

RESEARCH ARTICLE

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DOI: 10.21705/mcbs.v8i2.444***Andrographis paniculata* Ethanolic Extract Improved Doxorubicin-induced Cardiac Inflammation, Alterations in Liver Function Parameters and Anemia**Oluebube Magnificent Eziefule¹, Wawaimuli Arozal², Septelia Inawati Wanandi³, Melva Louisa², Puspita Eka Wuyung⁴, Syarifah Dewi³, Nafrialdi², Yulia Ratna Dewi¹, Deya Adiby Nabillah¹¹Master's Programme in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia²Department of Pharmacology and Therapeutics, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia³Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia⁴Department of Pathology Anatomy, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

Background: Doxorubicin (DOX), an efficacious chemotherapy drug is compromised by cardiotoxicity, myelosuppression, and hepatotoxicity. Due to the limited success of current treatments for DOX toxicity, there is a pressing need to explore alternative medical interventions, particularly from plant sources. This study was conducted to investigate the potential protective effect of ethanolic extract of *Andrographis paniculata* leaves (EEAP) against DOX-induced cardiac inflammation, liver toxicity, and anemia.

Materials and methods: Sprague-Dawley rats were intraperitoneally injected with DOX at a total dose of 16 mg/kgBW. EEAP was administered orally for 4 weeks at doses of 125, 250, and 500 mg/kgBW/day according to the assigned treatment groups. The mRNA expression levels of *interleukin-1 β* (*IL-1 β*) and *nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3* (*NLRP3*) in the heart tissue, along with the concentrations of nuclear factor kappa-light-chain-enhancer of activated B cells (NF κ B) and calcium level were examined. Additionally, the hematological parameters (including hematocrit, hemoglobin and red blood cells (RBCs)), aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and malondialdehyde (MDA) levels in blood were also analyzed.

Results: EEAP dose-dependently decreased the mRNA expressions of *IL-1 β* ($p < 0.05$), tended to decrease mRNA expression of *NLRP3* and the concentrations of NF κ B and calcium in heart tissue compared with the DOX-only group. Additionally, EEAP dose-dependently decreased ALP values ($p < 0.0001$) and tended to improve hematological parameters, as well as AST and MDA levels in serum.

Conclusion: This extract may prevent DOX-induced cardiac inflammation, anemia, and hepatotoxicity. However, further studies are needed to confirm these findings, including the efficacy profile of the extract in cancer rats treated with DOX.

Keywords: doxorubicin, *Andrographis paniculata*, inflammation, anemia, hepatotoxicity, herbal medicine

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Corresponding Author:

Wawaimuli Arozal
Department of Pharmacology and Therapeutics, Faculty of Medicine
Universitas Indonesia
Jl. Salemba Raya No.6, Jakarta 10430, Indonesia
e-mail: wawaimuli.arozal@ui.ac.id



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Institute



Introduction

Several research papers have corroborated the consensus that doxorubicin (DOX), a potent chemotherapy drug derived from *Streptomyces peucetius* bacterium is compromised by its unique toxicities including cardiotoxicity, hepatotoxicity, and myelosuppression.^{1,2} DOX is efficient in treating a wider range of cancers such as hematological cancers, melanoma, and sarcoma.^{2,3} Although DOX's exact mechanism of action to induce toxicities in normal cells remains unclear, many studies have shown that inflammation, oxidative stress, electrolyte imbalances, and hematological and biochemical alterations (mainly related to myelosuppression and liver toxicity respectively) are all associated with DOX toxicity.⁴⁻⁷

Cardiac inflammation is one of the signs of cardiotoxicity.⁴ One of the pro-inflammatory actions of DOX on the myocardium stems from its ability to activate nuclear factor kappa B (NFκB), a key regulator of inflammatory reactions.⁸ Depending on the cellular condition, NFκB activation may therefore lead to the activation of some other inflammatory proteins including *interleukin-1β* (*IL-1β*) and *nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3* (*NLRP3*). Calcium plays a vital role in the contraction of the heart muscles. The disruption of cardiac calcium homeostasis is associated with DOX-induced cardiotoxicity. DOX treatments in rats decrease Ca^{2+} ATPase activity and increase cardiac calcium levels, thereby disrupting normal cardiac function.⁶

DOX-induced myelosuppression results in a decline in bone marrow outputs and it has been linked to reactive oxygen species (ROS) generation that causes oxidative damage and apoptosis. The reduction of endogenous antioxidant levels and the accumulation of ROS can initiate the intrinsic apoptotic pathway in the blood stem cells thereby decreasing the red blood cell (RBC)³ count which is one of the signs of myelosuppression. Hemoglobin and hematocrit levels drop with less production of RBCs hence they are affected by RBCs levels in blood.^{9,10}

The liver (the body's chief organ of metabolism) is also affected by DOX toxicity. As much as 30.4% of hepatotoxicity cases was identified in patients who had undergone DOX treatments.¹¹ DOX metabolism leads to the accumulation of ROS in the liver cells leading to oxidative injury.¹² When the liver is injured or damaged, certain enzymes produced mainly by the hepatocytes such

as aspartate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) leak into the bloodstream. The increase of these enzymes in blood can be detected by biochemical assays. DOX causes an increase in the levels of these enzymes which is a sign of liver toxicity.⁵

In clinical practice, if myelosuppression is noticed during chemotherapy, the physician may either reduce or stop the treatment temporarily, perform a blood transfusion to replenish blood cells, or administer treatments that will boost bone marrow production of blood cells.¹³ Moreover, conventional drugs including dexrazoxane, beta-blockers, and statins have been tried to manage DOX toxicity.¹⁴⁻¹⁶ Unfortunately, these medical interventions had limited success^{13,15,16} thereby leaving a pressing need to explore alternative medical interventions, particularly from plant sources known for their potential therapeutic effects and relative safety.^{17,18}

Andrographis paniculata is one of the naturally derived medications being studied for the treatment of DOX-induced toxicity.¹⁹ It is an herbal plant and a member of the *acanthaceae* family, commonly referred to as "Sambiloto" in Indonesia.²⁰ It is largely used in many parts of the world because of its good medicinal properties including, anti-inflammation, antioxidants, anti-cancer activities, and hepatoprotective activities.²⁰ *A. paniculata* consists of numerous bioactive substances: Diterpene lactones (neo-andrographolide, deoxy-andrographolide, and 14-deoxy-11,12-didehydroandrographolide, andrographolide), diterpene glucoside (deoxyandrographolide 19-d-glucoside), and flavonoids (5,7,2',3'-tetramethoxyflavone) are found in *A. paniculata* leaves.²¹

The plant's major active constituent is andrographolide ($\text{C}_{20}\text{H}_{30}\text{O}_5$) which was identified in 1951.²² Due to *A. paniculata*'s largely documented anti-inflammatory effect on cardiovascular diseases²³, the need to check this effect on DOX-induced cardiac inflammation became imperative. In addition, although preliminary studies, *A. paniculata*'s effect on DOX-induced myelosuppression (with a focus on anemia) and hepatotoxicity were investigated; Interestingly, no studies on this exact subject were found. This study was therefore designed to show the protective effects of ethanolic extract of *A. paniculata* leaves (EEAP) against DOX-induced cardiac inflammation, anemia, and hepatotoxicity in rats.

Materials and methods

Study Design and Animal Treatment

Blood samples and heart tissue from 30 Sprague-Dawley male rats (6-8 weeks old, 150-200 g) obtained from the Indonesia National Agency of Drug and Food Control in Jakarta, Indonesia, were utilized specifically for this research. Animal care strictly followed the established guidelines and regulations for the humane treatment of experimental animals. Animal was housed in a controlled environment with a 21°C temperature, 55% relative humidity, and a light/darkness 12-hours cycle. Additionally, the liberty to access laboratory-standardized water and food was provided to the experimental animals.

The dose of DOX used to induce cardiac inflammation, as well as hematological and biochemical variations was 4 mg/kgBW/week for 4 weeks and administered intraperitoneally (i.p) using tuberculin syringe. The determination of DOX dosage administered in this study was consistent with the dose used in a previous study.²⁴ DOX was purchased from Kalbe Pharma (Jakarta, Indonesia) as DOX-hydrochloride (2 mg/mL).

Following a two-week acclimatization, 30 healthy rats were randomly divided into five groups of six each. The treatments administered to each of the 5 groups were as follows: Group 1 (Normal group) was treated with NaCl (0.9%) i.p. once a week for four weeks and it was labeled as a positive control group for baseline determination of values for various parameters; Group 2 (DOX group) was treated i.p. with 4 mg/kgBW/week DOX to make a cumulative dose of 16 mg/kgBW for the four weeks treatment period; Group 3 (DOX+EEAP125), Group 4 (DOX+EEAP250) and Group 5 (DOX+EEAP500) received doxorubicin i.p. at the same dose as group 2 but with the addition of either 125, 250 or 500 mg/kgBW/day of EEAP, orally for 4 weeks respectively. All experimental protocols were approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia. (No: KET-822/UN2.F1/ETIK/PPM.00.02/2022.)

Plant Materials

EEAP administered was formulated by PT Konimex (Sukoharjo, Indonesia) in the form of capsules that was specifically for research only. The leaf extract (standardized extract) contained 8.98% andrographolide (a main active compound of *A. paniculata*). Dried leaves were crushed

to fine particles; Percolation was done with 90% ethanol (solvent); solvent was evaporated at 60°C under vacuum; condensed extract was dried using a fluid bed granulator; dried extract was processed into capsules containing 125 mg each, with 48% native *A. paniculata* extract content. In a previous study, an acute toxicity test was performed for this extract. The LD₅₀ of the native extract was estimated to be greater than 2000 mg/kgBW.²⁵

Hematology and Biochemical Analysis

Hemoglobin, Hematocrit, and RBCs were measured using hematology analyzer (One Tech Medical, Guangzhou, China). AST, ALT, and ALP were measured using specific kits from DiaSys Diagnostic Systems (Holzheim, Germany) for each one of them according to the manufacturer's protocol. The concentration of NFκB in heart tissue was analyzed using an ELISA kit (Cat. No. BZ-22183961-EB; Bioenzy, Jakarta, Indonesia). Cardiac calcium concentration was analyzed using the Elabscience kit (Cat. No. E-BC-K207-M; Elabscience, Houston, TX, USA) and total cardiac protein was determined by the Bradford method.²² Malondialdehyde (MDA) levels in rat serum were determined using the Thiobarbituric acid reactive substance (TBARS) method and expressed as nmol/mL to assess lipid peroxidation (oxidative stress marker).²⁶

RNA Isolation, cDNA Synthesis, and qRT-PCR

The heart tissue was mechanically disrupted using an Ultra Turrax homogenizer (IKA, Selangor, Malaysia). Subsequently, total RNA was extracted from the homogenate employing the Trizol reagent (Zymo Research, Orange, CA, USA), following the manufacturer's recommended protocol. Spectrophotometric analysis at 260 nm determined the RNA concentration and purity, assessed by the A260/A280 ratio. Only samples exhibiting sufficient purity were employed for downstream cDNA synthesis. cDNA synthesis was performed using the ReverTra Ace® qPCR RT Master Mix (Lot. No. 245600; Toyobo BioTech, Osaka, Japan) following the manufacturer's protocol. The resulting cDNA concentration and purity were assessed spectrophotometrically at 260 nm. The genes were amplified using a well-established quantitative real-time polymerase chain reaction (qRT-PCR) method. The kit used was SensiFAST™ SYBR® No-ROX kit mix (Lot No. SF581-B112980; Meridian Bioscience Cincinnati, OH, USA). Uniform thermal cycling parameters were used

for all three genes (including the reference gene): 95°C denaturation, 60°C annealing, and 72°C extension. The genes and sequences were presented in Table 1.

Table 1. Gene-specific primers used for qRT-PCR.

Gene	Primer Sequence	Product Length (bp)
<i>β-Actin</i>	Forward: TAATGTCACGCACGATTTC	222
	Reverse: TGTTGTCCCTGTATGCCTCT	
<i>IL-1β</i>	Forward: CTGAAAGCTCTCCACCTCAAT	85
	Reverse: CGTTGCTTGTCTCTCCTTGTA	
<i>NLRP3</i>	Forward: CAGGATCTCGCATTGGTTCT	79
	Reverse: CTGAGTCTCCCAAGGCATTT	

Statistical Analysis

GraphPad Prism software 8.0 was used for the data analysis. Data were expressed as standard error of mean (SEM) on a bar plot. One-way analysis of variance (ANOVA) test was applied for comparing between groups, followed by the Bonferroni method for post hoc analysis. A p -value < 0.05 was considered statistically significant.

Results

EEAP Prevented Cardiac Inflammation and Cardiac Calcium Increase

The data for NFκB shows that the cardiac concentration of NFκB in rats of the DOX group tended to increase compared to the normal group. The cardiac concentration of NFκB in rats of both DOX+EEAP125 and DOX+EEAP250 groups showed tendencies to be reduced compared to the DOX group. DOX+EEAP500 did not show any favorable effect on the cardiac concentration of NFκB. Meanwhile, the data for *IL-1β* revealed that the mRNA expression of *IL-1β* in the DOX group was significantly increased compared to the normal group. While DOX+EEAP250 significantly decreased the mRNA expression of *IL-1β* compared to the DOX group, DOX+EEAP125, and DOX+EEAP500

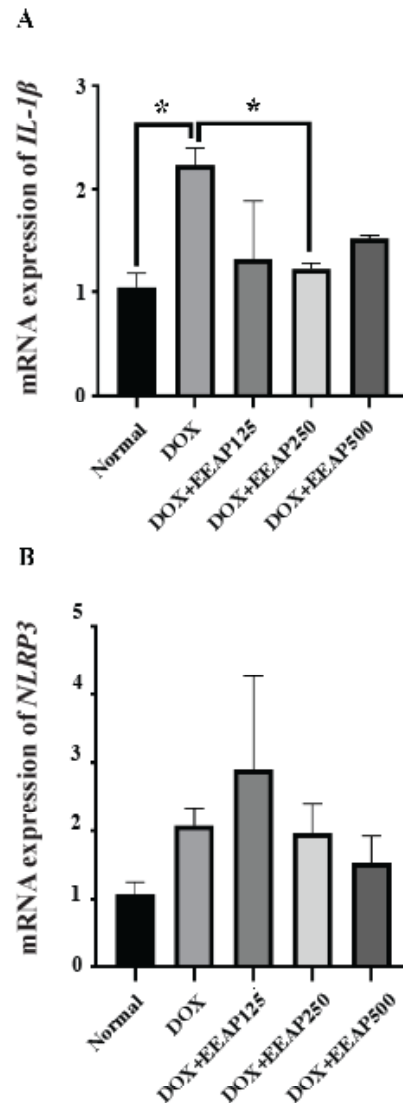


Figure 1. Effect of EEAP on DOX-induced elevations in the mRNA expression of *IL-1β* (A) and *NLRP3* (B). The values are presented as Mean±SEM. One-way analysis of variance (ANOVA) test was applied for comparing between groups, followed by the Bonferroni method for post hoc analysis (* p <0.05).

only showed tendencies to reduce the mRNA expression of *IL-1β* compared to the DOX group. The *NLRP3* data showed that the mRNA expression of *NLRP3* in the DOX group tended to be increased compared to the normal group. DOX+EEAP250 and DOX+EEAP500 but not DOX+EEAP125 showed tendencies to decrease the mRNA expression of *NLRP3* compared to the DOX group. These data were presented in Figure 1 and 2.

Moreover, the calcium data (Figure 2) revealed that the cardiac concentration of calcium in the DOX group tended to be increased compared to the normal group. DOX+EEAP125 and DOX+EEAP250 but not DOX+EEAP500 restored the concentration of cardiac calcium compared to the normal.

EEAP Reduced Serum MDA Level

The data for MDA level (Figure 2) shows that the DOX group was slightly elevated compared to the normal group. Only the DOX+EEAP500 group tended to decrease compared to the DOX group. Higher doses of EEAP resulted in a trend of decreasing MDA levels. Compared to the 125 mg dose, MDA levels tended to be lower at 250 mg, and further decreased at 500 mg.

EEAP Improved Liver Function Parameters

Figure 3 represented the result of liver function parameters. The data for aspartate aminotransferase (AST) showed an increased level between treatment groups, and the DOX group, compared to the normal group ($p<0.00001$). AST level in the DOX+EEAP500 group only, evidently reduced compared to the DOX group. Alanine transaminase (ALT) was elevated in the DOX group ($p<0.0001$) and all treatment groups compared to the normal group. The levels of ALP in the DOX group increased significantly compared to the normal group ($p<0.05$). Rats in all treatment groups decreased alkaline phosphatase (ALP) levels compared to the DOX group. Interestingly, in the DOX+EEAP125 and

DOX+EEAP250 groups, ALP was significantly reduced compared to the DOX group ($p<0.0001$ and $p<0.001$, respectively).

EEAP Prevented Anemia

The results for hematological parameters were presented in Figure 4. The data revealed that the percentage of hematocrit tended to increase in all the treatment groups except for DOX+EEAP500 after the four weeks of the treatment period compared with the DOX group. Rats in the normal and all treatment groups except DOX+EEAP500 showed a tendency to increase hemoglobin concentration compared to those in the DOX group. In addition, the levels of RBCs tended to be elevated in the normal group and all treatment groups except for the DOX+EEAP500 compared to the DOX group.

Discussion

Consistent with other studies^{5,8,27,28}, this study demonstrated that DOX treatment, induced toxicities as evidenced by cardiac inflammation and the alterations in the liver function (AST, ALT, and ALP) and hematological parameters (hematocrit, hemoglobin, and RBCs). Interestingly, co-treatment with EEAP showed tendencies to prevent these toxicities. EEAP have shown anti-inflammatory, hepato- protective and anti-myelosuppressive activities in previous studies.^{29,30} This study administered EEAP

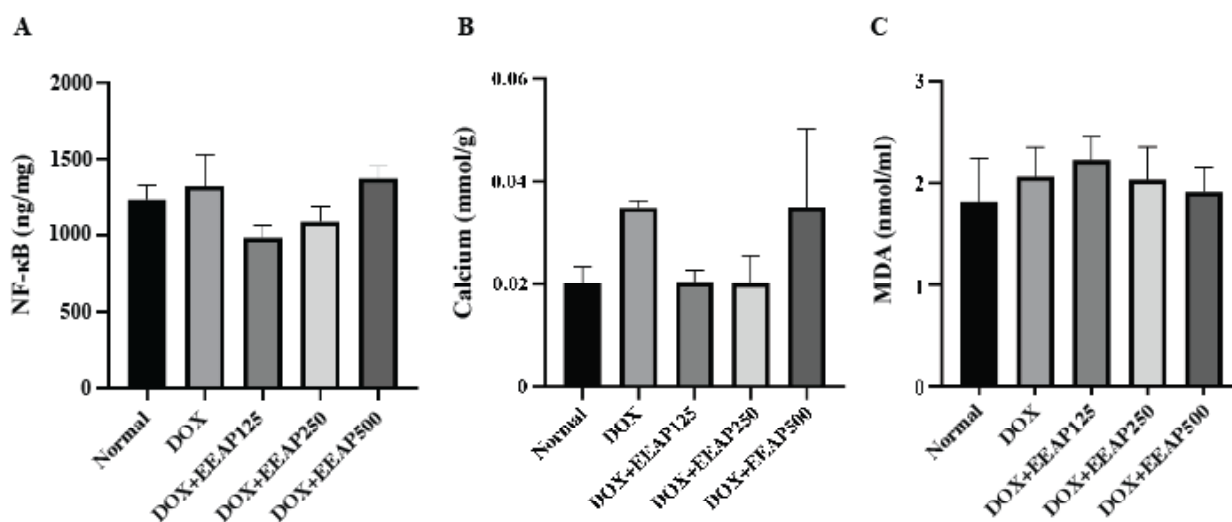


Figure 2. Effect of EEAP on DOX-induced elevations in the concentrations of NFκB (A), calcium (B), and serum MDA (C). The values are presented as Mean±SEM. One-way analysis of variance (ANOVA) test was applied for comparing between groups, followed by the Bonferroni method for post hoc analysis.

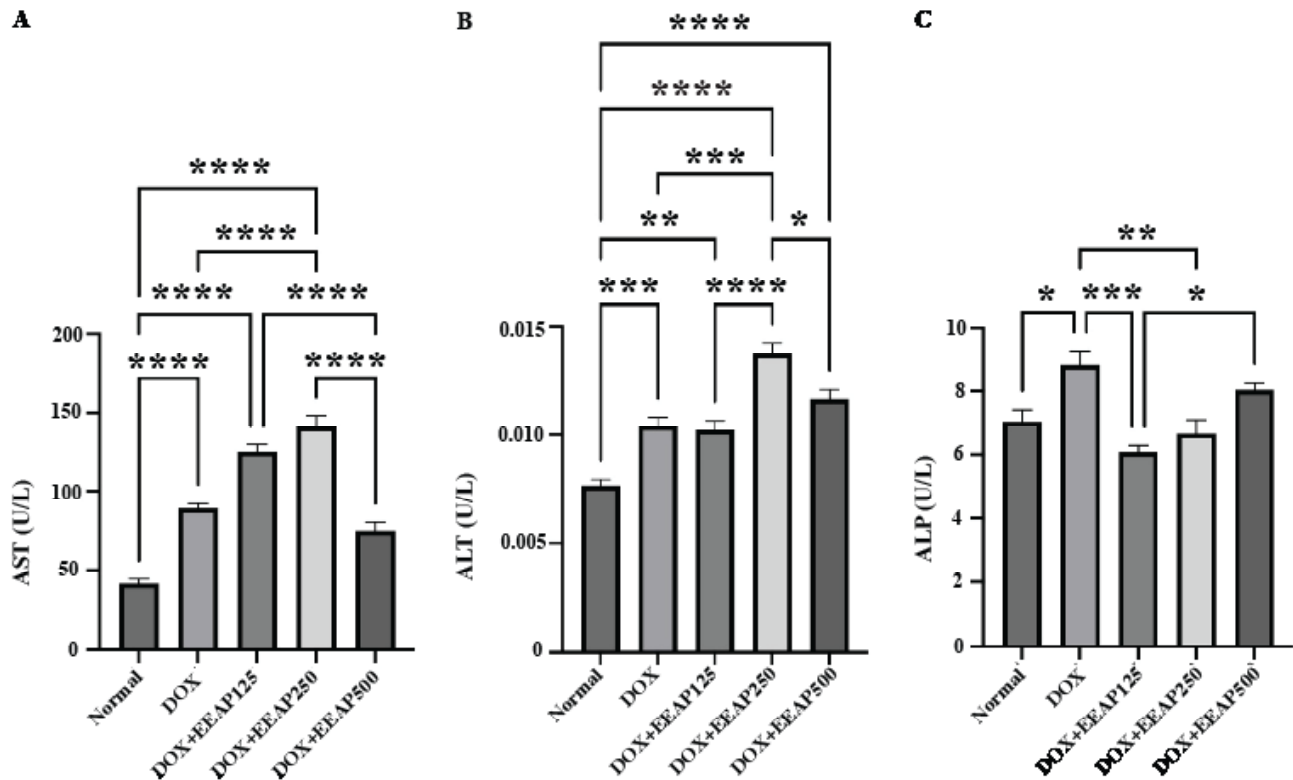


Figure 3. Effect of EEAP on DOX-induced variations on AST (A), ALT (B), and ALP (C) levels. The values are presented as Mean±SEM. One-way analysis of variance (ANOVA) test was applied for comparing between groups, followed by the Bonferroni method for post hoc analysis (* $p<0.05$; ** $p<0.001$; *** $p<0.0001$; **** $p<0.00001$).

at 3 different doses; 125 mg, 250 mg, and 500 mg. These doses were selected based on our preliminary study (unpublished data).

In this study, rats treated with DOX alone, although not statistically significant, slightly elevated the cardiac concentration of NFκB (Figure 2A). In addition, it also

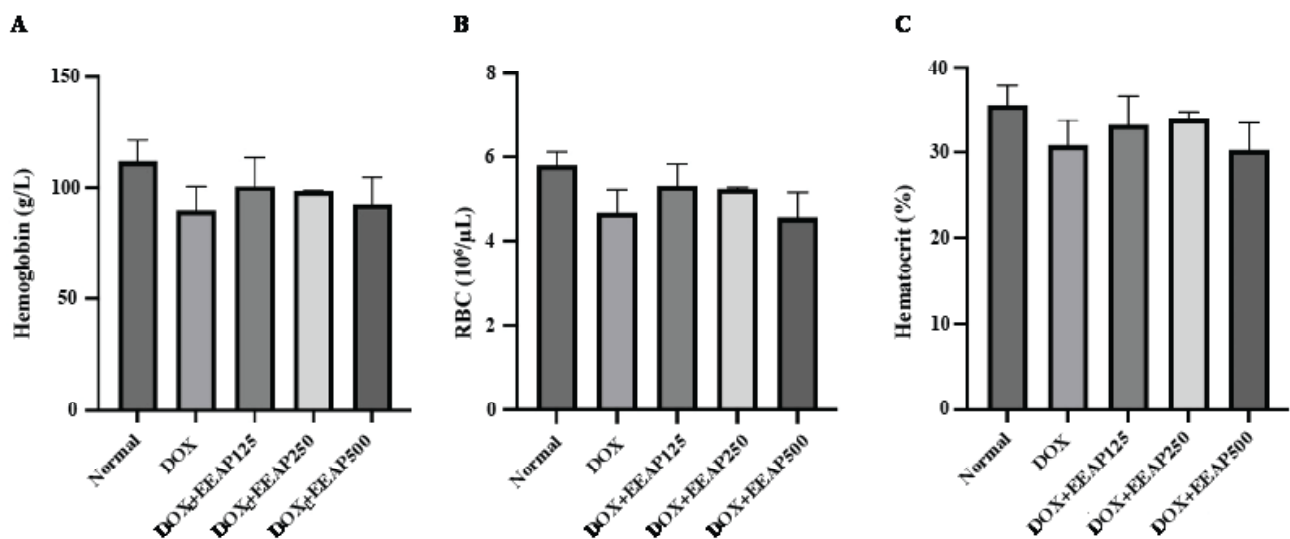


Figure 4. Effect of EEAP on DOX-induced variation on hemoglobin (A), RBCs (B), and hematocrit (C) levels. The values are presented as Mean±SEM. One-way analysis of variance (ANOVA) test was applied for comparing between groups, followed by the Bonferroni method for post hoc analysis.

observed that the cardiac mRNA expression of *IL-1 β* was significantly increased ($p<0.05$) but the mRNA expression of *NLRP3* was slightly increased (Figure 1A and 1B).

In a study using adult and infant mice, a cumulative dose treatment of 18 mg/kg of DOX for 3 weeks increased cardiac NF κ B levels in adult male mice but not in infant mice.⁸ This shows that the concentration of cardiac NF κ B in DOX-induced cardiac toxicity animal models may be influenced by age. Another study revealed that a cumulative dose treatment of 20 mg/kg of DOX for 4 weeks upregulated the cardiac mRNA expression of *NLRP3* in male mice.²⁸ A report also demonstrated that a cumulative dose of 12 mg/kg of doxorubicin treatment for 3 weeks elevated the mRNA expression of *IL-1 β* in male mice.²⁷ These three reports are in agreement with the results of this study.

The moderate elevation of NF κ B concentration and mRNA levels for *NLRP3* alongside the substantial increase in *IL-1 β* mRNA expression in this study, is reflective that some other factors in addition to NF κ B activation may contribute to the upregulation of *IL-1 β* and *NLRP3* mRNA expression in DOX-induced cardiotoxicity animal models. Meanwhile, co-treatment with EEAP in this study dose-dependently reduced the mRNA expression of *IL-1 β* ($p<0.05$) and showed tendencies to reduce NF κ B concentration and mRNA expression of *NLRP3* which is an indication that the standardized extract possesses great anti-inflammatory effect and is cardioprotective against DOX-induced toxicities.

Increase in cardiac calcium level is associated with DOX toxicity in rodents.⁶ Sodium influx depolarizes the cell, triggering voltage-gated calcium channels to open. Extracellular calcium floods and binds to ryanodine receptors on the sarcoplasmic reticulum, releasing calcium into the cytosol. This calcium binds to troponin, moving tropomyosin and enabling muscle cell contraction.^{31,32} This demonstrates that both extracellular and intracellular calcium are involved in cardiac muscle contraction. In a normal condition, during cardiac muscle relaxation, the Ca²⁺ ATPase regulates the transfer of calcium ions from the cytosol back to the sarcoplasmic reticulum.³¹ Several studies propose that DOX impairs Ca²⁺ ATPase activity, elevating cytosolic calcium and driving its uptake into mitochondria. This mitochondrial calcium overload disrupts function, leading to mitochondria dysfunction and cell death.^{33,34}

A study demonstrated that DOX-induced reduction in Ca²⁺ ATPase (raising cytosolic calcium) and increased total cardiac calcium,⁶ suggesting DOX disrupts both intracellular

and whole-organ calcium homeostasis. Similar to the previous study,⁶ DOX-only groups of rats modestly elevated cardiac calcium concentration (Figure 2B). Notably, co-treatment with EEAP demonstrated a dose-dependent trend toward restoring normal cardiac calcium levels, exhibiting its potential to restore calcium homeostasis and protect cells from damage. However, in the DOX+EEAP500 group (highest extract dose), calcium concentration increased to nearly the same level as the DOX alone group. This raises some concerns. As we know, diterpenoid lactones are one of the abundant classes of bioactive compounds present in *A. paniculata*.³⁵ Diterpenoid lactones, which include andrographolide, are poorly soluble in water.³⁶ Poorly soluble compounds administered at high doses may cause toxicity.³⁷ Although no research has directly linked this specific extract to such an effect (cardiac calcium elevation) at high doses (≥ 500 mg), it is possible that the observed elevation in calcium levels, or other negative consequences as observed in this study, could be due to the presence of abundant diterpenoid lactones. Further studies are therefore required to elucidate the exact mechanism behind this observation.

AST, ALT, and ALP, elevations in plasma caused by the DOX treatment are established signs of hepatotoxicity.⁵ In this study, DOX-only treatment significantly elevated the serum levels of AST, ALT, and ALP (Figure 3A, 3B and 3C), consistent with another study⁵ and proof of liver toxicity. Co-treatment with EEAP showed a favorable effect on AST and ALP by dose-dependently decreasing their values. However, the extract's inability to improve ALT values raises some concerns. It is imperative to note that ALT is mainly produced by hepatocytes, with only a trace of it in other organs. In contrast, AST exists in two forms: a mitochondrial version found in liver cells and a cytosolic version. Regular liver function tests may not separate between the two isoforms.³⁸ ALP which are the isoenzymes that are located on the outer layer of the cell membrane is found in numerous tissues throughout the body particularly common in the ileal mucosa, hepatic, skeletal, and renal tissue.³⁹ Therefore, considering AST and ALP's presence in other tissues alongside ALT's liver specificity, the extract might have protected tissues beyond the liver²⁹ from DOX damage, explaining the reduced AST and ALP values while only minimally impacting ALT. Figure 3A and 3B show a concerning elevation in AST levels for the DOX+EEAP125 and DOX+EEAP250 groups compared to DOX alone group, with similar increases in ALT observed

for the DOX+EEAP250 and DOX+EEAP500 groups. To explain this, further studies are needed, particularly, histopathological examination of liver tissues could be performed to identify any morphological changes in these groups. This would provide valuable insight into the effects of each of the administered doses of EEAP at the tissue level.

DOX-induced toxicity is also associated with reduced RBCs which is a sign of anemia.³ The RBCs contain a protein called hemoglobin which is responsible for oxygen delivery to tissues. In contrast, hematocrit compares the volume of RBCs to the total volume of blood (RBCs plus plasma). Hemoglobin and hematocrit levels will therefore drop with less production of RBCs.^{9,10} Although the effect of DOX on RBCs, hemoglobin, and hematocrit in this study was not statistically significant, it however, showed a tendency to decrease these three parameters which were consistent with the other studies that postulated that DOX treatment declined, RBCs, hemoglobin, and hematocrit in rat's plasma.^{3,5} Co-treatment with EEAP dose-dependently showed tendencies to increase these hematological parameters. The non-statistical result of these parameters upon DOX treatment is reflective that the toxicity in this study may not be localized in blood.

The mechanism by which DOX induces myelosuppression and hepatotoxicity is via free radicals/ROS generation, which affects normal cells.^{3,40} The consequence of ROS generation is oxidative stress and inflammation and then progress to other dysfunctions in normal cells and apoptosis.⁴⁰ Increased ROS production and a lack of endogenous antioxidants activate the intrinsic apoptotic pathway in hematopoietic cells which is a sign of myelosuppression.³

In this study, serum lipid peroxidation (oxidative stress marker) was investigated by measuring the MDA levels. DOX treatment only showed a tendency to increase MDA serum levels but wasn't significant compared to other models due to differences in the experimental design.⁴¹ Even though lipid peroxidation was not significant in this model, co-treatment with EEAP demonstrated tendencies to reduce lipid peroxidation as it dose-dependently tended to reduce MDA serum levels particularly in the DOX+ES500 group compared to the DOX group, and as a consequence, the liver toxicity was alleviated. The result for MDA compared to that of the hematological parameters suggests that the lack of significant reductions in these parameters after DOX treatment may be due to the lack of a substantial increase of

lipid peroxidation (oxidative stress marker) level in serum.

In summary, this study revealed localized DOX toxicity primarily in tissues, not blood. The standardized EEAP displayed promising cardioprotective effects, reducing inflammation and exhibiting the potential to restore normal calcium homeostasis in the heart. Additionally, it showed tendencies for managing DOX-induced anemia and protecting against liver toxicity. These findings warrant further investigation to explore EEAP's potential as a therapeutic agent for mitigating DOX-induced side effects, including the related parameters for ROS, mainly anti-oxidant enzymes that decreases as a result of toxicity. Further studies are needed to explore EEAP's impact on a wider range of hematological and liver function markers. Furthermore, elucidating the underlying mechanism of action for its protective effects against DOX-induced cardiotoxicity, myelosuppression, and liver toxicity is crucial for optimizing its prevention potential. Future studies should also ascertain the accurate dose for a significant therapeutic effect.

Conclusion

These findings suggest that the co-treatment with standardized EEAP has cardiac anti-inflammatory effects and promotes the restoration of some hematological parameters potentially mitigating DOX-induced anemia. Additionally, it demonstrates the tendency to mitigate DOX-induced liver toxicity by normalizing specific biochemical parameters.

Acknowledgment

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Authors Contributions

OME, WA, SIW, and PEW were involved in the conception and planning of the research. OME, YRD, SD, N and DAN performed data collection including animal experiments. SIW, OME, WA, ML, and PEW analyzed the data collected including statistical analysis. All authors contributed to drafting manuscript, designing figures, and revising manuscript before submission.

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