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Biochemical Evaluation of *Vernonia amygdalina* on the Haematology, Kidney Profile and Liver Function Tests in Wistar Rats

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Abstract:- Obesity and overweight contribute significantly to the burden of chronic diseases and complications globally. The rising prevalence of these conditions in Nigeria, especially among middle-class workers has been attributed to the acculturation of Western dietary patterns, reduced intake of dietary fibre, and sedentary lifestyles. Considering the risks associated with obesity and the associated side effects of orthodox medicine, a lot of studies have been dedicated to finding alternative treatments with limited side effects that are affordable hence, the use of medicinal plants such as Vernonia amygdalina. This study examined the therapeutic effects of V. amygdalina aqueous leaf extract on the biomarkers of metabolic syndrome in high-fat diet (HFD)-induced obese rats. 30 male Wistar rats were obtained and divided randomly into five groups containing six rats each. Group A was fed with only normal rat chow, while a high-fat diet was fed to groups B, C, D and E with V. amygdalina extract fed to groups C, D, and E alone after the induction of obesity. The animals were sacrificed on the 28th day and various analyses including growth performance, the concentration of blood glucose, serum lipid profile, and renal and hepatic function tests were performed. The group fed with only HFD had significantly high body weight, blood glucose concentration, serum levels of cholesterol, triglycerides, and liver and kidney biomarkers. However, posttreatment of groups C, D, and E with an aqueous extract of V. amygdalina significantly reduced these biomarkers to physiological levels and the organ tissue structure showed improvement. Thus, indicating the anti-obesity effects and the continuous role of V. amygdalina in weight loss management and prevention of obesity.

Keywords:- *Obesity, overweight, Vernonia amygdalina, high-fat diet (HFD), cholesterol, triglycerides, liver, kidney, anti-obesity, weight loss.*

I. INTRODUCTION

Obesity is a condition of the metabolic process where an excessive amount of fat is stored in the body, leading to negative health effects [1]. It is characterized by changes in food patterns, hormonal disturbances, genetic factors, and elevated fat and body mass [2]. Obesity has been linked with several metabolic syndrome abnormalities such as cardiovascular diseases, type 2 diabetes mellitus, cancer, pulmonary diseases, and musculoskeletal disorders [3].

Obesity and overweight contribute significantly to the global burden of chronic diseases and complications. Overweight and obesity occur due to an imbalance between consumed calories and utilized calories [1]. They were formerly concerns for higher-income countries, but are now prevailing in low and middle-income countries, especially in urban areas such as Nigeria [4]. Approximately 2.8 million deaths occur due to obesity annually, hence obesity is a global pandemic [5].

The rising prevalence of overweight and obesity in Africa, especially among middle-class workers has been attributed to the acculturation of Western dietary patterns, reduced intake of dietary fibre, and sedentary lifestyle. The consumption of a high-calorie diet is a major component of the Westernised diet, and this diet has been associated with the incidence of many metabolic syndromes such as obesity, dyslipidaemia, hyperinsulinaemia, hyperglycaemia, hypertension, and other cardiovascular diseases [6-8].

Body mass index (BMI) is an index used in describing the relationship between weight and height in adults. In addition, bioimpedance, waist/hip ratio, waist circumference, and skinfold thickness also help to assess overweight and obesity [9]. A BMI ≤18.5 is underweight, 18.5-24.9 is normal, 25-29.9 is overweight, 30-39.9 is obese, and BMI ≥40 is extremely obese [10].

Lifestyle modification aimed at reducing body weight is often advisable, but drug intervention is sometimes necessary [4, 11]. Antiobesity drugs have been classified into five categories which include digestion and absorption blockers, central appetite suppressants, obesity gene product inhibitors, metabolic promoters, and others [2]. However, these prescribed drugs are sometimes costly and may induce adverse side effects (ASEs) that could modulate monoamine neurotransmitters causing drug dependence or abuse [4, 12].

In patients with comorbidities, such as diabetes, obstructive sleep apnea, hypertension, and extremely obese patients (BMI ≥ 40), surgery is commonly used [13]. However, postoperative infection, postoperative anastomotic fistula, deep vein thrombosis, and long-term complications such as malnutrition and anaemia are common surgical complications [3, 14, 15]. Considering the risk associated with obesity and the ASEs of orthodox medicine, a lot of studies have been dedicated to finding alternative treatments with limited side effects that are affordable [16, 17], hence, the use of medicinal plants such as *Vernonia amygdalina* (VA) [3].

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Vernonia amygdalina Del. (Compositae) is a nutritionally important vegetable commonly called "bitter leaf" due to its bitter taste in West Africa, especially Nigeria. *V. amygdalina* is a multipurpose and rapidly regenerating mid-sized shrub ranging from 1 - 10m in height and dark green leaves of about 6mm in diameter [7, 18]. Known for its characteristic odour and taste [19], VA is a leafy vegetable commonly used as food ingredients to prepare delicacies such as “ofe ogbono”, “ofe onugbu” popularly called “ogbono” soup and “egusi” soup respectively [20]. Every plant parts of *V.amygdalina* are pharmacologically useful. Both the leaves and the roots are used in phytotherapy to treat stomach discomfort, kidney disease, hiccups, fever, etc. [21]. The root and stem divested of the bark are often chewed as chew-sticks in Nigeria [19].

The anti-nutritional phytochemicals present in *V. amygdalina* account for its bitter taste. These phytochemicals include bioactive compounds such as anthraquinone, steroidal glycosides, edotides, triterpenoids, flavonoids, saponins, lignans, alkaloids, phenolics, coumarins, terpenes, tannins, xanthones, and different types of sesquiterpene lactones. These bioactive compounds are responsible for different pharmacological properties like antioxidant [22], antimicrobial [23], anthelmintic [24], antimalarial [25], antibacterial [26], anticancer [27], antidiabetic [28], laxative [29], hypoglycemic [30], anti-inflammatory [31], cathartic [32], antifertility [33], antifungal [34], and antithrombotic effects of the plant [18].

V. amygdalina is used in the ethnomedicinal management of dyslipidaemic conditions [3, 28], hence it is a potential plant for anti-obesity studies. Therefore, this study examined the effect of dietary incorporation of *V. amygdalina* into high-fat diets on biological markers of metabolic syndrome.

II. MATERIALS AND METHODS

A. Plant materials

A large number of fresh leaves of V. amygdalina were bought from the local market in Oyo town, Nigeria. It was authenticated at the taxonomy department of the Federal Institute of Industrial Research Oshodi, Lagos Nigeria.

B. Chemicals and reagents

The Reagent kits such as total cholesterol kit, low-density lipoprotein kit, high-density lipoprotein, very low-density lipoprotein kit, aspartate transaminase kit, alkaline phosphatase kit, alanine transaminase kit, bilirubin assay kit, serum creatinine kit, and urea kit procured from Randox laboratories United Kingdom. All other chemicals and reagents used for this study were of analytical grade.

C. Study location

The experiments were carried out in the Toxicological laboratories of the Animal House at the Federal Institute of Industrial Research Oshodi, (FIIRO) Lagos Nigeria within the latitude 7°11’42” N, 3°20’54” E, 31 m above sea level. It is located in the derived savannah vegetation zone of southwestern Nigeria, with a humid climate. The relative humidity ranged between 63-96% in the rainy season (late March-October) and between 55-82% in the dry season (November-early March), with an annual average of 82%. It has a mean annual precipitation ranging between 2000 and 3000mm. The average annual temperature ranged between 21-33°C (NIMET, 2016).

D. Plant extract preparation

The leaves were washed thoroughly with clean water to remove any dirt (sand, insects, and dust). Washed leaves were air-dried at room temperature for a few days. The leaves were dried, milled, and separated then stored in an air-tight container till ready for infusion in water. For every 100g of the milled bitter leaf was diluted with 500ml of distilled water. The infused bitter leaf water was concentrated using a rotary evaporator at 45^{O}C under reduced pressure to obtain the extract. The weight of the extract was then used to do the calculations of the percentage yield for the concentrations. The extracts used were at 0, 2, 4, and 6% concentrations, making a total of four treatment groups [3].

E. Experimental animal, diets and feeding schedule

A total of twenty-four (24) male Wistar rats weighing between 91.18-93.75g were obtained from the Physiology Department of the College of Medicine, Idi-Araba, Lagos. Nigeria. They were weighed and randomly assigned to oral gavage (using 5mls syringes) of four experimental groups of the different extract concentrations of 2, 4, and 6% concentrations with the control (without any aqueous leaf extract but water). The animals were divided into four groups of six rats per group randomly. All the animals were humanely treated following the guidelines for animal care in a 12-h dark/12-h light cycle. Water and specific diets were offered ad libitum to the rats [3]. Two experimental diets were formulated;

Diet A was a normal basal/standard diet for the acclimatization period of seven days. It was a combination of different feed ingredients which includes corn, soybean, wheat offal, fish meal groundnut cake, brewery dry grain (BDG), methionine, lysine premix, limestone, and bone meal. These were formulated to give the rat a balanced diet with a crude protein content of 16.5% amongst other nutrients and energy.

Diet B was a high-fat diet (HFD) used to induce high cardiovascular and obesity conditions. It was made with high lard content from pigs, starch, etc. The treatments lasted four weeks. After this, blood samples were collected from the ocular vein using capillary tubes into anti-coagulant bottles EDTA for haematology and lithium heparin (blue-cap bottles) for serum biochemistry. The samples were refrigerated and sent to the laboratory for analysis within 24hrs.

F. Induction of cardiovascular/obesity condition

After the acclimatization period of seven days, the rats were randomly divided into four treatment groups of six rats each (n=6) and fed high-density fat to induce the high cholesterol condition with obesity. The high-fat diet (HFD) was fed to groups B, C, and D for four weeks to induce the condition which can be confirmed when the animals have a 20-25% weight increase from the initial weight. After the

induction, the rats in groups B, C, and D were treated with oral gavage of the concentrated bitter leaf extract daily for four weeks [3].

There were four (4) treatment groups of six (6) rats each. They are:

- Control (no leaf extract treatment, only water)
- 2% bitter leaf extract
- 4% bitter leaf extract
- 6% bitter leaf extract

G. Data collection

Data collection on haematology and serum chemistry was carried out by collecting blood samples from the ocular vein (eye) using capillary tubes into anti-coagulant bottles of Ethylene diamine tetraacetic acid (green cap bottles) and Lithium Heparin (blue cap bottles). The basal blood collection from the retro-orbital puncture (eye) was 24hrs before the introduction of HFD as baseline values.

At the end of the experimental period (4th week), animals were weighed and the readings were recorded. The blood was finally collected by retro-orbital puncture in serum vacutainers containing EDTA and Lithium heparin anti-coagulant bottles. The bottles were spun after blood collection to mix well and labelled for analysis after refrigeration in the laboratory. Centrifugation was done to obtain clear serum at 3000rpm. This serum was used for the analysis of various biochemical parameters like glucose, alanine transaminase, cholesterol, triglycerides, protein, albumin, urea, and creatinine.

Lipid function test was carried out on triglycerides, cholesterol, low-density lipoprotein, high-density lipoprotein, very low-Density lipoprotein, etc. Kidney function tests like Urea and creatinine were assessed while liver function tests like bilirubin, alkaline phosphatase, Aspartate transaminase, and alanine transaminase were also examined [18].

H. Statistical analysis

All data are presented as the mean ± standard error of mean (SEM). The data were analyzed by ANOVA, Tukey's HSD posthoc using SAS statistical software.

III. RESULTS AND DISCUSSION

The use of diet-induced animal models to evaluate the metabolic turnover of macro and micro nutrients and also the pathogenesis of disease conditions has proven to be quite useful in scientific studies. This model has been used to study diseases such as diabetes mellitus, cardiovascular diseases, obesity, and other metabolic syndromes. The dietary effect of food consumed on the body depends on the type and amount of food consumed, hence, food consumed in the wrong proportion could offset the balance of nutrients (e.g., calories, proteins, lipids, etc) within the body. This study was designed to evaluate the therapeutic effects of *V. amygdalina* aqueous extract on Wistar rats fed with high-fat diets.

Table 1: Quantitative proximate composition of *V. amygdalina*

Plant spp.	Crude fibre (%)	Crude protein (%)	Crude fat (%)	Ash (%)	NFE (%)	Dry matter (%)
V. amygdalina	17.32	23.78	4.89	12.64	33.21	91.84

The proximate composition of *V. amygdalina* leaf extract in this study (Table 3.1) showed that it consisted of 17.32% crude fibre, 23.78% crude protein, 4.89% crude fat, 12.64% ash, 33.21 % nitrogen-free extract (NFE) and 91.84 % dry matter. However, *V. amygdalina* has been reported to contain crude fibre within the range of 6.5-29.2%, crude protein within the range of 17-33%, and crude fat within the range of 2-15% [35]. Usunomena et al (2016) also reported 18.17% crude fibre, 22.81% crude protein, 16.65% ash content, 4.34% crude fat, and 90.68% dry matter from their proximate analysis of *V. amygdalina*, hence, the result from this study is an agreement with [19, 35].

Phytochemicals are natural substances produced by plants that serve the dual purpose of protecting them from environmental threats and regulating their growth and reproductive processes [36]. These metabolites are essential for plants to survive and thrive in their surroundings and are core indicators of desirable properties of plants such as their therapeutic potential [18]. The phytochemical constituent of *V. amygdalina* was presented in Table 3.2. The leaf extract is rich in tannins, alkaloids, flavonoids, saponins, and polyphenols. This result is in agreement with Usunomena et al (2016) whose phytochemical analysis of *V. amygdalina* reported the presence of polyphenols, flavonoids, saponins, alkaloids, tannins, cardiac glycosides, triterpenoids and reducing sugars. However, both results indicated the absence of anthraquinones.

Table 2: Phytochemicals of *V. amygdalina* leaves

Phytochemicals	Tannins (%)	Alkaloids (%)	Flavonoids (%)	Saponins (%)	Polyphenols (%)
	0.48±0.26	3.08±0.11	0.92±0.31	2.75±0.22	0.42±0.18

The biochemical and pharmacological actions of plants have been attributed to the phytochemicals present in their extracts, hence phytochemicals play a significant role in the therapeutic activities of plant extracts. Tannins, flavonoids, triterpenoids, and saponins have been reported to exhibit hypolipidaemic and antioxidant effects [37-41].

Table 3: Proximate analysis of the experimental diet

Nutrients	Rat chow values	High-fat diet values
Dry matter (%)	91.43	88.94
Crude protein (%)	16.21	15.87
Crude fibre (%)	4.69	3.76
Crude fat (%)	4.86	24.40
Ash (%)	5.67	2.98
NFE	60.00	45.38
Total energy Kcal/100g	300.2	423.97

Legends: nitrogen-free extract (NFE)

The proximate analysis of the experimental diet fed to the experimental animals is presented in Table 3.3. Crude fat is a representation of the fraction of the diet with the highest caloric value, and excess consumption of fat has been linked with diseases of the cardiovascular system [42]. The crude fat value in the HFD is 24% while its value in the normal rat chow was 4.86%. The moisture content in both diets is relatively low judging by the value of the dry matter in the normal rat chow and HFD which was 91.43% and 88.94% respectively.

The mean distribution of growth performance characteristics of the experimental animals is presented in Table 3.4. There was no significant difference in the initial mean weight of all the animal groups, however, after 28 days of feeding and supplementing with *V. amygdalina* extract, there was a significant difference in weight between the group fed with normal rat chow and those fed with HFD. This may be because the HFD acted as a source of saturated fats to those fed with HFD, resulting in an excessive supply of calories which increased their body weight [20]. However, supplementation of the HFD with different concentrations of *V. amygdalina* extract resulted in a significant decrease in the body weights of the animals compared to those fed with only HFD and normal rat chow. The result from this study is in tandem with the study of Olooto et al. (2017) who reported an increase in the body weight of animals fed with high sucrose diets, and Ekeleme-Egedigwe et al (2017) who also reported an increase in the body weight of animals fed with high-fat diets. Both studies later concluded with a significant decrease in the body weight of the experimental animals whose feed was supplemented with different concentrations of *V. amygdalina* extract [20, 43].

Table 4: Mean distribution of growth performance characteristics of the experimental animals

Parameters	Treatment groups				
	NC	HFD	HFD+2% V.a	HFD+4% V.a	HFD+6% V.a
Initial mean weight (g)	161.87±8.64^{a}	164.96±11.27^{a}	162.85±10.34^{a}	161.30±8.95^{a}	165.28±9.21^{a}
Final mean weight (g)	192.34±9.43^{c}	202.11±14.52^{a}	198.16±9.66^{ab}	196.43±9.63^{b}	197.58±11.08^{b}
Mean weight gain (g)	30.47±6.35^{b}	37.15±4.26^{a}	34.31±9.17^{a}	35.13±7.34^{a}	32.30±7.12^{a}

Values are expressed as mean ± SEM; n=6 replicates. Values with different superscripts (a-c) along the same rows are significantly different ($P < 0.05$). HFD=High Fat Diet; V.a = Vernonia amygdalina. a= significantly different from the control; ab = significantly different from HFD; b = significantly different from 2% v.a; c = significantly different from 4% v.a

The blood glucose concentration of the experimental animals from the beginning to the end of the study was presented in Table 3.5. It was observed that the concentration of blood glucose increased significantly in the groups fed with HFD compared to those fed with normal rat chow, however, there was a significant decrease across the groups fed with *V. amygdalina* extracts compared to those fed HFD and normal rat chow. The reduction in the serum glucose concentration could be a result of the insulin-like activity and inhibition of glycogenolysis and gluconeogenesis by terpenoids [44]. Flavonoids, tannins, and alkaloids have also been reported to exhibit α-glucosidase inhibitory activity while α-amylase inhibitory activity has been associated with tannins [45-47]. According to this study, these phytochemicals in *V. amygdalina* aqueous leaf extract contributed to the remarkable reduction of blood glucose levels. This result is similar to the blood glucose-lowering effect reported by [48] who investigated the antidiabetic effect of the ethanolic extracts of old and young leaves of *V. amygdalina* on streptozotocin (STZ) induced diabetic rats and reported the significant antihyperglycaemic effect of both leaf extracts after four weeks.

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Table 5: Blood glucose concentration levels before and after treatment with extracts of *V. amygdalina* in Wistar rats

GROUPS	Glucose conc. before Induction (mg/dL)	Glucose conc. at the end of study (mg/dL)	% differentials increase/decrease
Normal control (NC)	88±9.42^{b}	93±4.52^{c}	5.68
HFD (negative control)	162±11.76^{a}	132±9.56^{a}	-18.52
HFD+2% V.a	158±10.53^{a}	121±9.75^{ab}	-23.42
HFD+4% V.a	167±10.89^{a}	108±9.46^{b}	-35.23
HFD+6% V.a	151±9.72^{a}	101±8.97^{b}	-33.13

Values are expressed as mean ± SEM; n=6 replicates. Values with different superscripts (a-c) along the same column are significantly different (P < 0.05).

Studies conducted by scientists have demonstrated that diets that are rich in fat contribute to obesity as they cause an increase in energy stores, which is primarily because of the high energy density of HFDs. Consumption of these HFDs can lead to an increase in body weight, and the conversion of excess energy into lipids which get stocked as triglycerides in the fat cells resulting in obesity [49, 50]. The contributory effects of HFDs on weight gain, increase in blood glucose concentration and serum lipid profile have been presented in Table 3.4, Table 3.5, and Table 3.6 respectively.

Table 6: Serum lipid profile for the rats on *V. amygdalina* treatment

GROUPS	Serum lipid profile (mg/Dl)				
	TC	VLDL	LDL	HDL	TG
NC	93.89±4.16^{d}	13.42±0.78^{c}	37.42±2.63^{e}	43.08±3.47^{c}	67.63±6.16^{d}
HFD	224.56±6.43^{a}	32.42±6.62^{a}	137.89±9.48^{a}	41.23±5.37^{c}	162.10±4.16^{a}
HFD+2% V.a	208.37±8.37^{ab}	28.35±4.16^{b}	122.42±8.09^{b}	57.61±6.85^{b}	141.75±6.93^{b}
HFD+4% V.a	177.38±9.07^{b}	17.38±2.55^{c}	101.09±7.95^{c}	58.93±9.29^{ab}	86.85±5.87^{c}
HFD+6% V.a	145.06±7.32^{c}	9.83±3.78^{d}	70.23±3.84^{d}	65.02±5.42^{a}	50.06±3.63^{e}

Values are expressed as mean ± SEM; n=6 replicates. Values with different superscripts (a-d) along the same column are significantly different (P < 0.05). Legends: HFD=High-fat diet; V.a = *Vernonia amygdalina*. TC= Total cholesterol; VLDL= Very low-density lipoprotein; LDL= Low-density lipoprotein; HDL= High-density lipoprotein; TG = Triglycerides.

TC, LDL, VLDL, and TG values for the groups fed with HFD increased significantly relative to the normal control in Table 3.6, while there was a decrease in HDL. These are strong indicators for cardiovascular diseases and related metabolic syndromes [51]. The administration of *V. amygdalina* extract significantly decreased the values of TC, LDL, VLDL, and TG while significantly increasing the value of HDL across the treatment groups. HDL has been reported to possess an anti-atherogenic effect [52]. It has been reported that dietary cholesterol elevates the concentration of TC, LDL, and VLDL while decreasing HDL in the serum causing a build-up of fat in the body [20]. This explains the similar occurrence in this study. However, saponins are reported to exhibit serum-lowering activity through the production of a resin-like action by reducing the circulation of enterohepatic bile acids obtained from the conversion of cholesterol. The saponins present in the extracts of *V. amygdalina* extract play a significant role in the inhibition of fat accumulation in the liver by reducing the build-up of cholesterol in Wistar rats [53-55].

V. amygdalina extract through its flavonoid contents might have stimulated the hydrolysis of TG in those fed initially with HFD while enhancing cholesterol redistribution among the lipoprotein, thereby preventing obesity [56, 57]. This is evident by the significant increase in HDL and the significant decrease in TG across the groups relative to those fed with only HFD and normal rat chow. This result is in agreement with other studies [3, 20, 28, 58] that also examined the cardioprotective, hypolipidemic, and antiobesity effects of *V. amygdalina* extracts on diet-induced obesity in Wistar rats and suggested that the therapeutic mechanism of *V. amygdalina* extracts in HFD-fed animals could be as a result of a decrease in triglyceride synthesis and accumulation in the liver due to the hydrolysis of fat stores and inhibition of dietary triglyceride digestion and absorption.

The haematological indices of the experimental animals are presented in Table 3.7. There was a significant increase in the concentration of WBCs, RBCs and PCV in those fed with HFD, however, the administration of *V. amygdalina* ameliorated these counts significantly relative to the normal control groups. An increase in PCV could indicate an abnormal increase in RBC production (polycythemia vera disorder) or a lung or heart disease [59]. The efficiency of the immune system, as well as inflammation markers, can be identified by the white blood

cell count. This population of cells is crucial in determining the ability of the body to fight off infections and diseases [60]. It has been reported that individuals with central fat possess higher levels of inflammatory markers including higher percentages of WBC counts relative to those with normal fat distribution in their bodies [61]. The suppressing effect of *V. amygdalina* leaf extract on WBCs, RBCs and PCV in those initially fed with HFD suggests its anti-inflammatory action in obesity, a mechanism of some anti-obesity agents [62]. This supports the claim for the adoption of an anti-inflammatory diet as a therapeutic approach to obesity treatment and prevention [63].

Table 7: Effect of aqueous extract of *V. amygdalina* treatment haematological indices of Wistar rats

GROUPS	Haematological indices parameters				
	RBC(10^3/µl)	PCV (%)	Hgb (gdl)	WBC (10^3/µl)	MCV(fl)
NC	7.46±2.09^{c}	40.68±1.37^{b}	14.39±1.54^{b}	7.59±2.62^{c}	58.60±1.44^{a}
HFD	12.49±4.02^{a}	52.35±2.17^{a}	9.32±2.41^{c}	21.21±2.36^{a}	44.64±0.57^{c}
HFD+2% V.a	13.65±3.74^{a}	43.47±4.38^{b}	15.18±2.64^{a}	17.06±5.31^{ab}	51.08±3.87^{bc}
HFD+4% V.a	10.15±2.31^{b}	42.09±1.69^{b}	14.91±2.69^{b}	14.63±4.25^{b}	59.43±1.73^{a}
HFD+6% V.a	8.47±5.32^{c}	41.28±2.74^{b}	14.27±1.63^{b}	10.33±3.42^{bc}	53.68±1.71^{b}

Values are expressed as means ± standard error (SEM) for 6 rats per group (n = 6). Values in the same column with different superscript letters are significantly different at p< 0.05. Legends: HFD=High-fat diet; V.a = *Vernonia amygdalina*; RBC= Red blood cell; PCV = Packed cell volume; HGB=Haemoglobin, WBC=White blood cell; MCV=Mean cell volume.

Changes in the concentration of hepatic enzymes leading to liver dysfunction have been associated with individuals who are obese or with hyperlipidemic conditions [64]. Hence, this study evaluated the effect of *V. amygdalina* on AST, ALT and ALP, marker enzymes important in heart and liver damage (of HFD-fed Wistar rats and the result is presented in Table 3.8. There was a significant increase in the concentration of AST and ALT in HFD-fed rats implying an associated risk of liver dysfunction, however, the administration of *V. amygdalina* restored both of them to a concentration similar to those in the normal control. There were no significant changes in ALP across all groups except for those fed with a 6% aqueous concentration of the extract. The increase in the concentration of the hepatic enzymes in the serum could be a result of the increased generation and release of free radicals in the rats at a hyperlipidemic state which alters the semi-permeable status of the cell membrane causing the leakage of hepatic enzymes into the serum [65].

The ameliorative effect of *V.amygdalina* extract on the levels of AST, ALT and ALP indicates its hepatoprotective potentials in Wistar rats. The results of this study align with similar research conducted in other locations [28, 66-68], which also found that extracts from medicinal plants can aid in the repair of damaged liver cells. Each researcher used various injurious agents to induce cellular damage during their studies.

Table 8: Effect of aqueous extract of *V. amygdalina* treatment on liver function parameters

GROUPS	ALP (UL)	AST (U/L)	ALT (U/L)
NC	78.34±3.56^{a}	62.07±4.52^{b}	28.35±3.95^{b}
HFD	71.31±5.37^{a}	89.08±3.95^{a}	39.93±3.08^{a}
HFD+2% V.a	72.02±2.98^{a}	67.01±4.09^{b}	32.01±5.28^{ab}
HFD+4% V.a	74.43.±3.58^{a}	67.46±4.08^{b}	28.21±3.81^{b}
HFD+6% V.a	66.21±3.16^{b}	63.07±3.87^{b}	26.26±2.34^{b}

Values are expressed as means ± standard error (SEM) for 6 rats per group (n = 6), values in the same column with different superscript letters (a-b) are significantly different at p< 0.05. Legends: ALP = Alkaline phosphatase; ALT = Alanine aminotransferase; AST = Aspartate aminotransferase.

Urea and creatinine are the serum indices of kidney function that were measured in this study (table 3.9). There was no significant difference between the urea concentration of Wistar rats that were HFD-fed and those that were fed with normal rat chow, however, there was a significant decrease in those administered *V. amygdalina* extracts compared to the normal and the negative control groups. Acute nephropathy which is characterized by impaired tubular transport and acute cortical necrosis has been linked with an increase in kidney biomarkers [69]. Creatinine levels in the serum have also been described as the most reliable and sensitive indicator of kidney function [3]. In this

study, the creatinine level in those fed with HFD was significantly increased relative to the normal control group, however, the administration of *V. amygdalina* significantly reduced the creatinine levels of those fed HFD to a similar concentration of those in the normal control group indicating the ameliorative effect of *V. amygdalina* aqueous leaf extract on the potential case of nephropathy occurrence. This observation is similar to the study of [3] who reported a significant decrease in creatinine concentration after *V. amygdalina* treatment in rats with diet-induced obesity.

Table 9: Effect of aqueous extract of *V. amygdalina* treatment on kidney function biomarkers

GROUPS	Urea (mg/dl)	Creatinine (mg/dl)
Normal control (NC)	16.0 9±7.42^{a}	0.48±0.12^{b}
HFD (negative control)	17.25±5.37^{a}	0.80±0.31^{a}
HFD+2% V.a	11.38±0.33^{b}	0.50±0.09^{b}
HFD+4% V.a	13.53.±0.29^{b}	0.52±.08^{b}
HFD+6% V.a	12.24±0.30^{b}	0.50±0.3^{b}

Values are expressed as means ± standard error (SEM) for 6 rats per group (n = 6), values in the same column (a-b) with different superscript letters are significantly different at $p< 0.05$.

IV. CONCLUSION

The administration of *V. amygdalina* in HFD-induced obese rats ameliorated hyperglycemia, hyperlipidemia and overweight in Wistar rats by improving the metabolism of lipids, modulating inflammation, inhibiting fatty infiltration of liver tissues and preventing nephropathy, thus yielding potent antiobesity activities. The 6% extract concentration exhibited the most potent antiobesity effect and the phytochemicals present in this extract would continue to play significant roles in weight loss management and prevention of obesity.

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