



Cardiorenal protective effects of extracts of bitter leaf (*Vernonia amygdalina* L.) in animal model of metabolic syndrome

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Abstract:

Every year, metabolic syndrome and cardiorenal diseases cause many deaths worldwide. African bitter leaf (*Vernonia amygdalina* L.) is known for its numerous therapeutic effects. Potentially, it can lower plasma lipid and glucose levels, which, in turn, may improve the condition of patients with the abovementioned diseases. This research featured the antihyperlipidemic and antihyperglycemic effects of methanol extract of *V. amygdalina* in an animal model of metabolic syndrome.

Twenty albino rats were divided into four groups. Groups A to C were orally administered with ghee (3 mL/kg) + high-cholesterol diet (500 mg/kg) + high-sugar diet (10 mL/kg) to induce metabolic syndrome. Group A served as negative control and received no treatment with bitter leaf methanol extract. Groups B and C received 200 and 400 mg/kg of *V. amygdalina* methanol extract, respectively. Group D received no administration. The cardiorenal injuries and alterations in blood lipids and sugar levels were assessed via various biochemical analyses.

The combination of ghee + high-cholesterol diet + high-sugar diet triggered a significant elevation of creatine kinase myocardial band, lactate dehydrogenase, aspartate aminotransferase, triglycerides, total cholesterol, low density lipoprotein-cholesterol, glucose, urea, creatinine, and potassium levels. The histopathological results agreed with the biochemical findings. However, the treatment with 200 and 400 mg/kg of *V. amygdalina* methanol extract was able to inhibit the adverse alterations causing a dose-dependent significant antihyperlipidemic and antihyperglycemic effect ($p < 0.05$).

Bitter leaf (*V. amygdalina*) demonstrated cardiorenal protective effects and may be used to manage metabolic syndrome.

Keywords: Bitter leaf, *Vernonia amygdalina*, methanol extract, metabolic syndrome, animal model, hyperlipidemia, hyperglycemia

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INTRODUCTION

Metabolic syndrome is an umbrella term for such disorders as hyperlipidemia, hyperglycemia, hypertension, and obesity, rather than a single condition [1]. As a result, it is among the biggest causes of morbidity and mortality worldwide [2]. In economically developing countries like Nigeria, inadequate early diagnosis and poor management often lead to a sequence of complications and death. Metabolic syndrome triggers a group of risk factors that, if not addressed properly, can lead to more serious metabolic problems, such as Type II diabetes and nonalcoholic fatty liver disease [3–5].

Sedentary lifestyle and consumption of low-fiber, high-fat food products are two primary contributors to

this condition [2]. However, the pathogenic processes behind metabolic syndrome remain unclear and understudied. For instance, scientists cannot explain whether its numerous symptoms are evidence of separate diseases or of a single, shared pathogenic mechanism. The enormous geographic heterogeneity of metabolic syndrome highlights the extent to which social variables, including consuming too many calories and not exercising enough, contribute to its etiology [6]. Most of the mechanisms involved in metabolic syndrome are primarily triggered by visceral fat, which means that a high calorie intake is a primary cause of disease [7]. Out of all the potential mechanisms, chronic inflammation, neurohormonal activation, and insulin resistance seem

to be the key elements in the onset, development, and transformation of metabolic syndrome into cardiovascular diseases (Fig. 1) [8].

Numerous studies have established that metabolic syndrome can cause alterations in the cardiovascular system and renal structure [10]. Cardiovascular and kidney diseases are among the foremost causes of mortality in the world, despite the rapid progress in medical care [11]. Heart failure, hypertension, chronic kidney disease, high cholesterol, Type II diabetes, and cardiorenal metabolic disease are interconnected medical conditions [12, 13]. All these illnesses are associated with systemic inflammation, central obesity, and insulin resistance. Diets that are rich in fat and sugar cause degeneration in heart, kidney, and liver tissues [3, 14].

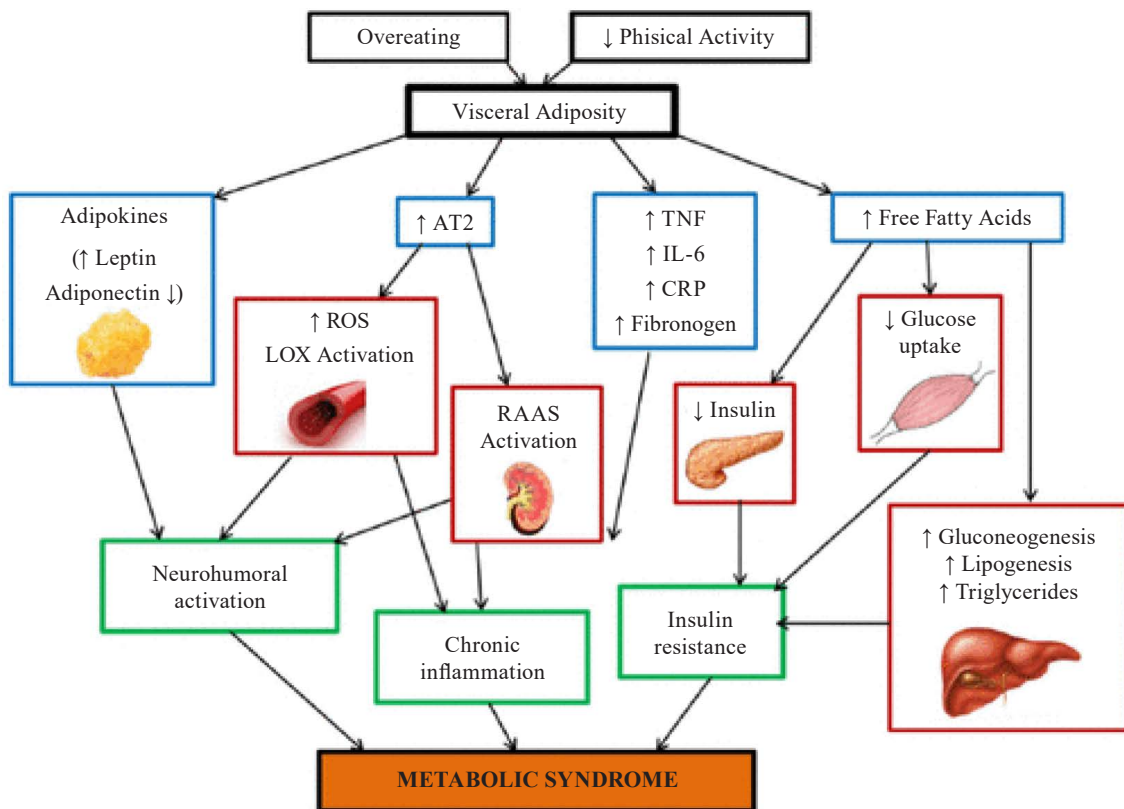
According to established scientific principles, a medical food is defined as a food prescribed by a physician and prepared to be eaten or delivered enterally. This food is designed specifically for dietary treatment of a particular medical condition or disease for which scientifically-proven dietary requirements are available [15].

Extracts of bitter leaf (*Vernonia amygdalina* L.) are known to have nutritional and therapeutic benefits that depend on its constituents [16–18]. Bitter leaf is a shrub or a small tree that can grow to be 23 feet tall when fully developed. Bitter leaf has flaking and gritty grey or brown bark. *V. amygdalina* is a native African plant

that can be found throughout Sub-Saharan Africa. Bitter leaf has long been used as a medicinal herb against renal toxicity and oxidative stress [18]. In addition, it contains vitamins and trace elements that minimize oxidative stress and promote healing. Cu, Fe, and Zn values in *V. amygdalina* were reported as 0.0375, 0.5352, and 0.1916 mg/kg, respectively [19]. African bitter leaf is a popular food component in eastern Nigeria. Tradition has it that bitter leaf is good for hyperglycemic patients due to its bitter taste, or that it can reduce cholesterol and relieve fever, joint pain, intestinal colic, stomach ache, and malaria. The total effect of the bitter leaf can be determined experimentally by analyzing various biochemical parameters.

This research featured the lipid profile and glucose level in a rat model with hyperlipidemia and hyperglycemia after treatment with *V. amygdalina* extract. We defined metabolic syndrome as three abnormal outcomes from five components: increased triglycerides, increased blood pressure, decreased high-density lipoprotein-cholesterol, increased fasting plasma glucose, and increased waist circumference [20].

We believe that a locally available herbal product derived from bitter leaf may be an effective solution to the metabolic syndrome epidemic, which affects population of all age in a country where most people cannot afford drugs required for the treatment and control of obesity, hypertension, and other complications.



CRP – C-reactive protein; ROS – reactive oxygen species; IL-6 – interleukin 6; LOX – lectin-like oxidized low-density lipoprotein; AT2 – angiotensin II type 2 receptor; RAAS – renin-angiotensin-aldosterone system; TNF – tumor necrosis factor

Figure 1 Pathophysiology of metabolic syndrome as adapted from [9]

STUDY OBJECTS AND METHODS

Plant raw materials. Fresh *Vernonia amygdalina* L. leaves were purchased on the Ogbete market, Enugu, Nigeria. A consulting taxonomist verified their authenticity. A voucher specimen (no. PCG/UNN/030) was deposited at the Department of Pharmacognosy and Environmental Medicines, University of Nigeria, Nsukka.

Chemicals and reagents. Vegetable oil ghee, coconut oil, sucrose, and cholesterol powder were acquired from the Ogbete Market, Enugu, Nigeria. All reagents were obtained from Randox Laboratory Ltd., UK.

High-fat, high-sucrose diet for inducing metabolic syndrome. We mixed vegetable oil ghee and coconut oil in a 3:1 (v/v) ratio to create a high-fat diet which we administered orally to rats at a dose of 3 mL/kg per day for 28 days. The high-sugar diet of 30% sucrose solution was given orally to rats at a dose of 10 mL/kg per day for 28 days. The high-cholesterol diet involved 50 g of readily available cholesterol powder plus 9 g of sodium deoxycholate with bile salt to improve bioavailability. The components were mixed together and dissolved in coconut oil before being diluted to 200 mL with coconut oil to produce 250 mg/mL. The rats received it at the amount of 500 mg/kg per day for 28 days.

Laboratory animals. Twenty adult albino Wistar rats (110 ± 10 g) were procured from the College of Veterinary Medicine, University of Nigeria. The rats lived in a metallic cage with a regular temperature of 22 ± 3°C and a 12-h light-dark cycle. The animals were monitored for 14 days prior to the experiment date in order to allow them to acclimatize to the environment. The experimental design and management complied with the institutional guidelines for the care and use of laboratory vertebrates published by the American Physiological Society [21].

Preparing the plant extracts. A 400-g fresh plant of *V. amygdalina* was cleaned, cut into little pieces, and fully homogenized in a Waring blender. The final mix was immersed in 2 L of 80% methanol followed by mixing at room temperature for 24 h while being shaken inter-

mittently. We used a low-pressure rotary evaporator to concentrate the filtrate to dryness at 40°C after filtration. After that, we extracted the residue once more in line with the same method. The entire amount of methanol was extracted from the collected filtrates using a rotary evaporator, and then the resulting crude extract was made and stored at 4°C until use.

Phytoconstituents analyses of bitter leaf. The sample of *V. amygdalina* was analyzed for flavonoids, glycosides, saponins, tannins, steroids, proteins, carbohydrates, and terpenoids at the Department of Pharmacognosy, University of Nigeria, Nsukka. We followed the methodology developed by Trease & Evans [22].

Acute toxicity. Tijjani *et al.* wrote that the intraperitoneal LD50 of ethanolic *V. amygdalina* leaf extract was 3721 mg/kg [23]. They reported no deaths after administering 5000 mg/kg extract orally, which means that the extract was deemed safe to use. In the current study, we applied methanol extract of bitter leaf as high and low doses of 400 and 200 mg/kg, respectively.

Experimental design. We divided 20 albino rats into four groups (A–D) of five rats in each. They received the following treatments for 28 days:

1. Group A (negative control) received ghee (3 mL/kg) + high-cholesterol diet (500 mg/kg) + sucrose solution (10 mL/kg), orally;
2. Group B rats were given ghee (3 mL/kg) + high-cholesterol diet (500 mg/kg) + sucrose solution (10 mL/kg), and low dose of *V. amygdalina* methanol extract (200 mg/kg), orally;
3. Group C rats were subjected to oral administration of ghee (3 mL/kg) + high-cholesterol diet (500 mg/kg) + sucrose solution (10 mL/kg), and high dose of *V. amygdalina* methanol extract (400 mg/kg); and
4. Group D (normal control) received no special treatment.

Sample collection. Blood samples for biochemical analysis were taken from the left ventricle of the heart under chloroform anesthesia. Heart and kidney tissues were excised for histopathological analyses.

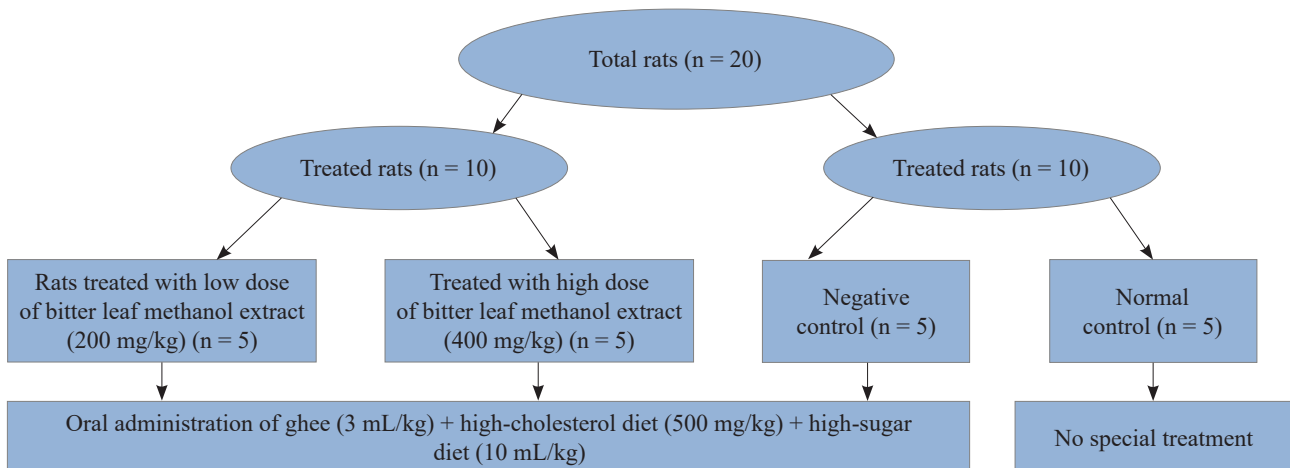


Figure 2 Research design: flow chart

Biochemical analyses. Serum lipid profile. We followed the cholesterol oxidase method described by Fredrickson *et al.* to measure total cholesterol [24]. The precipitation technique to determine high-density lipoprotein-cholesterol was described by Albers *et al.* [25]. The triglyceride test was in line with the glycerol phosphate oxidase method specified in [26]. The low-density lipoprotein-cholesterol was calculated using the Friedewald formula: Low-density lipoprotein-cholesterol = Total cholesterol – (Very low-density lipoprotein-cholesterol + High-density lipoprotein-cholesterol) [27].

Plasma glucose. The blood glucose levels were determined using the glucose oxidase method as in [28].

Measuring the cardiac biomarkers. The level of creatine kinase myocardial band was determined in line with the kinetic colorimetric technique [29]. A Randox kit made it possible to measure the lactate dehydrogenase. We adopted Reitman & Frankel’s colorimetric approach to determine the aspartate transaminase [30].

Measuring the renal function biomarkers. Electrolyte, urea, and creatinine levels in the blood were measured to assess the renal function. A Perlong Medical PL1000A electrolyte analyzer served to study serum K⁺ and Na⁺. The urea level was estimated using diacetyl monoxime technique with protein precipitation, while the creatinine level was estimated by the Jaffe creatinine method as described in [31, 32].

Histopathological analysis. The paraffin wax embedding method was employed to prepare the removed heart and kidney tissues. The sections of 5 μm were subjected to the hematoxylin and eosin staining and examined under an Olympus™ light microscope [33].

Statistical analysis. The data analysis involved Graph Pad Prism 7.0 (San Diego, CA, USA). The results of the biochemical experiments were presented as mean ± SEM (standard error of mean). We used the one-way analysis of variance (ANOVA) to determine the degree of significance with probability levels below 0.05 ($p < 0.05$) as significant.

RESULTS AND DISCUSSION

Phytochemical results. Table 1 illustrates the phytochemical examination of *Vernonia amygdalina* L. methanol extract, which appeared to be rich in alkaloids. Carbohydrates, glycosides, saponins, and tannins were present in moderate amounts. Flavonoids and resins were

detected in trace amounts while proteins, acidic compounds, oils, terpenoids, and steroids were found absent.

Biochemical results. This experiment featured the antihyperlipidemic and antihyperglycemic benefits of bitter leaf extract in albino Wistar rats with high-fat and high-glucose metabolic syndrome. The negative control rats that received ghee (3 mg/kg), high-cholesterol diet (500 mg/kg), and 30% sucrose solution (10 mg/kg) had the greatest levels of blood triglycerides, total cholesterol, and low-density lipoprotein (Table 2). This combination was able to induce significant dyslipidemia, thus giving the rats metabolic syndrome.

Low and high doses of bitter leaf methanol extract reduced triglyceride, total cholesterol, and low-density lipoprotein compared to the normal control group. Our results were comparable with those reported by Ogunrinola *et al.*, who detected a significant ($p < 0.05$) hypolipidemia in rats treated with bitter leaf extract at all doses [34]. Ogbuabu *et al.* also posited that *V. amygdalina* leaves could substantially reduce triglyceride and total cholesterol, with no effect on high-density lipoprotein-cholesterol [35].

The negative control rats that received a combination of ghee + high-cholesterol diet + high-sugar diet showed the highest plasma glucose levels (Fig. 3). Therefore, the treatment with ghee, high-cholesterol, and sucrose solution induced hyperglycemia, i.e., a metabolic syndrome condition, in the rats.

Table 1 Phytochemical analysis of *Vernonia amygdalina* L.

Compounds	Indication
Saponins	Moderately present
Reducing sugar	Absent
Alkaloids	Abundantly present
Tannins	Moderately present
Carbohydrates	Moderately present
Glycosides	Moderately present
Resins	Present (in trace amounts)
Flavonoids	Present (in trace amounts)
Steroids	Absent
Terpenoids	Absent
Oils	Absent
Acidic compounds	Absent
Proteins	Absent

Table 2 Serum lipid profile parameters of all experimental groups vs. normal control

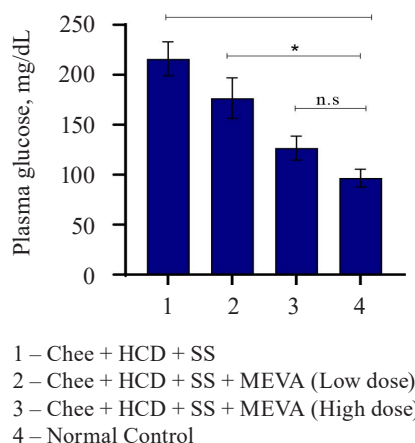
Groups	Triglyceride, mg/dL	Total cholesterol, md/dL	High-density lipoprotein, mg/dL	Low-density lipoprotein, mg/dL
Ghee + high-cholesterol diet + high-sugar diet (negative control)	131.00 ± 9.74**	74.13 ± 5.59*	20.50 ± 1.31*	27.43 ± 6.28**
Ghee + high-cholesterol diet + high-sugar diet + bitter leaf extract (low dose)	122.25 ± 8.31*	70.63 ± 6.55	21.25 ± 1.75	24.93 ± 5.96*
Ghee + high-cholesterol diet + high-sugar diet + bitter leaf extract (high dose)	105.63 ± 8.43	67.13 ± 5.17	24.25 ± 2.66	18.55 ± 7.94
Normal control	70.50 ± 7.21	57.75 ± 4.53	25.00 ± 2.39	17.90 ± 4.82

Compared to the normal control, ** ($p < 0.01$) or * ($p < 0.05$) were significant

In comparison with the normal control, the rats treated with low or high doses of bitter leaf extract demonstrated non-significant and significant declines in plasma glucose levels, respectively. This result also corroborates with other publications that reported the hypoglycemic properties of bitter leaf extract [36]. For instance, Olooto *et al.* studied the hypoglycemic potential of *V. amygdalina* in albino rats fed with a high-sucrose diet. In their study, the mean plasma glucose level was significantly elevated ($p < 0.05$) in the rats that followed a high-sucrose diet while those treated with bitter leaf demonstrated a small decrease [37]. Bawa & Ayobola proved the antidiabetic and anti-hyperlipidemic properties of *V. amygdalina* methanol extract [38]. Probably, that is why bitter leaf has historically been used to combat diabetes mellitus and its complications [39]. This beneficial effect may be attributed to such bioactive phytoconstituents as alkaloids, tannins, saponin, glycosides, and flavonoids [40].

Figure 3 clearly demonstrates that the bitter leaf extract was able to reduce blood glucose levels in a dose-dependent manner.

The negative control rats that received ghee + high-cholesterol diet + sucrose solution showed a statistically significant ($p < 0.05$) elevated levels of creatine kinase



MEVA – *V. amygdalina* methanol extract; HCD – high-cholesterol diet; SS – sucrose solution, i.e., high-sugar diet.

** ($p < 0.01$) or * ($p < 0.05$) were significant

Figure 3 Plasma glucose in experimental groups vs. normal control

myocardial band, low-density cholesterol, and aspartate transaminase when compared to the high-dose treatment group and the normal control, separately. Evidently, the combination of ghee, high-cholesterol diet, and sucrose solution could induce cardiac muscle damage in the rats. Both the low and high dose treatments caused a significant reduction of serum creatine kinase myocardial band, low-density cholesterol, and aspartate transaminase, compared to the negative control (Table 3).

Our finding is consistent with that made by Wijaya *et al.*, who also reported that rats treated with 500 mg/kg of ethanolic extract of *V. amygdalina* had the lowest levels of creatine kinase myocardial band and low-density cholesterol [41]. Syahputra *et al.* similarly observed that rats treated with *V. amygdalina* had lower creatine kinase myocardial band, low-density cholesterol, and aspartate transaminase [42]. The cardioprotective effect of bitter leaf ethanol extract could be attributed to luteolin. This flavonoid inhibits apoptosis, improves systolic and diastolic function of the heart, and potentiates nitric oxide synthesis, thereby improving the overall function of the heart [43].

The negative control rats that received a combination of ghee + high-cholesterol diet + sucrose solution for 28 days showed a significant ($p < 0.05$) increase in blood urea nitrogen, creatinine, and potassium (K^+), compared to the high-dose treatment group and the normal control, separately. Obviously, the combination of ghee, high-cholesterol diet, and sucrose solution was able to induce renopathy secondary to metabolic syndrome in the rats. When compared to the negative control, the treatment with both low and high doses of bitter leaf extract lowered the levels of blood urea nitrogen, creatinine, and potassium (K^+) (Table 4).

Our renal assessment was similar to that published by Barnes *et al.*, who fed renal dysfunctional rats with *V. amygdalina* extract for 3 weeks to achieve normal restoration of creatinine and urea level [44]. Onwubiko *et al.* also treated rats with 400 mg/kg of *V. amygdalina* extract and reported lower urea and creatinine levels [45].

Histopathological results. In this research, metabolic syndrome damaged cardiac muscles, which could potentially lead to a variety of cardiovascular diseases. Figure 4 shows the photomicrograph of heart sections in the experimental groups following the treatments. The rats that received no special treatment showed

Table 3 Cardiac biochemical markers of damage parameters in experimental groups vs. normal control

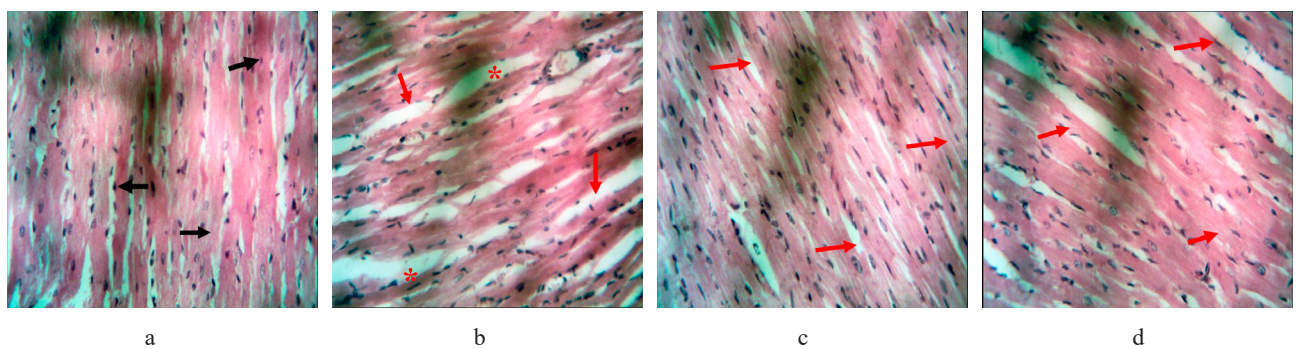
Groups	Creatine kinase myocardial band, U/L	Low-density cholesterol, U/L	Aspartate transaminase, U/L
Ghee + high-cholesterol diet + high-sugar diet	216.89 ± 17.87**	269.87 ± 15.01**	115.69 ± 9.26**
Ghee + high-cholesterol diet + high-sugar diet + bitter leaf methanolic extract (low dose)	169.37 ± 4.59*	219.12 ± 9.03	82.72 ± 9.58
Ghee + high-sugar diet + bitter leaf methanolic extract (high dose)	156.01 ± 7.71	211.24 ± 11.12	72.71 ± 4.62
Normal control	146.24 ± 11.05	208.45 ± 14.78	67.59 ± 7.13

Compared to the normal control, * ($p < 0.05$) and ** ($p < 0.01$) were significant

Table 4 Serum renal biochemical parameters in experimental groups vs. normal control

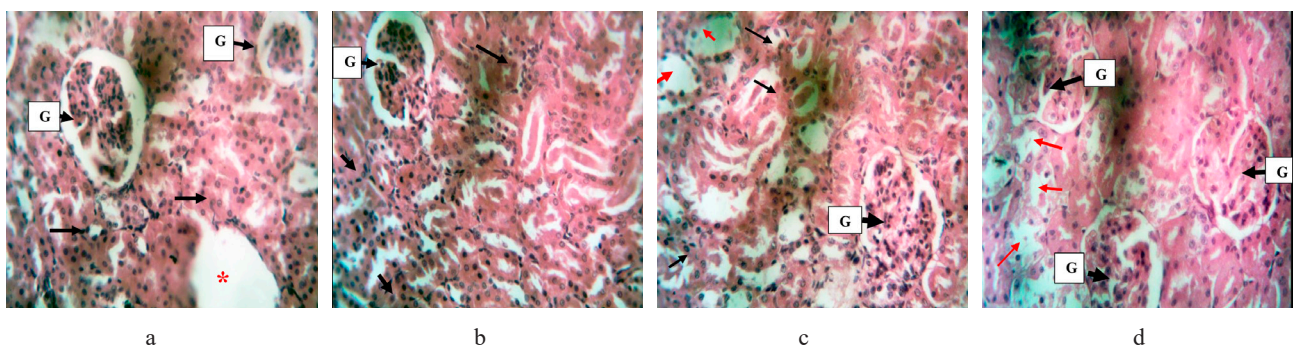
Groups	Blood urea nitrogen, mg/dL	Creatinine, mg/dL	K ⁺ , mmol/L	Na ⁺ , mmol/L
Ghee + high-cholesterol diet + high-sugar diet	37.92 ± 4.21	1.62 ± 0.04	7.68 ± 0.09	135.41 ± 0.23
Ghee + high-cholesterol diet + high-sugar diet + bitter leaf methanolic extract (low dose)	21.75 ± 3.48*	1.04 ± 0.05*	5.92 ± 0.24	140.02 ± 3.10
Ghee + high-sugar diet + bitter leaf methanolic extract (high dose)	21.68 ± 1.09*	0.92 ± 0.19*	6.19 ± 0.76*	143.88 ± 0.35*
Normal control	20.62 ± 1.31*	0.89 ± 0.30*	5.83 ± 0.12*	145.25 ± 1.42*

Compared to the group of ghee + high-cholesterol diet + high-sugar diet, *(*p* < 0.05) was significant



* – shows the evidence of oedema. The black arrows point at myocardial fibers undergoing degenerative changes while the red arrows mark undamaged tissues

Figure 4 Heart tissues of rats in different experimental groups (A–D): a representative micrograph (staining: hematoxylin and eosin; magnification 400×)



* – marks mildly constricted or eroded glomeruli (G). The black arrows point at normal tubules or those with intraluminal eosinophilic casts. The red arrows mark tubules with degenerated epithelia

Figure 5 Kidney tissues of rats in different experimental groups (A–D): a representative micrograph (staining: hematoxylin and eosin; magnification 400×)

unaffected myocardial fibers (Group D). The myocardial fibers of the negative control rats that received oral administration of ghee + high-cholesterol diet + sucrose solution showed some degenerative changes, e.g., poor striations (Group A). However, the rats subjected to the low dose of *V. amygdalina* treatment demonstrated normal myocardial fibers (Group B) with some evidence of edema seen as increased spaces between the fibers. Furthermore, the rats that received the high dose of *V. amygdalina* extract had normal-looking myocardial fibers (Group C).

The histopathological findings were in tandem with the biochemical results. This result was similar to the

research conducted by Syahputra *et al.*, where *V. amygdalina* extract also reduced cardiac degeneration [42].

In Fig. 5, the glomeruli appeared normal while some tubules seemed to have slightly degenerated epithelia in the untreated rats (Group D). The kidney section of the negative control rats that received ghee + high-cholesterol diet + sucrose solution showed some constricted or eroded glomeruli while the tubules appeared normal (Group A). However, the rats that were treated with the low-dose of bitter leaf extract demonstrated standard glomeruli while occasional tubules looked mildly eroded, some with intraluminal eosinophilic casts (Group B). The high-dose group had normal

glomeruli while the tubules appeared eroded with occasional intraluminal eosinophilic casts (Group C).

This histopathological finding showed a dose-dependent effect, with the high dose having a better renal protective function than the low one. This result was in tandem with the study published by Barnes *et al.*, where *V. amygdalina* extract improved the renal tissues of rats damaged by heavy metals [44]. The positive effect could be attributed to vitamins, antioxidants, and minerals in the plant.

CONCLUSION

The methanol extract of *Vernonia amygdalina* L. reduced hyperlipidemia and hyperglycemia in rats with metabolic syndrome caused by increased glucose and fat intake. In the test group, the bitter leaf extract ameliorated the negative effects. Its anti-cardiotoxic properties

provided important protection against cardiovascular diseases. In sufficient doses, *V. amygdalina* demonstrated a renoprotective effect.

CONTRIBUTION

The authors are equally liable for any plagiarism because they all contributed equally to writing the content.

CONFLICT OF INTEREST

There are no disclosed conflicts of interests regarding the publication of this article.

ETHICAL CONSIDERATIONS

Ethical approval was issued by the Ethics Committee of the Department of Veterinary Medicine, University of Nigeria (Approval number: UNN/eTC/14/67485).

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