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Original Research Article

Evaluating the Acute Toxicity and *In vivo* Protective Effect of Standardized *Andrographis paniculata* Extract against Doxorubicin-induced Cardiotoxicity in Sprague-dawley Rats

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ABSTRACT

Doxorubicin fights cancer effectively, but harms heart muscle. The current methods for managing doxorubicin's toxicity are limited. This study determined the acute toxicity and protective effect of a standardized *Andrographis paniculata* ethanolic leaf extract against doxorubicin- induced cardiotoxicity. The acute toxicity of the extract was first investigated before determining its protective effect. 24 Sprague-Dawley rats were allocated into four groups: normal, DOX, DOX+AP250, and DOX+AP125. Doxorubicin was administered intraperitoneally (4 mg/kg/week) for four weeks. Treatment groups received doxorubicin plus either 250 mg/kg or 125 mg/kg daily dose of *Andrographis paniculata* ethanolic extract for 4 weeks. The rats were subjected to echocardiography 24 hours prior to sacrifice. After sacrifice, blood and heart tissue were collected to analyze brain natriuretic peptide and troponin level by the enzyme-linked immunosorbent assay. Histopathology assessment of heart tissues was also performed using hematoxylin and eosin staining. *Andrographolide* content of the extract was determined by HPLC. The bulk capsule extract's lethal dose 50 (LD_{50}) was determined to be above 4166 mg/kg BW and the *andrographolide* content was 8.98%. *Andrographis paniculata* extract (125 or 250 mg) mitigated doxorubicin-induced cardiotoxicity by decreasing serum brain natriuretic peptide and troponin level ($p < 0.05$), reversing histopathological alterations in heart tissue ($p < 0.05$), and improving fractional shortening, ejection fraction and heart rate. This study demonstrates that the standardized extract may be a non-toxic herbal and holds promise as a therapeutic agent for mitigating doxorubicin-induced damage in rats. Further studies are needed to elucidate its exact protective molecular mechanism of action.

***Keywords*:** *Andrographis paniculata*; cardiotoxicity; doxorubicin; acute toxicity; biomarkers

Introduction

Over the last few decades, the subject of cardiotoxicity, its inducers, and curative therapies have been extensively studied.[1–3] Cardiotoxicity, a severe health consequence of exposure to toxic substances, poses a grave threat to life as the heart weakens and fails to fulfill its essential function within the circulatory system.[3] Chemotherapy drugs including doxorubicin (DOX) are popular inducers of cardiotoxicity and are widely studied in oncology.[3,4] DOX, or Adriamycin, is an anthracycline synthesized from the *Streptomyces peucetius* bacterium.[5]

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It is well-known and efficacious in treating several cancer types including ovarian, breast, thyroid, lung, gastric cancers, and pediatric cancers.[6] However, the consequence of its use is mainly cardiotoxicity. The frequency of DOX-induced cardiotoxicity, which varies with the dose and length of therapy along with the patient's age, sex, and pre-existing cardiovascular problems, has been described in investigations.[7] DOX-induced cardiotoxicity remains a challenge in the field of oncology because of the increased risk of mortality in patients. [4,7]

Researchers in the field of oncology have dedicated years of relentless effort to the pursuit of a ground breaking treatment for DOX-induced cardiotoxicity. The introduction of conventional drugs, including dexrazoxane, beta-blockers, angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers (ACEI/ARB), and statins, represented an initial advancement in managing DOX-induced cardiotoxicity. However, these drugs exhibited limitations and failed to substantially improve the life expectancy of affected patients.[8–12]

The limitations of current treatment options necessitate an ongoing search for alternative approaches to effectively manage DOX-induced cardiotoxicity. Herbal plants have shown promising potential and are being extensively investigated. [13]

Andrographis paniculata (AP) is an herbal plant and a member of the *Acanthaceae* family, locally referred to as "sambiloto" in Indonesia.[14] It has been used extensively as an herbal medicine in many parts of Asia including China, the Philippines, Indonesia, Malaysia, and Thailand

because of its good medicinal properties. These properties include anti-inflammatory, antioxidant, and anti-cancer, among many others.[14,15] AP possesses numerous bioactive compounds including *andrographolide* which is regarded as its major bioactive compound[16]

Although pure *andrographolide* possesses proven cardioprotective properties, some other studies have demonstrated the superior efficacy of whole or standardized AP extract, indicating that in addition to *andrographolide*, other bioactive compounds within AP contribute synergistically to its therapeutic effect.[14,17,18] It is important to consider that the choice of solvent used for extraction can influence the pharmacological activity of the extract, even though whole or standardized extracts may offer superior efficacy. For example, a previous study demonstrated that the ethanolic extract of AP exhibited greater pharmacological activity compared to the butanol extract.[19] This study therefore prepared a standardized ethanolic extract of AP (using *andrographolide* content as a standardised marker) and investigated its cardioprotective effect against DOX-induced cardiotoxicity, employing established diagnostic approaches, namely echocardiography, histopathology, and biochemical analysis of brain natriuretic peptide (BNP) and troponins in blood.[20–22] Since the standardized extract was newly prepared for this study, there was a need to check its safety at least by performing acute toxicity testing.

While animal studies have extensively explored the cardioprotective effects of AP in cardiovascular-related diseases,[14] to the best of our knowledge, only three studies have investigated its beneficial effect in ameliorating DOX-induced cardiotoxicity;[23–25] hence, more studies are warranted to confirm the results or report new findings. Among these three studies, two investigated the effects of *andrographolide*,[23,24] while the third employed an uncharacterized aqueous extract of AP, the preparation of which was detailed within the study. Notably, this third study utilized zebra fish as the animal model and has not been replicated in rodents, which limits its generalizability.[25] This study seeks to determine the acute toxicity of a standardized extract of AP and investigate its protective effects against DOX-induced cardiotoxicity in healthy rats.

Materials and Methods

Plants collection and Identification

The plant was collected by personnel from PT. Konimex (Pharmaceutical company), Indonesia in April 2022 from Tawamangu, Central Java, Indonesia. The voucher specimen (ANPS-05) was deposited in Faculty of Pharmacy, Universitas Gadjah Mada, Yogjakarta, Indonesia.

Plant Extraction

The extraction process of the simplicia was done using the percolation method with 90% ethanol as the solvent. The total volume of ethanol used was 10 litres for 1kg of the simplicia. The resulting extract was then evaporated under vacuum at a maximum temperature of 60°C until it achieved a maximum thickness with the total solid end-point parameter. The condensed extract was then dried using a fluid bed granulator with tapioca starch a filler. The dried extract was further processed into final product in the form of capsules, which were done by combining the extract with fillers. Each bulk capsule of 125 mg was prepared containing AP native extract content of 48%. The quality of the extract met the criteria of Indonesian Food and Drug Agency (BPOM), including the *andrographolide* concentration, organoleptic description, moisture content, and microbiology.

HPLC finger analysis for AP

Andrographolide in AP extract was determined using HPLC equipped with UV-Vis detector, Pursuit XRs C18 column (4,0 x 250 mm; 5 µm). The mobile phase employed comprised of a solution containing Methanol: Water (53:47) in isocratic elution method. The mobile phase underwent filtration using a Whatman filter membrane with a pore size of 0.45 µm and was subjected to sonication for 10 minutes before its utilization. The flow rate utilized in the experiment was 1 mL/min, while the injection volume was 10 µL. The separation process was monitored at a wavelength of 230 nm.

The preparation of the sample solutions involved the measurement of 500 mg of the extract, which was then diluted with 20 mL of methanol (HPLC grade). The resulting mixture was subjected to sonication for 10 minutes. Subsequently, 1 mL of the solutions were diluted in a 25 mL of methanol, vortexed and subjected to filtration through a 0.22-µm PTFE membrane filter before being injected into the High-Performance Liquid Chromatography (HPLC) system. The *andrographolide* concentration was then calculated by employing a calibration curve in a triplicate measurement.

Determination of the acute toxicity of AP

Fifty rats, 25 male and 25 female rats (5 rats of each sex per group, N=10) weighing 180-200 g were randomly allocated into 5 treatment groups (0, 10.5, 100.5, 625 and 4166 mg/kg BB of the standardized bulk capsule of AP extract, which were equivalent to 0, 5, 50, 300 and 2000 mg/kg BW native AP extract). The extracts were given in a 1% CMC suspension. The control group was only given 1% CMC suspension. All animals underwent weighing and were fasted for eight hours. Each group received a single oral gavage containing either a control substance or varying doses of the AP bulk capsule (0, 10.5, 100.5, 625, or 4166 mg/kg body weight). Following administration, all animals were observed daily for the first 24 hours (at ½, 1, 2, 4, and 24 hours) for any signs of toxicity, including changes in appearance (fur, skin, eyes) and behavior. This monitoring continued once daily for an additional 14 days. Throughout the study, animal weights were recorded daily. On Day 15, all animals were sacrificed after anesthetized with ketamine and xylazine, and gross necropsies were performed. The internal organs were examined and weighed. Afterward, 50% of lethal dose were determined.

Induction of cardiac toxicity by DOX in rats and treatment with AP

Twenty-four male Sprague-Dawley rats were used for animal experimentation for DOX-induced cardiac toxicity. The source of the rats was the Indonesian National Agency of Drug and Food Control in Jakarta, Indonesia, which were 6-8 weeks old and weighed between 150 and 200 grams. The instructions for the care and use of experimental animals were strictly adhered to while caring for the animals. Housing was offered in a controlled environment with a 21°C temperature, 55% relative humidity, and a light/darkness 12-hr cycle. Additionally, liberty to access laboratory standardized water and food was provided to them. The Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, gave their approval to all experimental protocols. The document number was KET-822/UN2.F1/ETIK/PPM.00.02/2022.

Four groups of six rats were randomly assigned. The Normal group (N) received saline injections, while the DOX group received doxorubicin at 4 mg/kg via intraperitoneal injection (i.p.) each week for four weeks, totaling 16 mg/kg BW. This well-established cardiotoxic dose served as the negative control. Two DOX+AP groups were treated with DOX alongside oral AP at different doses: 125 mg/kg BW (DOX+AP125) and 250 mg/kg BW (DOX+AP250). The AP treatment lasted for 30 days.

Echocardiographic analysis

At the end of the treatment period (day 31), all the rats underwent a heart ultrasound examination to observe changes in the heart function. Rats were anesthetized intraperitoneally using ketamine (60 mg/kg BW, i.p) and xylazine (6.0 mg/kg BW, i.p.). The rat hairs in the parasternal area were shaved and cleaned. The parasternal part was given a gel to facilitate echocardiographic examination. Ultrasound imaging of the rat heart was performed using a portable ultrasound Chison EBit60vet® (PT Mega Utama Medica, Indonesia). An Echocardiographic examination was performed in the right parasternal (RPS) position, with the short axis views (SA) transducer position. The transducer is positioned after the heartbeat is palpated. Heart rate was calculated by measuring between two wave crests on an echocardiographic monitor screen display. Interventricular septal diastole (IVSd) was calculated by measuring the distance of the interventricular septa at the end of the diastole while Interventricular septal systole (IVSs) was calculated by measuring the distance of the interventricular septa at the end of systole. We assessed the left ventricle's posterior wall thickness during two stages: end-diastole (LVPWd) and end-systole (LVPWs). Left ventricular internal diameter end-diastole (LVIDd) was calculated at the end of diastole while left ventricular internal diameter end-systole

(LVIDs) was calculated at the end of systole. EF and FS were also calculated.[26]

Serum and tissue preparation

To analyze both blood and heart tissue, samples were collected following specific protocols. Blood was drawn directly from the heart via puncture, then centrifuged at 3000 rpm for 10 minutes to separate serum. After euthanasia, hearts were harvested, weighed, and rinsed with ice-cold saline. Transverse sections were then made, with one half reserved for tissue homogenization and the apical portions of the other half underwent a fixation process in 10% neutral buffered formalin over a 48-hour period. Dehydration through a graded alcohol series, xylene clearing, and paraffin embedding were performed to prepare the specimens for sectioning. Finally, the paraffin blocks were sectioned into 5-µm slices using a microtome and stained with Hematoxylin-eosin (H&E). The histopathological changes were examined by light microscope.[27]

Analysis of brain natriuretic peptide and troponin levels by enzyme immunoassay

Brain natriuretic peptide (BNP) levels, a key indicator of cardiotoxicity, were measured in both serum and heart tissue using rat BNP 45 ELISA kit (Abcam, Catalog No: ab108816). Further assessment of potential cardiac injury was conducted by measuring serum levels of cardiac troponin I type 3 (cTnI) using an ELISA kit. (Elabscience, Catalog No:E-EL-R1253).

Histopathological analysis of heart tissues

Histological slides stained with H&E were examined under light microscopy to evaluate histomorphology changes in the cardiac tissue. To ensure unbiased assessment, two pathologists blinded to the experimental groups analyzed the slides using a slightly modified version of the scoring criteria from a previous study[27] https://doi.org/10.2147/JEP.S413256. Very little or no cardiac tissue abnormality (0) mild cardiac tissue abnormality such as very few zones of muscle cell degeneration accompanied with the presence of very few infiltration of inflammatory cells (1) (mild), moderate cardiac tissue abnormality characterized by muscle cell degeneration accompanied with infiltration of inflammatory cells and presence of few necrotic zones (2) (moderate), Severe tissue abnormality characterised by many zones of muscular degeneration, infiltration of inflammatory cells and severe necrosis (3) (severe).

Statistical analysis

Data are shown as mean ± standard error of the mean (SEM). Statistical analysis involved one-way analysis of variance (ANOVA) followed by Tukey's post hoc test, performed with GraphPad Prism software, version 5.0. Statistical significance was defined as a p-value of 0.05 or less.

Results and Discussion

Analysis of *Andrographolide* content in AP extract

Figure 1 presents the chromatographic profile of andrographolide in the extract. While *Andrographis paniculata* (AP) contains various bioactive compounds with potential health benefits,[16] numerous studies have consistently identified *andrographolide* as the main bioactive constituent responsible for AP's therapeutic effects.[14,28] *Andrographolide* has garnered significant attention due to its extensively studied beneficial effects in various branches of medicine,[29,30,31] including cardiology.[14] Additionally, it has demonstrated some cardio-protective activities against DOX-induced cardiotoxicity in rats.[23,24] Given its numerous beneficial effects, *andrographolide* was chosen as a marker compound to characterize the prepared plant extract. Its concentration, as shown in Figure 1, was determined to be 8.98% by HPLC.

Determination of Acute toxicity of AP

Figures 2 and 3 present the results of the acute toxicity test for the AP extract. Assessing the toxicity of herbal plants using animal models is crucial for validating their safety in clinical practice, as the effects observed in experimental animals exposed to chemical substances can inform potential outcomes in humans.[32–34] Therefore, to substantiate the safety of the AP ethanolic extract, an acute toxicity study was conducted.

The results showed no mortality in any animals at doses up to 4166 mg/kg body weight (BW) of the bulk extract capsule. No notable behavioral abnormalities or signs of toxicity were observed on the skin, fur, or eyes. Animals did not exhibit tremors, convulsions, salivation, diarrhea, weakness, or sleep disturbances. There were no signs of coma, movement abnormalities, or altered consciousness.

From the second to the fourteenth day, no physical or behavioral problems were identified in any of the experimental animals. There was no evidence of lacrimation, salivation, spasms, diarrhea, altered gait, or unusual body positions. Body weight remained relatively stable over the 14 days, except for the highest dose group (Figure 2). Liver weight in this group was significantly elevated compared to the control group. However, all other internal organs, including the lungs, kidneys, heart, spleen, brain, and intestines, appeared unchanged (Figure 3).

Based on these findings, the LD_{50} (lethal dose at which 50% of the test subjects die) of the AP extract capsule is likely greater than or equal to 4166 mg/kg BW. This suggests a relatively safe profile for use as an herbal medicine, conforming to the Hodge and Steiner toxicity scale.[35] A previous study reported that the LD_{50} of the native ethanolic leaf extract was even higher than 5000 mg/kg BW. These findings establishes that the ethanolic extract of AP exhibits low toxicity and may be considered a safe herbal product.[36]

Effect of AP on Echocardiography analysis

Left ventricular function was assessed using echocardiographic analysis. Representative images of heart function are presented in Table 1.

Echocardiography, a non-invasive method for assessing heart function, is widely used by clinicians to detect abnormalities in cardiac function during chemotherapy.[37] Left ventricular function is evaluated through measurements of left ventricular ejection fraction (LVEF), left ventricular fractional shortening (LVFS), and heart rate. Several clinical and animal studies have reported the use of echocardiographic parameters to diagnose DOX-induced cardiotoxicity.[37,38] Studies in rats have shown that DOX treatment leads to a decline in heart rate (beats/min), LVEF, and LVFS.[38,39]

Similarly, this study observed a significant decrease in heart rate (244±17), LVEF (69.8±3%), and LVFS (29.5±2%) following DOX treatment. Interestingly, co-treatment with the AP ethanolic extract significantly improved these parameters ($p < 0.05$ for both LVEF and LVFS compared to DOX alone). At a dose of 125 mg, heart rate, LVEF, and LVFS increased to 283±52 bpm, 70.28±5.9%, and 33.43±4.51%, respectively. Similarly, at the 250 mg dose, these parameters were 236±32 bpm, 78.5±1.3%, and 40.16±1.24%, respectively. These findings suggest that AP may restore normal left ventricular function at appropriate doses (Table 1).

DOX treatment did not significantly affect heart muscle thickness or chamber size. This includes measurements of the interventricular septum (diastole: IVSd, systole: IVSs), left posterior wall (diastole: LVPWd, systole: LVPWs), and left ventricle diameter (diastole: LVIDd, systole: LVIDs). Notably, all these values remained within the normal range.

Effect of AP on troponin and BNP level

To further validate the cardioprotective effects of AP against DOX-induced cardiotoxicity, the serum levels of cardiac troponin I (cTnI) and BNP together with the cardiac BNP level was investigated as shown in Figure 4.

Two interdependent proteins, myosin, and actin are fundamental for muscle contraction.[40] In a relaxed muscle, tropomyosin molecules hide the myosin binding sites on actin, preventing muscle contraction.[41] Troponins comprising three subunits (TnC, TnI, and TnT), serve as regulators of tropomyosin position, each subunit playing a distinct role in muscle contraction. [42,43]

Table 1: Echocardiography assessment AP effect on doxorubicin-induced cardiotoxicity analysis

Parameters	Normal	DOX	DOX+ES125	DOX+ES250
HR (beats/min)	277 ± 29	244 ± 17	283 ± 52	236 ± 32
IVSd (cm)	0.13 ± 0.01	0.14 ± 0.01	0.17 ± 0.03	0.14 ± 0.02
IVSs (cm)	0.22 ± 0	0.17 ± 0.03	0.19 ± 0.02	0.18 ± 0.01
LVPWd (cm)	0.2 ± 0.04	0.15 ± 0.01	0.18 ± 0.02	0.16 ± 0.04
LVPWs (cm)	0.22 ± 0	0.24 ± 0.02	0.26 ± 0.03	0.26 ± 0.04
LVIDd (cm)	0.77 ± 0.01	0.73 ± 0.08	0.72 ± 0.02	0.73 ± 0.07
LVIDs (cm)	0.5 ± 0.0	0.52 ± 0.06	0.49 ± 0.08	0.43 ± 0.04
EF (%)	74.5 ± 4.5	69.8 ± 3	70.28 ± 5.9	78.5 ± 1.3
FS (%)	34.8 ± 0.8	29.5 ± 2	33.43 ± 4.51	40.16 ± 1.24

Data are presented as mean ± SEM (n = 5). Abbreviations: HR, heart rate; IVSd, interventricular septal diastole; IVSs, interventricular septal systole; LVPWd, left ventricular posterior wall thickness at end-diastole; LVPWs, left ventricular posterior wall thickness at end-systole; LVIDd, left ventricular internal diameter at end-diastole; LVIDs, left ventricular internal diameter at end-systole; EF, ejection fraction; FS, fractional shortening.

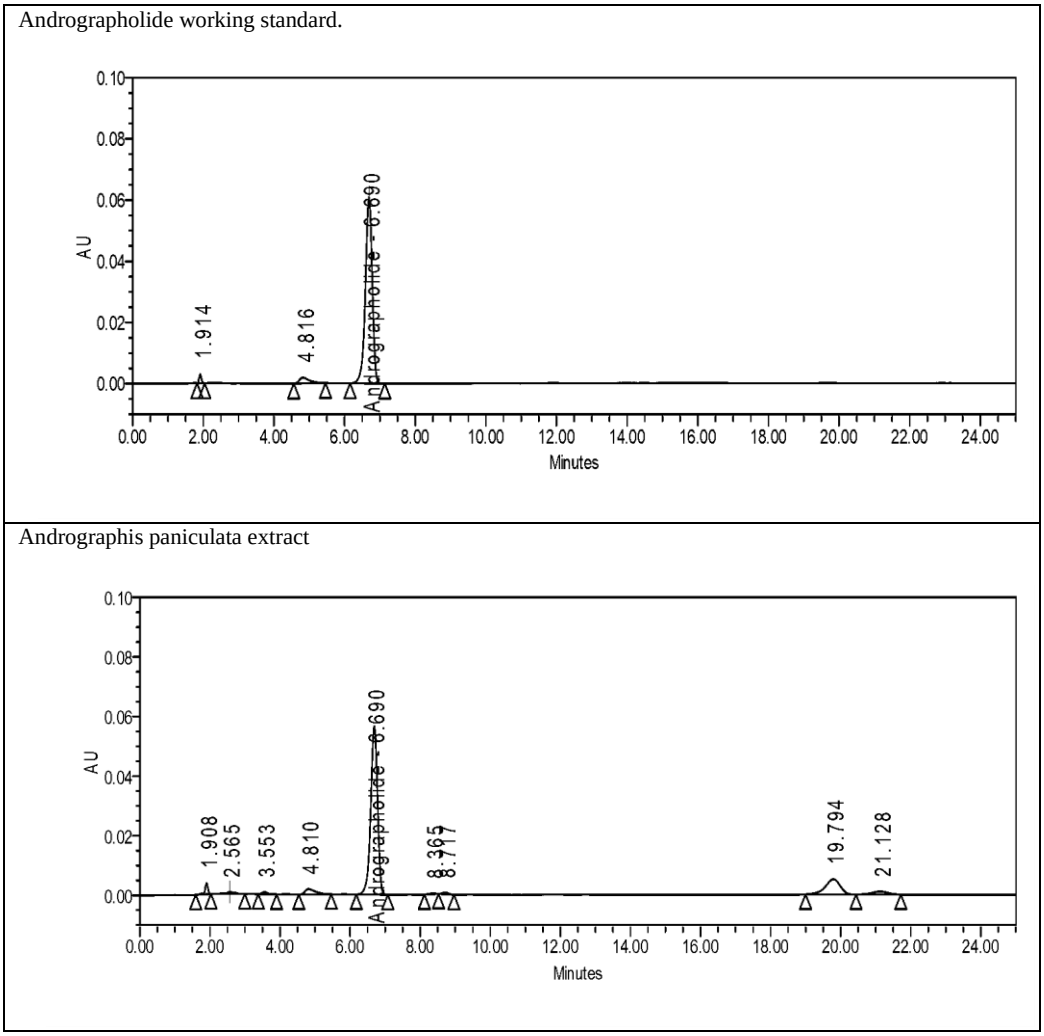

Figure 1: Analysis of andrographolide in *Andrographis paniculata* extract using HPLC-UV/Vis. Retention time of andrographolide was 6.690 minutes

TnT serves as a structural anchor for troponin on actin filaments, while TnI acts as a regulatory switch, inhibiting the ATPase activity of the actomyosin cross-bridge and preventing myosin from binding to actin.[42] Upon nerve stimulation, calcium ions (Ca^{2+}) are released from the sarcoplasmic reticulum, binding to troponin C (TnC). This interaction triggers a conformational change in tropomyosin, uncovering myosin-binding sites on actin and enabling myosin to bind, leading to muscle contraction.[43] Troponins therefore play a key role in heart muscle contraction and relaxation.

When cardiac myocytes (heart muscle cells) undergo necrosis (cell death) or injury, these troponin proteins are released into the bloodstream. This release of troponins into the blood allows for their detection and measurement in a blood test, serving as a valuable indicator of heart damage.[44] In this study, the level of cTnI was measured in serum. DOX administration significantly increased cTnI ($p < 0.05$) which was consistent with the other studies.[44,45] Interestingly, co-treatment with AP extract drastically reduced cTnI level ($p < 0.05$) (Figure 4).

BNP belongs to a group of naturally occurring peptides known as natriuretic peptides (NPs). Other members of this family include urodilatin, atrial natriuretic peptide (ANP), C-type natriuretic peptide (CNP), and D-type natriuretic peptide (DNP).[46] BNP is a bioactive peptide involved in cardiovascular regulation, and it is primarily produced and synthesized by myocytes in the left ventricles.[47] Consequently, when mechanical stress is induced by pressure overload or ventricular volume expansion, the left ventricle releases BNP, triggering vasodilation, promoting diuresis, and suppressing the renin-angiotensin-aldosterone system (RAAS).[48]

Research has found a close link between worsening left ventricle function and rising BNP levels in patients experiencing DOX-induced heart damage.[49] Many studies with rats have reported that plasma BNP levels are usually elevated in DOX-induced chronic cardiotoxicity.[50,51] Consistent with these studies, serum BNP was significantly elevated by DOX treatment ($p < 0.05$ DOX vs control). However, co-treatment with AP extract tended to cause a reversal. Further, cardiac BNP was measured in cardiac tissue. DOX treatment significantly decreased cardiac BNP levels ($p < 0.05$ DOX vs control). The lowered cardiac BNP levels reflected an increased release of BNPs into the blood and as a result, biochemical assays revealed elevated serum BNP levels in the DOX-treated group (Figure 4). The concurrent reduction of BNP and cTnI in blood, which aligns with the echocardiographic findings, suggests that AP offers protection against DOX-induced cardiotoxicity.

Effect of AP on the histopathological changes of the heart tissue

To evaluate DOX-induced morphological changes in the heart, tissue sections were stained with H&E and examined them under light microscopy. Figure 5 reveals the characteristic features of the normal group, DOX group and AP treatment groups.

Hematoxylin and eosin (H and E) staining of heart tissue has been used effectively for diagnosing DOX-induced cardiotoxicity, especially in experimental animals.[38] This study identified severe myocardial cell degeneration, inflammatory cell infiltration and necrosis in the DOX group. This finding aligns with reports from similar animal models,[28] supporting the established cardiotoxic effect of DOX. The observed necrosis likely contributed to the elevated plasma cTnI levels. Notably, co-treatment with AP leaf extract prevented DOX-induced lesions ($p < 0.05$) as demonstrated in Figure 5. which is confirmatory of AP's cardioprotective effect against cardiotoxicity induced by DOX.

In summary, histological analysis demonstrated muscle cell degeneration, inflammatory cell infiltration, and necrosis in the DOX group, consistent with cardiomyocyte damage. This finding was further supported by biochemical assays revealing elevated blood levels of cTnI (indicative of necrosis) and BNP (suggesting pressure overload). Echocardiography corroborated these findings, revealing a decline in left ventricular systolic function as evidenced by reduced ejection fraction (EF) and fractional shortening (FS), along with a decrease in heart rate. These findings further support DOX-induced cardiomyocyte damage. Interestingly, the AP ethanolic extract counteracted these harmful effects, suggesting its potential for clinical applications, especially as the LD_{50} data revealed that the extract may likely be non-toxic. Nevertheless, further research is still required to elucidate the molecular mechanisms underlying AP's protective effects. Additionally, further histological studies are warranted to assess the impact of AP on fibrosis, as fibrosis is a detrimental consequence of DOX-induced cardiotoxicity. Chronic toxicity testing should also be conducted in future.

Conclusion

This study demonstrates a beneficial effect of a standardized AP extract in ameliorating the heart toxicity caused by DOX as evidenced from biochemical, echocardiography profiles along with its histopathological assessments. In addition, based on the acute toxicity studies, this extract appears to have a good safety profile. However, chronic toxicity testing is necessary due to the intended chronological use of the extract as an adjunctive treatment for cancer patients receiving DOX

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

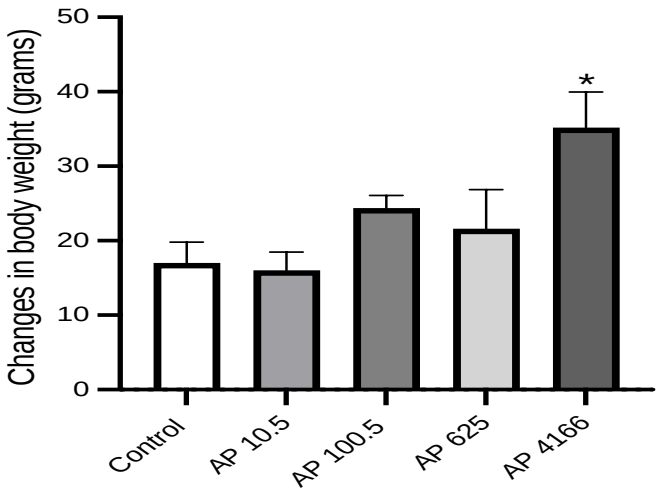

Figure 2: Changes in body weight in the rats 14 days after the administration of control or bulk capsule of AP ethanolic extract 10.5 mg/kg BW, AP 100.5 mg/kg BW, AP 625 mg/kg BW or AP 4166 mg/kg BW

* *p<0.05* vs control; Data are presented as mean ± SEM. AP: *Andrographis paniculate*

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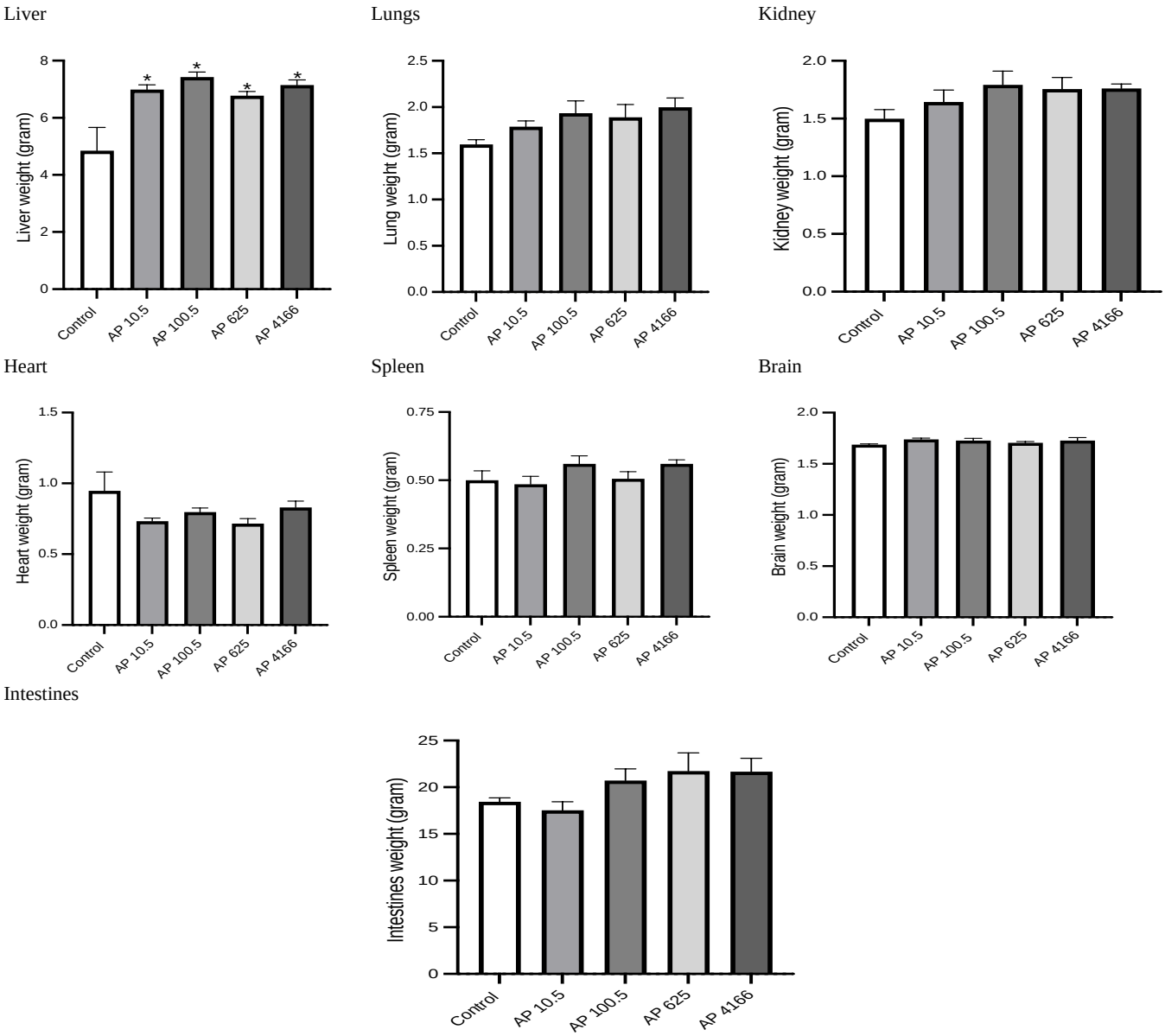

Figure 3: Internal organ weight of the rats 14 days after the administration of control or bulk capsule of AP ethanolic extract 10.5 mg/kg BW, AP 100.5 mg/kg BW, AP 625 mg/kg BW or AP 4166 mg/kg BW

* $p<0.05$ vs control; Data are expressed as mean ± SEM. AP: *Andrographis paniculata*

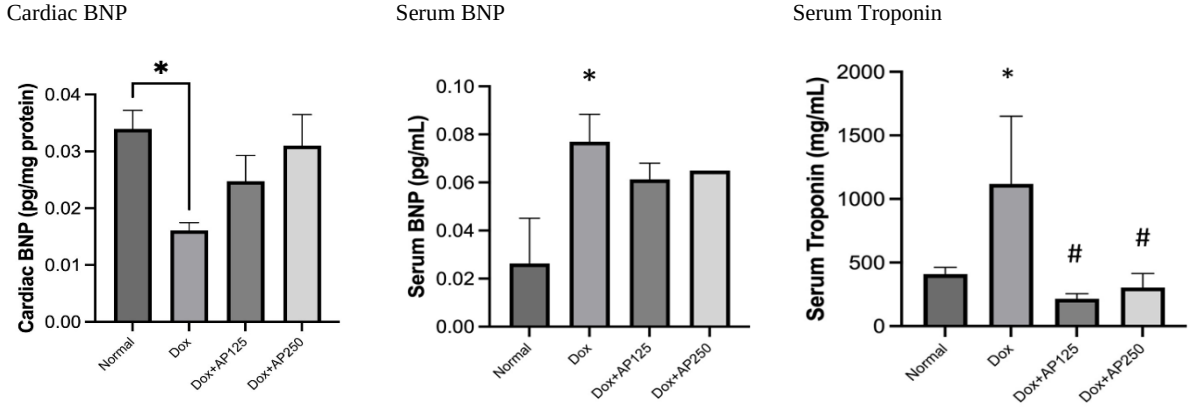

Figure 4: Cardiac BNP (A), Serum BNP (B) and Serum Troponin (levels after the administration of control or DOX treatment (4 mg/kg BW/week for 4 weeks) or/plus administration of bulk capsule of AP ethanolic extract (125 mg/kg BW/day or 250 mg/kg BW/day for 4 weeks)

* *p<0.05* vs control; Data are presented as mean ± SEM. BNP: B-type natriuretic peptide; DOX: doxorubicin; AP: *Andrographis paniculata*

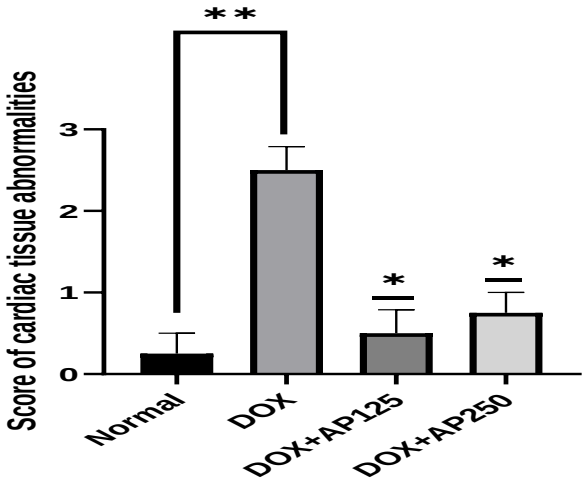

Figure 5: Examination of rat heart tissue stained with Hematoxylin and Eosin following treatment with doxorubicin alone or doxorubicin combined with AP leaf extracts**.** Normal group (A), normal tissue morphology; DOX only group (B), degeneration of muscle cell accompanied with infiltration of inflammatory cells and necrosis; DOX+AP125 (C) and DOX+ AP 250 (D), nearly normal tissue morphology with very little infiltration of inflammatory cells and muscle cell degeneration; score of cardiac tissue abnormalities (E). The levels of tissue abnormalities were classified as 0 (very little or none), 1 (mild), 2 (moderate), 3 (severe). AP: *Andrographis paniculate*

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