

Cardioprotective Potentials of Vernonia amygdalina on 1,2-Dimethylhydrazine Induced Toxicity in Rats

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Abstract

Background

Cardiotoxicity is an emerging condition that occurs due to systemic toxicity resulting from 1,2 dimethylhydrazine (DMH) exposure. Until now, nothing is known about the role of *Vernonia amygdalina* in suppressing lipid abnormalities and oxidative stress caused by DMH cardiotoxicity in rats. Our novel treatment measures tend to be beneficial in combating heart toxicity resulting from DMH exposure. This study was conducted to investigate the cardioprotective potentials of *Vernonia amygdalina* on 1,2-Dimethylhydrazine induced toxicity in rats.

Methods

Adult male Wistar rats were exposed to 1, 2 dimethylhydrazine (DMH) toxicity via the intraperitoneal route at a single dose of 40 mg/kg body weight before and after treatment with lower (200 mg/kg body weight) and higher (400 mg/kg body weight) doses of *Vernonia amygdalina* ethanol extract. Lipid profile parameters, cardiovascular disease risks factors and oxidative stress markers were measured in plasma and heart tissue homogenates of rats using standardized protocols, while portions of the excised heart specimens were subjected to histopathological examination. Data analysis and post-hoc test for this study were performed using SPSS version 20.

Results

The concentrations of heart tissue antioxidant enzymes (CAT, SOD, GPx, GSH, GR and GSH (%)) in the DMH group were significantly reduced ($p < 0.05$) relative to control and the other groups. However, administration of ethanol extract of *V. amygdalina* to rats significantly increased ($p < 0.05$) these levels when compared with the DMH group. Furthermore, there was a significant decrease ($p < 0.05$) in the mean values of cardiovascular disease risk factors, cardiac Nitric Oxide (NO), and Malondialdehyde (MDA) in the treatment groups when compared with control and DMH group. Heart tissues of DMH-induced rats treated with *Vernonia amygdalina* revealed a significant reduction ($p < 0.05$) in organ weight relative to the control group. Histological studies revealed the protective and regenerative effects of *Vernonia amygdalina* on heart tissues.

Conclusions

The findings from this study indicates that *Vernonia amygdalina* can effectively suppress irregularities in the lipid profile, cardiovascular disease risk factors, and oxidative stress parameters caused by DMH toxicity due to its antioxidant properties. This implies that *Vernonia amygdalina* holds promise as a novel therapeutic strategy for improving DMH systemic toxicity.

Background

Cardiotoxicity is the occurrence of heart dysfunction as electric or muscle damage, resulting in heart toxicity [1]. This can cause heart failure, arrhythmia, myocarditis, and cardiomyopathy, resulting in a weakened heart that is not as efficient at pumping blood. While some of these effects are reversible, others can cause permanent damage, requiring further treatment [2]. Cardiotoxicity is considered a subset of systemic toxicity, as it is one of the possible negative effects a drug or substance can have on the body [3]. Drug-induced cardiotoxicity is a primary concern in both drug development and clinical practice. Although the heart is not a common target for adverse drug reactions, some drugs still cause various adverse cardiac events, which sometimes lead to severe consequences. Direct cardiac toxicity encompasses functional and structural changes of the cardiovascular system due to possible exposure to medicines [4].

1, 2- Dimethyl hydrazine (DMH), is a potent colon toxicant, inducing colorectal tumors in experimental animals and is the most widely used model of chemically induced colon toxicity which leads to carcinogenesis [5]. 1, 2- Dimethyl hydrazine shares many resemblances to human colorectal toxicity, including resemblance in the response to some promotional and preventive agents [6]. Models used in DMH toxicity studies are developed for exploring the medicinal properties of plant-derived components, because it offers identical site and pathological changes, which can also be found in humans [7].

Medicinal plants have been serving as precious starting materials for drug development in both developing and developed countries and there has been a great deal of interest nowadays in the role of complementary and alternative medicines for the treatment of many acute and chronic diseases [8]. Most biologically active compounds isolated from natural plant products have been identified to be nontoxic or less toxic to normal cells, and this has gained attention from the scientific community and clinicians in the present drug discovery era [9].

Vernonia amygdalina, also known as bitter leaf is a medicinal plant widely used in traditional medicine across various parts of Nigeria. The leaves of this plant which have numerous bioactive compounds, offer a wide range of potential health benefits [10]. *Vernonia amygdalina* is rich in biologically-active compounds such as antioxidants and polyphenols which helps to reduce oxidative stress resulting from the accumulation of free radicals, thus leading to the prevention of diseases [11]. In this study we investigated cardioprotective potentials of *Vernonia amygdalina* on 1,2-Dimethylhydrazine induced toxicity in rats.

Methods

Chemicals and Reagents

Chemicals, reagents and solvents used in this study were of analytical grade. They were purchased from Sigma-Aldrich Ltd. (St. Louis Mo, USA), Randox Ltd. (UK), and Pyrex Ltd. (Nigeria). They include distilled water, phosphate buffer saline, ethanol, 1, 2 dimethylhydrazine powder, total cholesterol, triacylglycerol, nitric oxide, glutathione reductase, glutathione peroxidase, superoxide dismutase, catalase and malondialdehyde.

Equipment and Apparatuses

The equipment and apparatuses used in this study includes: micropipette (10 – 50µL and 100 – 10000µL), measuring cylinders, filter papers/cheese cloth, bunsen burner, plain bottles, test tubes, surgical blades, syringes, galvage tubes, cotton wools, methylated spirit, latex hand gloves, spectrophotometer, centrifuge, Soxhlet machine, laboratory fridge and electronic weighing balance.

Collection, Identification and Preparation of *Vernonia amygdalina* Extract

Fresh mature leaves of the plant were obtained from a vegetable farm in Benin City, Edo State, Nigeria. They were identified and authenticated in the Herbarium of the Department of Plant Biology and Biotechnology, University of Benin, Nigeria, with the voucher number given as: UBH-V342. Fresh leaves of *Vernonia amygdalina* were separated from the stalk, washed and air-dried at room temperature (24°C), pulverized, crushed into fine powder and weighed. Ethanol extract of *Vernonia amygdalina* leaves were prepared by soaking 400g of the dry powdered plant leaves in 1000ml of absolute ethanol at room temperature for 72 hrs. The extract was thereafter filtered first through a Whatmann filter paper No. 42(125mm) and then through cotton wool. The extract was concentrated using a rotary evaporator at 40°C to one-tenth its original volume and finally with a freeze drier. The dried residue was stored at 4°C. Portions of the crude plant extract were weighed, dissolved in distilled water and kept for experimental analysis.

Experimental Animals

Male Wistar albino rats weighing 150 – 200g, were purchased from the animal house, Department of Biochemistry, University of Benin, Benin City. They were accommodated in clean disinfected cages under standard laboratory conditions, with access to feeds (pelletized growers mash) and water ad libitum. They were acclimatized for two (2) weeks before the experiment began. The experimental protocol for this study was conducted following established guidelines as described by the National Institute of Health guidelines in the “Principle of Laboratory Animal Care” (NIH Publication No. 85 – 23) and approved by the Animal Care Ethical Committee of University of Benin, Benin City, Edo State, Nigeria.

Experimental Design of the Study

Forty (40) male Wistar albino rats weighing 150 – 200 g were randomly assigned to eight (8) groups (5 rats per group) as follows: control, DMH (40 mg/kg body weight), silymarin (100 mg/kg body weight), *Vernonia amygdalina* (300 mg/kg body weight), pretreatment (200 mg/kg body weight body weight), pretreatment (400 mg/kg body weight), post-treatment (200 mg/kg body weight), and post-treatment (400 mg/kg body weight) groups. With the exception of control and *Vernonia amygdalina* groups, the rats were exposed to DMH prior to or after treatment with *Vernonia amygdalina* via intraperitoneal route at a single dose of 40 mg/kg body weight [12]. Rats in silymarin group were treated with the standard hepato-/cardio-protective drug, silymarin (100 mg/kg body weight). The body weight of rats in all groups was recorded at weekly interval for 21 days and treatment lasted for the same period of time.

Table 2: Groupings and Treatments

EXPERIMENTAL DESIGN	
Groups	Treatments
I	Control
II	DMH only (40 mg/kg body weight)
III	Silymarin (100 mg/kg body weight)
IV	<i>Vernonia amygdalina</i> leaf extract only (300 mg/kg body weight)
V	Pretreatment – VA leaf extract (200 mg/kg bwt) for 18 days, then followed by DMH (40 mg/kg body weight).
VI	Pretreatment – VA leaf extract (400 mg/kg bwt) for 18 days, then followed by DMH (40 mg/kg body weight).
VII	Post treatment – DMH (40 mg/kg bwt) on day 0, then followed by VA leaf extract (200 mg/kg body weight) for 3 weeks.
VIII	Post treatment – DMH (40 mg/kg body weight) on day 0, then followed by VA leaf extract (400 mg/kg body weight) for 3 weeks.

Blood/Tissue Sample Collection and Preparation

At the closing stages of the experiment, blood samples were collected by cardiac puncture under mild ketamine anesthesia into plain and heparin/EDTA containers and centrifuged at 3500rpm for 15min to obtain plasma. The heart tissues were excised and used for histological studies, as well as homogenates preparation. Biochemical analysis were carried out for the following test: High density lipoprotein – cholesterol (HDL-C), Low density lipoprotein – cholesterol (LDL-C), Very low density lipoprotein – cholesterol (VLDL-C), Total cholesterol (TC), Triglyceride (TG), Cardiovascular disease risk factors - [(Atherogenic index of plasmas (AIP), Atherogenic coefficient (AC), Cardiac risk ratio (CRR)], Nitric oxide (NO), Superoxide dismutase (SOD), Catalase (CAT) and Malondialdehyde (MDA), Glutathione (GSH), Glutathione Peroxidase (GPx), Glutathione reductase (GR) and concentration of reduced glutathione (%GSH).

Biochemical Analysis

Heart tissues were homogenized in ice-cold phosphate buffer using a mechanical grinder. Tissue homogenates were centrifuged at 3,500 rpm in a refrigerated centrifuge at 4 °C for 10 min. Supernatants were aliquots which were stored at 20 °C for later determination of heart parameters of oxidative stress and lipid peroxidation.

Measurement of Lipid Profile and Cardiovascular disease risk factors

Plasma lipid profile parameters were determined: total cholesterol (TC), triglycerides (TG), and high-density-lipoproteins-cholesterol (HDL-C) assays were performed using the Randox Kit according to the manufacturer's instructions. Low-density lipoprotein-cholesterol (LDL-C) and very low-density lipoprotein-cholesterol (VLDL-C) were calculated from the values of triglycerides, total cholesterol, and HDL as earlier described [13,14,15]. Cardiovascular disease risk factors were determined by calculating the atherogenic index ((TC-HDL)/HDL) and coronary risk index.

Measurement of Nitric Oxide Concentration

To assess the nitrosative stress, the nitric oxide (NO) content of the heart homogenates was measured using the Griess reaction in which nitric oxide is transformed to nitrite as a more stable metabolite that is then converted to HNO_2 in an acidic environment. HNO_2 then forms a diazonium salt in reaction with sulfanilamide which reacts with N-(1-naphthyl) ethylenediamine to generate an azo dye detectable at 540nm. The NO content of the examined heart specimens was expressed as nmol per mg of protein in the sample [16].

Measurement of Lipid Peroxidation Index

Malondialdehyde (MDA) was estimated in heart tissues homogenates supernatants following the Thiobarbituric Acid Reacting Substance (TBARS) assay procedure [17]. 100 μL of supernatant was diluted twenty times in 0.15 M Tris-KCl buffer, and deproteinized with 500 μL trichloroacetic acid (30%). The mixture was centrifuged in a bench top centrifuge at 4000 rpm for 10 min at room temperature. 200 μL of the supernatant was removed into eppendorff tube, followed by addition of 200 μL thiobarbituric acid (0.75%), and the mixture was heated at 80 °C for 1 h. The tubes were cooled by placing on ice, 200 μL was removed into microtitre plate and absorbance measured at 532 nm. The concentration of TBARS in the tissues were expressed as $\eta\text{mol MDA/ mg protein}$.

Measurement of Oxidative Stress Parameters

Superoxide dismutase (SOD) enzyme activity was determined in heart tissues using the autooxidation of adrenaline assay method [18]. Assay for catalase enzyme activity in heart tissue supernatants was determined using the colorimetric assay based on the yellow complex formation with H_2O_2 and molybdate [19]. Glutathione content of tissue samples was determined as described by [20]. Briefly, heart homogenate (1.0mL) was mixed with 0.1mL of 25% trichloroacetic acid (TCA). The mixture was centrifuged at 5,000 $\times g$ for 10min to remove precipitate. Supernatant (0.1mL) was mixed with 2mL of 0.6mM DTNB prepared in 0.2M sodium phosphate buffer pH (8.0). Absorbance was read at 412nm. GSH Px activity was determined from the oxidation of NADPH to NADP^+ in the presence of H_2O_2 used as a substrate and monitored spectrophotometrically at 340nm for 3 minutes. GSH Px was expressed as (mol of NADPH/min/g protein using an extinction coefficient of 6.22 $\text{mM}^{-1} \text{ cm}^{-1}$) for NADPH.

Histopathological Examination of Heart Tissues

Heart specimens fixed in 10 % phosphosaline were examined microscopically for structural alterations in the tissues at the University of Benin Teaching Hospital (UBTH), Benin City, Edo State, Nigeria. The slides were processed and stained with hematoxylin and eosin (H&E). The photomicrographs of stained slides were captured using Olympus microscope (Olympus, New Jersey, USA) connected through a digital camera to a computer interface.

Statistical Analysis

Data obtained from this study were expressed as mean $\pm$ standard errors of means (SEM). They were evaluated using the one-way analysis of variance (ANOVA). Post-hoc comparison test was carried out using Turkey HSD to evaluate pair-wise differences among group means. Differences between groups were considered significant at $p < 0.05$. All statistical analysis were performed using the SPSS software tool (Version: 20, IBM Corporation, New York, USA).

Results

Effect of ethanol extract of *V. amygdalina* leaves on body weight of rats

Results obtained for the body weight of rats as shown in Table 1, indicated that the DMH group recorded significant reductions ($p<0.05$) in body weight when compared with control and the other groups. Consequently, there was a significant increase ($p<0.05$) in body weight of rats in the treatment groups when compared with the DMH group. Furthermore, the silymarin group recorded a significant increase ($p<0.05$) in final body weight when compared with the other groups.

Table 1: Body Weight of Rats

Groups	Initial Weight (g)	Final Weight (g)
Control	170.87 $\pm$ 8.12 ^a	200.38 $\pm$ 14.48 ^a
DMH	195.03 $\pm$ 12.01 ^b	178.95 $\pm$ 11.17 ^b
Silymarin	182.39 $\pm$ 5.20 ^c	212.42 $\pm$ 13.56 ^{ac}
VA only	180.44 $\pm$ 3.42 ^c	216.08 $\pm$ 11.94 ^c
Pretreatment (200 mg/kg bwt)	170.36 $\pm$ 35.09 ^a	188.86 $\pm$ 41.39 ^d
Pretreatment (400 mg/kg bwt)	168.15 $\pm$ 34.62 ^d	187.66 $\pm$ 16.35 ^{d^e}
Post-Treatment (200 mg/kg bwt)	177.66 $\pm$ 6.87 ^{ae}	195.44 $\pm$ 7.48 ^f
Post-Treatment (400 mg/kg bwt)	166.50 $\pm$ 6.47 ^{df}	204. 12.37 ^{ag}

Values are stated as Mean $\pm$ SEM (n=5)

For each parameter, values having different superscripts between groups differ significantly ($p<0.05$).

Effect of ethanol extract of *V. amygdalina* leaves on body weight change in rats

As shown in Table 2, there was a percentage decrease ($p<0.05$) in the body weight change of rats in the DMH group when compared with control and the other groups. Furthermore, groups treated with ethanol extract of *V. amygdalina* leaves, before and after DMH administration recorded percentage increase ($p<0.05$) in the body weight change relative to the DMH group.

Table 2: Change in Body Weight of Rats

Groups	Weight Change (g)	% Weight Change (g)
Control	29.51 $\pm$ 6.36 ^a	17.27 $\pm$ 0.78 ^a
DMH	16.08 $\pm$ 0.84 ^b	8.24 $\pm$ 0.06 ^b
Silymarin	30.03 $\pm$ 8.36 ^c	16.46 $\pm$ 1.60 ^c
VA only	35.64 $\pm$ 8.52 ^c	19.75 $\pm$ 2.49 ^c
Pretreatment (200 mg/kg bwt)	18.50 $\pm$ 6.30 ^b	13.18 $\pm$ 0.17 ^d
Pretreatment (400 mg/kg bwt)	41.51 $\pm$ 18.27 ^e	30.04 $\pm$ 0.53 ^e
Post-Treatment (200 mg/kg bwt)	17.78 $\pm$ 0.61 ^b	10.00 $\pm$ 0.88 ^c
Post-Treatment (400 mg/kg bwt)	38.02 $\pm$ 5.90 ^d	22. $\pm$ 0.91 ^f

Values are stated as Mean $\pm$ SEM (n=5)

For each parameter, values having different superscripts between groups differ significantly ($p<0.05$).

Effect of Ethanol Extract of *V. amygdalina* leaves on weight of cardiac tissues

The relative weight of the heart tissues in the DMH group revealed significant increase ($p<0.05$) in weight as shown in Table 3 relative to control and the other groups. Consequently, there were insignificant changes ($p<0.05$) in the various groups administered *V. amygdalina* extract before and after DMH induction when compared especially with silymarin and *V. amygdalina* groups respectively.

Table 3: Relative Weight of Heart in Rats

Groups	Heart weight (x 10 ⁻³)	Relative Heart weight (x 10 ⁻³)
Control	0.60 ± 0.12 ^a	3.00 ± 0.10 ^a
DMH	0.72 ± 0.03 ^b	4.00 ± 0.30 ^b
Silymarin	0.64 ± 0.04 ^c	3.00 ± 0.10 ^a
VA only	0.68 ± 0.03 ^a	3.10 ± 0.10 ^a
Pretreatment (200 mg/kg bwt)	0.62 ± 0.03 ^a	3.90 ± 0.20 ^{a, c}
Pretreatment (400 mg/kg bwt)	0.69 ± 0.03 ^a	3.80 ± 0.10 ^{a, d}
Post-Treatment (200 mg/kg bwt)	0.65 ± 0.03 ^a	3.30 ± 0.30 ^{a, b}
Post-Treatment (400 mg/kg bwt)	0.61 ± 0.05 ^a	2.90 ± 0.20 ^d

Values are stated as Mean ± SEM (n=5)

For each parameter, values having different superscripts between groups differ significantly ($p < 0.05$).

Effect of Ethanol Extract of *V. amygdalina* on lipid profile of rats

As shown in Table 4 and Table 5, results obtained from the lipid profile of rats in the DMH group recorded a high significant increase ($p < 0.05$) in the plasma levels of total cholesterol (TC), triglyceride (TG), very low-density lipoprotein-cholesterol (VLDL-C) and low-density lipoprotein-cholesterol (LDL-C), while high density lipoprotein-cholesterol (HDL-C) was significantly reduced ($p < 0.05$) relative to control and the other groups. Consequently, the pre- and post-treatment groups revealed significant decreases ($p < 0.05$) in levels of TC, TG, LDL-C and VLDL-C, and an increase ($p < 0.05$) in HDL-C levels when compared with the DMH groups.

Table 4: Lipid Profile of the Rats

Groups	Plasma Total Cholesterol (mg/dL)	Plasma Triglyceride (mg/dL)	HDL-Cholesterol (mg/dL)
Control	26.88 ± 3.30 ^a	42.52 ± 2.01 ^a	54.38 ± 1.68 ^a
DMH	92.75 ± 0.44 ^b	93.61 ± 4.56 ^b	16.01 ± 0.98 ^b
Silymarin	47.83 ± 1.68 ^c	48.54 ± 1.83 ^a	47.93 ± 0.89 ^c
VA only	42.62 ± 0.18 ^c	49.09 ± 0.92 ^a	51.11 ± 2.48 ^a
Pretreatment (200 mg/kg bwt)	50.03 ± 2.00 ^d	54.32 ± 3.11 ^a	66.04 ± 4.10 ^d
Pretreatment (400 mg/kg bwt)	54.63 ± 1.14 ^d	56.01 ± 1.51 ^a	49.66 ± 4.56 ^e
Post-Treatment (200 mg/kg bwt)	57.03 ± 1.51 ^d	62.41 ± 1.10 ^c	55.70 ± 2.12 ^a
Post-Treatment (400 mg/kg bwt)	52.52 ± 0.18 ^d	45.99 ± 17.89 ^a	49.51 ± 5.66 ^e

Values are stated as Mean ± SEM (n=5)

For each parameter, values having different superscripts between groups differ significantly (p<0.05).

Table 5: Lipid Profile of the Rats (Cont'd)

Groups	Plasma VLDL-Cholesterol (mg/dL)	LDL- Cholesterol (mg/dL)
Control	8.50 ± 1.04 ^a	44.77 ± 4.02 ^a
DMH	18.72 ± 1.53 ^b	179.32 ± 7.53 ^b
Silymarin	9.71 ± 1.09 ^c	111.06 ± 3.84 ^c
VA only	9.82 ± 0.88 ^c	115.92 ± 4.11 ^c
Pretreatment (200 mg/kg bwt)	10.32 ± 0.41 ^d	59.10 ± 11.56 ^d
Pretreatment (400 mg/kg bwt)	7.51 ± 1.11 ^e	59.85 ± 8.81 ^d
Post-Treatment (200 mg/kg bwt)	12.48 ± 0.93 ^b	59.87 ± 2.58 ^d
Post-Treatment (400 mg/kg bwt)	9.20 ± 1.27 ^c	89.59 ± 2.82 ^e

Values are stated as Mean ± SEM (n=5)

For each parameter, values having different superscripts between groups differ significantly ($p < 0.05$).

Effect of ethanol extract of *V. amygdalina* on cardiovascular disease risk factors of rats

In this study, there were high significant increases ($p < 0.05$) in the mean values of cardiovascular disease risk factors [atherogenic index of plasma (AIP), atherogenic coefficient (AC), cardiac risk ratio (CRR)], but these parameters were markedly reduced ($p < 0.05$) after treatment with ethanol extract of *V. amygdalina* (Table 6).

Table 6: Cardiovascular Disease Risk Factors

Groups	Atherogenic Index of Plasma	Atherogenic Coefficient	Cardiac Risk Ratio
Control	44.93 ± 2.37 ^a	0.98 ± 0.06 ^a	1.98 ± 0.08 ^a
DMH	180.49 ± 8.26 ^b	12.37 ± 1.16 ^b	13.37 ± 0.85 ^b
Silymarin	111.26 ± 5.84 ^c	2.52 ± 0.46 ^c	3.52 ± 0.28 ^c
VA only	116.11 ± 5.03 ^c	2.46 ± 0.18 ^c	3.46 ± 0.56 ^c
Pretreatment (200 mg/kg bwt)	66.04±4.10 ^d	3.19 ± 0.29 ^d	2.47 ± 0.64 ^b
Pretreatment (400 mg/kg bwt)	49.66±4.56 ^a	2.10 ± 9.46 ^c	2.20 ± 0.82 ^b
Post-Treatment (200 mg/kg bwt)	60.09 ± 1.62 ^d	1.30 ± 0.09 ^e	2.30 ± 0.40 ^b
Post-Treatment (400 mg/kg bwt)	89.78 ± 2.84 ^e	2.00 ± 0.21 ^c	3.00 ± 0.50 ^c

Values are stated as Mean ± SEM (n=5)

For each parameter, values having different superscripts between groups differ significantly ($p<0.05$).

Effect of ethanol extract of *V. amygdalina* on the activities of cardiac antioxidant enzymes in rats

Exposure of Wistar albino rats to DMH led to significant reductions ($p<0.05$) in the concentrations of cardiac antioxidant enzymes (CAT, SOD, GPx, GSH, GR and % GSH) relative to control and the other groups as shown in Table 7 and Table 8. However, treatment of rats before and after DMH administration with ethanol extract of *V. amygdalina* significantly increased ($p<0.05$) the levels of the antioxidant enzymes when compared with the DMH group.

Table 7: Activities of Antioxidant Enzymes in Cardiac Tissues

Groups	Catalase (U/min) $\times 10^{-2}$	SOD (U/min) $\times 10^{-2}$	GPx (U/min) $\times 10^{-4}$
Control	41.45 $\pm$ 1.25 ^a	39.00 $\pm$ 2.00 ^a	14.50 $\pm$ 0.50 ^a
DMH	11.50 $\pm$ 0.10 ^b	13.50 $\pm$ 3.50 ^b	0.65 $\pm$ 0.15 ^b
Silymarin	36.10 $\pm$ 1.20 ^c	28.50 $\pm$ 0.50 ^c	11.50 $\pm$ 1.50 ^c
VA only	35.45 $\pm$ 0.25 ^c	25.50 $\pm$ 0.50 ^c	11.00 $\pm$ 1.00 ^c
Pretreatment (200 mg/kg bwt)	35.60 $\pm$ 1.82 ^c	24.50 $\pm$ 0.50 ^c	8.00 $\pm$ 1.00 ^d
Pretreatment (400 mg/kg bwt)	36.37 $\pm$ 1.49 ^c	30.50 $\pm$ 2.50 ^a	9.00 $\pm$ 0.00 ^e
Post-Treatment (200 mg/kg bwt)	36.00 $\pm$ 0.90 ^c	28.50 $\pm$ 1.50 ^c	8.50 $\pm$ 0.50 ^d
Post-Treatment (400 mg/kg bwt)	37.70 $\pm$ 1.40 ^c	31.00 $\pm$ 4.00 ^a	9.50 $\pm$ 0.50 ^e

Values are stated as Mean $\pm$ SEM (n=5)

For each parameter, values having different superscripts between groups differ significantly ($p < 0.05$).

Table 8: Glutathione Reductase Activity and Concentration of Reduced Glutathione in Cardiac Tissues

Groups	GR (U/min) $\times 10^{-3}$	GSH (mg/mL)	% GSH
Control	47.50 $\pm$ 0.50 ^a	48.77 $\pm$ 0.47 ^a	72.87 $\pm$ 0.46 ^a
DMH	33.00 $\pm$ 0.00 ^b	34.15 $\pm$ 0.05 ^b	38.16 $\pm$ 1.32 ^b
Silymarin	44.50 $\pm$ 0.50 ^a	46.77 $\pm$ 0.29 ^a	66.79 $\pm$ 1.61 ^c
VA only	43.50 $\pm$ 1.50 ^a	46.75 $\pm$ 0.11 ^a	64.17 $\pm$ 2.28 ^c
Pretreatment (200 mg/kg bwt)	44.00 $\pm$ 1.00 ^a	44.93 $\pm$ 0.68 ^a	61.09 $\pm$ 4.09 ^c
Pretreatment (400 mg/kg bwt)	45.50 $\pm$ 0.50 ^a	46.54 $\pm$ 0.11 ^a	64.95 $\pm$ 3.66 ^c
Post-Treatment (200 mg/kg bwt)	43.00 $\pm$ 1.00 ^a	46.59 $\pm$ 1.09 ^a	53.20 $\pm$ 5.14 ^d
Post-Treatment (400 mg/kg bwt)	44.50 $\pm$ 0.50 ^a	47.86 $\pm$ 0.44 ^a	61.35 $\pm$ 3.01 ^c

Values are stated as Mean $\pm$ SEM (n=5)

For each parameter, values having different superscripts between groups differ significantly ($p < 0.05$).

Effect of ethanol extract of *V. amygdalina* on concentrations of malondialdehyde and nitric oxide in cardiac tissues of rats

In this study, there were significant increase ($p < 0.05$) in the mean values of cardiac NO and MDA in the DMH group when compared with control and the other groups as shown in Table 9. Consequently, treatment of rats with ethanol extract of *V. amygdalina* significantly decreased ($p < 0.05$) these values close to normal levels (Table 9)

Table 9: Levels of Malondialdehyde and Nitric Oxide in Cardiac Tissues

Groups	MDA (mole/mg tissue) $\times 10^{-3}$	Nitric Oxide ($\mu\text{mol/L}$)
Control	2.50 ± 0.50^a	36.95 ± 12.55^a
DMH	14.50 ± 1.50^b	59.95 ± 2.05^b
Silymarin	3.50 ± 0.50^c	30.10 ± 5.40^a
VA only	2.67 ± 0.33^a	41.80 ± 0.60^c
Pretreatment (200 mg/kg bwt)	5.00 ± 1.00^d	40.55 ± 18.25^c
Pretreatment (400 mg/kg bwt)	3.67 ± 0.33^c	40.25 ± 3.35^c
Post-Treatment (200 mg/kg bwt)	5.00 ± 1.00^d	41.50 ± 0.80^c
Post-Treatment (400 mg/kg bwt)	4.00 ± 1.00^e	40.85 ± 1.85^c

Values are stated as Mean $\pm$ SEM ($n=5$)

For each parameter, values having different superscripts between groups differ significantly ($p < 0.05$).

Heart

Discussion

In this current research, the rats were exposed to 1, 2 dimethylhydrazine (DMH) before and after *Vernonia amygdalina* administration. Results obtained for the body weight of rats as shown in Table 1, indicated that the DMH group recorded significant reductions ($p < 0.05$) in the final body weight when compared with control and the other groups. However, there was a significant increase ($p < 0.05$) in the relative weight of the heart when compared with other groups as shown in Table 3. When rats are exposed to toxicants, they typically experience a decrease in body weight when compared to normal rats, meaning the exposure to DMH can lead to weight loss in the animals; this is often used as an indicator

of toxicity and is frequently studied in research investigating potential preventive treatments for diseases [21]. The percentage increases in body weight of rats treated with ethanol extract of *V. amygdalina* leaves were significantly increased ($p < 0.05$), relative to the DMH group as shown in Table 2. This implies that the extract possesses beneficial properties for weight management. This agrees with a similar study carried out by [22].

Dyslipidemia is a condition of abnormal lipids, such as high triacylglycerol, cholesterol, and low-density lipoprotein cholesterol levels, which is characterized by metabolic disorders highly associated with obesity [23]. Lipid profile in plasma and tissues are important diagnostic tools, particularly in conditions such as obesity, diabetes mellitus, coronary heart disease, atherosclerosis, hypertension, infertility, obstructive jaundice, hepatitis and other diseases characterized by abnormalities of lipid metabolism [24]. Results obtained from the lipid profile of rats in the DMH group as shown in Table 4 and Table 5, recorded a high significant increase ($p < 0.05$) in the plasma levels of total cholesterol (TC), triglyceride (TG), very low-density lipoprotein-cholesterol (VLDL-C) and low-density lipoprotein-cholesterol (LDL-C), while high density lipoprotein-cholesterol (HDL-C) was significantly reduced relative to control and the other groups. This implies that DMH administration can lead to dyslipidemia which is a known condition of abnormal lipids in the body [25]. Consequently, the pre- and post-treatment groups revealed significant decreases ($p < 0.05$) in plasma levels of TC, TG, LDL-C and VLDL-C, and an increase ($p < 0.05$) in HDL-C levels when compared to the DMH groups. These observed effects of *V. amygdalina* in the plasma levels of lipid profile in DMH rats suggest that the extract is effective in ameliorating complications such as cardiovascular disease, arteriosclerosis, coronary artery disease etc. owing to its high content of polyphenols which are bioactive components in the extract [26, 27]. The elevated levels ($p < 0.05$) of the cardiovascular disease risk factors [atherogenic index of plasma (AIP), atherogenic coefficient (AC) and cardiac risk ratio (CRR)], in the DMH group as shown in Table 6, implies that there might be an increased risk of coronary heart disease and arteriosclerosis [28]. On the other hand, these changes were significantly reverted ($p < 0.05$) especially in the pre- and post-treatment groups relative to the control, silymarin and DMH groups respectively. This implies that *V. amygdalina* can reduce complications of cardiovascular disease such as blood pressure in rats, further contributing to its cardiovascular benefits [29, 30].

Antioxidant enzymes are crucial for protecting the body against the damaging effects of reactive oxygen species (ROS) or free radicals. They neutralize these highly reactive molecules, preventing them from causing harm to cells and tissues. By reducing ROS, antioxidant enzymes help maintain a healthy balance in the body, supporting overall well-being [31]. Exposure of Wistar albino rats to DMH led to significant reductions ($p < 0.05$) in the concentrations of cardiac antioxidant enzymes (CAT, SOD, GPx, GSH, GR and % GSH) relative to control and the other groups as shown in Table 7 and Table 8. The decrease observed in these parameters occurred due to the formation of free radicals caused by oxidative stress thereby leading to decrease in membrane fluidity, cell injury and cell death [32]. However, treatment of rats before and after DMH administration with ethanol extract of *V. amygdalina* significantly increased ($p < 0.05$) the levels of the antioxidant enzymes when compared with the DMH group. This suggests that the extract relieves heart tissues from inflammation and minimizes oxidative stress and

cell injury [33]. Other studies further shows that the extract contains vitamins and trace elements that promote healing in heart tissue [34].

In cardiac tissues, elevated levels of both malondialdehyde (MDA) and nitric oxide (NO) can be indicators of oxidative stress and potential heart damage. MDA, a product of lipid peroxidation, reflects oxidative stress, while NO, a vasodilator, can be affected by oxidative stress in the heart [35]. In this study, there were significant increase ($p < 0.05$) in the mean values of cardiac NO and MDA in the DMH group when compared with control and the other groups as shown in Table 9. The remarkable elevations of these parameters suggests that there is an increase in lipid peroxidation, loss of vascular integrity, coronary artery disease, atherosclerosis, blood pressure and smooth muscle cell proliferation [36]. Consequently, treatment of rats with ethanol extract of *V. amygdalina* significantly decreased ($p < 0.05$) these values close to normal levels which means that the extract may have protective effects against oxidative stress and inflammation in the heart [37].

In the histology studies, sections of heart tissues in rats exposed to DMH alone showed severe congestion of blood vessels and derangement of muscle fiber when compared with control and other groups (Plate 1 and Plate 2). Sections of cardiac tissues of rats that received only *V. amygdalina* and silymarin revealed normal myocytes, coronary artery and mild congestion with peripherally placed nuclei surrounded by normal eosinophilic cytoplasm (Plate 3 and Plate 4). Segments of cardiac muscles of the pre- and post-treatment groups at lower and higher doses revealed moderate myocytes, mild vascular architecture and mild vascular dilation with peripherally placed nuclei surrounded by eosinophilic cytoplasm which is consistent with the control group (Plate 5, Plate 6, Plate 7 and Plate 8). The defects in heart tissue histoarchitecture seen in the DMH group is due to edema, vasoconstriction, severe glassy or waxy hyaline degeneration, and necrosis of skeletal muscles [38]. *V. amygdalina* treatment prior to or after DMH induction at lower and higher doses suggests that the extract might have cardioprotective effects in rats, potentially by reducing oxidative stress and apoptosis, and improving heart histology [39]. This shows that the extract enhances the antioxidant defense system in rats, potentially reducing oxidative stress-induced damage in heart histology [40].

Conclusion

The results of this study have shown that *Vernonia amygladina* is able to suppress or reduce oxidative stress associated with cardiac toxicity in rats due to phytochemicals such as saponins and polyphenols present in the plant extract. Thus, further research on *Vernonia amygladina* may reveal its potential as a source of phyto-medicine which can be presented as a recommendation for clinical trials.

Abbreviations

%GSH – Concentration of Reduced Glutathione

AC – Atherogenic coefficient

AIP – Atherogenic index of plasmas

ANOVA – Analysis of variance

AOM – Azoxymethane (AOM)

CRR – Cardiac risk ratio

CVD – Cardiovascular disease

DMH – 1, 2- Dimethyl hydrazine

DNA – Deoxyribonucleic Acid

EDTA – Ethylenediaminetetraacetic acid

GPx – Glutathione Peroxidase

GR – Glutathione reductase

GSH – Glutathione

HDL-C – High Density Lipoprotein Cholesterol

LDL-C – Low Density Lipoprotein Cholesterol

MDA – Malondialdehyde

ROS – Reactive Oxygen Species

SEM – Standard Error Mean

SOD – Superoxide Dismutase

TAG – Triacylglycerol

TP – Total Protein

UBTH – University of Benin Teaching Hospital

VA – *Vernonia amygdalina*

VLDL – Very Low-Density Lipoprotein

WHO – World Health Organization

Declarations

Ethics approval and consent to participate

The experimental protocol for this study was approved by the Faculty of Life Science Ethical Committee of University of Benin, Benin City, Edo State, Nigeria.

Consent for publication

Not applicable

Availability of data and material

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

AOO conceived the idea for the work, Methodology, AOO and ODA designed the study, AO and ODA were involved in carrying out the analysis and interpretation of data generated in this study, AO and ODA provided resources such as assay kits and laboratory equipment for this study, AOO prepared the original draft/manuscript of this work, ODA substantively reviewed and edited the work, ODA approved the submitted version of the work.

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Plate

Plates 3.1 to 3.8 are available in the Supplementary Files section.

Figures



Figure 1

(a) *Vernonia amygdalina* plant (b) Washed bitter leaves

Supplementary Files

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