

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

ABSTRACT

Aim: The aim of this study is 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 as the solvent. The methanol was evaporated 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 solution 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. 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 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 associated with increased morbidity and mortality and 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 WHO [3], there are over 150 million diabetics worldwide and this is likely to increase to about 300 million by the year 2023, in spite of major in understanding the

pathophysiology and treatment of the disease. Lipids may be broadly defined as hydrophobic or amphiphilic small molecules; the amphiphilic nature of some lipids 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 encompass molecules such as fatty acids and their derivatives (including tri-, di-, monoglycerides, and phospholipids), as well as other sterol-containing metabolites such as cholesterol [8]. Although humans and other mammals use various biosynthetic pathways to synthesize and break down lipids, some essential lipids cannot be made this way and must be obtained from the diet [9]. Lipids have been reported to have several roles in the body [10].

Vernonia amygdalina is a shrub of 2–5 meter tall with petiolate green leaves of about 6 mm diameter that grows in African tropics and other parts of Africa, particularly, Nigeria, Cameroon, and Zimbabwe. The taxonomic classification of *Vernonia amygdalina* is as follows: Kingdom: plantae, Division: Angiosperms, Order: Asterales, Family: Asteraceae, Genus: Vernonia, Species: *V. amygdalina*. In Nigeria, it has a variety of names in various languages. It is commonly called “Bitter leaf” in English language, “Ewuro” in Yoruba language, “Shuwaka” or “Chusadoki” in Hausa language, “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 soaking in several changes of 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 in some parts of Nigeria [13].

All parts of the plant are pharmacologically useful. The roots and the leaves are used in ethnomedicine to treat fever, hiccups, kidney problems and stomach discomfort among several other uses [12, 14]. Both aqueous and alcoholic extracts of the stem, bark, roots and leaves are reported to be extensively 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 possess potent antimalarial and antihelminthic properties [16] as well as antitumorigenic properties [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 vernodaline and vernonamygdaline and steroid glycosides like vernonioside B₁ and vernoniol B₁ [15].

Nutritionally, *V. amygdalina* is used mainly 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 of Wistar rats.

2. MATERIALS AND METHODS

2.1 Collection and Extraction of Plant Material

Fresh plants of *V. amygdalina* were harvested from the Institute of Agricultural Research and Training, 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 (Moulinex). The extraction was done using soxhlet apparatus 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 °C with a yield of 2.24 g which represents a percentage yield of 8.96%. The extract was preserved 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 feed and drinking water and 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 rats each. Animals in group 1 were administered normal saline solution 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 rats 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 locust bean 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 (before the administration).

2.4 Determination of Lipids

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

2.5 Statistical Analysis

Data were subjected to analysis of variance using Graph Pad Prism. Results were presented as Mean $\pm$ 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

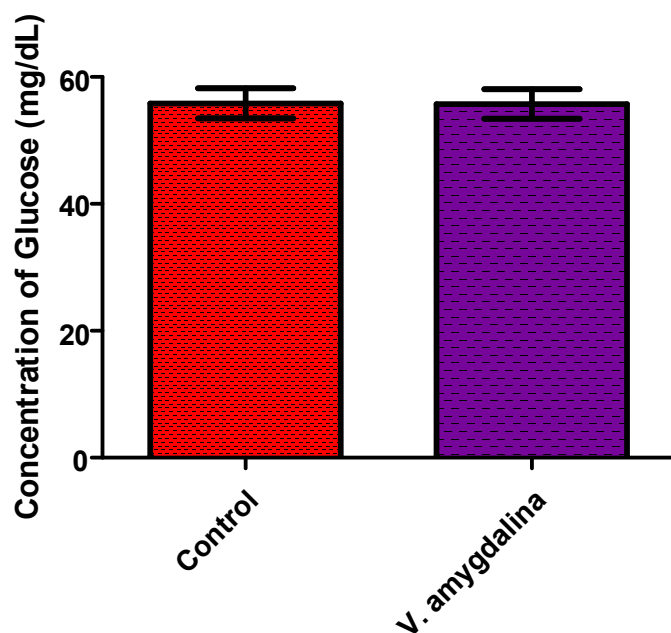


Fig. 1. Fasting blood sugar of animals before treatment

Results are presented as mean $\pm$ SEM with n = 5

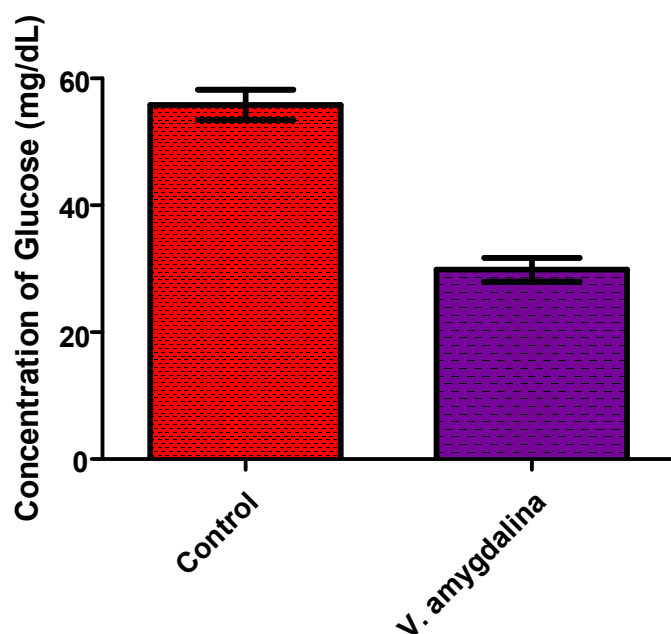


Fig. 2. Fasting blood sugar after 14 days treatment

Results are presented as mean $\pm$ 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 *.

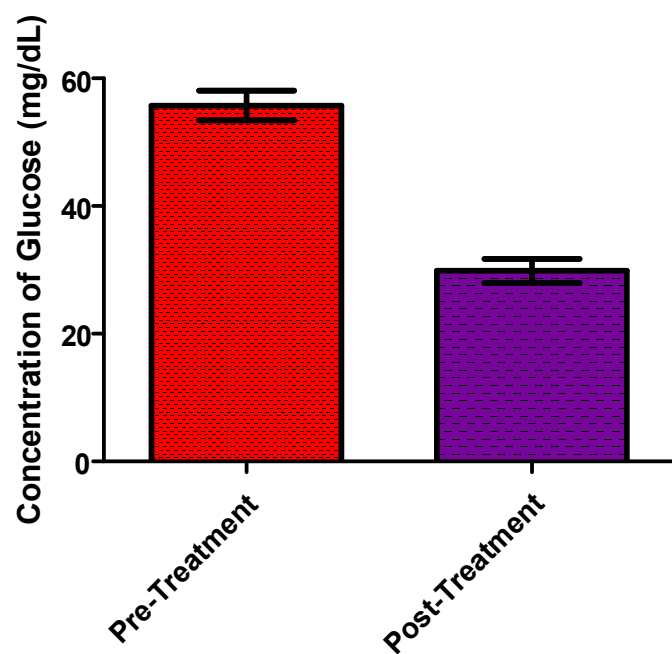


Fig. 3. Effect of *V. amygdalina* leaves extract administration on the fasting blood sugar of animals after 14 Days

Results are presented as mean $\pm$ 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 *.

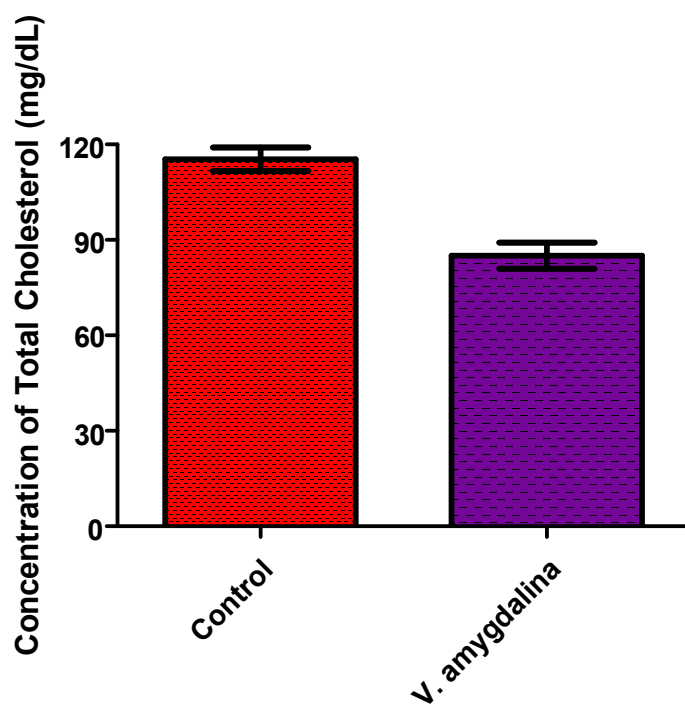


Fig. 4. Effect of *V. amygdalina* leaves extract administration on the total cholesterol of animals after 14 Days

Results are presented as mean $\pm$ 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 *

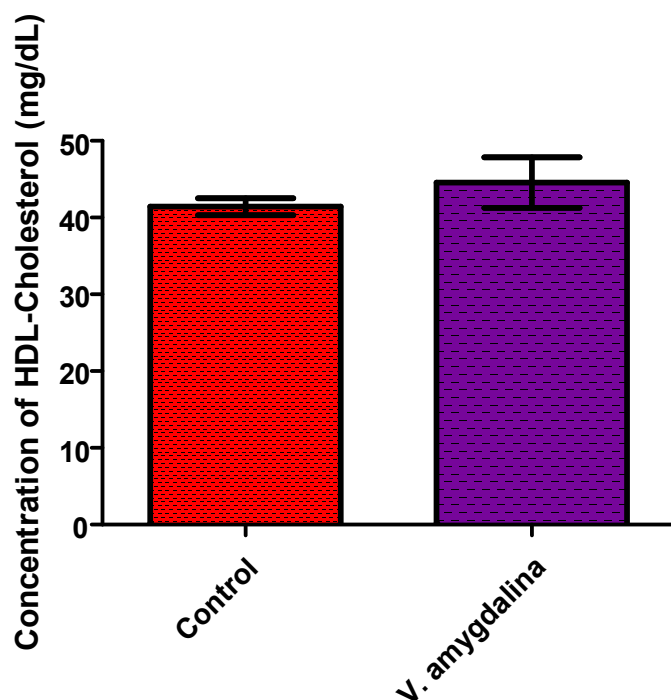


Fig. 5. Effect of *V. amygdalina* leaves extract administration on the HDL-cholesterol of animals after 14 days

Results are presented as mean $\pm$ SEM with $n = 5$.

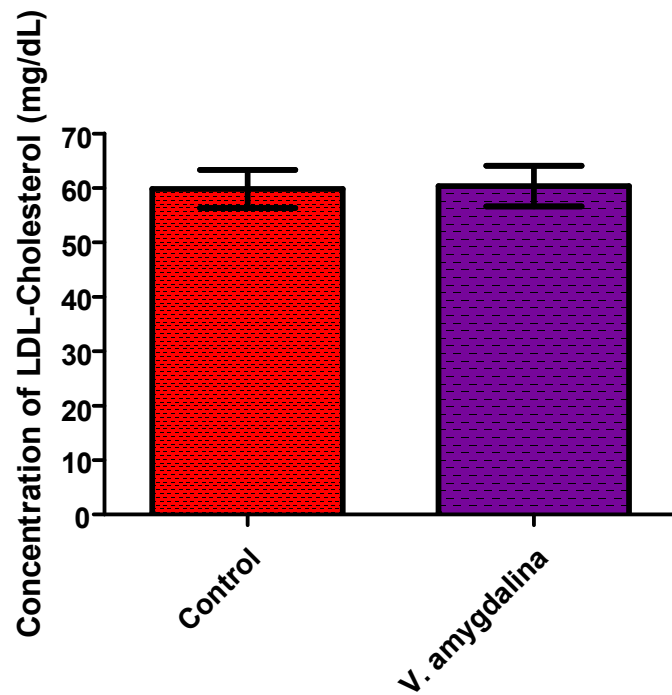


Fig. 6. Effect of *V. amygdalina* leaves extract administration on the LDL-cholesterol of animals after 14 days

Results are presented as mean $\pm$ SEM with $n = 5$.

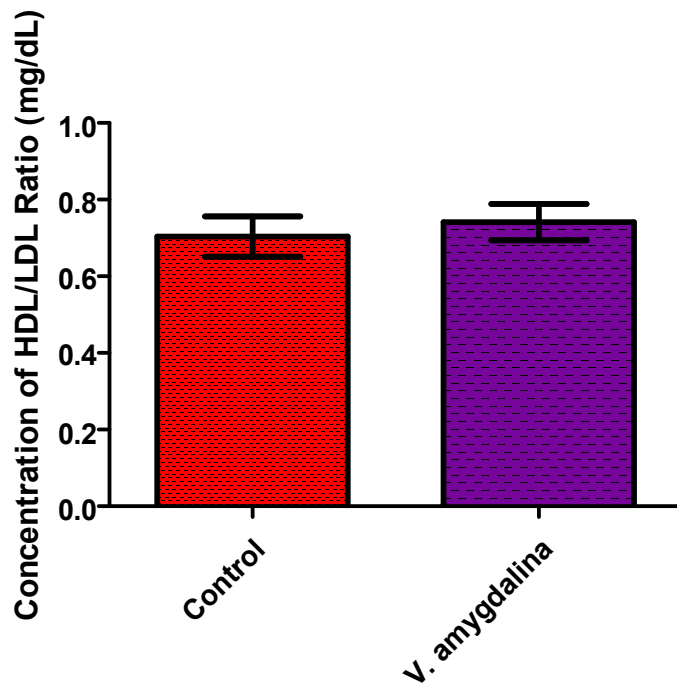


Fig. 7. Effect of *V. amygdalina* leaves extract administration on the HDL/LDL-cholesterol ratio of animals after 14 days

Results are presented as mean $\pm$ SEM with n = 5.

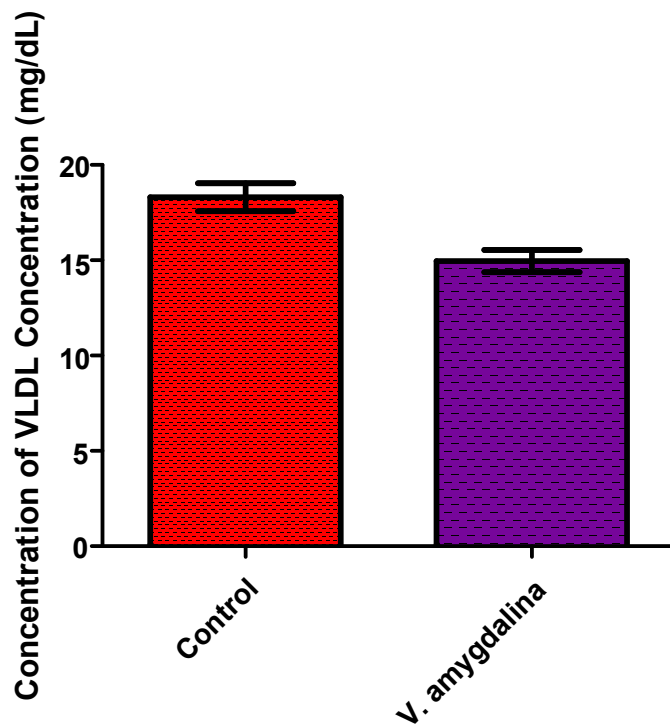


Fig. 8. Effect of *V. amygdalina* leaves extract administration on the VLDL-cholesterol of animals after 14 days

Results are presented as mean $\pm$ 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 *

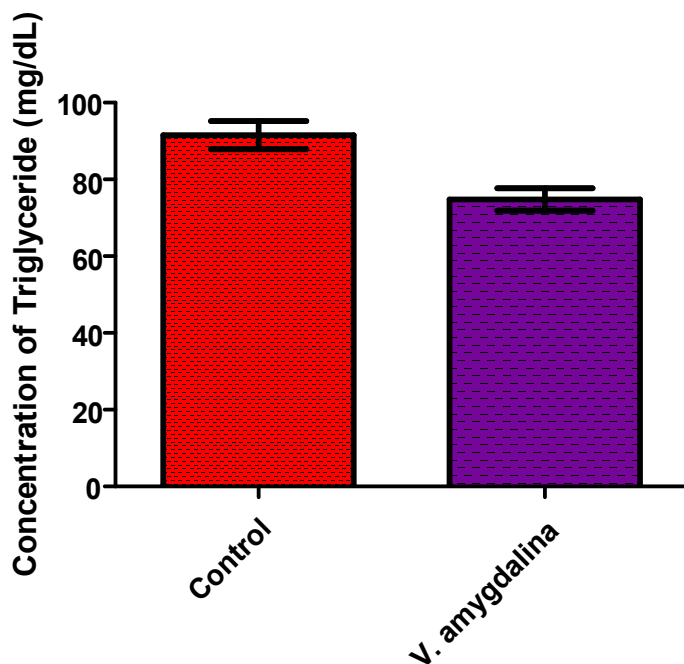


Fig. 9. Effect of *V. amygdalina* leaves extract administration on the Triglyceride of animals after 14 days

Results are presented as mean $\pm$ 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 cause of diabetes which has become very rampant. Among the multiple risk factors underlining the incidence and progression of diabetes, diet is the main modifiable factor. Both experimental and epidemiological evidences have shown that consumption of vegetables rich in phenolic compounds and possess high antioxidant capacity may 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 chronic degenerative diseases

such as diabetes and cardiovascular diseases. The growing number of diabetics coupled with the harsh side effects of some synthetic drugs has led to the increasing search for alternatives which are relatively cheap with minimal side effects. Green leafy vegetables and fruits have been reported to have some health benefits [29, 30]. Consequently, *V. amygdalina* leaf is a green leafy vegetable popularly used as food and in traditional medicine for the 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 suggests 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. A number of other plants and extracts have also been reported to have an antihyperglycemic and an insulin-stimulatory effect. [24, 31, 32, 33]. Most of the plants with hypoglycemic properties have been found to contain metabolites such as glycosides, alkaloid and flavonoids [29, 30, 34]. These metabolites may be responsible for the hypoglycemic effect of *V. amygdalina* leaves observed in this study. The fasting blood sugar lowering effect of *V. amygdalina* leaves may also indicate that it possesses antidiabetic agents which could control hyperglycemia. This is in consonance 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. Consequently, inhibitors of these enzymes determine a reduction in the rate of glucose absorption and consequently 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, exerting an important protective role in the prevention of the development and progression of vascular complications caused by the

hyperglycemic state. In fact, studies have shown that polyphenolic compounds present in some plant foods can inhibit the process of thrombus formation [38, 39].

Apart from the regulation of carbohydrate metabolism, insulin plays an important role in lipid metabolism. Insulin insufficiency, as in diabetes mellitus, is associated with hypercholesterolemia and hypertriglyceridemia, which have been reported to occur in experimental diabetic rats [40, 41, 42]. Hypercholesterolemia could result in a relative molecular ordering of the residual phospholipids, resulting in a decrease in membrane fluidity [43]. Accumulation of triglycerides is 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 conditions [44].

In this study, total cholesterol and triglycerides concentrations were observed to decrease significantly when animals treated with methanolic extract of *V. amygdalina* leaves were compared with those of the control group at $p < 0.05$ (Fig. 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, remedies from medicinal plants are used with increasing success to treat this disease and manage its complications better [45]. Furthermore, it has been suggested that plant drugs and herbal formulations are less toxic and are free from side-effects compared with synthetic drugs, leading to an increasing preference for traditional plants over synthetic drugs [46-50]. Increased evidence of therapeutic effectiveness of herbal medicines may have influenced the interest of world health organization (WHO) in hypoglycemic agents of plant origin used in the traditional treatment of diabetes [51]. Hypertriglyceridaemia has been reported in diabetic animals [52]. This was reported to be due to an increased absorption and formation of triglycerides in the form of chylomicrons following exogenous consumption of diet rich in

fat or through increased endogenous production of triglyceride-enriched hepatic VLDL-cholesterol and decreased triglyceride uptake in peripheral tissues [52]. Hypercholesterolaemia has also been reported in diabetic animals [52]. This was attributed to the increased dietary cholesterol absorption from the small intestine following the intake of high fat diet in a diabetic condition [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 conjectured that the lipid lowering effects of *V. amygdalina* leaves could 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 reported 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 may lead to a reduction in the risk of developing heart diseases since a high HDL-cholesterol/LDL-cholesterol ratio has been shown to be beneficial and is indicative of a lower risk of cardiovascular diseases [56].

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

Epidemiological studies have also shown that elevated concentrations of total cholesterol and/or LDL-cholesterol in the blood are powerful risk factors for coronary heart disease [61]. Most extra-hepatic tissues, although having a requirement for cholesterol, have low activity of the cholesterol biosynthetic pathway. 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 β -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 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.

REFERENCES

1. Lebovitz HE. Oral Anti-Diabetic Agents. In: Joslin's Diabetes Mellitus. Khan, C.R. and G.C. Weir, (Eds). 13th Edn., Lea and Febiger, Philadelphia. 1994;29: 508-524
2. Bhatena SJ, Velasquez MT. Beneficial role of dietary phytoestrogens in obesity and diabetes. *Am J Clin Nutr.*, 2002;76: 1191-1201.
3. World Health Organization (WHO). Definition, Diagnosis and classification of Diabetes Mellitus and its complication www.who.org.1999.
4. Subramaniam S, Fahy E, Gupta S, Sud M, Byrnes RW, Cotter D, Dinasarapu AR, Maurya MR. Chemical Reviews. 2011;111(10):6452–6490.
5. Fahy E, Subramaniam S, Murphy R, Nishijima M, Raetz C, Shimizu T, Spener F, Van Meer G., Wakelam M, Dennis EA. Journal of Lipid Research. 2009; 50(Supplement):S9–S14.
6. Page RA, Okada S, Harwood JL. AcetylCoA carboxylase exerts strong flux control over lipid synthesis in plants. *Biochim. Biophys. Acta.* 1994;1210:369-372.
7. Small DM. The physical chemistry of lipids. Handbook of Lipid Research. D. J. Hanahan, Editor. Plenum Press, New York. 1986;4.
8. Michelle A, Hopkins J, McLaughlin CW, Johnson S, Warner MQ, LaHart D, Wright JD. Human biology and health. Englewood Cliffs, New Jersey, USA: Prentice Hall; 1993.
9. Airaodion AI, Adejumo PR, Njoku CO, Ogbuagu, EO, Ogbuagu U. Implication of sugar intake in haemorrhoid and menstruation. *International Journal of Research and Reports in Hematology.* 2019;2(2):1-9.
10. Airaodion AI, Ogbuagu U, Ogbuagu EO, Oloruntoba AP, Agunbiade AP, Airaodion EO, Mokelu IP, Ekeh SC. Mechanisms for controlling the synthesis of lipids. *International Journal of Research.* 2019;6(2):123-135.
11. Ojiako OA, Nwanjo HU. Is *Vernonia amygdalina* hepatotoxic or hepatoprotective? Response from biochemical and toxicity studies in rats. *African Journal of Biotechnology* 2006;5(18):1648-1651.
12. Olufunmilayo LA, Oshiobugie MJ, Iyobosa AI. Acute toxicity and

- hypoglycemic properties of ethanolic root extract of *Vernonia amygdalina* (bitter leaf) in alloxan- induced diabetic rats". International Journal of Current Research. 2017; 9, (05), 50132- 50138
13. Aregheore EMK, Makkar HPS, Becker K. Feed value of some browse plants from the central zone of Delta State, Nig. Trop. Sci. 1998;38(2): 97-104.
 14. Hamowia AM, Saffaf AM. Pharmacological studies on *Vernonia amygdalina* (Del) and *Tithonia diversifolia* (Gray). Vet. Med. J. Giza 1994;2: 91-97.
 15. Kupcham SM, Hernichway RJ, Karim A, Wermer D. Tumor inhibitors. XLVII. Vernodaline and vernomygdin, two new cytotoxic sesquiterpene lactones from *Vernonia amygdalina* Del. J. Org. Chem. 1969;34 (12):3908-11.
 16. Abosi AO, Raseroka BH. *In vivo* antimalarial activity of *Vernonia amygdalina*. Br. J. Biomedical Sci. 2003;60(2): 89-91.
 17. Izevbigie EB, Bryant JL, Walker A. A novel natural inhibitor of extracellular signal-regulated kinases and human breast cancer cell growth. Experimental Biol. Med. (Maywood). 2004;229(2):163-169.
 18. Huffman MA. Animal self-medication and ethno-medicine: exploration and exploitation of the medicinal properties of plants. Proceedings of the Nutr. Society. 2003;62(2):371-81.
 19. Ijeh I, Nwugo VO, Obidoa O. Comparative studies on the nutritive Phyto-chemical and antimicrobial properties of two varieties of *Vernonia amygdalina*. Plant Pros. Res. Comm. 1996;1: 71-75.
 20. Iwu MM, Okunji E, Akah PA, Tempesta MS, Carley DG. Dioscoretin: The hypoglycaemic principles of *Dioscorea dumetorum*. Plant Med. 1996;56: 119-126.
 21. Ejoh AA, Djuikwo VN, Gouado I, Mbofung CM. Nutritional components of some non-conventional leafy vegetables consumed in Cameroon. Pakistan Journal of Nutrition. 2007;6(6):712-717.
 22. Okafor JC. Horticulturally promising indigenous wild plant species of the Nigerian forest zone. Acta Hort. 1983;123:165-176.
 23. Onwuka CFI, Akinsoyinu AO, Tewe OO. Feed value of some Nigerian browse plants: chemical composition and *in vitro* digestibility. East African Agriculture and Forestry J. 1989;54:157-163.
 24. Airaodion AI, Ogbuagu EO, Ogbuagu U, Awosanya OO, Airaodion EO. Hypoglycemic and hypolipidaemic effect of methanolic extract of *Corchorus olitorius* leaves in Wistar rats. Asian Plant Research Journal. 2019;7(6):1-12.
 25. Airaodion AI, Airaodion EO, Ogbuagu EO, Ogbuagu U, Osemwowa EU. Effect of Oral Intake of African Locust Bean on Fasting Blood Sugar and Lipid Profile of Albino Rats. *Asian Journal of Research in Biochemistry*. 2019;4(4), 1-9.
 26. Owoade AO, Adetutu A, Airaodion AI, Ogundipe OO. Toxicological assessment of the methanolic leaf extract of *Bridelia ferrugelia*. The Journal of Phytopharmacology. 2018;7(5):419-424.
 27. Owoade AO, Airaodion AI, Adetutu A, Akinyomi OD. Levofloxacin-induced dyslipidemia in male albino rats. Asian Journal of Pharmacy and Pharmacology. 2018;4(5):620-629.
 28. Bahadoran Z, Golzarand M, Mirmiran P, Saadati N, Azizi F. The association of dietary phytochemical index and cardio-metabolic risk factors in adults: Tehran lipid and glucose study. *Journal of Human Nutrition and Diet*. In print. 2013.
 29. Airaodion AI, Olatoyinbo PO, Ogbuagu U, Ogbuagu EO, Akinmolayan JD, Adekale OA, Awosanya OO, Oloruntoba AP, Agunbiade AP, Airaodion EO, Adeniji AR, Obajimi OO. Comparative assessment of phytochemical content and antioxidant potential of *Azadirachta indica* and *Parquetina nigrescens* leaves. Asian Plant Research Journal. 2019;2(3):1-14.

30. Airaodion AI, Ibrahim AH, Ogbuagu U, Ogbuagu EO, Awosanya OO, Akinmolayan JD, Njoku OC, Obajimi OO, Adeniji AR, Adekale OA. Evaluation of Phytochemical Content and Antioxidant Potential of *Ocimum gratissimum* and *Telfairia occidentalis* Leaves. *Asian Journal of Research in Medical and Pharmaceutical Sciences*. 2019; 7(1):1-11.
31. Prince P, Stanely M, Menon PV, Pari L. Hypoglycaemic activity of *Syzigium cumini* seeds: Effect on lipid peroxidation in alloxan diabetic rats. *J. Ethnopharmacol*. 1998;61:1-7.
32. Venkateswaran S, Pari L, Saravenan G. Effect of *Phaseolus vulgaris* on circulatory antioxidants and lipids in streptozotocin-induced diabetic rats. *J. Med. Food*. 2002;5:97-104.
33. Latha M, Pari L. Preventive effects of *Cassia auriculata* L. flowers on brain lipid peroxidation in rats treated with streptozotocin. *Mol. Cell. Biochem*. 2003;243:23-28.
34. Loew S, Kaszkin M. Approaching the problem of bioequivalence of herbal medicinal products. *Phytother. Res*. 2002;16:705-711.
35. Zhang L, Yang J, Chen X, Zan K, Wen X, Chen H, Wang Q, Lai M. Antidiabetic and antioxidant effects of extracts from *Potentilla discolor* Bunge on diabetic rats induced by high fat diet and streptozotocin. *Journal of Ethnopharmacology*. 2010;132:518-524.
36. Balamurugan R, Ignacimuthu S. Antidiabetic and Hypolipidemic effect of methanol extract of *Lippia nodiflora* L. in streptozotocin induced diabetic rats. *Asia Pacific Journal of Tropical Biomedicine*. 2011; S30-S36.
37. Chen X, Zheng Y, Shen Y, Voglibose BAO. One of the most important α -glucosidase inhibitors. *Current. Medical. Chemistry*. 2006;13: 109-116.
38. Dohadwala MM, Vita JA. Grapes and cardiovascular disease. *Journal of Nutrition*. 2009;139(9):1788-1793.
39. Gresele P, Cerletti C, Guglielmini G, Pignatelli P, de Gaetano G, Violi F. Effects of resveratrol and other wine polyphenols on vascular function: *An update*. *Journal of Nutritional Biochemistry*. 2011;22(3):201-211.
40. Loci AS, Shaabha M, Khazraji AL, Husain A, Twaija A. Hypoglycemic effect of a valuable extract on some blood parameters in diabetic animals. *J. Ethnopharmacol*. 1994;43:167-171.
41. Ahardh CD, Bjorgell P, Nilson EP. The effect of tolmetamide in lipoproteins and lipoprotein lipase and hormone sensitive lipase. *Diabetes Res. Clin. Pract*. 1999;46: 99-108.
42. Frayn KN. Insulin resistance and lipid metabolism. *Curr. Opin. Lipidol*. 1993;4: 197-204.
43. Bopanna KN, Kannan J, Suchma G, Balaraman R, Ranthod SP. Antidiabetic and antihyperlipidemic effect of neem seed, kernel powder on alloxan diabetic rabbits. *Ind. J. Pharmacol*. 1997;29:162-167..
44. Rotimi OS, David AO, Olusola AT, Regina NU, Elizabeth AB, Oladipo A. Amoxillin and pefloxacin-induced cholesterogenesis and phospholipidosis in rat tissues. *Lipids in Health and Disease*. 2015;14:13-30.
45. Bhattaram VA, Cercefe M, Cohlest C, Vest M, Deundo FH. Pharmacokinetics bioavailability herbal medicinal products. *Phytomedicine*. 2002;9:1-36.
46. Airaodion AI, Ogbuagu U, Ogbuagu EO, Airaodion EO, Agunbiade AP, Oloruntoba AP, Mokelu IP, Ekeh SC. Investigation of aqueous extract of *Zingiber officinale* root potential in the prevention of peptic ulcer in albino rats. *International Journal of Research and Innovation in Applied Science*. 2019;4(2):64-67.
47. Airaodion AI, Obajimi OO, Ezebuiro CN, Ogbuagu U, Agunbiade AP, Oloruntoba AP, Akinmolayan JD, Adeniji AR, Airaodion EO. Prophylactic efficacy of aqueous extract of *Curcuma longa*

- against indomethacin-induced ulcer. International Journal of Research. 2019;6(1):87-91.
48. Airaodion AI, Olayeri IM, Ewa AO, Ogbuagu EO, Ogbuagu U, Akinmolayan JD, Agunbiade AP, Oloruntoba AP, Airaodion EO, Adeniji AR, Obajimi OO, Awosanya OO. Evaluation of *Moringa oleifera* leaf potential in the prevention of peptic ulcer in wistar rats. International Journal of Research. 2019;6(2):579-584.
 49. Saravanan R, Pari L. Antihyperlipidemic and antiperoxidative effect of diasulin, a polyherbal formulation in alloxan induced hyperglycemic rats. BMC Complement. Alternative Med. 2005;5:14–34.
 50. Airaodion AI, Adekale OA, Airaodion EO, Ogbuagu EO, Uloaku Ogbuagu U, Osemwowa EU. Efficacy of Combined crude Extract of *Curcuma longa* and *Moringa oleifera* in the Prevention of Peptic Ulcer in Albino Rats. Asian Journal of Research in Medical and Pharmaceutical Sciences. 2019; 7(2):1-8.
 51. Shoback DG, Gardner D, Eds. "Chapter 17". Greenspan's Basic & Clinical Endocrinology (9th Ed.). New York: McGraw-Hill Medical; 2011. ISBN: 978-0-07-162243-1.
 52. Saliu JA, Obboh G, Schetinger MR, Stefanello N, Rocha JBT. Antidiabetic Potentials of Jute Leaf (*Vernonia amygdalina*) On Type-2 Diabetic Rats. Journal of Emerging Trends in Engineering and Applied Sciences (JETEAS). 2015;6(7):223- 230.
 53. Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. *Pharmacological Research*. 2005;52:313–320.
 54. Allen RR, Carson LA, Kwik-Urbe C, Evans E, Erdman JW. Daily consumption of a dark chocolate containing flavanols and added sterol esters affects cardiovascular risk factors in normotensive population with elevated cholesterol. J Nutr. 2008;138: 725-731.
 55. Greene CM, Zern TL, Wood RJ, Shrestha S, Fernandez ML. Maintenance of the LDL-cholesterol/HDL-cholesterol ratio in an elderly population given a dietary cholesterol challenge. J Nutr 2005; 135: 2793-2798.
 56. Perona JS, Covas MI, Fito M, Cabello-Moruno R, Aros F, Corella D, Ros E, Garcia M, Estruch R, Martinez-Gonzalez MA, Ruiz-Gutierrez V. Reduction in systemic and VLDL triacylglycerol concentration after a 3-month Mediterraneanstyle diet in high-cardiovascular-risk subjects. J Nutr Biochem. 2010; 9: 892-898.
 57. Gordon DJ, Rifkind BM. High-density lipoprotein: The clinical implications of recent studies. New England Journal of Medicine. 1989;321(19):1311-1316.
 58. Sviridiv D. Intracellular cholesterol trafficking. Histology and Histopathology. 1999;14:305-319.
 59. Navab M, Berliner JA, Watson AD, Hama SY, Territo MC, Lusis AJ, Shih DM, Van Lenten BJ, Frank JS, Demer LL, Edwards PA, Fogelman AM. The Yin and Yang of oxidation in the development of the fatty streak. A review based on the George Lyman Duff Memorial Lecture. Arteriosclerosis and Thrombosis in Vascular Biology. 1994;16:831-842.
 60. Oram JF, Lawn RM. ABCA1: The gatekeeper for eliminating excess tissue cholesterol. Journal Lipid Research. 2001;42:1173-1179.
 61. Law MR. Lowering heart disease risk with cholesterol reduction: Evidence from observational studies and clinical trials. European Heart Journal. 1999;1:S3-S8.
 62. Stein O, Stein Y. Atheroprotective mechanisms of HDL- Atherosclerosis. 1999;144:285-303.

63. Das DK. Cardioprotection with high density lipoproteins. Fact or friction? *Circulation Research*. 2003;92:258-260.
64. Cromwell WC, Otvos JD (2004). Low Density Lipoprotein Particle Number and Risk for Cardiovascular Disease. *Curr. Atheroscler. Rep.*, 6(8): 381-387.

UNDER PEER REVIEW