organ systems as well. Therefore, we first evaluated the activity of the catalase, an enzyme involved in neutralizing hydrogen peroxide. Doxorubicin impaired the activity of catalase. *A. paniculata* extract strongly induced the activity of catalase in a dose-dependent manner. The elevated activity of catalase by *A. paniculata* extract implies effective scavenging reactive oxygen species induced by doxorubicin.

Glutathione is a tripeptide and is an indicator of cellular oxidative stress which switches between oxidised and reduced states. Glutathione contains a thiol group and is the only non-protein thiol bearing compound in a cell. We estimated the level of reduced glutathione in the tissues. Doxorubicin triggered a decline in the quantity of reduced glutathione compared to the control. On the other hand, treatment with the extract of *A. paniculata* increased the amount of reduced glutathione. Woo *et al.*[18] have shown that *A. paniculata* is cardioprotective through its anti-oxidant potential by upregulating reduced glutathione level. In our experiment also it has been observed the same manner. In addition, several lines of evidence exist in the literature supporting the anti-oxidant potential of *A. paniculata* by increasing reduced glutathione level[19,20].

We further quantified cellular nitric oxide levels from the cardiac tissue of zebrafish treated with doxorubicin. There was a significant increase in the nitric oxide level in doxorubicin treated samples. Our observation is in agreement with earlier findings[21]. However, treatment with the extract of *A. paniculata* reduced the amount of nitric oxide indicating the effective free radical scavenging system. Nitric oxide is a molecule that possesses high reactivity. In the heart, nitric oxide is involved in contractility and is also involved in maintaining heart rate[22]. Restoration of nitric oxide levels implies a restoration of cardiac function to normalcy.

Superoxide dismutase is an enzyme that catalyzes the conversion of superoxides into molecular oxygen and hydrogen peroxides. The majority of complications of doxorubicin mediated cellular toxicities can be directly correlated to impaired levels of superoxide dismutase[23]. Superoxide radicals are elevated upon treatment with doxorubicin whereas treatment with the extract of *A. paniculata* significantly reduced the levels. This observation could be directly correlated to the restoration of superoxide dismutase enzyme activity. The chances of survival of cardiomyocytes are enhanced when the superoxide dismutase activity is enhanced[24]. Therefore, treatment with the extract of *A. paniculata* enhances the survival rate of cardiomyocytes.

The release of lactate dehydrogenase by a cell is an indication of cellular damage, especially the loss of integrity of plasma membrane[25-27]. We evaluated the release of LDH from the tissue treated with doxorubicin at three different time points (1, 2 and 3 min). There was a time-dependent increase in LDH release from the tissues treated with doxorubicin. However, the release of LDH was suppressed by the extract of *A. paniculata* in a dose-dependent manner. This indicates that the membrane integrity was restored by the plant extract of *A. paniculata*.

Serum creatinine kinase is one of the markers for doxorubicin-mediated toxicity in the heart. Clinically, it is possible to correlate the extent of cardiac damage by doxorubicin by estimating Creatinine Kinase (CK)[28,29]. We, therefore, performed a quantitative analysis of CK activity. The activity of CK was elevated in doxorubicin treated samples. A total of 5 µg mL^{-1} of *A. paniculata* extract showed little effect in the activity of CK. A total of 10 µg mL^{-1} of the extract displayed a moderate decrease in the activity of CK. However, significant restoration of CK activity was found at 15 µg mL^{-1} of *A. paniculata* extract indicating the protective effect of the extract on cardiac function.

Total protein content from serum is an indicating factor of a pathological condition in doxorubicin-induced cardiomyopathy[30]. There was a significant reduction in the total protein levels upon treatment with doxorubicin. However, even the lowest dose (5 µg mL^{-1}) of *A. paniculata* plant extract strongly restored the total protein content. Further, 10 and 15 µg mL^{-1} of the extract strongly increased the total protein content. Our results are in agreement with the findings by Afsar *et al.*[31].

Histopathological analysis revealed abnormalities induced by doxorubicin compared to control. Treatment with doxorubicin revealed tissue damage and accumulation of neutrophils in cardiac tissue[32]. The presence of neutrophils in cardiac tissue marks the status of inflammation[33]. Doxorubicin-induced a heavy infiltration of neutrophils into the cardiac tissue as evidenced by the tissue sectioning. *A. paniculata* markedly recovered the tissue damage induced by doxorubicin. (Fig. 8). The higher dose (15 μg mL^{-1}) of *A. paniculata* repaired the tissue derangement induced by doxorubicin. Moderate and a little recovery could be observed with 10 and 5 μg mL^{-1} of the extract, respectively. Most importantly, infiltration of neutrophils was restricted by the extract in a dose-dependent manner which implies that it prevents inflammation in doxorubicin treated cases also. This view is supported by the anti-inflammatory activity of *A. paniculata* reported by Zou *et al*.[34]. Flavonoids have been shown to prevent the respiratory burst of neutrophils[35]. Therefore, it may be concluded that the flavonoids present in the extract of *A. paniculata* could have altered the neutrophil accumulation in the cardiac tissue. It is noteworthy that, presence of neutrophils could also increase the ROS through respiratory burst and neutrophil extract by *A. paniculata* clearance might have also contributed to reduced oxidative stress.

From our studies, it is evident that the cardiotoxicity induced by doxorubicin could be recovered by supplementing the extract of *A. paniculata*. The extract strongly reduces oxidative stress by activating enzymes such as SOD. The extract also reduces the cellular damage induced by doxorubicin as evidenced by LDH release and CK activity assay. The histopathological study also reveals the recovery of tissue damage induced by the extract. Reduction of neutrophil infiltration into cardiac tissue implies the anti-inflammatory activity of *A. paniculata* and its role in reducing the toxicity induced by doxorubicin. In conclusion, the extract of *A. paniculata* has a beneficial effect in reducing the toxicity induced by doxorubicin.

CONCLUSION

Doxorubicin, though an effective anti-cancer drug, causes life-threatening cardiomyopathy which limits the clinical utility of the drug. Doxorubicin-induced ROS is one of the major contributors to the adverse effects of the drug. Here in the current work, we used *A. paniculata* extract to neutralize the ROS induced by doxorubicin. In conclusion, our extract is very effective in reducing the toxicity of the drug by activating antioxidant enzymes such as catalase and SOD. Moreover, it was also evident that the extract was able to recover the cellular damage induced by the drug which is supported by the histopathological examination.

SIGNIFICANCE STATEMENT

This study proves the ameliorating effect of *A. paniculata* extract on doxorubicin-induced cardiomyopathy. Our results will help scientist to explore the application of *A. paniculata as a* cardio protective agent in doxorubicin-induced complication in cancer chemotherapy patients.

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