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Effect of Methanolic Extract of *Vernonia amygdalina* Leaves on Glycemic and Lipidaemic Indexes of Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study sought to investigate the effect of methanolic extract of *Vernonia amygdalina* leaves on glycemic and lipidaemic indexes of Wistar rats.
Methods*:* Fresh plants of *V. amygdalina* were harvested from the Institute of Agricultural Research and Training, Ibadan. The leaves were dried and extracted using soxhlet apparatus and methanol was used as the solvent. The solvent was recovered in a rotary evaporator at 35°C with a yield of 2.24 g which represents a percentage yield of 8.96%. Ten adult male Wistar rats with body weight between 100 and 120 g were used for this study. They were randomly divided into two groups of five rats each. Animals in group 1 were administered normal saline while those in group 2 were administered *V. amygdalina* extract. The animals were exposed to the extract and saline solution at a dose of 3 mL per 100 g body weight 12 hourly via oral route of administration. After fourteen days of administration, the animals were fasted overnight and anaesthetized using diethyl ether.

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Blood samples were collected by cardiac puncture. Fasting blood sugar and lipid profile were determined using standard methods.
Results: *V. amygdalina* leaves was observed to significantly lower fasting blood sugar, total cholesterol, triglyceride and VLDL-cholesterol but had no significant effect on the concentrations of HDL-cholesterol, LDL-cholesterol and HDL/LDL-cholesterol ratio when compared to those of the control group at $p<0.05$.
Conclusion: The lowering effect of *V. amygdalina* leaves on fasting blood sugar indicated that it has the propensity to be antidiabetic. Its effect on total cholesterol and triglyceride showed that *V. amygdalina* leaves could be useful in the control of obesity and hypertension.

Keywords: Vernonia amygdalina leaves; methanolic extract, fasting blood sugar; lipid profile; diabetes.

1. INTRODUCTION

Diabetes mellitus is a common disorder which has been associated with increased morbidity and mortality. It can be defined as a group of metabolic diseases characterized by chronic hyperglycemia due to defective insulin secretion, insulin action, or both, resulting in impaired carbohydrate, lipid, and protein metabolism [1, 2]. World Health Organization (WHO) has defined diabetes mellitus based on laboratory findings as a fast venous plasma glucose concentration greater than 7.8 mmol/L (140 mg/dL) or greater than 11.1 mmol/L (200 mg/dL) two hours after a carbohydrates meal or two hours after an oral ingestion of the equivalent of 75 g glucose. According to the WHO [3], there are over 150 million diabetic patients worldwide and this is likely to increase to about 300 million by the year 2023, in spite of the understanding of the pathophysiology and treatment of the disease. Lipids can be broadly defined as hydrophobic or amphiphilic small molecules; which allows them to form structures such as vesicles, liposomes, or membranes in an aqueous environment [4]. Lipids are a large and diverse group of naturally occurring organic compounds that are related by their solubility in nonpolar organic solvents (e.g. ether, chloroform, acetone & benzene) and general insolubility in water [5]. They constitute a group of naturally occurring molecules that include fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E, and K), monoglycerides, diglycerides, triglycerides, phospholipids, etc. [6]. Although the term lipid is sometimes used as a synonym for fats, fats are a subgroup of lipids called triglycerides [7]. Lipids also include molecules such as fatty acids and their derivatives (including tri-, di-, monoglycerides, and phospholipids). It also includes other sterol-containing metabolites such as cholesterol and sex hormones [8]. Although humans and other mammals use various biosynthetic pathways to metabolize lipids, some essential lipids must be obtained from the diet [9]. Lipids have been reported to play vital functions in the body [10].

Vernonia amygdalina is a shrub of 2–5 meter tall with petiolate green leaves of about 6 mm in diameter that grows in parts of Africa, particularly, Nigeria, Cameroon, and Zimbabwe. The taxonomic classification of *V. amygdalina* is as follows: Kingdom: plantae, Division: Angiosperms, Order: Asterales, Family: Asteraceae, Genius: Vernonia, Species*: V. amygdalina.* In Nigeria, it has a variety of names in different languages due to multi-ethnicity. It is commonly referred to as “bitter leaf” in English language, “Ewuro” in Yoruba language, “Shuwaka” or “Chusadoki” in Hausa language, “Olubu” or “Onugbu” in Igbo language. It is called “Etidot”*,* in Efik, Ijaw and Ibibio, and “Oriwo” in Edo [11]. The leaves are characteristically bitter but the bitterness can be abated by boiling or by squeezing in clean water [12]. The stem and root divested of the bark are used as chew-sticks in Nigeria. More importantly, the leaves are a very popular soup vegetable and have even been reported to be consumed by goats and other animals in some parts of Nigeria [13].

All parts of the plant have been reported to be pharmacologically useful. The roots and the leaves have been used in ethnomedicine to treat fever, hiccups, kidney problems and stomach discomfort among several other uses [12,14]. Both water and solvent extracts of the stem, bark, roots and leaves have been reported to be used as a purgative, antimalarial and in the treatment of eczema [15]. The plant has acquired special relevance recently, having been proved in human medicine to be a potent antimalarial and antihelminthic agent [16] as well as anticarcinogenic agent [17] with an amazing antiparasitic efficacy in zoopharmacognosy as it is easily recognized and used for self-medication by parasitized chimpanzees [18]. The active

components of the plant have been shown to be mainly sesquiterpene lactones like vernodalin and vernoamygdalin and steroid glycosides like vernonioside B_1 and vernoniol B_1 [15].

Nutritionally, *V. amygdalina* have been reportedly used in soup making in the tropics and also as an appetizer and febrifuge [19,20] and has proven to be a successful supplement in weaning foods [21]. In Nigeria, as in other tropical countries of Africa where the daily diet is dominated by starchy staple foods, vegetables are the cheapest and most readily available sources of important proteins, vitamins, minerals and essential amino acids [22]. The importance of *V. amygdalina* in animal nutrition in Nigeria has also been well documented [13,23]. The WHO has recommended the use of alternative therapy, especially in countries or nations where accesses to conventional management procedures are inadequate. This has led to the search for more effective antihyperglycemic and antihyperlipidaemic agents. This study is therefore design to demonstrate experimentally, the effect of methanolic extract of *V. amygdalina* leaves on glycemic and lipidaemic indexes using Wistar rats.

2. MATERIALS AND METHODS

2.1 Collection and Extraction of Plant Material

Fresh plants of *V. amygdalina* were collected from the Institute of Agricultural Research and Training (IAR&T), Moor Plantation, Ibadan and were identified by a botanist. The leaves were carefully removed from the stem and washed in running water to remove contaminants. They were air dried at room temperature in an open laboratory space for 14 days and milled into powder using an electronic blender. The extraction was done using soxhlet extractor and methanol as the solvent according to the method described by Airaodion et al. [24]. About 25 g of the powder was packed into the thimble of the soxhlet extractor and 250 mL of methanol was added to a round bottom flask, which was attached to the soxhlet extractor and condenser on a heating mantle. The solvent was heated using the heating mantle and began to evaporate moving through the apparatus to the condenser. The condensate dripped into the reservoir housing the thimble containing the sample. Once the level of the solvent reached the siphon, it poured back into the round bottom flask and the cycle began again. The process was allowed to run for a total of 18 hours. Once the process was completed, the methanol was evaporated in a rotary evaporator at 35^{o}C with a yield of 2.24 g which represents a percentage yield of 8.96%. The extract was stored in the refrigerator for further analysis.

2.2 Animal Treatment

Ten adult male Wistar rats (*Rattus norvegicus*) with body weight between 100 and 120 g were purchased from the Animal Holding Unit of the Department of Physiology, University of Ibadan, Nigeria. They were housed in Imrat animal house, Ibadan. They were acclimatized for seven (7) days during which they were fed *ad libitum* with standard animal feed and drinking water. They were housed in clean cages placed in well-ventilated housing conditions (under humid tropical conditions) throughout the experiment. All the animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health. They were randomly divided into two groups of five animals each. Animals in group 1 were administered normal saline while those in group 2 were administered *V. amygdalina* extract. The animals were exposed to the extract and saline solution at a dose of 3 mL per 100 g body weight 12 hourly via oral route of administration. After fourteen days of administration, the animals were fasted overnight and anaesthetized using diethyl ether. Blood samples were collected by cardiac puncture.

2.3 Determination of Fasting Blood Sugar

After the acclimatization period, animals used in this study were allowed to fast for twelve (12) hours before the administration of saline solution and *V. amygdalina* extract to groups 1 and 2 respectively, the fasting blood sugar was determined according to the method described by Airaodion et al. [25]. The tails of the animals were sterilized with 10% alcohol, and cutting the tails using scissors then allowing the blood to touch the test strip which was inserted into a calibrated glucose meter (One touch Glucometer, Acon Laboratory INC. San Diego, USA). This gave a direct reading after 5 seconds in mg/dL. The blood glucose level of the animals before the administration of locust bean was measured in order to know the normal blood glucose of the rats in each group. After the administration of *V. amygdalina* leaves on the last day, all the rats in the groups were fasted again for 12 hours and their fasting blood sugar was determined using

glucose meter. This was done in order to check and observe the effect of *V. amygdalina* leaves on blood glucose level when compared to their initial glucose level.

2.4 Determination of Lipids

Lipids were extracted and determined according to methods earlier described [26,27].

2.5 Statistical Analysis

Data were subjected to analysis of variance using Graph Pad Prism version 6.0. Results were presented as Mean ± Standard Error of the Mean (SEM). 2–tailed t–test was used for comparison of the means. Differences between means were considered to be significant at $p<0.05$.

3. RESULTS

One major finding of this study was that *V. amygdalina* leaves indeed unhinged and perturbed the concentrations of fasting blood sugar and lipids in the animals used. These perturbations were reflected as up/down regulation of the concentrations of these metabolites as shown in Figs. 1-8.

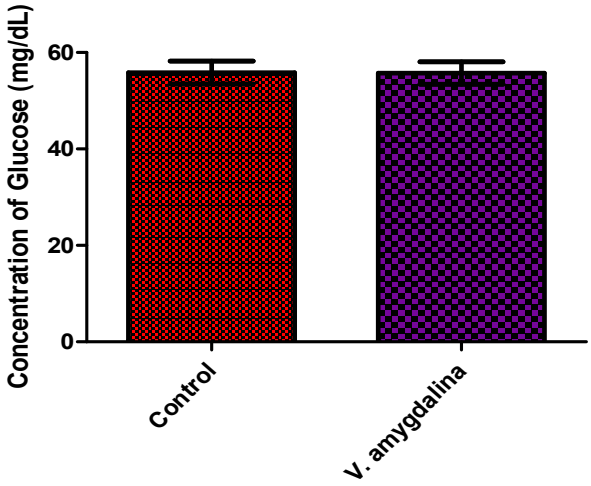

Fig. 1. Fasting blood sugar of animals before treatment
Results are presented as mean±SEM with n = 5

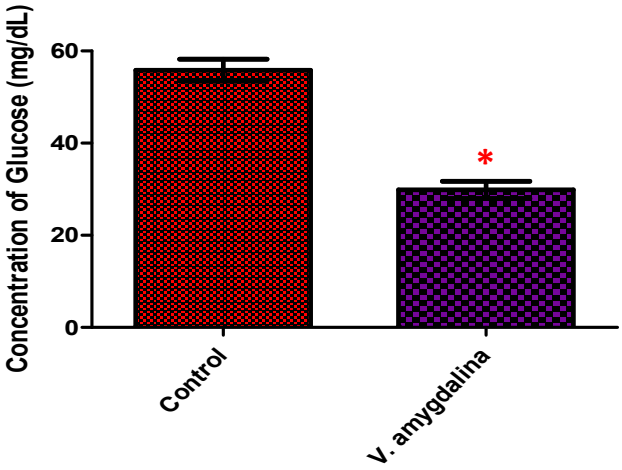

Fig. 2. Fasting blood sugar after 14 days treatment
*Results are presented as mean ± SEM with n = 5. The significant difference between the V. amygdalina leaves extract–treated animals and control group at p<0.05 is represented by **

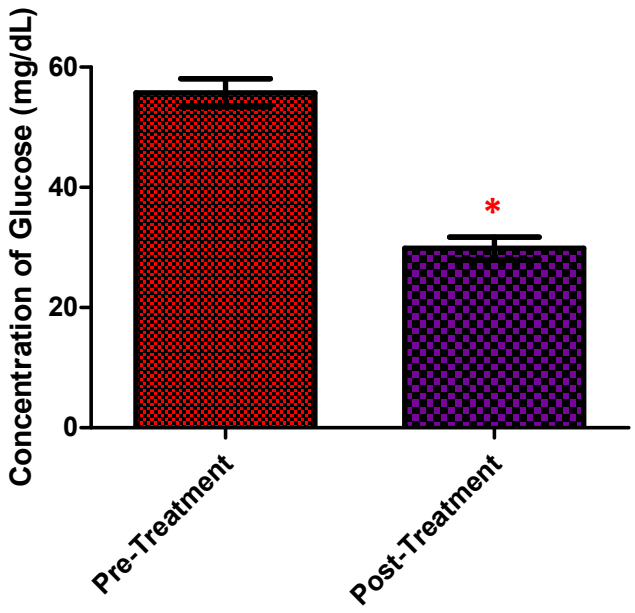

Fig. 3. Effect of *V. amygdalina* leaves extract administration on the fasting blood sugar of animals after 14 days

*Results are presented as mean ± SEM with n = 5. The significant difference between the V. amygdalina leaves extract–treated animals and control group at p<0.05 is represented by **

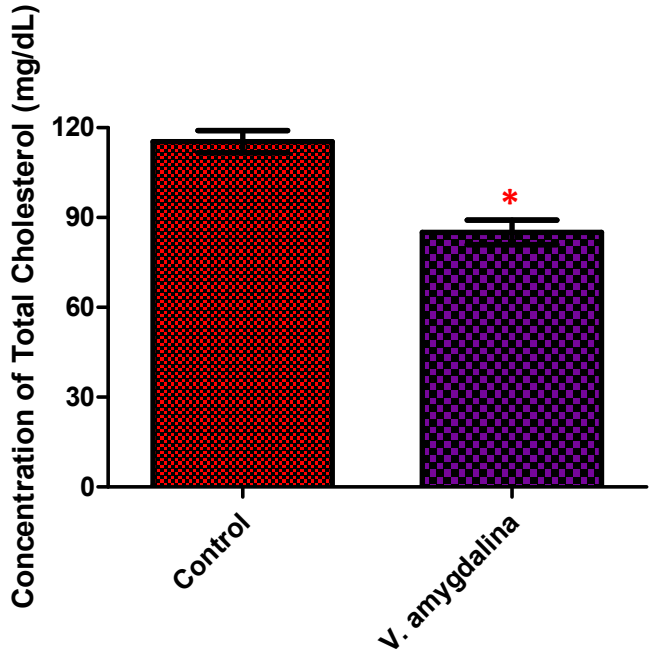

Fig. 4. Effect of *V. amygdalina* leaves extract administration on the total cholesterol of animals after 14 days

*Results are presented as mean ± SEM with n = 5. The significant difference between the V. amygdalina leaves extract–treated animals and control group at p<0.05 is represented by **

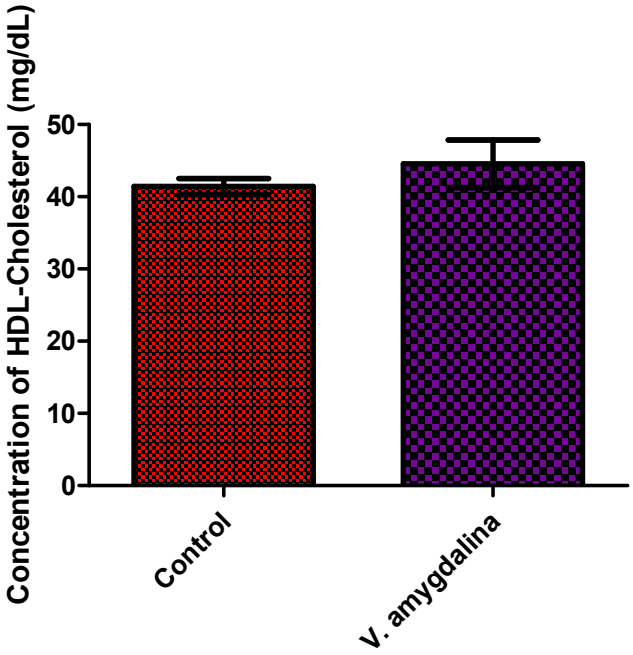

Fig. 5. Effect of *V. amygdalina* leaves extract administration on the HDL-cholesterol of animals after 14 days

Results are presented as mean ± SEM with n = 5

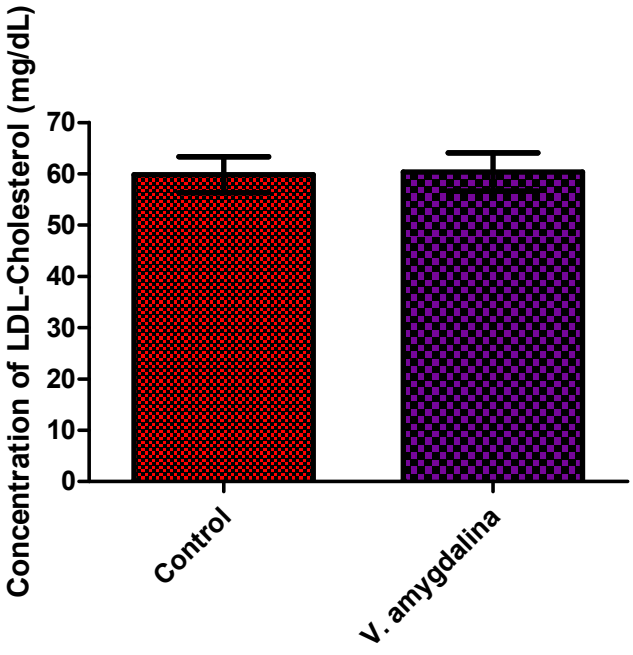

Fig. 6. Effect of *V. amygdalina* leaves extract administration on the LDL-cholesterol of animals after 14 days

Results are presented as mean ± SEM with n = 5

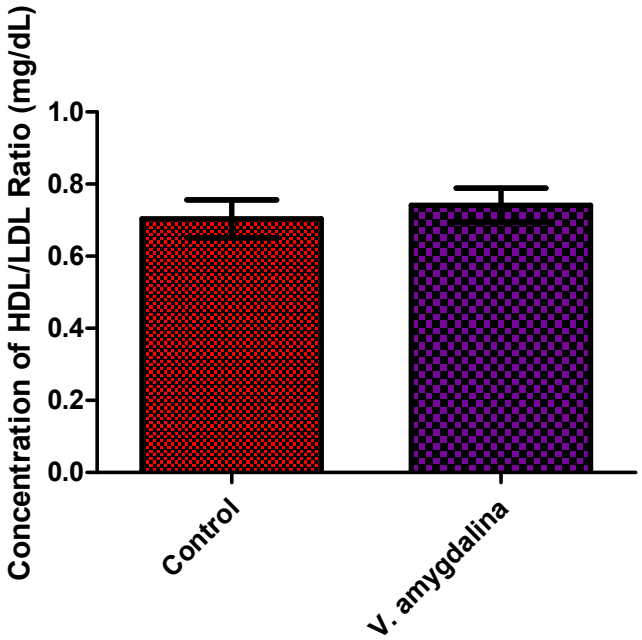

Fig. 7. Effect of *V. amygdalina* leaves extract administration on the HDL/LDL-cholesterol ratio of animals after 14 days

Results are presented as mean ± SEM with n = 5

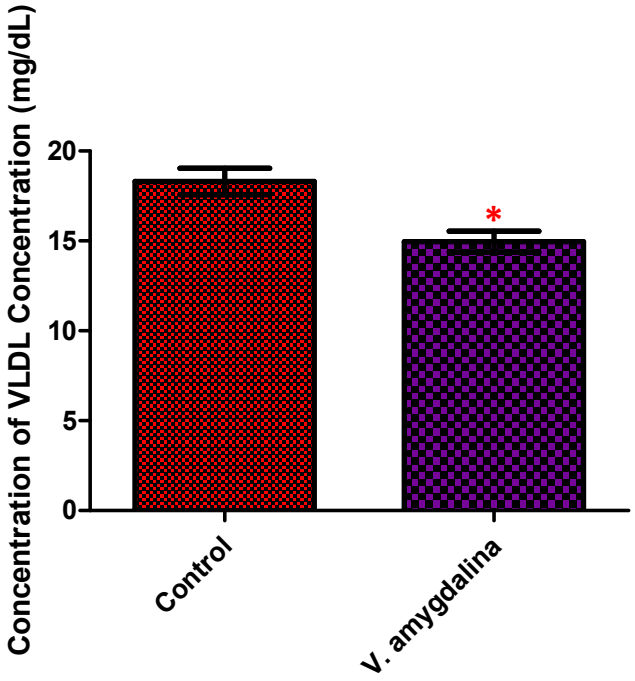

Fig. 8. Effect of *V. amygdalina* leaves extract administration on the VLDL-cholesterol of animals after 14 days

*Results are presented as mean ± SEM with n = 5. The significant difference between the V. amygdalina leaves extract–treated animals and control group at p<0.05 is represented by **

4. DISCUSSION

Hyperglycaemia and hyperlipidaemia are the main causes of diabetes which has become very rampant in the world today. Among the multiple risk factors underlining the incidence and progression of diabetes, diet is the major modifiable factor. Both experimental and epidemiological evidences have proved that consumption of vegetables rich in phytochemicals (especially phenolic compounds) as well as possess high antioxidant potential have inverse relationship with the incidence and prevalence of diabetes [28]. Dietary control remains one of the most desirable avenues for the prevention and management of most chronic degenerative diseases such as diabetes and cardiovascular diseases which arises from hyperglycaemia and hyperlipidaemia. The growing number of diabetes coupled with the harsh side effects of some synthetic drugs has led to the increasing search for alternatives which are relatively cheap with negligible side effects. Studies revealed that green leafy vegetables and fruits have some health benefits [29,30]. Thus, *V. amygdalina* leaf is a green leafy vegetable popularly used as food and in traditional medicine for the prevention and management of some diseases. However, there is dearth of information on the possible mechanisms of action by which these vegetables exert their health benefits. This study is therefore design to demonstrate experimentally, the effect of methanolic extract of *V. amygdalina* leaves on glycemic and lipidaemic indexes of Wistar rats.

In this study, no significant difference was observed in the fasting blood sugar when animals in the *V. amygdalina* leaves group were compared with those of the control group prior to treatment (Fig. 1). After fourteen days of treatment, animals treated with *V. amygdalina* leaves had significantly lowered fasting blood sugar when compared with control and pretreated groups (Figs. 2 and 3) at $p<0.05$ respectively. This is suggestive that *V. amygdalina* leaves may have an extrapancreatic antihyperglycemic mechanism of action. This is in agreement with the study of Airaodion et al. [25] on the effect of oral intake of African locust bean on fasting blood sugar and lipid profile of albino rats. Several studies has shown that a number of other plants and extracts have an antihyperglycemic and an insulin-stimulatory effect [24,31,32,33]. Most of these plants have been reported to be rich in phytochemicals and contain metabolites such as glycosides, alkaloid and flavonoids [29,30,34]. These metabolites could be responsible for the hypoglycemic effect of *V. amygdalina* leaves observed in this study. The fasting blood sugar lowering effect of *V. amygdalina* leaves could also indicate that it possesses antidiabetic agents which could control hyperglycaemia. This is consistent with earlier reports that green leafy vegetables possess antidiabetic properties [24,35,36]. One therapeutic approach for treating early stage of diabetes is to decrease post-prandial hyperglycaemia. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate-hydrolyzing enzymes, α-amylase and α-glucosidase, in the digestive tract. Thus, inhibitors of these enzymes determine a reduction in the rate of glucose absorption and therefore blunting the post-prandial plasma glucose rise [37]. Based on these findings, it could be suggested that *V. amygdalina* leaves may inhibit platelet aggregation and promote vasodilatation, exhibiting a crucial protective role in the prevention of the development and progression of vascular complications caused by the hyperglycemic state. In fact, studies have proved that polyphenols present in some vegetables have the propensity of inhibiting the process of thrombus formation [38,39].

Apart from the regulation of glucose metabolism, insulin has been reported to play a vital role in the anabolism and catabolism of lipid. Deficiency of insulin is associated with hypercholesterolemia and hypertriglyceridemia, which have been shown to occur in experimental diabetic animals [40,41,42]. Hypercholesterolemia could result in a relative molecular ordering of the residual phospholipids, resulting in a decrease in the fluidity of the membrane [43]. Accumulation of triglycerides has been reported to be one of the leading risk factors in coronary heart disease (CHD). Lipid and lipoprotein abnormalities have been shown to play a major role in the pathogenesis and progression of several disease state [44].

Total cholesterol and triglycerides concentrations were observed to decrease significantly in this study, when animals treated with methanolic extrat of *V. amygdalina* leaves were compared with those of the control group at $p<0.05$ (Figs. 4 and 9) respectively. This could be that *V. amygdalina* leaves may prevent the progression of CHD. Despite the availability of known anti-diabetic medications, treatments with medicinal plants are used with increasing success to treat this disease and better manage its complications

[45]. Moreover, it has been suggested that medicinal plants and herbal medications are less toxic with little or no side-effects compared with synthetic drugs, leading to an increasing preference for herbal medications over synthetic formulations [46-50]. Increased evidences of therapeutic effectiveness of herbal drugs may have motivated the decision of the WHO in hypoglycemic agents of from medicinal plants used in the traditional treatment of diabetes [51]. Hypertriglyceridaemia has been implicated in diabetic animals [52]. This was associated to an increased formation and absorption of triglycerides in the form of chylomicrons following exogenous consumption of lipid-rich diet or through enhanced endogenous production of triglyceride-enriched hepatic VLDL-cholesterol and decreased triglyceride uptake in peripheral tissues [52]. Hypercholesterolaemia has also been implicated in diabetes [52]. This has been linked to the increased dietary cholesterol absorption from the small intestine following the intake of high fat diet in diabetic conditions [53]. However, the levels of serum triglyceride, VLDL-cholesterol and total cholesterol were significantly reduced in animals treated with extracts of *V. amygdalina* leaves when compared with those of the control group in the present study. Moreover, it can be suggested that the lipid lowering effects of *V. amygdalina* leaves might be due to the inhibition of hepatic cholesterol, triglyceride and possibly fatty acid synthesis by the phenolic constituents of *V. amygdalina* leaves [36].

Hypertriglyceridaemia has also been proved to be a predictor of hypertension risk [54]. In the peripheral vascular system, endothelial cells rely on lipoproteins for the transfer of neutral sterols at this site. Although free cholesterol is transferred to HDL-cholesterol particles through the functioning of a designated HDL-cholesterol receptor, lecithin cholesterol acyl transferase (LCAT) serves to maintain the concentration toward the HDL core and preserve the hydrophobic nature that facilitates the transfer. Esterification of cholesterol produces cholesterol ester (CE), which is concentrated in HDL core, and may be transferred by cholesterol ester transfer protein (CETP) in the plasma compartment to apo-B containing lipoproteins in exchange for triglyceride. Increased CETP activity would suggest an enrichment of apo-B lipoproteins in plasma, while simultaneously decreasing HDL-cholesterol, and has generally been considered pro-atherogenic [55]. This probably explains why *V. amygdalina* leaves could lead to a reduction in the risk of developing heart diseases since a high HDL-cholesterol/LDL-cholesterol ratio has been proved to be beneficial and is indicative of a lower risk of cardiovascular diseases [56].

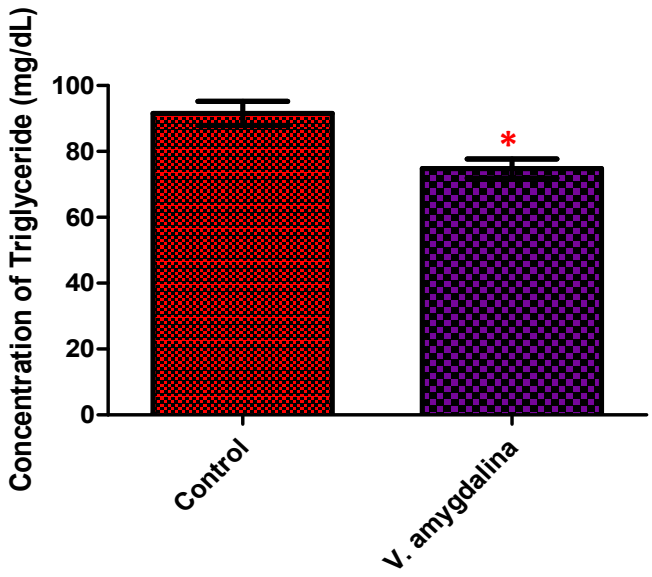

Fig. 9. Effect of *V. amygdalina* leaves extract administration on the Triglyceride of animals after 14 days

*Results are presented as mean ± SEM with n = 5. The significant difference between the V. amygdalina leaves extract–treated animals and control group at p<0.05 is represented by **

LDL- and HDL-cholesterol are the two among four main groups of plasma lipoproteins that are involved in the metabolism of lipid and the exchange of cholesterol, cholesterol ester and triglycerides between tissues [57,58]. Several studies have shown an inverse correlation between plasma HDL-cholesterol levels and a risk of cardiovascular disease, implying that factors associated with HDL-cholesterol protect against atherosclerosis and thus vascular diseases. Some of these factors appear to have antioxidant and anti-inflammatory effects which may obstruct processes that initiate atherogenesis [59,60].

Epidemiological studies also shown that elevated concentrations of total cholesterol and/or LDL-cholesterol in the blood are powerful risk factors for atherosclerosis and coronary heart disease [61]. Their cholesterol requirements are supplied by LDL, which is internalized by receptor-mediated endocytosis. A major function of HDL-cholesterol is to enhance reverse cholesterol transport by scavenging excess cholesterol from peripheral tissues followed by esterification through lecithin: cholesterolacyltransferase and delivering it to the liver and steroidogenic organs for subsequent synthesis of bile acids and lipoproteins and eventual elimination from the body [62,63]. This role of HDL-cholesterol has been shown to be responsible for its atheroprotective properties. HDL-cholesterol also regulates the exchange of proteins and lipids between various lipoproteins.

In addition, HDL-cholesterol provides the protein components required to activate lipoprotein lipase which releases fatty acids that can be oxidized by the ß-oxidation pathway to release energy [57,58]. Most importantly, HDL-cholesterol can inhibit oxidation of LDL-cholesterol as well as the atherogenic effects of oxidized LDL-cholesterol by virtue of its antioxidant property [63]. LDL is a lipoprotein that transports cholesterol and triglyceride from the liver to peripheral tissues. It enables fat and cholesterol to move within the water-blood solution of the blood stream. LDL is often called bad cholesterol; hence low levels are beneficial [64].

Interestingly, the administration of *V. amygdalina* leaves in this study caused an insignificant increase in the serum level of HDL-cholesterol when compared with the control animals (Fig. 5) at $p<0.05$. This contradicts the findings of Airaodion et al. [24] who reported a significant increase in the HDL-cholesterol concentration when animals were treated with methanolic extract of *Corchorus olitorius* leaves for 14 days. HDL-cholesterol is usually referred to as the 'good cholesterol' [26]. Again, *V. amygdalina* leaves administration did not cause any significant difference in the concentration of LDL-cholesterol (bad cholesterol) when compared with animals treated with normal saline solution at $p<0.05$ (Fig. 6). This result is in agreement the findings of Airaodion et al. [25] who reported a nonsignificant difference in the LDL-cholesterol concentration when animals were treated with African locust bean for 14 days but contradicts that of Airaodion et al. [24] who reported a significant decrease in the LDL-cholesterol concentration when animals were treated with methanolic extract of *Corchorus olitorius* leaves for 14 days.

5. CONCLUSION

The lowering effect of *V. amygdalina* leaves on fasting blood sugar indicates that it has the propensity to be antidiabetic. Its effect on total cholesterol and triglyceride showed that *V. amygdalina* leaves could be useful in the control of obesity and hypertension, thus its consumption is recommended to those predisposed to these health conditions.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All the animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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