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Effect of Bambara Nut Consumption on Blood Glucose Level and Lipid Profile of Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author AIA designed the study and wrote the manuscript. Author AUM carried out the analyses of the study. Authors PNA and OCN managed the literature searches, Author NOO managed the statistical analysis while, author OJA wrote the protocol of the study. All authors read and approved the final manuscript

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ABSTRACT

Aim: This study sought to investigate the effect of Bambara nut consumption on blood glucose level and lipid profile of Wistar rats.

Methodology: The Songkhla 1 variety (red seed coat) of Bambara nuts were locally sourced from Obinze area of Owerri, Imo State, Nigeria. The seeds were peeled and ground to a fine powder using a coffee grinder and extracted using soxhlet apparatus and methanol as the solvent. Twenty-four adult male Wistar rats were acclimatized for seven days during which they were fed *ad libitum* with standard feed and drinking water. They were randomly divided into four groups of six rats each. Rats in group A were administered distilled water while those in groups B, C and D were administered 100, 200 and 400 mg/kg body weight of Bambara nut extract 12 hourly for twenty-one days *via* oral route of administration. At the end of 21 days of treatment, animals were sacrificed

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under diethyl ether as anaesthesia and blood samples were collected by cardiac puncture. Blood glucose level and lipid profile were determined using standard methods.
Results: Bambara nut was observed to reduce weight gain and blood glucose level of treated animals when compared with those in the control group. The lipid profile was favourably regulated when animals treated with Bambara nut were compared with those in the control group. The effect of Bambara nut on measured parameters was observed to be dose dependent.
Conclusion: The result of this study revealed that Bambara nut consumption is of significant health benefit as far as hyperglycemia and hyperlipidemia is concerned. It could also be exceedingly helpful in the control of diabetes, obesity and cardiovascular diseases arising from hyperglycemia and hyperlipidemia. This pharmacological study is a useful tool for further drug development from the natural plant products.

Keywords: Bambara nut; blood glucose level; cardiovascular diseases; diabetes; lipid profile.

1. INTRODUCTION

Diabetes mellitus is defined as a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism; resulting from defects in insulin secretion, insulin action, or both [1]. The effects of diabetes mellitus include long–term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as polyphagia, polyuria, blurring of vision, and weight loss [2]. Diabetic complications are linked to hyperglycemia induced oxidative stress, which eventually overcomes the endogenous antioxidant defense system within the human and animal body. Long term effect of diabetes mellitus has been reported to cause some chronic complications like high blood pressure, heart attack and stroke. Clinical and animal studies on type 2 diabetes have shown inverse correlation between oxidative stress and insulin sensitivity [3]. The management of diabetes mellitus includes strategies to reduce hyperglycemia; the strategies includes regular physical exercises, eating carefully controlled diet, taking medications as prescribed and losing excessive weight.

Plant foods and products which are used trado-medically are now receiving research attention to be able to ascertain and document their therapeutic abilities. In the management of diabetes, the therapeutic properties of these plants have been hinged on the abundance of phytochemicals like polyphenols which possess strong antioxidant properties [4]. Legumes are known to be rich in polyphenols [5]. Legumes abound in many parts of Africa, where they are traditionally major components of many food preparations; in African trado-medical practice, legumes are components of many preparations used for the management of diabetes [6]. Legumes which are well utilized in Nigeria include cowpea (popularly called beans), groundnut, and soybeans; however, many other legumes are still underutilized or unexploited. Bambara nut is a legume that is still underutilized.

Bambara nut (*Vigna subterranea*) is classified under the family *Leguminosae*, sub-family *Faboidea* and genus *Vigna*. It is a seed of Africa origin used locally as a vegetable and it was first found in West Africa [7]. Bambara nut is a crop with great potential to sustain the dietary needs of both urban and rural communities [6]. Its seed consist of 49.0 to 63.5% carbohydrate, 15.0 to 25% protein, 4.5 to 7.4% fat, 5.2 to 6.4% fibre, 3.2 to 4.4% ash and 2% mineral [7].

It might be surprising to say that most people in Nigeria may not be conversant with the name Bambara nut as the local name is commonly used but it forms most parts of some families' daily meal. Locally, it is called ‘Okpa’ in Igbo, ‘Epa-Roro’ in Yoruba, ‘Kwaruru’ or ‘Gurjiya’ in Hausa [8]. The traditional uses of Bambara nut to treat several ailments are noteworthy, and present a gap for detailed study on the therapeutic and pharmaceutical value of the crop [9]. Jideani and Diedrick [6] reported that the medicinal role of Bambara nut is mainly based on information obtained from communities in several parts of Africa where this crop is reportedly responsible and useful for treatment of various ailments. For example as a treatment for diarrhoea, a mixture of Bambara nut and water from boiled maize are consumed; to alleviate the nausea associated with pregnancy, Bambara nut seeds are chewed and swallowed by pregnant women. Other prophylactic and therapeutic use of Bambara nut includes use against protein deficiency kwashiorkor, treatment of veneral diseases, treatment of polymenorrhea (roasted Bambara nut seeds are used); treatment for internal bruising, treatment of cataracts (mixture of water and crushed Bambara nut seeds are used [10]. Recently, Megwas et al. [8] reported that Bambara nut ameliorated ethanol-induced oxidative stress in Wistar rats. This study is therefore aimed at evaluating the effect of Bambara nut on blood glucose level and lipid profile of Wistar rats.

2. MATERIALS AND METHODS

2.1 Collection and Extraction of Plant Material

Bambara nut, the Songkhla 1 variety (red seed coat) were locally sourced from Obinze area of Owerri, Imo State, Nigeria and were identified by a botanist. Immature and damaged seeds were removed. The seeds were peeled and ground to a fine powder using a coffee grinder and stored in screw-cap bottle at -20°C. The extraction was done using soxhlet apparatus and methanol as the solvent according to the methods described by Airaodion et al. [11,12]. About 25 g of the powder was packed into the thimble of the soxhlet extractor. 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 evaporate at 35 ^{o}C with a yield of 2.17 g which represents a percentage yield of 8.68%. The extract was preserved in the refrigerator until when needed.

2.2 Animal Treatment

Twenty-four (24) adult male Wistar rats with body weight between 140 and 160 g were used for the experiment. 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 four (4) groups of six (6) rats each. Animals in group A were administered distilled water while those in groups B, C and D were administered 100, 200 and 400 mg/kg body weight of Bambara nut extract for twenty-one (21) days, 12 hourly via oral route of administration. At the end of 21 days of treatment, animals were anaesthetized using diethyl ether and were sacrificed and blood samples were collected *via* cardiac puncture.

2.3 Determination of Blood Glucose Concentration

Blood glucose concentration was determined according to the method described by Airaodion et al. [13] and Ogbuagu et al. [14] using glucose oxidase with the aid of a glucometer (Accu-chek active) .

2.4 Determination of Lipids

Lipids were extracted and determined according to previously described methods [15,16].

2.5 Statistical Analysis

Results are expressed as mean ± standard deviation. The levels of homogeneity among the groups were assessed using One-way Analysis of Variance (ANOVA) followed by Tukey's test. All analyses were done using Graph Pad Prism Software Version 5.00 and P values < 0.05 were considered statistically significant.

3. RESULTS

Bambara nut was observed to reduce weight gain and blood glucose level of treated animals when compared with those in the control group (Figs. 2 and 3 respectively). The lipid profile was favourably regulated when animals treated with Bambara nut were compared with those in the control group as presented in Figs. 4-9.

4. DISCUSSION

The growing number of diabetic cases 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. The effect of Bambara nut on weight gain of rats used in this study is presented in Fig. 2. The results suggest that administration of Bambara nut significantly decreased weight gain of Wistar rats when compared with those of the control group at P<0.05. Significant changes in body weights have been used as an indicator of effects of drugs and chemicals. Nevertheless, the growth of an organism comprises many factors including physiological, biological and cellular processes [17].

Fig. 1. Bambara nut

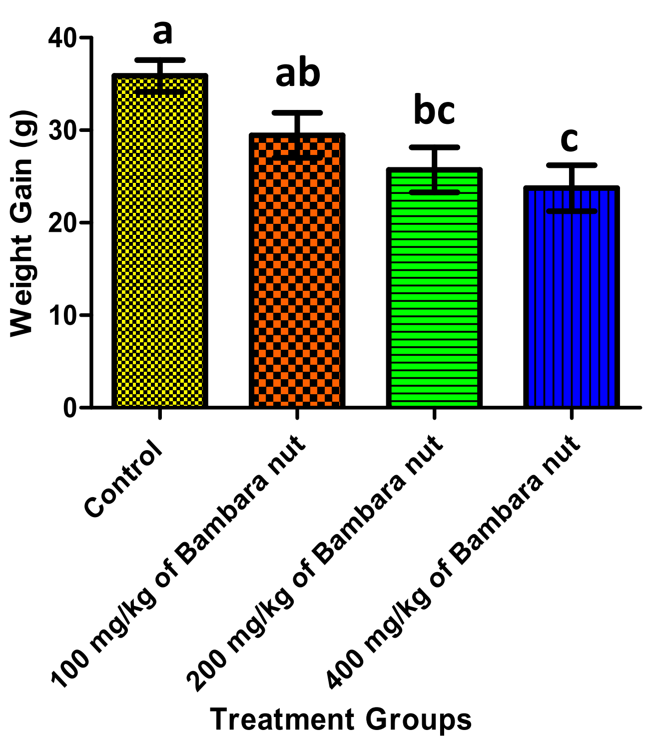

Fig. 2. Effect of Bambara nut on Weight Gain by animals after 21 days of Administration
Results are presented as mean ± standard deviation with n = 6. Bars with different letters are significantly different at P<0.05

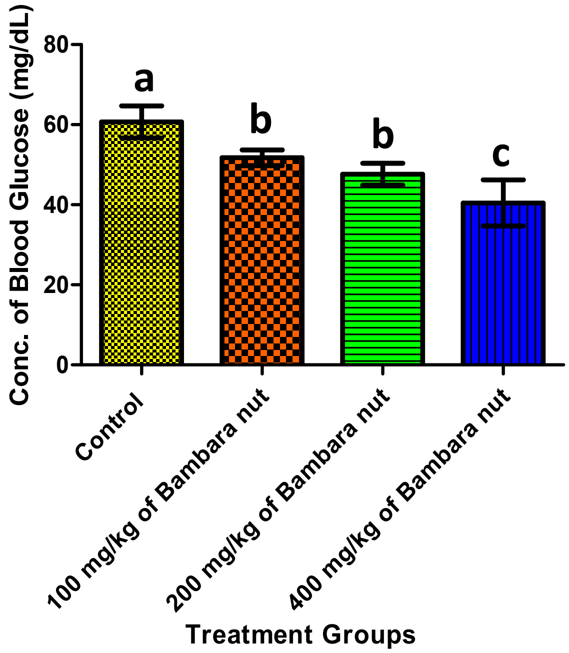

Fig. 3. Effect of Bambara nut on the concentration of Blood Glucose Level of animals after 21 days of administration

Results are presented as mean ± standard deviation with n = 6. Bars with different letters are significantly different at P<0.05

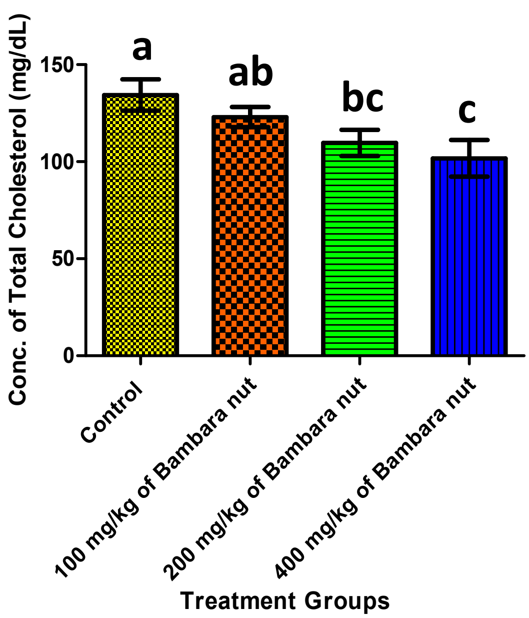

Fig. 4. Effect of Bambara nut on the concentration of Total Cholesterol of animals after 21 days of administration

Results are presented as mean ± standard deviation with n = 6. Bars with different letters are significantly different at P<0.05

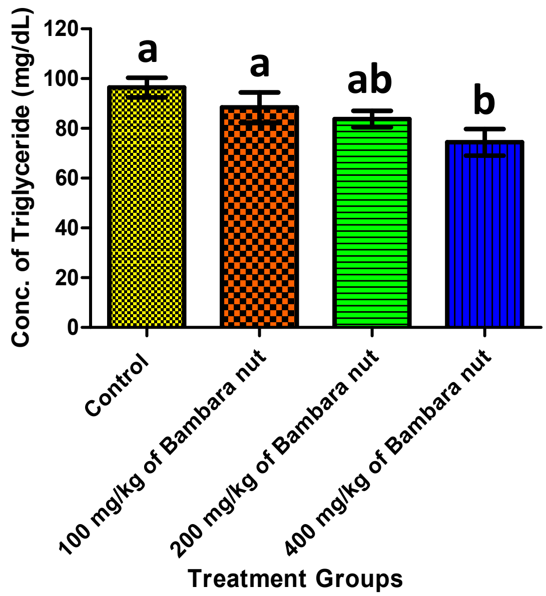

Fig. 5. Effect of Bambara nut on the concentration of Triglyceride of animals after 21 days of administration

Results are presented as mean ± standard deviation with n = 6. Bars with different letters are significantly different at P<0.05

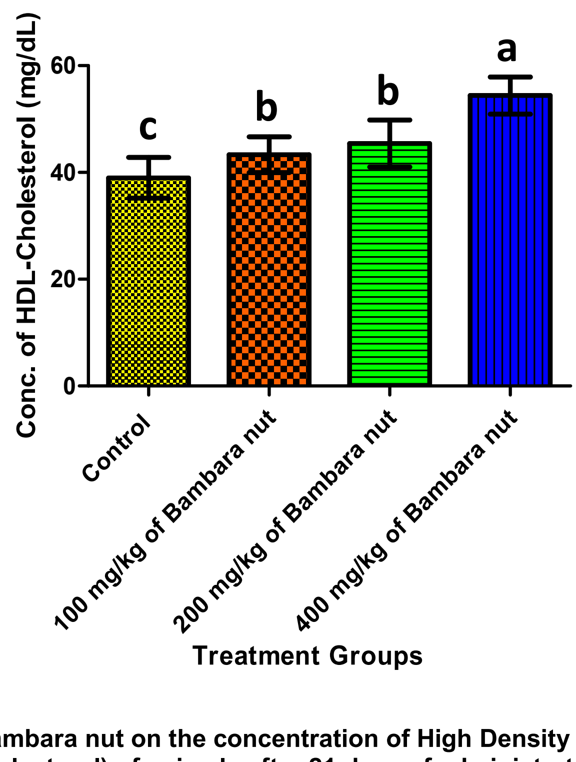

Fig. 6. Effect of Bambara nut on the concentration of High Density Lipoprotein (HDL-Cholesterol) of animals after 21 days of administration

Results are presented as mean ± standard deviation with n = 6. Bars with different letters are significantly different at P<0.05

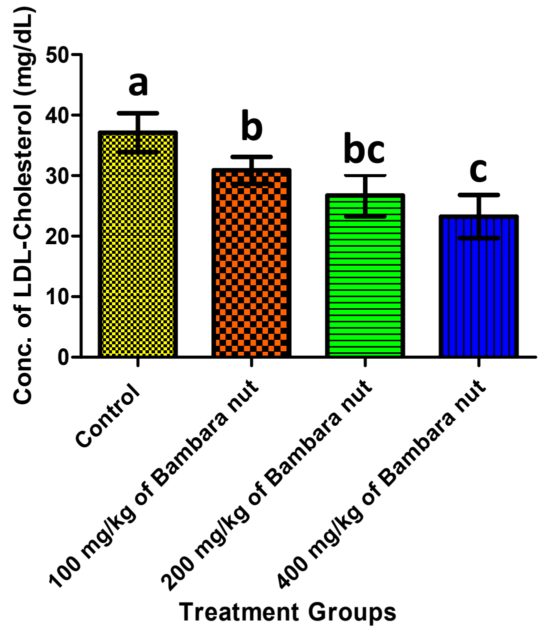

Fig. 7. Effect of Bambara nut on the concentration of Low Density Lipoprotein (LDL-cholesterol) of animals after 21 days of administration

Results are presented as mean ± standard deviation with n = 6. Bars with different letters are significantly different at P<0.05

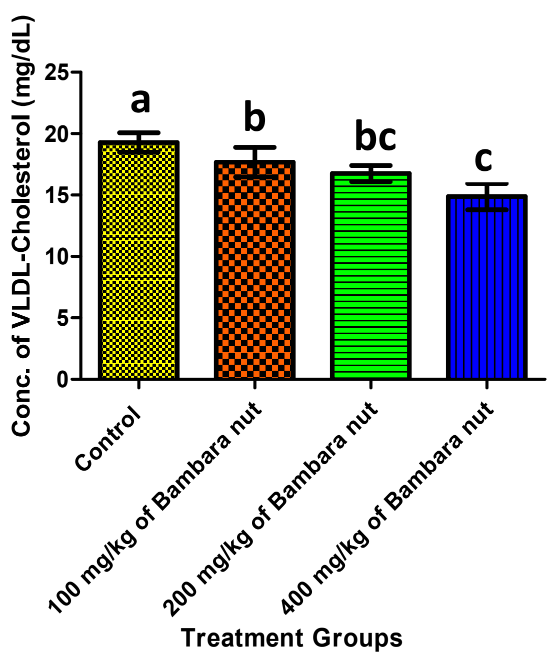

Fig. 8. Effect of Bambara nut on the concentration of Very Low Density Lipoprotein (VLDL-cholesterol) of animals after 21 days of administration

Results are presented as mean ± standard deviation with n = 6. Bars with different letters are significantly different at P<0.05

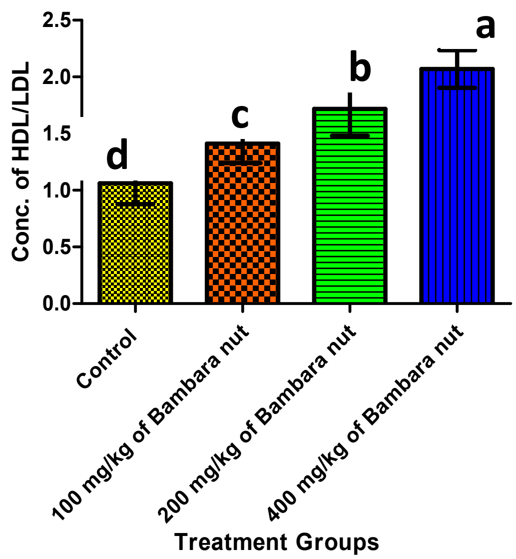

Fig. 9. Effect of Bambara nut on the concentration of HDL/LDL ratio of animals after 21 days of administration

Results are presented as mean ± standard deviation with n = 6. Bars with different letters are significantly different at P<0.05

The results of this study revealed that Bambara nut extract caused a dose dependent decrease in the blood glucose level of treated rats when compared with those of the control group as presented in Fig. 3. This might be suggestive that Bambara nut possesses hypoglycemic property. The mechanism could be that the nut decreases gluconeogenesis by decreasing the activities of key enzymes such as glucose-6-phosphatase, fructose-1,6-bisphosphate phosphoenol pyruvate carboxykinase [18]. Glucose-6-phosphatase is an important enzyme in homeostasis of blood glucose as it catalyzes the terminal step both in gluconeogenesis and glycogenolysis, while fructose-1,6-bisphosphatase is one of the key enzymes of gluconeogenic pathway [19,20]. Hence, the ability of Bambara nut to decrease the activities of these enzymes probably makes it potential hypoglycemic agent. This result is similar to the hypoglycemic effect of Bambara nut reported by Olanipekun et al. [21]. A number of other plant extracts have also been reported to have a hypoglycemic properties and an insulin-stimulatory effect [22,23]. Most of the plants with hypoglycemic properties have been found to contain metabolites such as glycosides, alkaloid and flavonoids [24,25]. Phytochemical analysis of Bambara nut has been shown to contain flavonoids, alkaloids, anthrocyanins, coumarin, oxalate, saponins, steroid, glycoside, tannin and phenolic compounds [26]. It has also been proven to possess remarkable free radical scavenging ability [21,27]. In fact, Megwas et al. [8] reported that Bambara nut ameliorated the effect of ethanol-induced oxidative stress in Wistar rats. Some of these chemical substances such as flavonoids, alkaloids and glycosides may be responsible for the hypoglycemic effect of Bambara nut observed in this study. The blood glucose lowering effect of Bambara nut may also indicate that it possesses an antidiabetic agent which could control hyperglycemia. One therapeutic approach for treating early stage of diabetes is to decrease post-prandial hyperglycemia. 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 [28]. Consequently, inhibitors of these enzymes determine a reduction in the rate of glucose absorption and consequently blunting the post-prandial plasma glucose rise [29]. Based on these findings, it could be suggested that Bambara nut 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 [30,31].

Beside the regulation of carbohydrate metabolism, insulin plays an integral role in the metabolism of lipid. Insulin deficiency, as in diabetes mellitus, is associated with hypercholesterolemia and hypertriglyceridemia, which have been reported to occur in experimental diabetic rats [29]. Hypercholesterolemia could result in a relative molecular ordering of the residual phospholipids, resulting in a decrease in membrane fluidity [30]. 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 [24].

In this study, total cholesterol and triglycerides concentrations were observed to decrease significantly when animals treated with Bambara nut extract were compared with those of the control group at P<0.05 (Figs. 4 and 5). This could suggest that Bambara nut possess the propensity to prevent the progression of Coronary Heart Disease (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 [25]. 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 [32,33]. 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 [34]. Hypertriglyceridaemia has been reported in diabetic animals [35]. This was reported to be due to 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 [15,16]. Hypercholesterolaemia has also been reported in diabetic animals [22].This was attributed to the increased dietary cholesterol absorption from the small intestine following the intake of high fat diet in a diabetic condition. However, the levels of serum triglyceride, VLDL-cholesterol and total cholesterol were significantly reduced in animals treated with Bambara nut when compared with those in animals of the control group. Moreover, it can be conjectured that the lipid lowering effects of Bambara nut could be due to the inhibition of hepatic cholesterol, triglyceride and possibly fatty acid synthesis by the phenolic constituents of Bambara nut reported by Nwadi et al. [26].

Hypertriglyceridaemia has also been reported to be a predictor of hypertension risk [23]. 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 [19]. This probably explains why Bambara nut 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 [29].

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 [29]. 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 [16,25].

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 [30,31]. 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. 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 [23,25].

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 [15]. 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 [36]. 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 [30].

Excitingly, administration of Bambara nut for 21 days in this present study resulted in a significant increase in the serum level of HDL-cholesterol when compared with the control animals at $P<0.05$ as presented in Fig. 6. HDL-cholesterol is usually referred to as the 'good cholesterol' [28]. Conversely, Bambara nut administration significantly decreased the concentration of LDL-cholesterol (bad cholesterol) when compared with that of animals in the control group at $P<0.05$ (Fig. 7).The combined effect of increased HDL-cholesterol (good cholesterol) and decreased LDL-cholesterol (bad cholesterol) in the present study resulted in an increased HDL-cholesterol/LDL-cholesterol ratio in animals treated with Bambara nut when compared with the control group at $P<0.05$ (Fig. 9). This strongly supports the notion that dietary supplementation with the extract of some medicinal plants may lead to a reduction in the risk of developing heart diseases, because a high HDL-cholesterol/LDL-cholesterol ratio has been shown to be beneficial and is suggestive of a lower risk of CHD [37]. In fact, the mechanism leading to lipid alterations in Bambara nut-treated rats could be that there is reduction in stimulation of sympathetic adrenal system leading to decreased secretion of catecholamine resulting in decreased concentration of plasma free fatty acids which might further result in decreased secretion of hepatic free fatty acids [38]. Hence, the decreased level of total cholesterol, triglyceride and LDL-cholesterol. The alterations in lipids observed in this study might also be mediated via the speculated Bambara nut-induced inhibition of dietary lipid absorption in the gastrointestinal tract, which is thought to be achieved via the reduction in the bile salts which are required for cholesterol absorption in the small intestine.

5. CONCLUSION

The result of this study revealed that Bambara nut consumption is of significant health benefit as far as hyperglycemia and hyperlipidemia is concerned. It could also be exceedingly helpful in the control of diabetes, obesity and cardiovascular diseases arising from hyperglycemia and hyperlipidemia. This pharmacological study is a useful tool for further drug development from the natural plant products.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal ethic Committee approval has been collected and preserved by the author.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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