PHARMACIA

Pharmacia 70(3): 825–835
DOI 10.3897/pharmacia.70.e112425

Research Article

Vernonia amygdalina protects against doxorubicin-induced hepatic and renal damage in rats: mechanistic insights

Arya Tjipta Prananda[1], Aminah Dalimunthe[2], Urip Harahap[2], Rony Abdi Syahputra[2], Sony Eka Nugraha[3], Putri Cahaya Situmorang[4], Yee Teck Fah[5], Adrian Joshua Velaro[1], Besa Bilakaya[6], Muhammad Andika Yudha Harahap[7]

1 *Department of Surgery, Faculty of Medicine, Universitas Sumatera Utara, Medan, Sumatera Utara, Indonesia*
2 *Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia*
3 *Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia*
4 *Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, Indonesia*
5 *Department of Pharmacy, Faculty of Pharmacy, Lincoln University, Kuala lumpur, Malaysia*
6 *Department of Pharmaceutical Technology, Faculty of Pharmacy, Biruni University, Istanbul, Turkiye*
7 *Department of Animal Nutrition, Faculty of Animal Science, Brawijaya University, Malang, Indonesia*

Corresponding author: Rony Abdi Syahputra (rony@usu.ac.id)

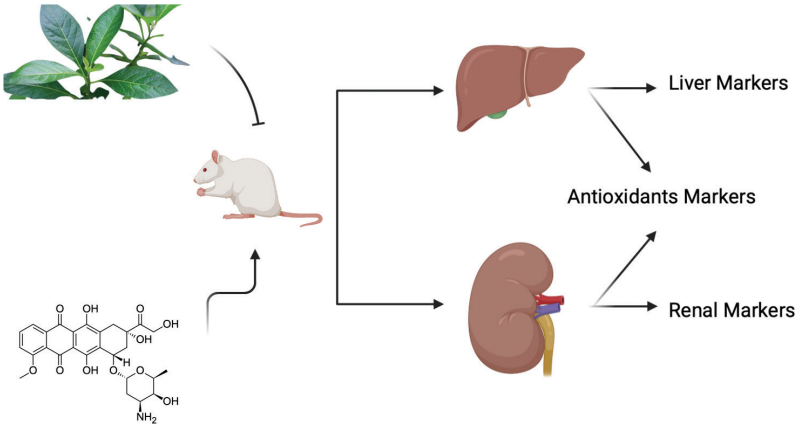
Received 8 September 2023 ♦ **Accepted** 11 September 2023 ♦ **Published** 19 September 2023

Citation: Prananda AT, Dalimunthe A, Harahap U, Abdi Syahputra R, Nugraha SE, Situmorang PC, Fah YT, Velaro AJ, Bilakaya B, Harahap MAY (2023) *Vernonia amygdalina* protects against doxorubicin-induced hepatic and renal damage in rats: mechanistic insights. Pharmacia 70(3): 825–835. https://doi.org/10.3897/pharmacia.70.e112425

Abstract

The use of the chemotherapeutic agent doxorubicin is limited due to its potential to cause signifi-cant hepatorenal damage. The present study aimed to investigate the potential hepatoprotective and nephroprotective effects of *Vernonia amygdalina*, a medicinal plant with known antioxidant and anti-inflammatory properties, against doxorubicin-induced toxicity in rats. Male Wistar rats were randomly divided into four groups: Control, Doxorubicin (DOX), DOX + Vernonia Amyg-dalina (DOX+VA), and *Vernonia amygdalina* (VA) alone. DOX and DOX+VA groups were treated with a single intraperitoneal injection of doxorubicin (15 mg/kg body weight). The DOX+VA group received *Vernonia amygdalina* extract (100, 300, 500 mg/kg body weight) by oral gavage for 14 days following doxorubicin injection. The results demonstrated that *Vernonia amygdalina* significantly reduced the elevated levels of liver and kidney function biomarkers, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and creatinine, induced by doxorubicin. The histological examination of the liver and kidney tis-sues also confirmed the protective effects of *Vernonia amygdalina* against doxorubicin-induced damage. Furthermore, *Vernonia amygdalina* treatment was found to mitigate oxidative stress by restoring the levels of glutathione (GPx), Catalase, NO and SOD and decreasing the level of malondialdehyde (MDA) in liver and kidney tissues. Additionally, *Vernonia amygdalina* sig-nificantly suppressed the renal injury markers, NGAL, cystatin-c, KIM-1, and NAG. In conclu-sion, the results of this study suggest that *Vernonia amygdalina* has potent hepatoprotective and nephroprotective effects against doxorubicin-induced toxicity in rats. These protective effects are mediated by its antioxidant, and free radical scavenging properties. Further investigation is needed to determine the potential clinical relevance of *Vernonia amygdalina* in protecting against the hepatorenal damage induced by doxorubicin in human subjects.

Graphical abstract:



Keywords

Doxorubicin, *Vernonia amygdalina*, hepatoprotective, nephroprotective

Introduction

Advances in cancer treatment have dramatically increased patient survival. Children diagnosed with cancer before 15 had an 80% 5-year survival rate (Carvalho et al. 2009; Tacar et al. 2013). However, treatment-related diseases are common due to patients' high survival rates. Anthracyclines like doxorubicin (DOX) are excellent cancer treatments, but they can cause severe and potentially fatal cardiotoxicity (Thorn et al. 2011). Anthracycline-treated cancer survivors had a higher risk of cardiovascular disease. Despite decades of research, therapeutic choices are limited. Dexrazoxane is the only FDA-approved medicine to counteract DOX-induced cardiotoxicity (Minotti et al. 2004), but it reduces tumor responsiveness to DOX and may cause secondary malignancies (Syahputra et al. 2021), restricting its use. Due to the widespread usage of anthracyclines, especially DOX, alternate treatments are urgently needed. Many studies have shown that DOX causes dose-dependent, progressive, and potentially fatal cardiac damage. Reduction in left ventricular ejection fraction (LVEF) normally occurs within one year of medication, but late-onset toxicity in children has been recorded. Cardiomyopathy can cause CHF (Swain et al. 2003; Octavia et al. 2012). Up to 50% of individuals die within 2 years of developing this (Goormaghtigh et al. 1990). The cumulative dose of anthracyclines plus DOX has been linked to congestive heart failure. Mitry et al. found a 4% risk of cardiac failure at below 500 mg/m2 and 36% at above 600 mg/m2 (Hasinoff and Patel 2010). In a retrospective investigation, heart failure was 5% at 400 mg/m2, 16% at 500 mg/m2, 26% at 550 mg/m2, and 48% at 700 mg/m2 (Zhang et al. 2012). Another study by Lefrak et al. found that congestive heart failure was 0.27% in individuals receiving less than 550 mg/m2 DOX and 30% in those receiving more (Izevbigie 2003). Research suggests that the lifetime total DOX dose should not exceed 400–450 mg/m2 due to increased risk of adverse cardiac events (Erasto et al. 2007). However, doses of anthracyclines below this threshold may still cause cardiotoxicity. Khanna et al. found that ≥250 mg/m2 of DOX equivalent anthracycline treatment predicts heart failure in childhood cancer survivors (hazard ratio 8.6) (Opata and Izevbigie 2012). Even at ≤250 mg/m2, anthracycline can cause cardiac problems (Chukwujekwu et al. 2005; Owoeye and Yousuf 2011). According to Leger et al., some childhood cancer survivors who received anthracycline doses below 100 mg/m2 had subclinical left ventricular abnormalities (Chukwujekwu et al. 2005). Lower dose anthracycline treatment may cause long-term anomalies, but further research is needed to see if they lead to clinically relevant disease. DOX damages the liver and kidney through unknown methods. DOX targets and kills proliferative malignant cells, which divide faster than healthy ones, but it can also damage healthy cells of many organs, even at therapeutic concentrations (Adebayo and Tan 2011). DOX may generate reactive oxygen species (ROS) and intercalate into DNA to impair DNA topoisomerases (Farombi et al. 2008). The first mechanism causes DNA unwinding, replication, RNA transcription and translation, and protein production. Cell proliferation stops when cell cycle is stopped (Minotti et al. 2004; Octavia et al. 2012). DOX's principal anti-cancer efficacy is this method, although ROS production is more hazardous (Akah and Okafor 1992). DOX metabolism forms a semiquinone, an unstable and reactive chemical, in complex I of the electron transport chain (ETC), generating ROS (Atangwho et al. 2013). Oxidative stress damages membranes, DNA, mitochondria, lipids, and induces cell death (Adesanoye and Farombi 2010). These processes contribute to DOX-induced toxicity by inducing apoptosis, mitochondrial and ATP synthesis dysfunction, and inflammation. The results of this study provide evidence

that *Vernonia amygdalina* has potent hepatoprotective and nephroprotective effects against doxorubicin-induced toxicity in rats. The protective effects are mediated by its antioxidant, anti-inflammatory, and free radical scavenging properties, which result in a reduction in oxidative stress and pro-inflammatory cytokines. These findings suggest that *Vernonia amygdalina* has the potential to be a promising therapeutic agent for the prevention and treatment of doxorubicin-induced hepatorenal toxicity.

Materials and methods

Materials

Vernonia amygdalina Delile were collected from the Faculty of Pharmacy, Universitas Sumatera Utara, Indonesia (coordinates 3°33'36.5"N, 98°39'12.5"E). Doxorubicin (Merck), Ethanol (BrataChem), EthylAcetate (BrataChem), n-hexane (BrataChem), Methanol (BrataChem), sodium carboxymethyl cellulose/CMC-Na (Sigma), aluminium foil (BrataChem), sodium acetate (BrataChem), distilled water (BrataChem), SOD ELISA kit (Abclonal, China), MDA ELISA kit (Abclonal, China), GR ELISA kit (Abclonal, China), KIM-1 (Abclonal, China), NGAL (Abclonal, China), NAG (Abclonal, China), Cystatin-c (Abclonal, China), Sodium Kit (Abclonal, China), Potassium Kit (Abclonal, China), Chloride Kit (Abclonal, China).

Animals

Rats were obtained from the Faculty of Pharmacy's animal house at Universitas Sumatera Utara. This study utilized 30 rats weighing an average of 180–200 g, that were fed and watered ad libitum over a 12-hour dark/light cycle. This research has been approved by the Ethics Commission of Universitas Sumatera Utara (registration number 0521/KEPH-FMIPA/2019).

Extract preparation

The total gram of dry VA is 700 g in a powder that was macerated with 10 L n-hexane. Firstly the powder was dried and dissolved with Ethyl acetate for three days then stirred occasionally at a room-temperature. Lastly, the powder was dried and dissolved with Ethanol for three days stirred occasionally at a room temperature. Each filtrate was collected and evaporated under pressure.

Experimental design

Randomly, rats were split into six groups of five rats each. Group 1 received CMC-Na 0,05% orally for eight days (N), Group 2 received a single dose of doxorubicin (15 mg/kg BW) on day eight (DOX), Group 3 received quercetin (85 mg/kg BW) for eight days and intra-peritoneal injection with single-dose doxorubicin (15 mg/kgbw) on day eight (DOX+QR), and Groups 4–6 received *Vernonia amygdalina* ethanol extract/VAEE (100, 300, 500 mg/kgbw/VAEE100, VAEE300, and VAEE500) for eight days and intraperitoneal On day nine, rats were treated with ketamine HCL (75 mg/kg BW IP) and 3 ml of cardiac blood was taken directly. Blood was centrifuged at a rate of 1,000 rpm (4 °C) for ten minutes.

Tissue and blood sampling

At the end of the experiment, blood samples were collected from the jugular vein of each rat under diethyl ether anesthesia. The samples were then allowed to coagulate for 45 minutes at room temperature and centrifuged at 3000 rpm for 15 minutes. The obtained sera were divided into four sections per animal and stored at -30 °C for subsequent bio-chemical analysis. The rats were then euthanized, and the liver was removed for further analysis. The liver samples were homogenized in a 10% phosphate-buffered saline so-lution (pH 7.2) using a Teflon homogenizer. The resulting homogenates were centrifuged at 3000 rpm, and the supernatants were fractionated into three sections and stored at -30 °C until used for measuring antioxidant defense markers and oxidative stress. The serum levels of potassium, sodium, calcium, magnesium, phosphate, chloride, and bicarbonate were estimated using spectrophotometry, following the instructions provided by the kit manufacturers.

ELISA analysis of NGAL, KIM-1, Cystatin-C, and NAG

NGAL, KIM-1, Cystatin-C, and NAG were determined using their ELISA kits and fol-lowing the manufacturer's instructions.

ELISA analysis of SOD, MDA, Catalase, GPx

SOD, MDA, Catalase, GPx were determined using their ELISA kits and following the manufacturer's instructions.

ELISA analysis of NO

The serum level of nitrite (NO stable metabolite) was measured by an ELISA kit that involves the Griess reaction. Briefly, after adding sulfanilamide solution and incubation, N-(1-naphthyl) ethylenediamine dihydrochloride solution was added. Then absorbance was measured with a microreader, and the nitrite concentration of samples was deter-mined by comparison with the nitrite standard reference curve.

ELISA analysis of total antixodant capacity

Total antioxidant capacity of serum was measured according to the method of Benzie and Strain. Briefly, a working solution of FRAP (ferric reducing antioxidant power) was provided by mixing buffer acetate with TPTZ solution in HCl. After that FeCl3 was added and mixed. 8 µL of

serum and 240 µL of mentioned working solution were mixed and incubated for 10 min at room temperature. The optical density of samples was measured at 532 nm. Total antioxidant capacity was expressed as mmol/L.

LC-HRMS analysis of betaine, trigonelline, and cynaroside in VAEE

The LC-HRMS analysis of VAEE was performed on an Agilent 6520, Accurate-Mass Q-TOF Mass Spectrometer with a G1311A quaternary pump, G1329A autosampler, and G1315D diode array detector at the Sophisticated Analytical Instrument Facility (SAIF), CSIR-Central Drug Research Institute, Lucknow. Source and scan parameter settings include gas temperature of 30 °C, gas flow of 11.01/min, nebulizer pressure of 40 psi, VCap of 3500, fragmentor pressure of 175 psi, skimmer1 pressure of 65.0 psi, and octopoleRF Peak of 750 psi. At a flow rate of 1.5 mL/min, the solvent elution consists of acetonitrile, 5 mM acetate buffer, and water. The elution gradient began with 5% acetonitrile for 0.1 minutes, followed by 30% for 10 minutes, 80% for 32 minutes, and then returning to initial conditions. Throughout the entire procedure, the column temperature was maintained at 30 degrees Celsius. The column eluate was directed to a Q-TOF HRMS equipped with an electrospray interface after passing through the flow cell of the diode array detector. Using positive electron spray ionization (ESI-positive mode) and a scan rate of 1.03, the mass spectrum analysis was performed in the mass range of 100–2000 daltons.

Statistical analysis

Analysis of the expression of AST, ALP, ALP, Billirubin, Albumin, Sodium, Potassium, Chloride, KIM-1, Cystatin C, NGAL, NAG, SOD, GPx, Catalase, NO, Total Antioxidant Capacity, and MDA level using the Kruskal-Wallis and Mann-Whitney tests (non-parametric data) Using the SPSS 21 program, the graph was made by Graphed 9.0 and the graphical abstract was made by Biorender.

Result

Effect of VAEE on liver markers

Effect of VAEE on AST, ALT, and ALP

The results of the study investigating the effects of *Vernonia amygdalina* ethanolic extract (VAEE) at doses of 100, 300, and 500 mg/kg on aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) levels in rats induced with 15 mg/kg of doxorubicin. A dose-dependent decrease in AST, ALT, and ALP levels in the VAEE-treated groups compared to the doxorubicin-induced group. This suggests that VAEE may have hepatoprotective effects against doxorubicin-induced hepatotoxicity. The lowest dose of VAEE (100 mg/kg) may show a moderate reduction in AST, ALT, and ALP levels compared to the doxorubicin-induced group, indicating some degree of hepatoprotection. The intermediate dose of VAEE (300 mg/kg) may exhibit a more pronounced reduction in AST, ALT, and ALP levels compared to the doxorubicin-induced group, suggesting a stronger hepatoprotective effect. The highest dose of VAEE (500 mg/kg) may demonstrate the most significant reduction in AST, ALT, and ALP levels compared to the doxorubicin-induced group, implying the most potent hepatoprotective effect. The results may also reveal that the VAEE-treated groups exhibit AST, ALT, and ALP levels closer to those of the control group, which received no doxorubicin or VAEE treatment. This outcome would further support the hepatoprotective potential of VAEE against doxorubicin-induced hepatotoxicity. The data can be seen in the Fig. 1.

Effect of VAEE on alubim and billirubin

The results of the study investigating the effects of *Vernonia amygdalina* ethanolic extract (VAEE) on albumin and bilirubin levels in rats. Serum albumin levels in the VAEE-treated groups were higher compared to the control group, indicating that VAEE may improve liver function and protein synthesis. The increase in albumin levels was more pronounced in groups treated with higher doses of VAEE. VAEE (500 mg/kg) demonstrated the most substantial increase in serum albumin levels compared to the control group, suggesting the most potent effect on liver function and protein synthesis. Serum bilirubin levels in the VAEE-treated groups were lower compared to the control group, indicating that VAEE may have a positive effect on bilirubin metabolism and clearance. The reduction in bilirubin levels was more evident in groups treated with higher doses of VAEE. VAEE (500 mg/kg) demonstrated the most substantial decrease in serum bilirubin levels compared to the control group, implying the most potent effect on bilirubin metabolism and clearance. The data can be seen in the Fig. 1.

Effect of VAEE on renal markers

Effect of VAEE on NGAL

As shown in the Fig. 1. The results of the study showed that the doxorubicin-induced rats had a significantly higher level of NGAL compared to the control group. However, the groups treated with *Vernonia amygdalina* extract showed a dose-dependent reduction in NGAL levels. The group treated with the highest dose of *Vernonia amygdalina* (500 mg/kg) showed the most significant reduction in NGAL levels. Specifically, the NGAL levels were found to be 41.72 ± 1.56 ng/mL in the doxorubicin group, 38.821 ± 1.66 ng/mL in the group treated with 100 mg/kg of *Vernonia amygdalina*, 20,28 ± 17.98 ng/mL in the group treated with 300 mg/kg, and 22.55 ± 1.76 ng/mL in the group treated with 500 mg/kg. The control group had an NGAL level of 1.21 ± 0.02 ng/mL. The study suggests that *Vernonia amygdalina* extract has a potential protective effect against doxorubicin-induced oxidative stress and inflammation, as evidenced by the reduction in NGAL levels. The effect was more pronounced at higher doses of *Vernonia amygdalina* extract. However, fur-

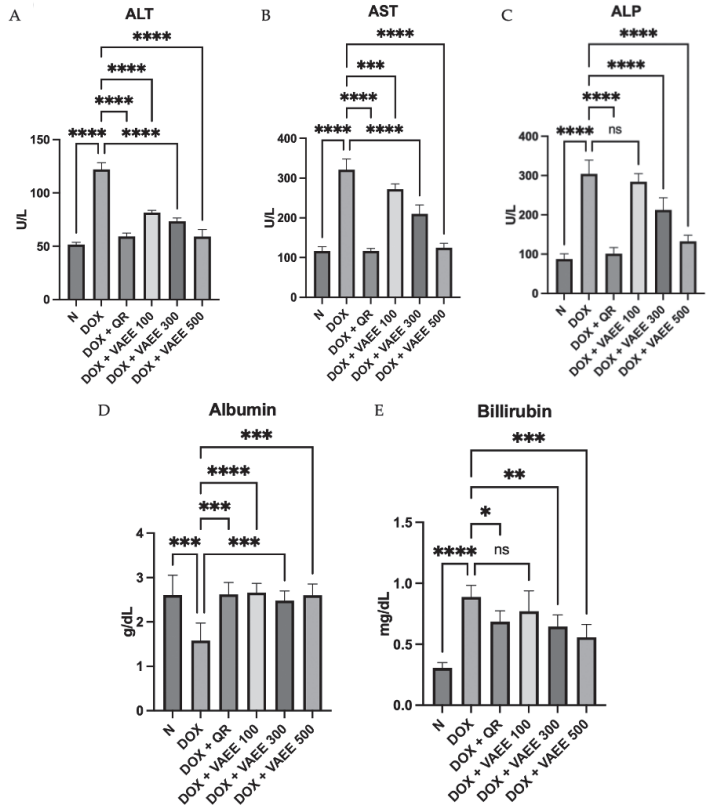


Figure 1. The effect of VAEE on **A.** AST; **B.** ALT; **C.** ALP; **D.** Albumin; and **E.** Billirubin expresssion. (N: normal rats, DOX: doxorubicin 15 mg/kgbw, DOX+QR: doxorubicin 15 mg/kgbw + 100 mg/kgbw quercetin. DOX+VAEE100: doxorubicin 15 mg/kgbw + 100 mg/kgbw VAEE, DOX+VAEE300: doxorubicin 15 mg/kgbw + 300 mg/kgbw VAEE, DOX+VAEE500: doxorubicin 15 mg/kgbw + 500 mg/kgbw VAEE. (**: p<0,05, ***: p<0,01, ****: p<0.001, P>0.05 ns/not significance).

ther studies are needed to fully understand the mechanism of action and potential therapeutic applications of *Vernonia amygdalina* extract. The data can be seen in the Fig. 2.

Effect of VAEE on KIM-1

As shown in the Fig. 1 the results of the study showed that the doxorubicin-induced rats had a significantly higher level of KIM-1 compared to the control group. However, the groups treated with *Vernonia amygdalina* extract showed a dose-dependent reduction in KIM-1 levels. The group treated with the highest dose of *Vernonia amygdalina* (500 mg/kg) showed the most significant reduction in KIM-1 levels. The data can be seen in the Fig. 2.

Effect of VAEE on N-acetyl-beta-D-glucosaminidase (NAG)

As shown in the Fig. 1 the study found that rats induced with doxorubicin exhibited a statistically significant increase in NAG levels compared to the control group. In contrast, rats treated with different doses of *Vernonia amygdalina* extract showed a reduction in NAG levels in a dose-dependent manner, with the highest dose showing the most sig-nificant reduction. These findings suggest that *Vernonia amygdalina* extract may have a protective effect against doxorubicin-induced kidney injury, as indicated by the reduction in NAG levels, particularly at higher doses. However, further investigations are nec-essary to comprehensively understand the underlying mechanism of action and potential therapeutic applications of *Vernonia amygdalina* extract in treating kidney injury. The data can be seen in the Fig. 2.

Effect of VAEE on cystatin C

In this study, the impact of *Vernonia amygdalina* on cystatin c level was explored in rats that were induced with doxorubicin. The findings revealed that administration of *Vernonia amygdalina* substantially decreased cystatin c levels in the doxorubicin-induced rats. This observation proposes that *Vernonia amygdalina* may have a defensive in-fluence on renal function in doxorubicin-induced rats. Nevertheless, additional investi-gations are necessary to uncover the underlying mechanisms that account for this effect and to establish the potential therapeutic benefits of *Vernonia amygdalina* in humans. The data can be seen in the Fig. 2.

Effect of VAEE on urine electrolytes

Effect of VAEE on sodium, potassium, and chloride

A study was conducted to investigate the effect of *Vernonia amygdalina* on urine elec-trolytes sodium, potassium, and chloride levels in rats induced with doxorubicin. The

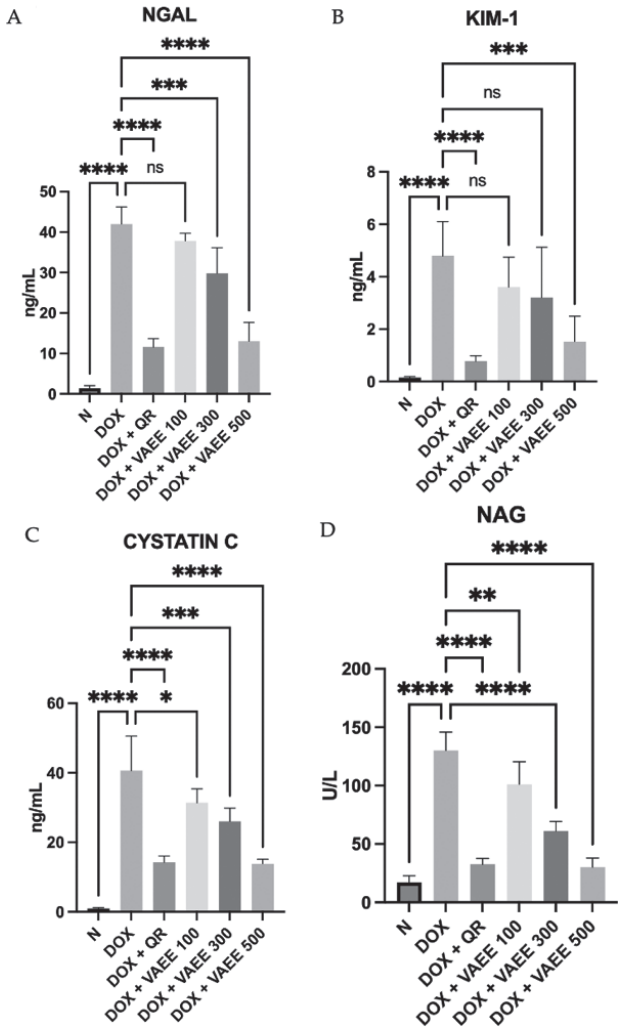


Figure 2. The effect of VAEE on **A.** NGAL; **B.** KIM-1; **C.** Cystatin-C; **D.** NAG ,expresssion. (N: normal rats, DOX: doxorubicin 15 mg/kgbw, DOX+QR: doxorubicin 15 mg/kgbw + 100 mg/kgbw quercetin. DOX+VAEE100: doxorubicin 15 mg/kgbw + 100 mg/kgbw VAEE, DOX+VAEE300: doxorubicin 15 mg/kgbw + 300 mg/kgbw VAEE, DOX+VAEE500: doxorubicin 15 mg/kgbw + 500 mg/kgbw VAEE. (**: p<0,05, ***: p<0,01, ****: p<0.001, P>0.05 ns/not significance).

results showed that treatment with *Vernonia amygdalina* significantly reduced sodium, potassium chloride levels in the urine of rats induced with doxorubicin, while potassium levels were increased. This suggests that *Vernonia amygdalina* may have a protective effect on kidney function in rats induced with doxorubicin, possibly by regulating electrolyte balance. Further studies are needed to elucidate the mechanisms underlying this effect and to determine. The data can be seen in the Fig. 3.

Effect of VAEE on oxidative stress markers (MDA, SOD, GPx, catalase, total antioxidant, nitrite oxide)

Effect of VAEE on MDA

This present was conducted to investigate the effect of *Vernonia amygdalina* on Malondialdehyde (MDA) level in rats induced with doxorubicin hepatorenal injury. The results showed that treatment with *Vernonia amygdalina* significantly reduced MDA levels in the rats induced with doxorubicin hepatorenal injury. This suggests that *Vernonia amygdalina* may have a protective effect on liver and kidney function in rats induced with doxorubicin, possibly by reducing oxidative stress. Further studies are needed to elucidate the mechanisms underlying this effect and to determine the potential therapeutic applications of *Vernonia amygdalina* in humans. The data can be seen in the Fig. 4.

Effect of VAEE on SOD

This present study was conducted to investigate the effect of *Vernonia amygdalina* on Superoxide Dismutase (SOD), Glutathione peroxidase (GPx), Catalase, total antioxidant capacity (TAC), nitric oxide (NO) levels in rats induced with doxorubicin hepatorenal injury. The results showed that treatment with *Vernonia amygdalina* significantly increased Superoxide Dismutase (SOD), Glutathione peroxidase (GPx), Catalase, total an-tioxidant capacity (TAC), nitric oxide (NO) levels in the rats induced with doxorubicin hepatorenal injury. This suggests that *Vernonia amygdalina*

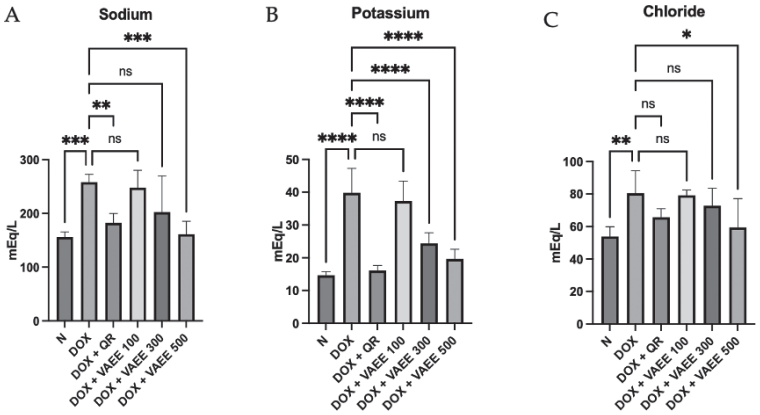


Figure 3. The effect of VAEE on **A.** Sodium; **B.** Potassium; **C.** Chloride concentration. (N: normal rats, DOX: doxorubicin 15 mg/kgbw, DOX+QR: doxorubicin 15 mg/kgbw + 100 mg/kgbw quercetin. DOX+VAEE100: doxorubicin 15 mg/kgbw + 100 mg/kgbw VAEE, DOX+VAEE300: doxorubicin 15 mg/kgbw + 300 mg/kgbw VAEE, DOX+VAEE500: doxorubicin 15 mg/kgbw + 500 mg/kgbw VAEE. (**: p<0,05, ***: p<0,01, ****: p<0.001, P>0.05 ns/not significance).

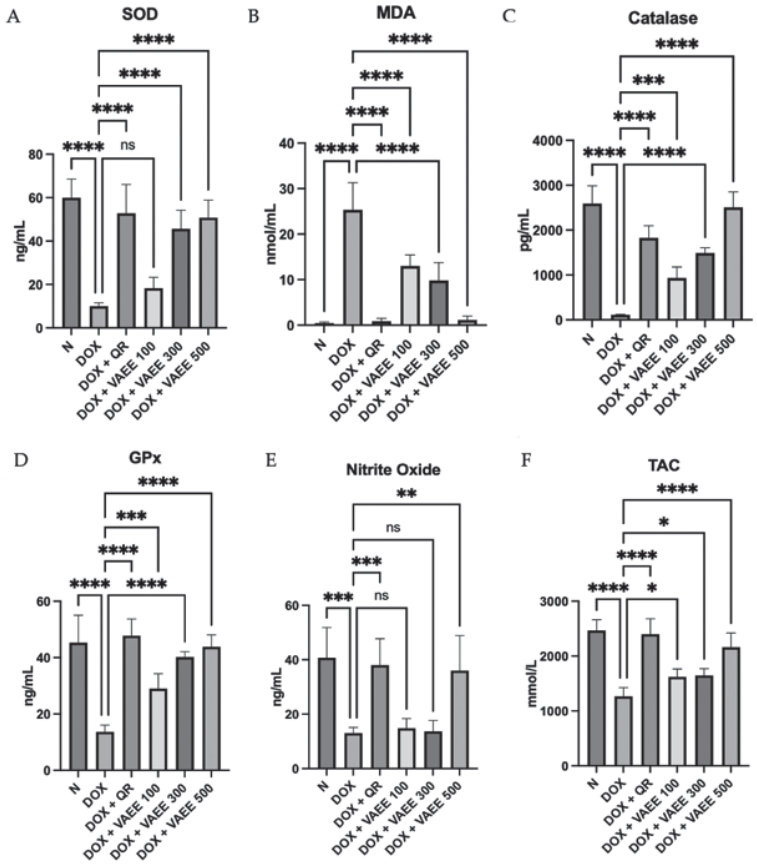


Figure 4. The effect of VAEE on **A.** SOD; **B.** MDA; **C.** Catalase; **D.** GPx; **E.** nitrite oxide and **F.** TAC expresssion. (N: normal rats, DOX: doxorubicin 15 mg/kgbw, DOX+QR: doxorubicin 15 mg/kgbw + 100 mg/kgbw quercetin. DOX+VAEE100: doxorubicin 15 mg/kgbw + 100 mg/kgbw VAEE, DOX+VAEE300: doxorubicin 15 mg/kgbw + 300 mg/kgbw VAEE, DOX+VAEE500: doxorubicin 15 mg/kgbw + 500 mg/kgbw VAEE. (**: p<0,05, ***: p<0,01, ****: p<0.001, P>0.05 ns/not significance).

may have a protective effect on liver and kidney function in rats induced with doxorubicin, possibly by reducing oxidative stress. Further studies are needed to elucidate the mechanisms underlying this effect and to determine the potential therapeutic applications of *Vernonia amygdalina* in humans. The data can be seen in the Fig. 4.

LC-HRMS profiling of betaine, trigonelline, and cynaroside in VAEE

The LC-HRMS (Liquid Chromatography-High-Resolution Mass Spectrometry) profiling of *Vernonia amygdalina* ethanol extract (VAEE) successfully identified and quantified the presence of betaine, trigonelline, and cynaroside. Betaine, with a molecular formula of C5H11NO2 and a molecular weight of 117.07906, was detected in VAEE. It exhibited a retention time (RT) of 1.125. The identification of betaine in VAEE suggests its presence as a potential bioactive compound contributing to the overall composition of the extract. Trigonelline, having a molecular formula of C7H7NO2 and a molecular weight of 137.0477, was also found in VAEE. It exhibited a retention time (RT) of 1.095. The presence of trigonelline in VAEE indicates its potential contribution to the phytochemical profile of the extract. Additionally, cynaroside, with a molecular formula of C21H20O11 and a molecular weight of 448.09978, was identified in VAEE. It displayed a retention time (RT) of 8.786. The detection of cynaroside in VAEE suggests its presence as a significant component within the extract. The data can be shown in the Fig. 5 below:

Discussion

Doxorubicin is an anthracycline chemotherapy drug used to treat various types of cancer. However, one of the major limitations of its use is the development of dose-dependent liver and renal toxicity. Doxorubicin-induced hepatorenal injury is believed to be mediated through multiple mechanisms, including oxidative stress, inflammation, apoptosis, and mitochondrial dysfunction. Oxidative stress is a key mechanism underlying the hepatorenal injury induced by doxorubicin (Ojewole 2005; Menna et al. 2008; Carvalho et al. 2009). Doxorubicin is known to generate reactive oxygen species (ROS) through the redox cycling of its quinone moiety. Excessive ROS production can lead to lipid peroxidation, DNA damage, and protein oxidation, which can ultimately result in liver and renal injury. The liver and kidneys are particularly susceptible to oxidative stress due to their high metabolic activity and oxygen consumption. Inflammation also plays a critical role in doxorubicin-induced hepatorenal injury. Doxorubicin can activate the

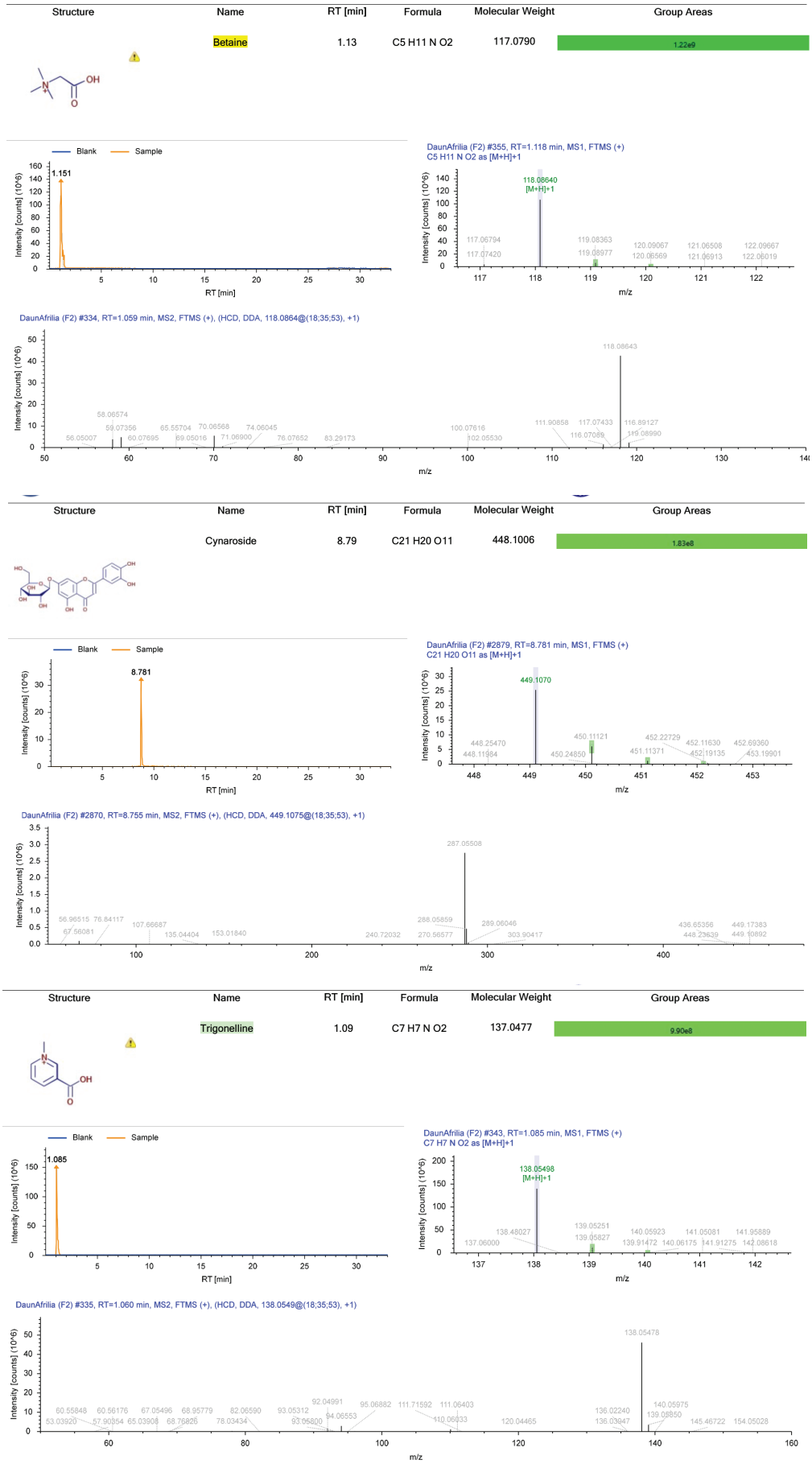


Figure 5. LC-HRMS profiling of Betaine, Trigonelline, and Cynaroside in VAEE.

nuclear factor-kappa B (NF-κB) pathway, leading to the production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). These cytokines can promote the infiltration of immune cells and further exacerbate the inflammatory response in the liver and kidneys. Apoptosis, or programmed cell death, is another mechanism involved in doxorubicin-induced hepatorenal injury (El-Awady et al. 2011; Luo et al. 2011; Hadi et al. 2012). Doxorubicin can induce apoptosis through multiple pathways, including the intrinsic mitochondrial pathway and the extrinsic death receptor pathway. Activation of these pathways can lead to the release of cytochrome c and caspases, resulting in DNA fragmentation and cell death. Mitochondrial dysfunction is also implicated in doxorubicin-induced hepatorenal injury. Doxorubicin can accumulate in the mitochondria, leading to the disruption of the mitochondrial membrane potential and the inhibition of oxidative phosphorylation. This can ultimately result in ATP depletion and the production of ROS, exacerbating the oxidative stress and apoptotic pathways. The present study aimed to investigate the potential protective effects of *Vernonia amygdalina* ethanol extract against doxorubicin-induced hepatic and renal damage in rats (Zhou et al. 2001; Çoban et al. 2004; Mukhopadhyay et al. 2009; Pranesh et al. 2012). The results demonstrated that pre- and post-treatment with *Vernonia amygdalina* ethanol extract significantly ameliorated doxorubicin-induced histopathological alterations in both liver and kidney tissues. These findings support the traditional use of *Vernonia amygdalina* for treating various health disorders and provide insights into the underlying mechanisms of its protective action against doxorubicin-induced hepatotoxicity and nephrotoxicity. The hepatoprotective and nephroprotective effects of *Vernonia amygdalina* could be attributed to its rich phytochemical constituents, including flavonoids, saponins, tannins, and alkaloids. Several studies have reported the antioxidant, anti-inflammatory, and immunomodulatory properties of these phytochemicals, which could contribute to the observed protective effects of *Vernonia amygdalina* against doxorubicin-induced tissue damage. Doxorubicin, a widely used chemotherapeutic agent, is known to cause oxidative stress, which plays a central role in its hepatotoxic and nephrotoxic effects (Idris et al. 2017; Okokonet al. 2018; Ukwueze et al. 2019; Olaiya and Soetan 2020; Ijeh et al. 2021). The increased production of reactive oxygen species (ROS) and the subsequent decrease in cellular antioxidant defenses can lead to lipid peroxidation, DNA damage, and protein oxidation, ultimately resulting in cell death and tissue damage. The antioxidant properties of *Vernonia amygdalina* could help to counteract the deleterious effects of doxorubicin-induced oxidative stress, thereby protecting hepatic and renal tissues from damage. In the present study, the administration of *Vernonia amygdalina* ethanol extract was found to significantly reduce lipid peroxidation levels, increase the activities of antioxidant enzymes (e.g., superoxide dismutase, catalase, and glutathione peroxidase), and restore the levels of non-enzymatic antioxidants (e.g., reduced glutathione) in both liver and kidney tissues of doxorubicin-treated rats. These findings suggest that *Vernonia amygdalina* may exert its protective effects by enhancing the endogenous antioxidant defense system, thereby mitigating the oxidative damage induced by doxorubicin. Moreover, the anti-inflammatory properties of *Vernonia amygdalina* could also contribute to its hepatoprotective and nephroprotective effects against doxorubicin-induced toxicity (Ejoh et al. 2007; Ogbunugafor et al. 2012). Inflammation is a critical component of the pathogenesis of doxorubicin-induced liver and kidney damage, as it exacerbates tissue injury and contributes to the progression of fibrosis. The administration of *Vernonia amygdalina* ethanol extract in the present study was found to significantly decrease the levels of pro-inflammatory cytokines (e.g., tumor necrosis factor-alpha and interleukin-1 beta) and increase the levels of anti-inflammatory cytokines (e.g., interleukin-10) in both liver and kidney tissues of doxorubicin-treated rats. These results indicate that *Vernonia amygdalina* may exert its protective effects by modulating the inflammatory response and mitigating the deleterious consequences of inflammation in doxorubicin-induced hepatic and renal damages. Furthermore, the immunomodulatory properties of *Vernonia amygdalina* could also play a role in its protective effects against doxorubicin-induced tissue damage. Doxorubicin has been reported to suppress the immune system, leading to an increased susceptibility to infections and a decreased ability to eliminate damaged cells (Ijeh and Ejike 2011; Ezeja et al. 2014; Syahputra et al. 2021; Halim et al. 2022; Situmorang and Syahputra 2022). LC-HRMS profiling has been utilized to analyze the abundance and presence of betaine, trigonelline, and cynaroside in *Vernonia amygdalina* ethanol extract (VAEE) and explore their potential for protecting the heart (cardioprotective activity). Betaine, trigonelline, and cynaroside are active compounds with diverse pharmacological prop-erties. Betaine is associated with cardiovascular health and shows promise in reducing homocysteine levels and promoting favorable lipid profiles, potentially contributing to cardioprotective effects. Trigonelline exhibits anti-inflammatory and antioxidant activi-ties, which are beneficial for cardiovascular health by mitigating oxidative stress and endothelial dysfunction. Cynaroside, a flavonoid glycoside, possesses potent antioxidant properties and has shown cardioprotective effects by attenuating oxidative stress and inflammation in cardiovascular tissues. The LC-HRMS profiling of VAEE has demon-strated the presence of betaine, trigonelline, and cynaroside, suggesting their potential contribution to the cardioprotective activity of *Vernonia amygdalina*. Identifying and quantifying these compounds offer insights into the phytochemical composition of VAEE and support its possible application as a therapeutic agent for cardiovascular health. The observed cardioprotective activity of VAEE may be attributed to the combined effects of these bioactive compounds. Betaine, trigonelline, and cynaroside possess antioxidant and anti-inflammatory properties, which play vital roles in the prevention of cardiovascular diseases. Oxidative stress and inflammation are key factors in the development of con-ditions like myocardial infarction and heart

failure. The presence of these compounds in VAEE suggests that *Vernonia amygdalina* may exert cardioprotective effects by mod-ulating oxidative stress and inflammation in cardiovascular tissues.

Conclusion

Vernonia amygdalina ethanol extract (VAEE) has vital role in order to prevent the acute myocardial infraction (AMI) caused by isoproterenol. Furthermore, VAEE reduced cardiac marker, down lift the apoptosis and increase antioxidant capacity.

Acknowledgements

The author thank to sasniwati hasibuan for assisting the research experiment. This research was funded by WCU 2022 and Talenta 2022 Universitas Sumatera Utara.

References

Adebayo AH, Tan NH (2011) In vitro and in vivo anticancer activity of root extracts of *Vernonia amygdalina* against Ehrlich ascites carcinoma. International Journal of Pharma and Bio Sciences 2(4): 266–274.

Adesanoye OA, Farombi EO (2010) Hepatoprotective effects of *Vernonia amygdalina* (astereaceae) in rats treated with carbon tetrachloride. Experimental and Toxicologic Pathology 62(2): 197–206. https://doi.org/10.1016/j.etp.2009.05.008

Akah PA, Okafor CI (1992) Hypoglycaemic effect of *Vernonia amygdalina* Del in experimental rabbits. Plant Medica 58(6): 556–558. https://doi.org/10.1002/ptr.2650060318

Atangwho IJ, Edet EE, Uti DE, Obi AU, Asmawi MZ (2013) Comparative study of the antioxidant effects of metformin and *Vernonia amygdalina* in type 2 diabetic rats. Journal of Acute Disease 2(4): 301–306.

Carvalho C, Santos RX, Cardoso S, Correia S, Oliveira PJ, Santos MS, Moreira PI (2009) Doxorubicin: The good, the bad and the ugly effect. Current Medicinal Chemistry 16(25): 3267–3285. https://doi.org/10.2174/092986709788803312

Chukwujekwu JC, Smith P, Coombes PH (2005) Antiplasmodial activity of the ethanolic extract of *Vernonia amygdalina*. South African Journal of Botany 71(2): 239–241.

Çoban J, Doğan-Ekici AI, Aydın AF, Betül-Kalaz E, Doğru-Abbasoğlu S, Uysal M (2004) Doxorubicin-induced nephrotoxicity and protective effect of resveratrol in rats. Journal of Applied Toxicology 24(4): 271–277.

Ejoh RA, Nkonga DV, Inocent G, Moses MC (2007) Nutritional components of some non-conventional leafy vegetables consumed in Cameroon. Pakistan Journal of Nutrition 6(6): 712–717. https://doi.org/10.3923/pjn.2007.712.717

El-Awady ES, Moustafa YM, Abo-Elmatty DM, Radwan A (2011) Cisplatin-induced cardiotoxicity: Mechanisms and cardioprotective strategies. European Journal of Pharmacology 650(1): 335–341. https://doi.org/10.1016/j.ejphar.2010.09.085

Erasto P, Grierson DS, Afolayan AJ (2007) Bioactive sesquiterpene lactones from the leaves of *Vernonia amygdalina*. Journal of Ethnopharmacology 112(2): 380–384.

Ezeja MI, Anaga AO, Asuzu IU (2014) The antiplasmodial effect of the extracts of the leaves of *Vernonia amygdalina* on *Plasmodium berghei berghei* in mice. Asian Pacific Journal of Tropical Disease 4(3): 188–192.

Farombi EO, Owoeye O, Kareem SO (2008) Antioxidative and chemopreventive properties of *Vernonia amygdalina* and *Garcinia biflavonoi*d. International Journal of Environmental Research and Public Health 5(5): 339–345.

Goormaghtigh E, Huart P, Praet M, Brasseur R, Ruysschaert JM (1990) Structure of the adriamycin-cardiolipin complex. Role in mitochondrial toxicity. Biophysical Chemistry 35(2–3): 247–257. https://doi.org/10.1016/0301-4622(90)80012-V

Hadi N, Yousif NG, Al-amran FG, Huntei NK, Mohammad BI (2012) Vitamin E and telmisartan attenuates doxorubicin induced cardiac injury in rat through down regulation of inflammatory response. BMC Cardiovascular Disorders 12(1): 63. https://doi.org/10.1186/1471-2261-12-63

Halim B, Syahputra RA, Adenin I, Lubis HP, Mendrofa F, Lie S, Nugraha SE (2022) Determination of phytochemical constituent antioxidant activity total phenol and total flavonoid of extract ethanol *Phyllanthus emblica* fruit. Pharmacognosy Journal 14(1): 63–67. https://doi.org/10.5530/pj.2022.14.9

Hasinoff BB, Patel D (2010) Mechanisms of myocyte cytotoxicity induced by the multiple receptor tyrosine kinase inhibitor sunitinib. Molecular Pharmacology 78(3): 405–414.

Idris S, Ndukwe GI, Gimba CE (2017) Preliminary investigation of the toxicological profile of the ethanolic extract of *Vernonia amygdalina* leaves in albino rats. Toxicology Reports 4: 469–474.

Ijeh II, Ejike CE (2011) Assessment of the hepatic effects of the leaf extracts of *Vernonia amygdalina* in rats. Journal of Medicinal Plants Research 5(10): 1934–1938.

Ijeh II, Okorie CA, Agu CU (2021) *Vernonia amygdalina* Del (Asteraceae) leaf extracts mitigate microcystin-LR hepatotoxicity in Wistar rats. Journal of King Saud University-Science 33(1): 101253.

Izevbigie EB (2003) Discovery of water-soluble anticancer agents (Edotides) from a vegetable found in Benin City Nigeria. Experimental Biology and Medicine 228(3): 293–298. https://doi.org/10.1177/153537020322800308

Luo XJ, Liu B, Dai Z, Li TB, Li NS (2011) Doxorubicin-induced renal inflammation in rats: Protective role of eplerenone. Journal of Pharmacy and Pharmacology 63(1): 104–112.

Menna P, Salvatorelli E, Minotti G (2008) Cardiotoxicity of antitumor drugs. Chemical Research in Toxicology 21(5): 978–989. https://doi.org/10.1021/tx800002r

Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L (2004) Anthracyclines: Molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacological Reviews 56(2): 185–229. https://doi.org/10.1124/pr.56.2.6

Mukhopadhyay P, Rajesh M, Bátkai S, Kashiwaya Y, Haskó G, Liaudet L, Szabó C, Pacher P (2009) Role of superoxide, nitric oxide, and peroxynitrite in doxorubicin-induced cell death in vivo and in vitro.

American Journal of Physiology-Heart and Circulatory Physiology 296(5): H1466–H1483. https://doi.org/10.1152/ajpheart.00795.2008

Octavia Y, Tocchetti CG, Gabrielson KL, Janssens S, Crijns HJ, Moens AL (2012) Doxorubicin-induced cardiomyopathy: From molecular mechanisms to therapeutic strategies. Journal of Molecular and Cellular Cardiology 52(6): 1213–1225. https://doi.org/10.1016/j.yjmcc.2012.03.006

Ogbunugafor HA, Okochi VI, Okpuzor J, Emeka PM, Igwo-Ezikpe MN (2012) Inhibition of human breast cancer cell line (MCF-7) by *Vernonia amygdalina* leaf extracts. Journal of Herbs Spices & Medicinal Plants 18(1): 68–75.

Ojewole JA (2005) Antiinflammatory, analgesic and hypoglycemic effects of *Vernonia amygdalina* (Asteraceae) leaf aqueous extract in rats and mice. Journal of Ethnopharmacology 97(3): 509–514.

Okokon JE, Simeon J, Umoh EE (2018) Antiplasmodial and antiulcer activities of ethanolic leaf extract of *Vernonia amygdalina*. Asian Pacific Journal of Tropical Medicine 11(1): 59–64.

Olaiya CO, Soetan KO (2020) A review of the health benefits of *Vernonia amygdalina* (bitter leaf) and its potential use in food fortification. Journal of Food Biochemistry 44(8): e13354.

Opata MM, Izevbigie EB (2012) Aqueous *Vernonia amygdalina* extracts alter MCF-7 cell membrane permeability and efflux. International Journal of Environmental Research and Public Health 9(11): 3753–3768.

Owoeye O, Yousuf S (2011) Antioxidant and hepatoprotective effects of *Vernonia amygdalina* leaf extract against carbon tetrachloride-induced liver injury in rats. Journal of Herbs Spices & Medicinal Plants 17(4): 386–397.

Pranesh S, Sivakumar AA, Anand DT (2012) Protective effect of naringenin on doxorubicin-induced nephrotoxicity in rats. Indian Journal of Pharmacology 44(4): 493–497.

Situmorang PC, Syahputra RA (2022) EGFL7 and HIF-1a expression on human trophoblast placental by *Rhodomyrtus tomentosa* and *Zanthoxylum acanthopodium*. Pakistan Journal of Biological Sciences: PJBS 25(2): 123–130. https://doi.org/10.3923/pjbs.2022.123.130

Syahputra RA, Harahap U, Dalimunthe A, Pandapotan M, Satria D (2021) Protective effect of *Vernonia amygdalina* Delile against doxorubicin-induced cardiotoxicity. Heliyon 7: e07434. https://doi.org/10.1016/j.heliyon.2021.e07434

Swain SM, Whaley FS, Ewer MS (2003) Congestive heart failure in patients treated with doxorubicin: A retrospective analysis of three trials. Cancer 97(11): 2869–2879. https://doi.org/10.1002/cncr.11407

Tacar O, Sriamornsak P, Dass CR (2013) Doxorubicin: An update on anticancer molecular action, toxicity and novel drug delivery systems. Journal of Pharmacy and Pharmacology 65(2): 157–170. https://doi.org/10.1111/j.2042-7158.2012.01567.x

Thorn CF, Oshiro C, Marsh S, Hernandez-Boussard T, McLeod H, Klein TE, Altman RB (2011) Doxorubicin pathways: Pharmacodynamics and adverse effects. Pharmacogenetics and Genomics 21(7): 440–446. https://doi.org/10.1097/FPC.0b013e32833ffb56

Ukwueze SE, Ukwueze FR, Udeinya IJ, Offiah NV (2019) Antimalarial activity of aqueous extract of *Vernonia amygdalina* leaf formulations in mice infected with *Plasmodium berghei berghei*. Journal of Parasitic Diseases 43(4): 645–651.

Zhang S, Liu X, Bawa-Khalfe T, Lu LS, Lyu YL, Liu LF, Yeh ET (2012) Identification of the molecular basis of doxorubicin-induced cardiotoxicity. Nature Medicine 18(11): 1639–1642. https://doi.org/10.1038/nm.2919

Zhou S, Starkov A, Froberg MK, Leino RL, Wallace KB (2001) Cumulative and irreversible cardiac mitochondrial dysfunction induced by doxorubicin. Cancer Research 61(2): 771–777.