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Research Article

Phytochemical profiling and cardioprotective activity of *Vernonia amygdalina* ethanol extract (VAEE) against ISO-induced cardiotoxicity in rats

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[6], Jekson Martiar Siahaan[7], Adrian Joshua Velaro[8], Besa Bilakaya[9], Muhammad Andika Yudha Harahap[10]

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 Surgery, Faculty of Medicine, Universitas Methodist Indonesia, Medan, Indonesia*
7 *Department of Physiology, Faculty of Medicine, Universitas Methodist Indonesia, Medan, Indonesia*
8 *Department of Surgery, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia*
9 *Department of Pharmaceutical Technology, Faculty of Pharmacy, Biruni University, Istanbul, Turkiye*
10 *Department of Animal Nutrition, Faculty of Animal Science, Brawijaya University, Malang, Indonesia*

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

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Abstract

Cardiovascular disorder is the leading cause death in the world, one of them are acute myocardial infarction (AMI) which associated with hypertension and cardiac remodeling. ISO may cause inflammation, enhance the production of oxidative stress while decrease the antioxidant defensive system, myocardium impairment, calcium overload, enhanced cyclic adenosine monophosphate level, intracellular acidosis, and altered membrane permeability. *Vernonia amygdalina* (*VA*) is a medicinal plant with antioxidant and anti-inflammatory properties. This study investigated the potential cardioprotective effect of *VA* on ISO-induced cardiac toxicity in rats. Male Wistar rats were randomly divided into six groups: ISO (ISO), quercetin 100 mg/kg plus ISO (ISO+QR), VA ethanol extract 100, 300, 500 mg/kg plus ISO (ISO+VA100, ISO+VA300 and ISO+VA500). ISO was administered subcutaneously (85 mg/kg) on days 15 while quercetin and VA extract and was given orally for 14 days. At the end of the experiment, the blood was taken from the heart were analyzed for markers of cardiac, oxidative stress and inflammation. The ISO group exhibited significant ($p<0.05$) elevation of cardiac biomarkers such as lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB), troponin-T, and BNP as well as increased oxidative stress markers such as malondialdehyde (MDA) and reduced antioxidant enzyme superoxide dismutase (SOD), catalase (CAT), and Glutathione peroxidases (GPx). Additionally, the ISO group had elevated levels of pro-inflammatory cytokines interleukin-1 (IL-1), interleukin-6 (IL-6), Highly sensitive c reactive protein (HsCRP) and tumor necrosis factor-alpha (TNF-α). Treatment with VA extract significantly ($p<0.001$) reduced these parameters in the VA+ISO group compared to the ISO group. These findings suggest that VA has a potential protective effect against ISO-induced cardiotoxicity by reducing oxidative stress, apoptosis, and inflammation. (The graphiccal abstract can be seen in the Fig. 1).

Keywords

Cardioprotective, Isoproterenol, GC-MS, *Vernonia amygdalina*

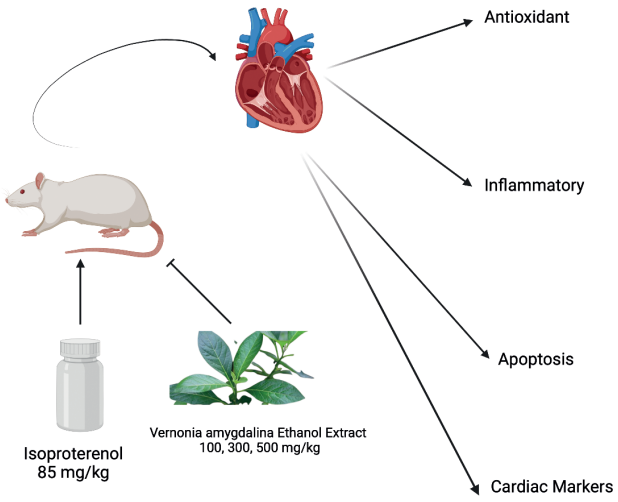

Figure 1. Graphical abstract.

Introduction

Cardiovascular disorder is the leading cause death in the world, one of them are acute myocardial infarction (AMI) which associated with hypertension and cardiac remodeling. The prevalence of AMI reaches up to three million people in worldwide specifically in United States (US) bring more than one million deaths, while in Indonesia AMI also dominantly caused death among the older (Zhang et al. 2014; Kumar and Agarwal 2019). AMI divided into 2 which are non-ST-segment elevation MI (NSTEMI) and ST-segment elevation MI (STEMI). Seriously AMI may cause damage on the cardiomyocytes that result lack of oxygen supplies, this condition brings systolic and diastolic dysfunction. Normally, AMI condition generates the elevation of cardiac markers such as troponin I and T, creatinine kinase MB that specific to myocardium, lactate dehydrogenase (LDH), and Brain natriuretic peptide (BNP). The ECG diagnosis of AMI markedly ST elevation that indicating early ischemia and T wave changes, these diagnostic tools can be used to identifying STEMI or NSTEMI (Zhang et al. 2019; Zhao et al. 2019). AMI triggers morphological changes of myocardium and myocardial fibroblasts due to oxidative stress, inflammatory response, neuroendocrine regulation, and other secondary lesions, thus affecting ventricular size, structure and function which can be assessed by histology evaluation (Yang et al. 2015; Li et al. 2020; Ma et al. 2020; Tian J et al. 2020; Tian W et al. 2020; Zhao et al. 2021; Chen et al. 2022). Isoproterenol (ISO) or isoprenaline is nonselective beta-adrenergic receptor agonist that often use to treat cardiac arrest, hypovolemic shocks, septic shock, hypoperfusion, congestive heart failure, and cardiogenic shock. ISO enhance the heart rate as well as the cardiac output without vasoconstriction and ISO has been approved by FDA. ISO usually used as AMI model in vivo model experiment; the injection of ISO may cause irreversible cardiomyocytes damage in rats (Naseem and Parvez 2014; Rajesh et al. 2014; Kumar et al. 2015). Moreover, the rat's model of ISO induced AMI offers non-invasive technique to study the cardioprotective effect of compound or extract. Numerous studies have been revealed that ISO may cause inflammation, enhance the production of oxidative stress while decrease the antioxidant defensive system, myocardium impairment, calcium overload, enhanced cyclic adenosine monophosphate level, intracellular acidosis, and altered membrane permeability. Inflammation during AMI cause the activation of many pro-inflammatory cytokines like tumor necrosis factor α (TNF-α) and Interleukin-6 (IL-6) as well as other inflammation-related proteins that induce various pathophysiological changes, the TNF-α trigger the neutrophil shifted into the ischemic region of infarcted myocardial tissue while IL-6 involved response to myocardium stress. Additionally, AMI caused by ISO characterized by the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and inducible nitrite oxide (iNOS) (Ghosh and Das 2010; Chen et al. 2013; Guo et al. 2016; Jin et al. 2020; Liu et al. 2017; Gu et al. 2018; Zhang et al. 2019; Halim et al. 2022). Herbal plant has been widely explored the cardioprotective activity one of them are *Vernonia amygdalina*, our previously study shown that *Vernonia amygdalina* ethanol extract has potential cardioprotective activity via TGFβ, cytochrome c, and apoptosis. In tropical areas like Indonesia and Malaysia, *Vernonia amygdalina* (VA) is frequently encountered and has long been used medicinally. VA is frequently referred to as bitter leaves, numerous secondary metabolites, including sesquiterpene lactone, vernolide, vernodalol, vernoamygdalin, vernolepin, lutein, luteolin 7-O-beta-glucoronoside, and luteolin 7-O-glucoside, Vernonioside A1, Vernonioside A2, Vernonioside A3, Vernonioside A4, Vernonioside B1, Vernonioside B2, Vernonioside B3, Vernonioside C, Vernonioside D, Vernonioside 3, Vernoniamyoside A, Vernoniamyoside B, Vernoniamyoside C, Vernoniamyoside D, veramyoside A, veramyoside B, veramyoside C, veramyoside D, veramyoside E, veramyoside F, veramyoside G, veramyoside H, veramyoside I, and veramyoside J. *Vernonia amygdalina* have numerous of pharmacological activities like cardioprotective, antidiabetic, anticholesterolemic, antihypertensive, antirheumatic, hepatoprotective, nephroprotective, immunomodulator, antileptic, antimalaria, and antoxidant (Igile et al. 1994; Ezuruike and Prieto 2014; Mbaebie et al. 2015; Nartey et al. 2016; Attah et al. 2020; Gbashi et al. 2020; Mbabazi et al. 2021; Syahputra et al. 2021; Sotirova et al. 2023; Stoeva et al. 2023). This study aims to determine

the cardioprotective activity of *Vernonia amygdalina* ethanol extract in rats induced ISO which determine the cardiac markers, apoptosis marker, inflammation markers and antioxidant markers. (Isoprenaline strcuture can be seen in

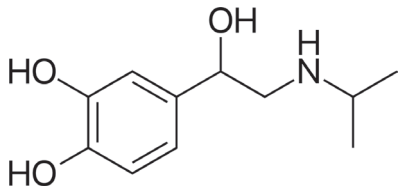

Figure 2. Chemical structure of isoproterenol.

the Fig. 2).

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) and identified under herbarium Medanese (MEDA), Laboratoirum Taxonomy of Plants, Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Sumatra, Medan (7313/MEDA/2023). Isoproterenol (Merck), Ethanol (BrataChem), Ethyl Acetate (BrataChem), n-hexane (BrataChem), Methanol (BrataChem), sodium carboxymethyl cellulose/CMC-Na (Sigma). CK-MB ELISA kit, LDH ELISA kit, Troponin T ELISA kit, BNP ELISA kit, TNF alpha ELISA kit, HsCRP ELISA kit, IL-6 ELISA kit, IL-1 ELISA kit, Caspase-3 ELISA kit, Bcl-2 ELISA kit, p53 ELISA kit, SOD ELISA kit, GR ELISA kit, rat Bcl-2 antibody for IHC, rat Collagen antibody for IHC. All the ELISA kit used in the study were purchased from Abclonal (China).

Animals

Rats were obtained from the Faculty of Pharmacy animal house at Universitas Sumatera Utara. This study utilized 30 rats weighing an average of 180–200 g, that were provided food and water 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

1000 g of *Vernonia amygdalina* (VA) leaves powder was dissolved in 10 L of n-hexane for 7 days and evaporated, after that the powder residue was dissolved 10 L of ethyl acetate for 7 days and evaporated, after that the powder residue was dissolved 10 L of ethanol for 7 days and evaporated. Each filtrate was collected and evaporated under pressure by rotary evaporator (Syahputra et al. 2021).

Experimental design

All the rats were randomly classified into 6 groups (five rats/group) which are normal group (N), Isoproterenol group (ISO) rats were inject 85 mg/kgbw on the day 15th day, ISO+QR group rats orally pre-administered of 100 mg/kgbw of quercetin according to previously study for 14th consecutive days plus injection of ISO 85 mg/kgbw on the day 15th day, ISO+VAEE (100, 300, 500 mg/kgBB) rats were pre-administered of *Vernonia amygdalina* ethanol extract 100, 300, 500 mg/kgBB for 14th consecutive days plus injection of ISO 85 mg/kgbw on the day 15th day. After 24 h of the last ISO (15th day), the rats were anesthetized using ketamine hydrochloride (24 mg/kg bw) and euthanized by cervical dislocation and the heart were taken for histopathological evaluation.

Serum preparation

Blood samples were collected in a dry test tube and allowed to coagulate at ambient temperature for 30 min. The serum was separated by centrifugation at 2000 r/min for 10 min. We measured the levels of creatine kinase-myocardial band (CK-MB), cardiac troponin T (cTnT), brain natriuretic peptide, and inflammatory cytokines (IL-1, IL-6, HsCRP, TNF alpha), apoptosis-related protein (caspase-3 and p53), antixoidant (SOD, GPx, and Catalase)

Cardiac marker activities determination

The troponin T are the major regulatory markers that control cardiac actin and myosin interaction. CK-MB is an isoenzyme that mainly present in the cardiac muscle. BNP 32 amino acid peptide named after its initial detection in the porcine brain is specific which produced after left ventricular dysfunction. The serum Troponin T, CK-MB, LDH, and BNP were measured using a commercial kit abclonal. The data were quantitatively calculated, as per the kit provided by the manufacturer (ELISA).

Measurement of apoptosis-related protein

Table 1. Experimental design.

Mark	Normal group
ISO	Injection 85 mg/kgbw of ISO (on the day 15th)
ISO + QR	Injection 85 mg/kgbw of ISO (on the day 15th) + 100 mg/kgbb of quercetin (orally for 14th days)
ISO + 100 mg/kgbw VAEE	Injection 85 mg/kgbw of ISO (on the day 15th) + 100 mg/kgbb of VAEE (orally for 14th days)
ISO + 300 mg/kgbw VAEE	Injection 85 mg/kgbw of ISO (on the day 15th) + 300 mg/kgbb of VAEE (orally for 14th days)
ISO + 500 mg/kgbw VAEE	Injection 85 mg/kgbw of ISO (on the day 15th) + 500 mg/kgbb of VAEE (orally for 14th days)

The levels of pro-inflammatory cytokines, that is TNF- IL-1, IL-6, and HsCRP, were generally elevated during AMI. The serum level of TNF-α and IL-6 was measured by ready to use commercial ELISA kit, as per the instructions given by the manufacturer (Abclonal, Wuhan, China). Briefly, the monoclonal antibody specific for TNF- IL-1, IL-6, and HsCRP precoated microtiter plates was added with the experimental samples. A reaction of biotin-conjugated antibody specific for TNF- IL-1, IL-6, and HsCRP with avidin-conjugated HRP was measured using a substrate solution. The developed color intensity was measured using a microplate reader (thermos fisher, Germany).

Measurement of apoptosis-related protein

The levels of caspase-3 and p53 were were measured by ready to use commercial ELISA kit, as per the instructions given by the manufacturer (Abclonal, Wuhan, China).

Determination of antioxidant

Antioxidant enzymes play a major role in the redox homeostasis of tissues that scavenges ISO-induced free radicals. Superoxide dismutase (SOD) scavenges ROS by converting superoxide to hydrogen peroxide and molecular oxygen. Catalase (CAT) degrades hydrogen peroxide into water and oxygen, thereby maintaining redox status in the cellular milieu. Glutathione peroxidase (GPx) catalyzes the reduction of hydrogen peroxide to water via the oxidation of reduced glutathione (GSH) into glutathione disulfide. The SOD, CAT, and GPx were measured by ready to use commercial ELISA kit, as per the instructions given by the manufacturer (Abclonal, Wuhan, China).

Cardiac histopathology

The hearts that had been fixed were dehydrated using varying concentrations of alcohol (70–100%), cleaned using xylene, and paraffin-fixed at a temperature of 56 °C before the 4 μm thick slices were deparaffinized and subjected to Hematoxylin and Eosin (H&E) staining. Digital photographs were captured at 4× and 10× magnification using a microscope. The dimensions of total cell count, diameter, and size were obtained via ImageJ software (ImageJ, Version 1.44p, NIH, USA). To assess cardiac fibrosis, Masson's trichrome staining was carried out with some adjustments. The heart slices were subjected to prewarmed Bouin's solution incubation for 120±10 minutes, following which the nuclei were stained with Weigert's hematoxylin for 20 minutes, whereas the cytoplasm and muscle were stained with Biebrich scarlet-acid fuchsin solution for 20 minutes. The tissue slices were then treated with phosphomolybdic-phosphotungstic acid solution for 10 minutes, followed by a blue aniline solution for 10 minutes. After a 30-second incubation with 1% acetic acid, the slices were dehydrated using ethanol and xylene, and digital photos were taken using a microscope at 4× and 10× magnification. Using ImageJ software (ImageJ, Version 1.44p, NIH, USA), necrosis, congestion, and oedema was evaluated.

Immunohistochemistry of Bcl-2 and collagen

The heart tissue was fixed in formalin and sliced into 4 μm thickness to obtain paraffin blocks. Immunohistochemical staining was carried out on slides using Bcl-2 and Collagen mouse monoclonal antibodies (NOK-1):sc-19681 (dilution 1:50 in PBS, Santa Cruz Biotechnology, Santa Cruz, CA, USA) for TGFβ detection and monoclonal mouse anti-cytochrome C antibody (ready-to-use) 7H8.2C12 (Me-daysis Enable Innovation Company) in PBS pH 7.4, containing BSA and 0.09% (NaN3) for cytochrome c detection. The heart tissue was rehydrated with 3% hydrogen peroxide and distilled water for 5 minutes, followed by heat pretreatment in citrate buffer at pH 6.0 and 350 W for 10 minutes. After washing with PBS, the tissue was incubated with Bcl-2 and Collagen antibodies for 15 minutes and 120 minutes, respectively, at 37 °C before avidin–biotin peroxidase was applied. The chromogenic visualization reaction was carried out using 3,3-Diaminobenzidine (DAB) hydrochloride and hematoxylin staining for 30 s. The slides were then passed through an ethanol series and xylene before being coated with Canadian balm and a glass cover (Merck, Darmstadt, Germany). The scoring system used was as follows: a score of 0 indicated staining in fewer than 10% of the cells, a score of 1 indicated staining in 10%–25% of the cells, a score of 2 indicated staining in 25%–50% of the cells, a score of 3 indicated staining in 50%–75% of the cells, and a score of 4 indicated staining in more than 75% of the cells. The staining intensity was graded as weak, moderate, or strong.

The gas chromatography-mass spectrometry (GC-MS) analysis of VAEE

The gas chromatography-mass spectrometry (GC-MS) analysis was conducted using a combined 7890A gas chromatograph system (Agilent 19091-433HP, USA) and mass spectrophotometer. The system was equipped with an HP-5 MS fused silica column (5% phenyl methyl siloxane, 30.0 m × 250 μm, film thickness 0.25 μm) and a 5675C Inert MSD with Triple-Axis detector. Helium gas was utilized as the carrier gas and adjusted to a column velocity flow of 1.0 ml/min. The GC-MS conditions included an ion-source temperature of 250 °C, an interface temperature of 300 °C, a pressure of 16.2 psi, an outlet time of 1.8 mm, and a 1 μl injector in split mode with a split ratio of 1:50. The injection temperature was set at 300 °C. The column temperature started at 36 °C for 5 minutes and then increased to 150 °C at a rate of 4 °C/min. It was further raised to 250 °C at a rate of 20 °C/min and held for 5 minutes. The total elution time was

47.5 minutes. The relative percentage of each component was determined by comparing its average peak area to the total areas. The MS solution software provided by the supplier was employed to control the system and acquire the data.

Statistical analysis

Analysis of the expression of CK-MB, LDH, Troponin T, BNP, IL-1, IL-6, HsCRP, TNF alpha, caspase-3, Bcl-2, p53, SOD, GPx, Catalase, and MDA level using the Kruskal-Wallis and Mann-Whitney tests (non-parametric data) Using the SPSS 21 program.

Result

Effect of VAEE on cardiac injury markers (CK-MB, LDH, Troponin T, Troponin I, BNP)

As shown in the Fig. 3, the administration of ISO significantly increasing the serum level of CK-MB compared to the control group ($p<0.001$). However, treatment with VAEE significantly attenuated the rise of CK-MB level induced by ISO ($p<0.001$). These findings suggest that VAEE may have a cardioprotective effect against ISO-induced cardiac injury as evidenced by a reduction in CK-MB level. In the present study also show the effect of VA on LDH level in rats induced with ISO was evaluated. The LDH level was signifi-cantly increased in the ISO-induced group when compared to the control group ($p<0.001$). However, treatment with VA extract at a dose of 500 mg/kg significantly decreased the LDH level when compared to the ISO-induced group ($p<0.001$). These results suggest that VAEE has a cardioprotective effect on ISO-induced cardiotoxicity by reducing LDH level in rats. The results showed that the Troponin T level significantly increased in the ISO group compared to the control group ($p<0.001$). However, treatment with VA significantly reduced the Troponin T level in the treatment group compared to the ISO group. In this study treatment with VA was found to significantly reduce the BNP concentration compared to the control group ($p<0.001$). This indicates that VA may have a protective effect against ISO-induced cardiac damage by reducing BNP levels.

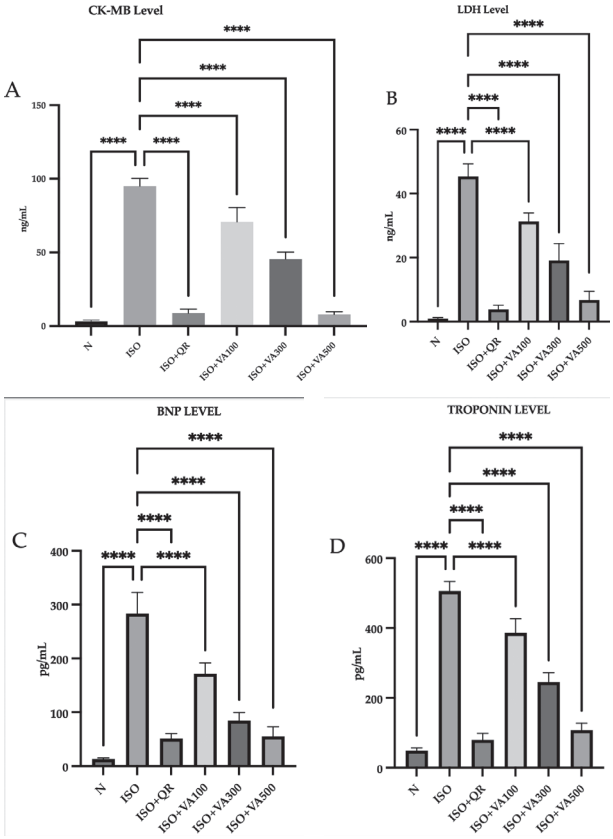

Figure 3. The effect of VAEE on CK-MB (**A**), LDH (**B**), Troponin T (**C**), and BNP (**D**) expresssion. (N: normal rats, ISO: ISO 85 mg/kgbw ,ISO+QR: ISO 85 mg/kgbw + 100 mg/kgbw quercetin. ISO+VA100: ISO 85 mg/kgbw + 100 mg/kgbw VAEE, ISO+-VA300: ISO 85 mg/kgbw + 300 mg/kgbw VAEE, ISO+VA500: ISO 85 mg/kgbw + 500 mg/kgbw VAEE, the data were presented in mean ± standard error of the mean (SEM). (**: p<0,05, ***: p<0,01, ****: p<0.001, P>0.05 ns/not significance).

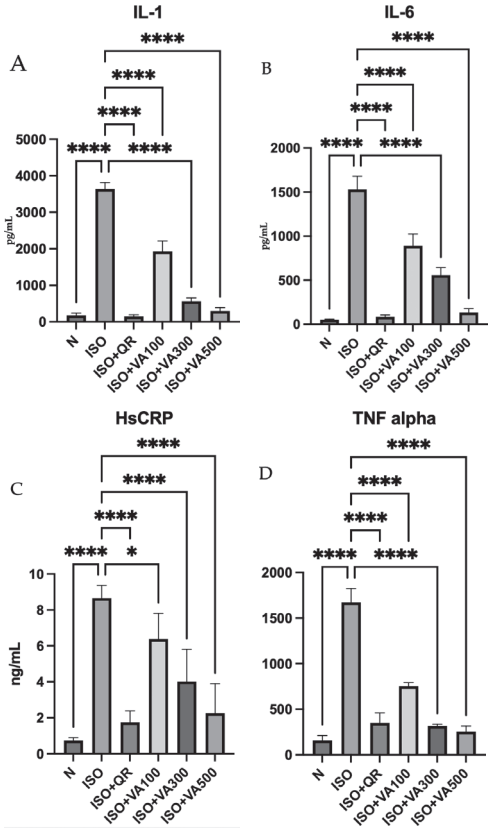

Figure 4. The effect of VAEE on IL-1 (**A**), IL-6 (**B**), HsCRP (**C**), and TNF alpha (**D**) expresssion. (N: normal rats, ISO: ISO 85 mg/kgbw ,ISO+QR: ISO 85 mg/kgbw + 100 mg/kgbw quercetin. ISO+VA100: ISO 85 mg/kgbw + 100 mg/kgbw VAEE, ISO+-VA300: ISO 85 mg/kgbw + 300 mg/kgbw VAEE, ISO+VA500: ISO 85 mg/kgbw + 500 mg/kgbw VAEE, the data were presented in mean ± standard error of the mean (SEM). (**: p<0,05, ***: p<0,01, ****: p<0.001, P>0.05 ns/not significance).

Effect of VAEE on pro-inflammatory cytokines (IL-1, IL-6, Hs-CRP, TNF alpha)

As shown in the Fig. 4. In the present study, the effect of VAEE on the level of in-terleukin-1 (IL-1), interleukin-6 (IL-6), and TNF alpha in rats induced with ISO was evaluated. The results showed that the IL-1, IL-6, TNF alpha level was significantly in-creased in the ISO-induced group compared to the control group ($p<0.001$). However, treatment with VAEE significantly decreased the IL-1, IL-6, and TNF alpha levels com-pared to the ISO-induced group ($p<0.001$). These results suggest that VA may have an anti-inflammatory effect in the heart and can potentially reduce the risk of cardiac damage induced by ISO. Furthermore, the effect of VA on high-sensitivity C-reactive protein (HsCRP) level in rats induced with ISO was evaluated. The results showed that the HsCRP level was significantly increased in the ISO-induced rats when compared to the control group ($p<0.001$). However, treatment with VA significantly decreased the HsCRP level when compared to the ISO-induced group ($p<0.001$).

Effect of VAEE on apoptosis markers (Caspase-3 and p53)

As shown in the Fig. 5. A study was conducted to investigate the effect of VA on caspase-3 level in rats induced with ISO. The results showed that the ISO group had a significant increase in caspase-3 level compared to the control group ($p<0.001$). Treatment with VA significantly reduced caspase-3 level compared to the ISO group ($p<0.001$). The caspase-3 level in the VA group was also significantly lower than the control group ($p<0.001$). These results suggest that VA may have a protective effect against ISO-induced cardiac apoptosis through the inhibition of caspase-3 activation. In this study conducted on rats induced with ISO, treatment with VA was found to significantly reduce the con-centration of p53 protein compared to the control group ($p<0.001$). This suggests that VA may have a cardioprotective effect by reducing oxidative stress and apoptosis of cardiac cells, which is mediated by the p53 protein. The study highlights the potential of p53 protein concentration as a biomarker to assess the cardioprotective activity of VA in the treatment of cardiovascular diseases.

Effect of VAEE on oxidative stress markers (SOD, GPx, Catalase)

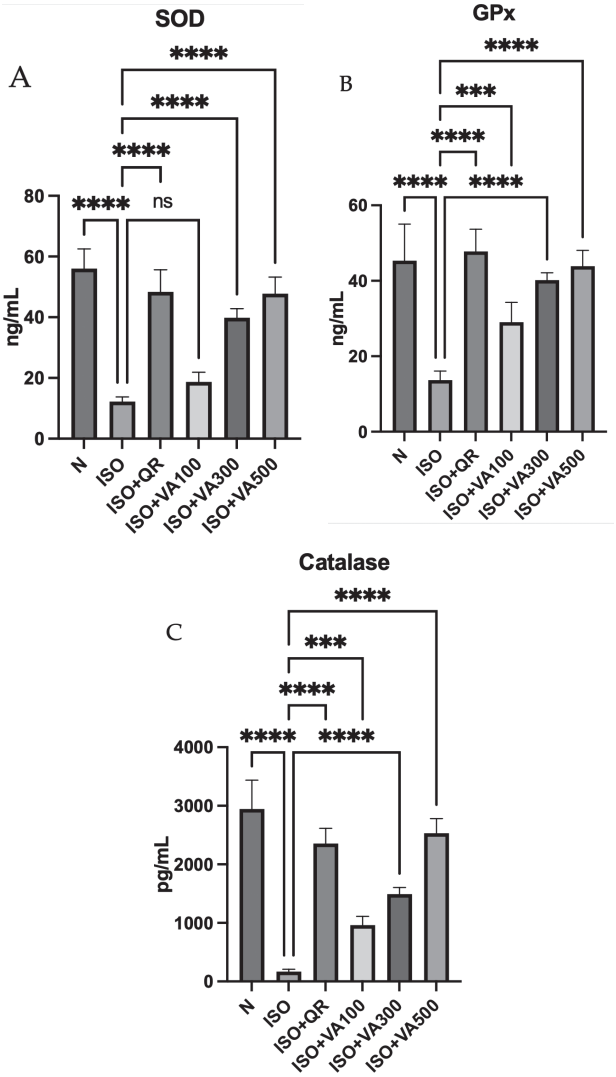

Figure 6. The effect of VAEE on SOD (**A**), GPx (**B**), and Catalase (**C**) expresssion. (N: normal rats, ISO: ISO 85 mg/kgbw ,ISO+QR: ISO 85 mg/kgbw + 100 mg/kgbw quercetin. ISO+VA100: ISO 85 mg/kgbw + 100 mg/kgbw VAEE, ISO+VA300: ISO 85 mg/kgbw + 300 mg/kgbw VAEE, ISO+VA500: ISO 85 mg/kgbw + 500 mg/kgbw VAEE, the data were presented in mean ± standard error of the mean (SEM). (*: $p<0,05$, **: $p<0,01$, ***: $p<0.001$, $P>0.05$ ns/not significance).

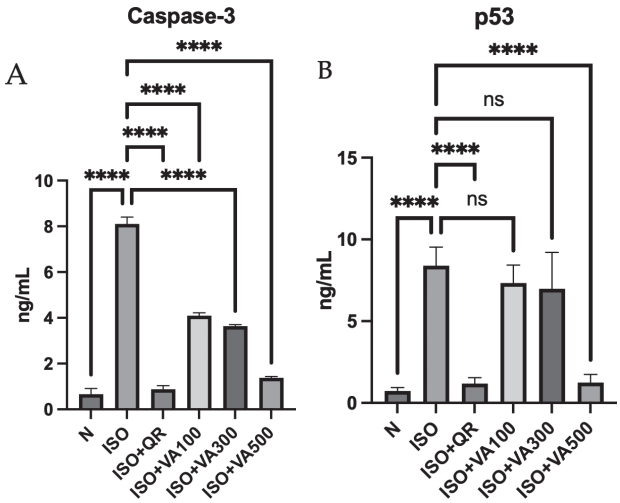

Figure 5. The effect of VAEE on caspase-3 (**A**), Bcl-2 (**B**), p53 (**C**) expresssion. (N: normal rats, ISO: ISO 85 mg/kgbw ,ISO+QR: ISO 85 mg/kgbw + 100 mg/kgbw quercetin. ISO+VA100: ISO 85 mg/kgbw + 100 mg/kgbw VAEE, ISO+VA300: ISO 85 mg/kgbw + 300 mg/kgbw VAEE, ISO+VA500: ISO 85 mg/kgbw + 500 mg/kgbw VAEE, the data were presented in mean ± standard error of the mean (SEM). (*: $p<0,05$, **: $p<0,01$, ***: $p<0.001$, $P>0.05$ ns/not significance).

As shown in the Fig. 6. This study investigating the effects of VAEE on rats induced with ISO may report a significant increase in SOD activity in rats treated with VA compared to the control group. This result suggests that VA may have potential cardioprotective effects by enhancing the antioxidant defense system and reducing oxidative stress-induced damage. Additionally, this study shown a dose-dependent response, where increasing doses of VA lead to a greater increase in SOD activity. This finding provides further evidence of the potential benefits of VA in reducing oxidative stress and enhancing antioxidant defense in rats induced with ISO. In this study shown the effects of VAEE on rats induced with ISO may report a significant increase in GPx activity in rats treated with VAEE compared to the control group. This result suggests that VAEE may have potential cardioprotective effects by enhancing the antioxidant defense system and reducing oxidative stress-induced damage. This study shown the positive correlation between SOD and GPx levels in rats treated with VA, indicating a coordinated antioxidant response to reduce oxidative stress. Present study shown the effects of VAEE on rats induced with ISO may report a significant increase in CAT activity in rats treated with VA compared to the control group. This result suggests that VA may have potential cardioprotective effects by enhancing the antioxidant defense system and reducing oxidative stress-induced damage.

Effect of VAEE on cardiac histology

As shown in the Fig. 7. The induction of ISO in rats shown to cause cardiac injury, including necrosis, fibrosis, and apoptosis. VAEE, a medicinal plant, shown possess potent antioxidant and anti-inflammatory properties, which may protect the heart against injury induced by ISO. This study showed that VAEE pretreatment prevented ISO-induced cardiac injury by preserving the histological architecture of the heart tissue. The rats treated with VA had signifi-

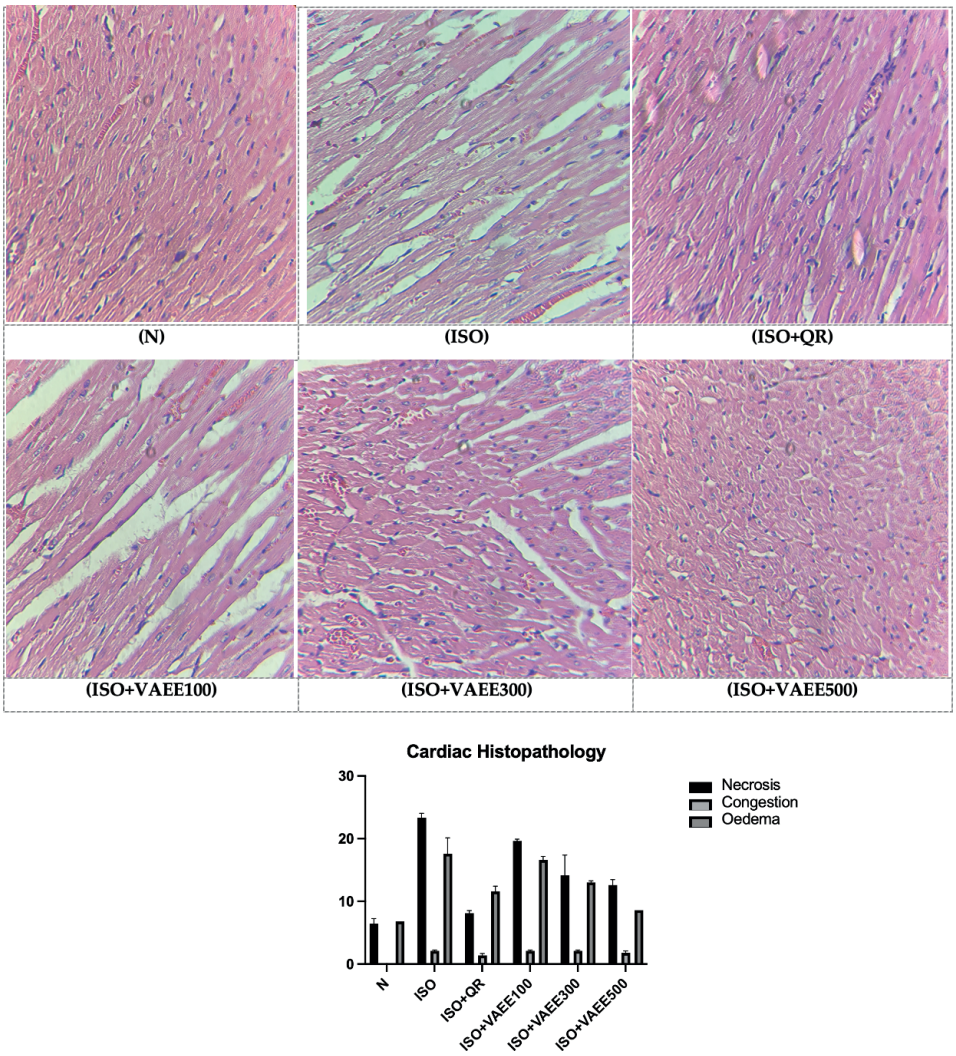

Figure 7. Histology of cardiac tissue stained with HE which assess the necrosis, congestion, and oedema (N: normal rats, ISO: ISO 85 mg/kgbw ,ISO+QR: ISO 85 mg/kgbw + 100 mg/kgbw quercetin. ISO+VA100: ISO 85 mg/kgbw + 100 mg/kgbw VAEE, ISO+-VA300: ISO 85 mg/kgbw + 300 mg/kgbw VAEE, ISO+VA500: ISO 85 mg/kgbw + 500 mg/kgbw VAEE, black arrow show aggregation while red arrow show necrotic, 400× magnification, , the data were presented in mean ± standard error of the mean (SEM). (*: p<0,05, **: p<0,01, ***: p<0.001, P>0.05 ns/not significance).

cantly reduced levels of necrosis, congestion and oedema compared to the rats that were not treated with VAEE ($p<0.001$). This study showed that VA treatment significantly reduced the levels of oxidative stress and inflammation in the heart tissue.

The effect of VAEE on the expression of bcl-2 and collagen on cardiac histology

As shown in the Fig. 8. This study investigating the effects of VA on the expression of bcl-2 in rats with cardiac histopathology may show an increase in bcl-2 expression ($p<0.001$). Bcl-2 is an anti-apoptotic protein that plays a key role in regulating cell death in cardiac tissue. Histopathological changes in the heart can lead to a decrease in bcl-2 expression and an increase in cell death, which can lead to the development of cardiovascular disease. Moreover, in the group of rats induced with ISO and treated with

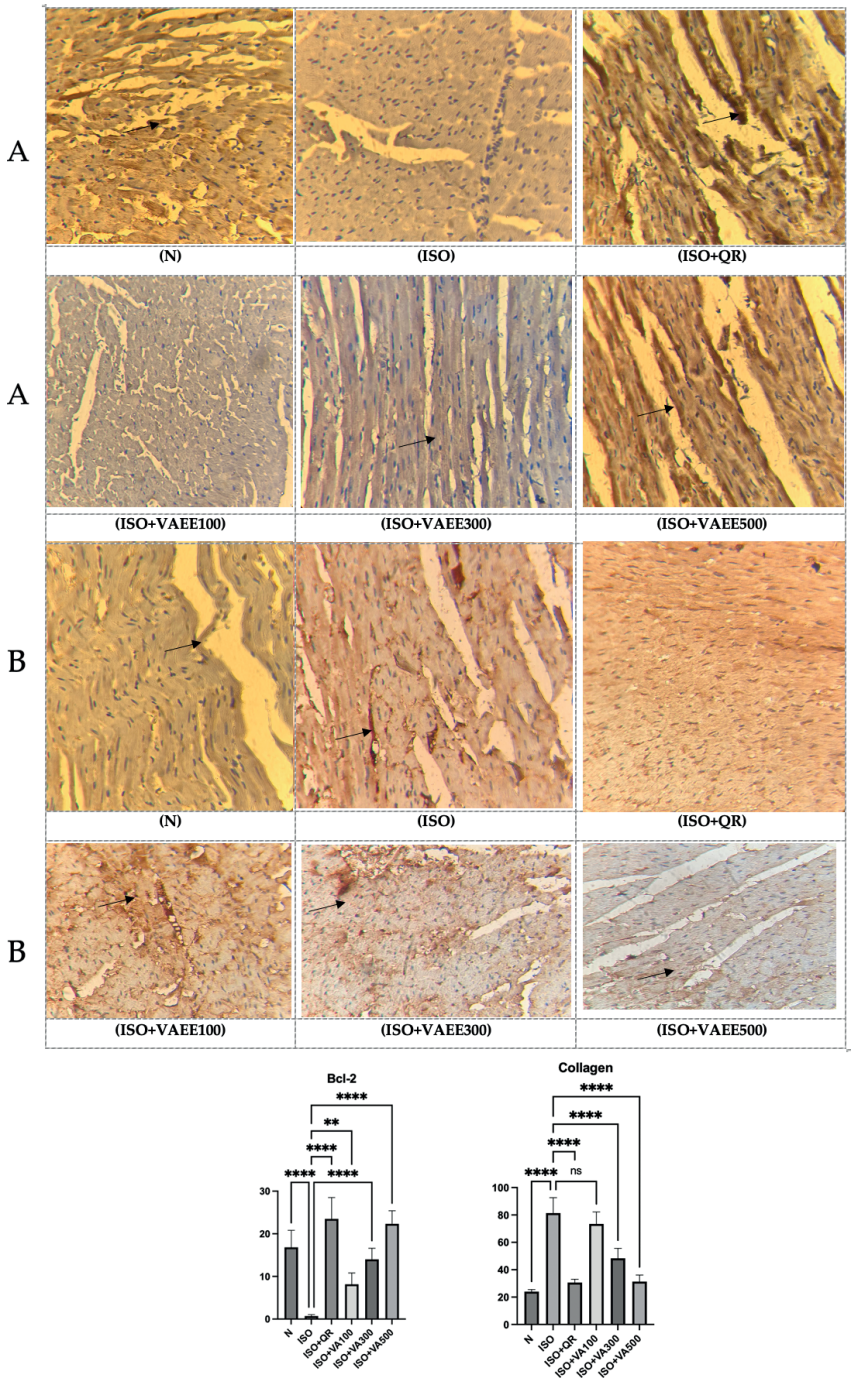

Figure 8. Histology of cardiac tissue stained with Bcl-and Collagen (B) (N: normal rats, ISO: ISO 85 mg/kgbw, ISO+QR: ISO 85 mg/kgbw + 100 mg/kgbw quercetin. ISO+VA100: ISO 85 mg/kgbw + 100 mg/kgbw VAEE, ISO+VA300: ISO 85 mg/kgbw + 300 mg/kgbw VAEE, ISO+VA500: ISO 85 mg/kgbw + 500 mg/kgbw VAEE, the arrows show the yellow color of the expression bcl-2 in coloum A and collagen in coloum B, 400× magnification, the data were presented in mean ± standard error of the mean (SEM). (*: p<0,05, **: p<0,01, ***: p<0.001, P>0.05 ns/not significance).

VAEE, the expression of collagen in the cardiac tissues was significantly reduced compared to the untreated group. The histological examination showed a significant reduction in collagen deposition and fibrosis in the cardiac tissues of the VAEE-treated group. This suggests that VAEE may have a protective effect against ISO-induced cardiac fibrosis.

Gas chromatography-mass spectroscopy profiling of VAEE

The GC-MS analysis of VAEE revealed the presence of 20 different compounds with diverse phytochemical activities. Fig. 8 shows the chromatogram obtained from the analysis. Table 2 and Fig. 9 provides information on the retention time (RT), molecular formula, molecular weight (MW), and concentration (%) of the identified chemical constituents in VAEE. The analysis identified several bioactive compounds in VAEE, which are listed below Carbetapentane Trans-β-Ionone, Bornyl Acetate, Methyl pentadecanoate, 2-furancarboxaldehyde, 5-methyl-, Cyclohexane, 1,1'-(1,2-dimethyl-1, 2-ethanediyl)bis-, 2-Pentadecanone, 6,10,14-trimethyl, Trans-Farnesol, EthylHexadecanoate, 1,6-Anhydro-beta.-D-Glucopyranose (levoglucosan), 6,8-Tioxa-3-thiabicyclo(3,2,1)octane 3,3-dioxide, 1-Heneicosanol, 2,4-Di-Tert-Butylphenol, Hexadecanoic acid, methyl ester, Phytol, Trihexadecyl borate, 3a-Hydroxy-1,2,3,3a,8,8a-Hexahydropyrrolo (2,3b)

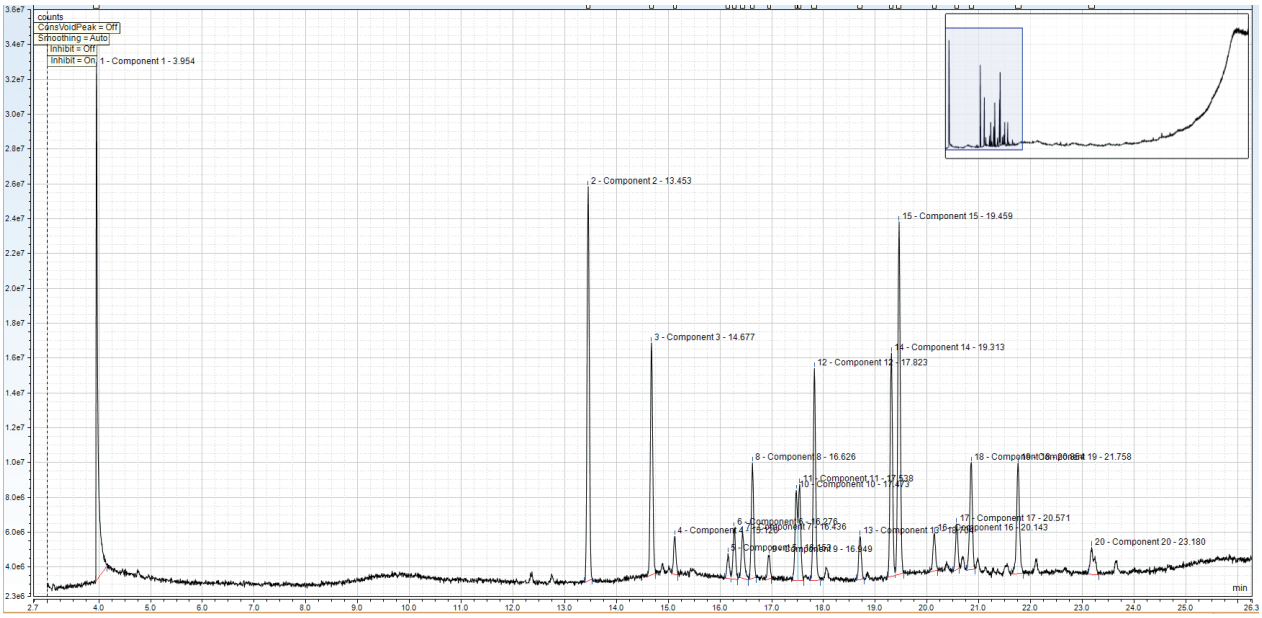

Figure 9. GC-MS chromatogram of VAEE.

Table 2. Bioactive compounds found in VAEE.

No.	Chemical name	Molecular weight (g/mol)	Molecular formula	Retention time (Min)	Relative area (%)
1.	Carbetapentane	221.34	C14H23NO	3.954	13.44
2.	Trans-β-Ionone	192.30	C13H20O	13.453	13.58
3.	Bornyl Acetate	196.29	C12H20O2	14.677	8.53
4.	Methyl pentadecanoate	270.45	C17H34O2	15.126	1.45
5.	2-furancarboxaldehyde, 5-methyl-	110.11	C6H6O2	16.153	0.91
6.	Cyclohexane, 1,1'-(1,2-dimethyl-1, 2-ethanediyl)bis-	194.35	C14H26	16.276	2.02
7.	2-Pentadecanone, 6,10,14-trimethyl	296.53	C20H40O	16.436	2.10
8.	Trans-Farnesol	222.37	C15H26O	16.626	4.21
9.	EthylHexadecanoate	284.48	C18H36O2	16.949	0.98
10.	1,6-Anhydro-beta.-D-Glucopyranose (levoglucosan)	162.14	C6H10O5	17.473	3.52
11.	6,8-Tioxa-3-thiabicyclo(3,2,1)octane 3,3-dioxide	208.30	C7H12O3S2	17.538	3.49
12.	1-Heneicosanol	312.59	C21H44O	17.823	7.87
13.	2,4-Di-Tert-Butylphenol	206.32	C14H22O	18.704	1.77
14.	Hexadecanoic acid, methyl ester	270.45	C17H34O2	19.313	8.51
15.	Phytol	296.53	C20H40O	19.459	13.36
16.	Trihexadecyl borate	742.19	C48H99BO3	20.143	1.56
17.	3a-Hydroxy-1,2,3,3a,8,8a-Hexahydropyrrolo (2,3b)Indole	192.26	C11H16N2O	20.571	1.69
18.	Alpha-cedrol	222.37	C15H26O	20.854	4.58
19.	Methyl 10-trans,12-cis-octadecadienoate	294.47	C19H34O2	21.758	5.00
20.	Benzyl benzoate	212.25	C14H12O2	23.180	1.75

Indole, Alpha-cedrol, Methyl 10-trans,12-cis-octadecadienoate, Benzyl benzoate.

This study contributes to the prediction of the formula and structure of 20 biomolecules. Subsequent research could potentially involve isolating bio-active compounds, determining their structure, and conducting pharmacological screening. These findings would be beneficial for advancing drug development efforts in the future.

Discussion

As CVDs are the primary cause of mortality and morbidity on a global scale, there is a crucial need to develop efficient treatments for CVDs, particularly MI. Since oxidative tissue injury and inflammation are key components implicated in the pathological processes of MI, novel compounds with antioxidant and anti-inflammatory effects may prevent the development of cardiovascular dysfunction in AMI ISO as beta adrenergic agonist produce abundant of oxidate stress in myocardium in which cause myocardial destruction. Several mechanism of ISO cause myocardial dysfunction have been revealed and one the most studied is production of ROS. In this study show that ISO-induced generates free radical stress which increase lipid peroxidation and the result of irreversible demerge of myocardial membrane (Kalpana et al. 2003; Menon and Sudheer 2007; Lee et al. 2010; Kumar et al. 2011; Muthu et al. 2011; Maheswari et al. 2013; Amsterdam et al. 2014). Endogenous antioxidant such as GPX, catalase dan SOD structurally consist of hydroxy radical and superoxide anion which has advantage effect protective the cell from oxidative stress by ISO, the existence of this antioxidant is essential neutralizing the free radical. Abnormally, the ISO group depleted the production of GPX, catalase dan SOD while in the group pre-administered by *Vernonia amygdalina* extract increase the level of GPX, catalase dan SOD and restore it to normal level. Similar study also show that rat were pre-treatment with quercetin or extract show the increasing level of GPX, catalase dan SOD. In short, elevating the antioxidant capacity and neutralizing the free radical are essential for defensive system of myocardium in rats induced SOD. In line with this study show that *Vernonia amygdalina* extract contain secondary metabolite such as rutin and luteolin which has protective effect on the heart. This study show dose dependent manner that increase the dosage of the extract may increase the production of GPX, catalase dan SOD (O'gara et al. 2013; Anderson et al. 2016; Roffi et al. 2016; Ibanez et al. 2018; Thygaesen et al. 2018). In the current study, ISO induced AMI showed significant increase of cardiac markers enzymes like CK-MB, LDH, Troponin T, and BNP. Meanwhile in the group of orally pre-treatment of VAEE 100, 300, 500 mg/kgbw were reduced in the level of those enzyme, this is indicating that myocardium dysfunction event in ISO group, this data also supported by histoarchitecture of the myocardium that showed necrosis in ISO group. Our previously study confirmed that VAEE attenuating the cardiac dysfunction in rats induced doxorubicin which shown depletion of CK-MB, LDH, Troponin T, and BNP levels in group given *Vernonia amygdalina*. ISO-induced ROS production promotes myocardial inflammation by activating NF-B and releasing pro-inflammatory mediators, resulting in cardiac apoptosis and injury. Several studies have found that the hearts of ISO-intoxicated mice have decreased Bcl-2 expression while increasing NF-B p65, Bax, and caspase-3 expression, as well as TNF- and IL-6 levels [43-45]. Increased pro-inflammatory cytokines, such as TNF- and IL-6, can directly or indirectly affect the myocardium via changes in hemodynamic loading conditions, causing additional oxidative stress, myocyte contractility and viability changes, endothelial and myocardial dysfunction, and myocardial necrosis. The execution phase of caspase-3-dependent apoptotic cell death appears to be triggered by persistent ROS generation, which facilitates the dissipation of mitochondrial membrane potential and the release of cytochrome c. As a result, the inflammatory response and subsequent apoptosis are regarded as one of the most important therapeutic components associated with cardiac injury and dysfunction. Oxidative stress also upregulated pro-inflammatory cytokine synthesis, demonstrated the mechanistic link between ROS overproduction and inflammation (Lopes et al. 2010; Levine et al. 2016). ROS release during MI increased pro-inflammatory mediator levels and stimulated caspase-mediated apoptosis. In the present study, this was demonstrated by the increased caspase-3 level in the ISO group. In this present study show that ISO group increase the pro-inflammatory cytokines IL-1, IL-6, TNF alpha and also increase Hs-CRP, meanwhile in the group pre-treatment orally with VAEE reduce the pro-inflammatory cytokines IL-1, IL-6, TNF alpha. Additionally, to confirmed apoptosis incidence on the myocardium caspase-3, Bcl-2, and p53 protein were measured which shown that in the group ISO increase the level of caspase-3, Bcl-2, and p53. Histopathological investigations provided additional support for these biochemical findings. Mice treated with ISO demonstrated degenerative changes in the myocardium, including a decreased number of myocardial cells, partial absence of the basement membrane, and necrosis of cardiac tissue (Sunahara et al. 1996). This indicates a significant amount of injury at higher magnification. The protective effect of VAEE against ISO-induced AMI was demonstrated by a regenerative effect and reduced pathological changes in rodents that were pretreated with VAEE. VAEE contain several flavononid compound such as luteolin, rutin, kaemperol, and betanin. Moreover, the study reveal that VAEE has phytol compound, Phytol (PYT) is classified as a diterpene and belongs to the group of long-chain unsaturated acyclic alcohols. PYT and its many derivatives, such as phytanic acid (PA), exhibit a diverse array of biological impacts. Phytol exhibits robust antioxidant properties, which can effectively mitigate oxidative stress. Consequently, its administration following isoproterenol-induced cardiac damage could potentially confer cardioprotective benefits (Islam et al. 2018).

Conclusion

Vernonia amygdalina ethanol extract (VAEE) has vital role in order to prevent the acute myocardial infraction (AMI) caused by isoproterenol. Furthermore, VAEE reduced car-

diac marker, down lift the apoptosis and increase antioxidant capacity.

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