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Biochemical evaluation of the effects of Nigerian polyherbal preparation on Wistar rabbits

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ABSTRACT

Background: Diabetes mellitus is a metabolic disorder with multiple etiologies. Its sufferers are generally at high risk of dyslipidemia characterized by hypercholesterolemia, hyper-triglyceridemia, hyperlipoproteinemia and low levels of high-density lipoprotein cholesterol. Globally, the estimated cost of diabetes care was $376 billion in 2010, representing 12% of health expenditures for that year.

Methods: The effects of the aqueous extracts of a little known Nigerian traditional polyherbal formula consisting of *Emilia coccinea*, *Acanthus montanus*, *Hibiscus rosasinensis* and *Asystasia gangetica* on serum glucose concentration, amylase activity and lipid profiles of normal, diabetic, and liver-damaged rabbits were studied using standard procedures. The mixture of the aqueous extracts of the four plants was orally administered in two doses – 120mg/kg body weight and 240mg/kg body weight for 28 days.

Results: The drug elicited dose- and duration-of-administration-dependent, significant ($p<0.05$) reductions in serum levels of glucose, total cholesterol, triacylglycerol and LDL-cholesterol; and significant ($p<0.05$) increases in the HDL-cholesterol concentrations with no changes in amylase activity.

Conclusion: These results confirm the hypoglycemic, antihyperlipidemic and hepatoprotective potentials of the crude drug and thus justify its application in ethnomedicine in the management of diabetes.

Key words: *Emilia coccinea, Acanthus montanus, Hibiscus rosasinensis, Asystasia gangetica*, antidiabetic, hepatoprotection.

INTRODUCTION:

Diabetes mellitus is a metabolic disorder with multiple etiologies. Its sufferers are generally at high risk of dyslipidaemia characterized by hypercholesterolemia, hyper-triglyceridemia, hyperlipoproteinemia, (mostly low-density lipoproteins) and low levels of high-density lipoprotein cholesterol [1]. Globally, the estimated cost of diabetes care was $376 billion in 2010, representing 12% of health expenditures for that year. Many individuals with diabetes make use of functional foods, nutritional supplements, and/or herbal remedies to manage the disease [2]. For thousands of years prior to the advent of modern allopathic medicine, herbs and substances derived from plants have been the mainstay of traditional medicine around the world. Several herbs, for instance, have been reported to counter the peroxidative stress due to some stressors [3]. Despite the presence of registered orthodox antidiabetic drugs in the pharmaceutical market, remedies from medicinal plants have been used with success to manage this disease in Nigeria [4]. Even in the western world, many individuals with diabetes make use of functional foods, nutritional supplements, and/or herbal remedies to manage their disease [2]. In the present study, the spotlight is on *Ọgwụ mmamiri*, a polyherbal antidiabetic drug from South Eastern Nigeria. Its four plant components are *Emilia coccinea, Acanthus montanus*, *Hibiscus rosasinensis* and *Asystasia gangetica* which are all well known in the study area.

Emilia coccinea (Sims) G. D (*Compositae*) is called "Cupids shaving brush", or "Tassel flower" in English, and *Ọgbaewuabụba* or *Osiisi* by the Igbos of South Eastern Nigeria [5]. It is reportedly used in folkloric medicine for the treatment of tumor, inflammation, cough, rheumatism, fever, dysentery, and wounds. The juice of the edible leaves is reportedly used in treating eye inflammations, night blindness, and earches. The root is used in the treatment of diarrhea [6,7].

Acanthus montanus, commonly known in English as "mountain thistle" or "devil's fig", is a perennial plant that belongs to the *Acanthaceae* family. It is known as *Ogwuduburu-Ọkụkọ*, or *Agamebu*, among the Igbos of South Eastern Nigeria. The plant extract is reported to be diuretic, purgative and anthelmintic. It is also reported to be analgesic, antispasmodic, hallucinogenic and useful for the treatment of leprosy, inflammation, bilious fever, warts, cold, skin diseases, cataracts, dropsy and jaundice [6,8] and is also found to be useful in soap making, as a lubricant, and for protection from termites [9].

Hibiscus rosasinensis (*Malvaceae)* is called "Chinese hibiscus", "Rose of China", "Hawaiian rose", or "Hawaiian hibiscus" in English and "habiskus" in Igbo. The flowers are reportedly used for the treatment of excessive and painful menstruation, cystitis, venereal diseases, feverish conditions, bronchial catarrh, coughs, carbuncles, mumps and sores, and to promote hair growth. Juice from the petals is used in China as a shoe-blacking and mascara agent [6,7,10].

Asystasia gangetica (*Acanthaceae*) is known as "creeping foxglove" or "Chinese violet" in English, "isihobo" in Zulu, and "*Añara ọhia"* in Igbo [11]. *Asystasia* means 'inconsistency' and relates to the fact that the corolla is more or less regular; an unusual phenomenon in the *Acanthaceae* family. *Gangetica*, on the other hand, is derived from the Ganges River in India

where the species is presumed to have originated from. The leaves, which are eaten as spinach by local people, are important sources of micronutrients, vitamins, and minerals. The young leaves and shoots, whether raw or cooked as a pot herb, are often added to soups [11, 12].

The major aims of this work are tripartite: initially, to test the potential of the combined aqueous extracts of these plants to reverse hyperglycemia, as claimed by traditional practitioners. In addition, the research is aimed at assessing the possible use of these extracts to reverse dyslipidemia, which is usually associated with diabetes. Finally, the work is also intended to study the effects of the extract on the pancreas (using amylase activity) as well as on the normal and abnormal liver. The ability of this drug to achieve any or all of these objectives may precipitate testing of its use in other applications such as management of other metabolic diseases, and even management of fatigue since these conditions are all associated with oxidative stress.

MATERIALS AND METHODS:

Collection and Preparation of Plant Materials: Sample collection and preparation followed standard botanical field collection methodology [13, 14]. Apparently healthy leaves of *E. coccinea, A. montanus, H. rosasinensis*, and *A. gangetica*, were harvested from their normal farmland habitats in Owerri, Nigeria. The leaves were authenticated at the Department of Forestry and Wildlife Technology, Federal University of Technology, Owerri, Nigeria. They were then washed under running tap water, kept out to drain, and dried overnight to a constant weight in an oven at 60^0C. Twenty grams (20g) of each set of dried leaves were ground into a fine powder and stored at 4^0C in a labeled, air-tight, glass container. A 5g portion of each sample powder was extracted overnight at room temperature with 100ml of distilled water. Each extract was then filtered through Whatmann No.1 filter paper. Next, the filtrates from the four herbs were mixed together and concentrated to 200ml on a water bath at 100^oC. The crude drug was then stored in a labeled air-tight container and preserved in a refrigerator at 4^0C after which, it solidified [14].

Experimental Animals and Induction of Diabetes and Liver Damage: Thirty-six weaned male Wistar rabbits, aged 6 weeks and weighing between 650-750g, were kept in laboratory cages for one-week to acclimate. The animals were maintained on a standard pellet diet (Bendel Feed and Flour Mill Ltd, Nigeria) and given water *ad libitum.* Diabetes was induced in 12 rabbits by a single intraperitoneal injection of 150mg alloxan monohydrate (Sigma-Aldrich, U.S.A) per kg body weight, while liver damage was induced in another 12 rabbits by an oral administration of 50mg/kg body weight of trichloromethane (chlorofrom) [15, 16]. Treatment began four days after confirmation of diabetic status and liver damage. Diabetes was confirmed by retrieving an elevated fasting blood glucose of > 7.78mmol/l, while liver damage was confirmed by a combination of abnormal serum activities of alanine aminotransferase (>108.40U/l), and aspartate aminotransferase (>110.10U/l), in combination with high bilirubin concentrations (>12.65µmol/l) [17, 18]. All the rabbits were divided into 9 groups (I-IX) of 4 rabbits each and arranged as follows:

Group I: Normal (control) rabbits without any disease induced and no crude drug given.
Group II: Normal rabbits administered 120mg crude drug per kg body weight.

Group III: Normal rabbits administered 240mg crude drug per kg body weight.
Group IV: Diabetic (control) rabbits without any administration of crude drug.
Group V: Diabetic rabbits administered 120mg crude drug per kg body weight.
Group VI: Diabetic rabbits administered 240mg crude drug per kg body weight.
Group VII: Liver-damaged (control) rabbits without any administration of crude drug.
Group VIII: Liver-damaged rabbits administered 120mg crude drug per kg body weight.
Group IX: Liver-damaged rabbits administered 240mg crude drug per kg body weight.
The animals were administered their respective group drug treatments for 28 days by oral compulsion using sterilized disposable 5ml syringes without needles.

Sample Collection and Determination of Biochemical Parameters: Fasting blood samples were drawn (3ml) from the animals through their ear veins on days 0, 7, 14, 21, and 28 into labeled plain containers and allowed to clot. The clotted and retracted blood samples were then centrifuged at 3000rpm. Supernatant sera samples were analyzed using microliter Human 80 Automatic Chemistry Machine (Human, Germany).

Serum glucose levels were determined by glucose oxidase method [19]. Amyloclastic method was used to determine the serum amylase activity [20]. Serum total cholesterol was determined according to the method of Richmond [21]. The quantitative method of Bucalo and David [22] was used to determine the triacylglycerol concentrations of the experimental animals. HDL-cholesterol concentrations of the sera samples were determined by Grove's method [23]. LDL-cholesterol was calculated using the formula of Friedewald *et al* [24].

Statistical Analysis: The data generated were analyzed using ANOVA and Duncan's New Multiple Range Tests. Differences at $p<0.05$ were considered statistically significant.

RESULTS:

The administration of the drug caused a dose-dependent significant ($p<0.05$) reduction in blood glucose concentrations of both untreated (normal) and diabetic rabbits, thus confirming the hypoglycemic activity of the drug. When these animals' fasting blood glucose concentrations on the 28^{th} day were compared with their corresponding baseline values (i.e. zero day), the crude drugs (120mg and 240mg/kg b.wt.) exhibited higher hypoglycemic effects on the diabetic rabbits (35.2% and 44.8% respectively) relative to the normal rabbits (9.8% and 16.2% respectively) (Figure 1). Crude drug administrations to liver-damaged rabbits non-significantly ($p>0.05$) reduced the blood glucose concentration by 0.02% only at both 120mg and 240mg/kg treatments when compared with their respective baseline values (Figure 2). Meanwhile, liver-damage by administration of chloroform had minimal effect on blood glucose concentration (0.07%; $p>0.05$; Figure 2), unlike the administration of alloxan monohydrate, which caused a profound rise in serum glucose (76.3%; $p<0.05$; Figure 1).

The serum amylase activities of the normal and diabetic rabbits were not significantly ($p>0.05$) affected by the crude drug administrations at 120mg and 240mg/kg in comparison with their respective baseline values, and the values obtained for the control animals (Figure 3). Similarly, crude drug administration did not significantly ($p>0.05$) change the amylase activities of liver-damaged animals (Figure 4).

Administration of the drug at the two doses of 120mg and 240mg/kg significantly ($p<0.05$) reduced the serum total cholesterol concentrations of the normal (by 21.2% and 27.2%) and diabetic (by 19.6% and 28.3%) rabbits respectively, in relation to their respective baseline values (Figure 5). On the other hand, reductions of 6.6% and 16.6% in serum total cholesterol concentrations of liver-damaged rabbits were recorded at 120mg, and 240mg/kg, respectively ($p>0.05$), compared to values obtained for the animals at zero day (Figure 6).

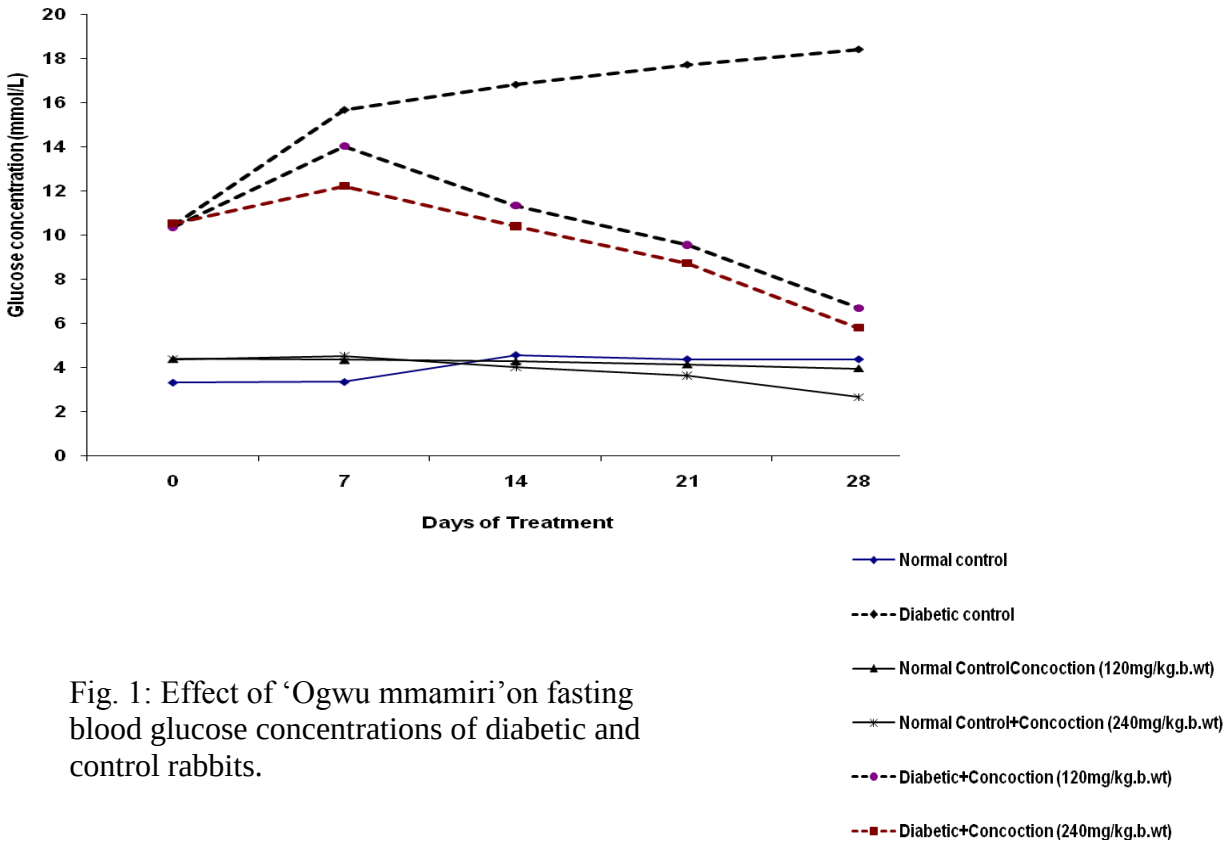

Fig. 1: Effect of 'Ogwu mmamiri'on fasting blood glucose concentrations of diabetic and control rabbits.

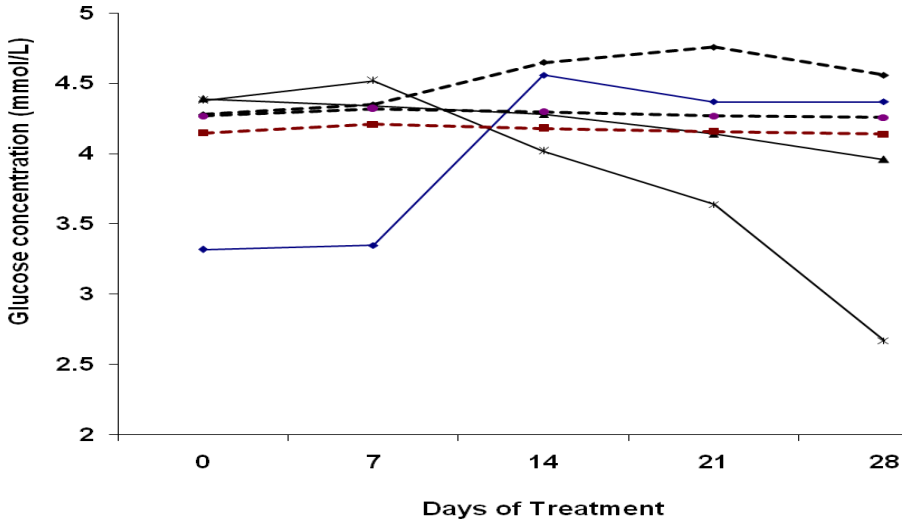

Fig. 2: Effect of 'Ogwu mmamiri'on fasting blood glucose concentrations of liver-damaged and control rabbits.

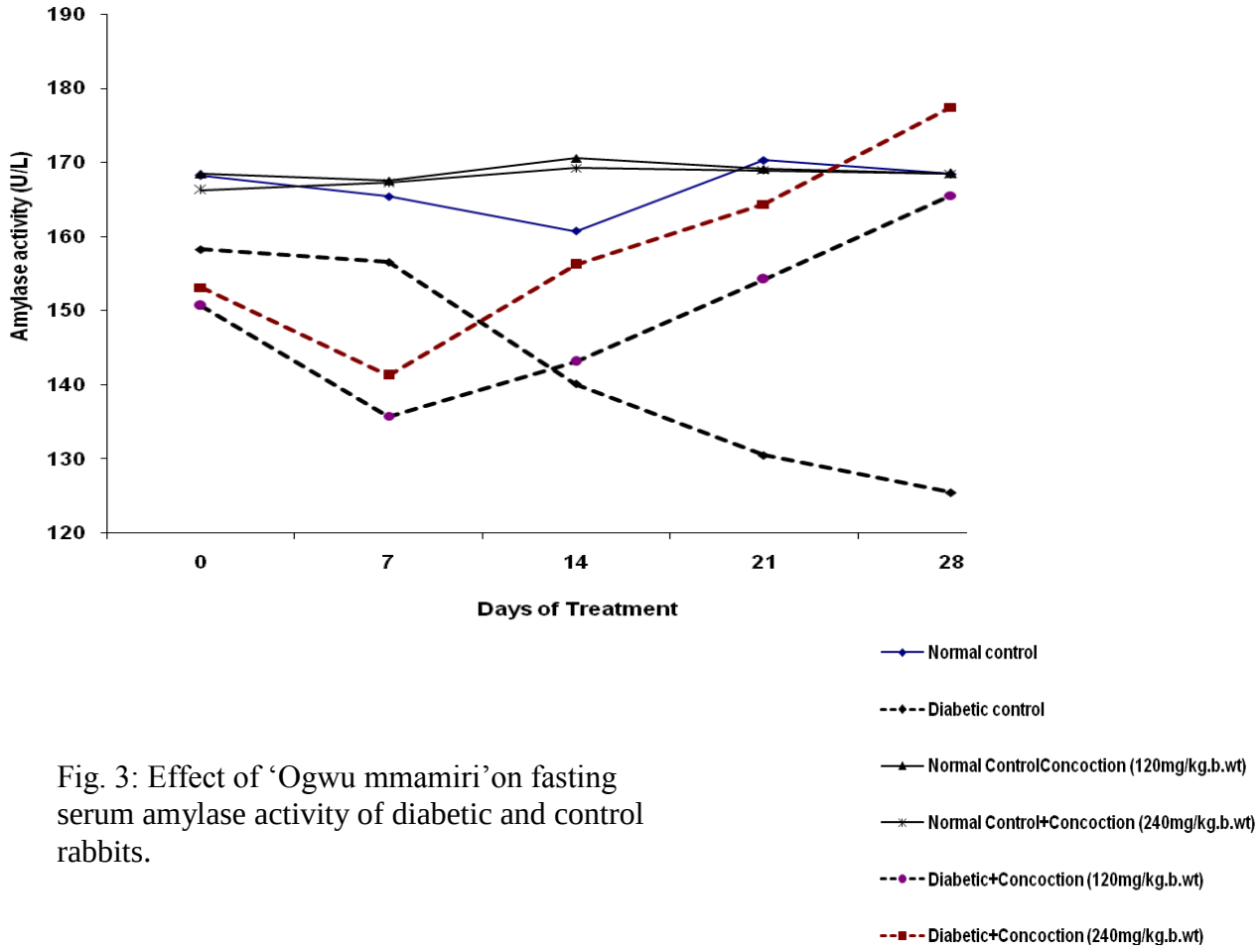

Fig. 3: Effect of 'Ogwu mmamiri'on fasting serum amylase activity of diabetic and control rabbits.

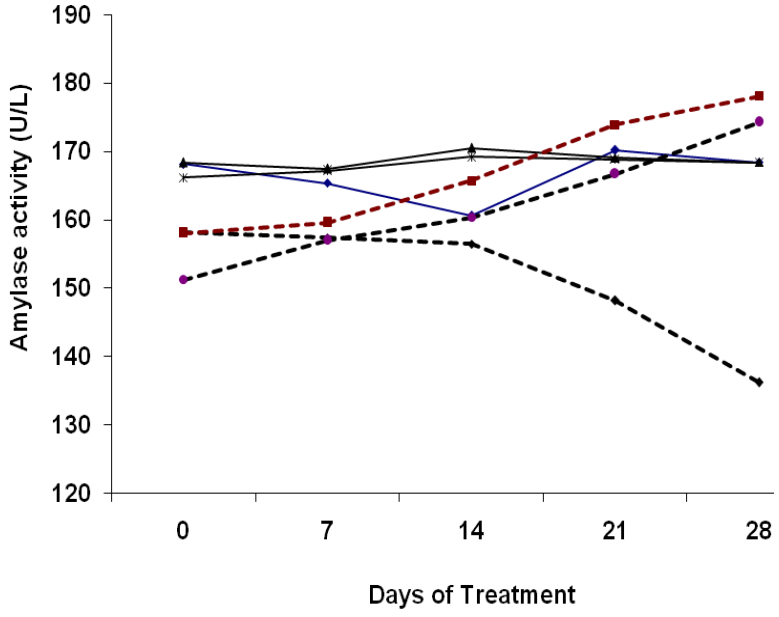

Fig. 4: Effect of 'Ogwu mmamiri'on fasting serum amylase activity of liver-damaged and control rabbits.

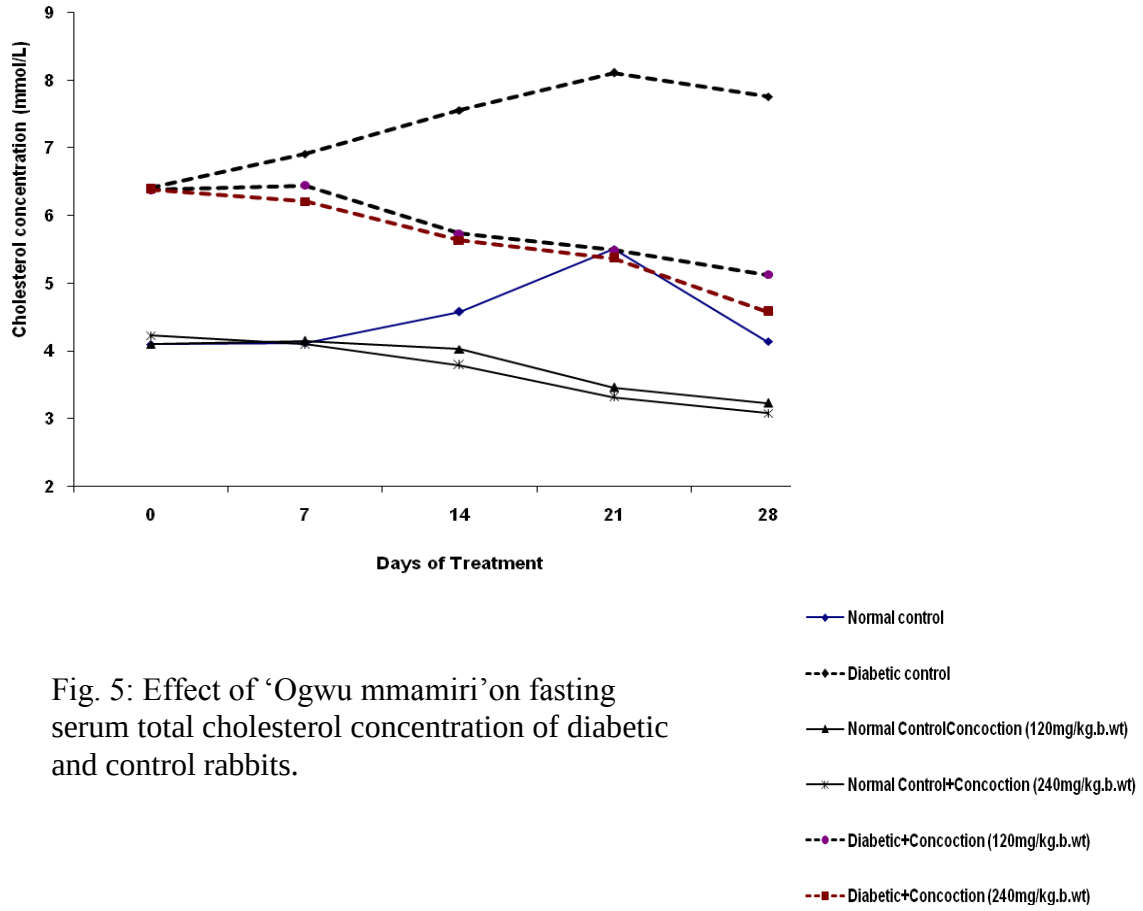

Fig. 5: Effect of 'Ogwu mmamiri'on fasting serum total cholesterol concentration of diabetic and control rabbits.

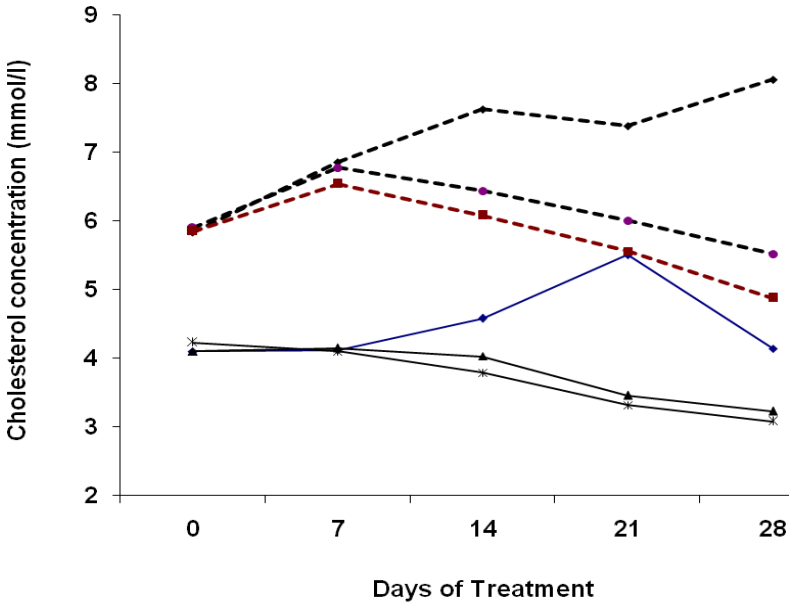

Fig. 6: Effect of 'Ogwu mmamiri'on fasting serum total cholesterol concentration of liver-damaged and control rabbits.

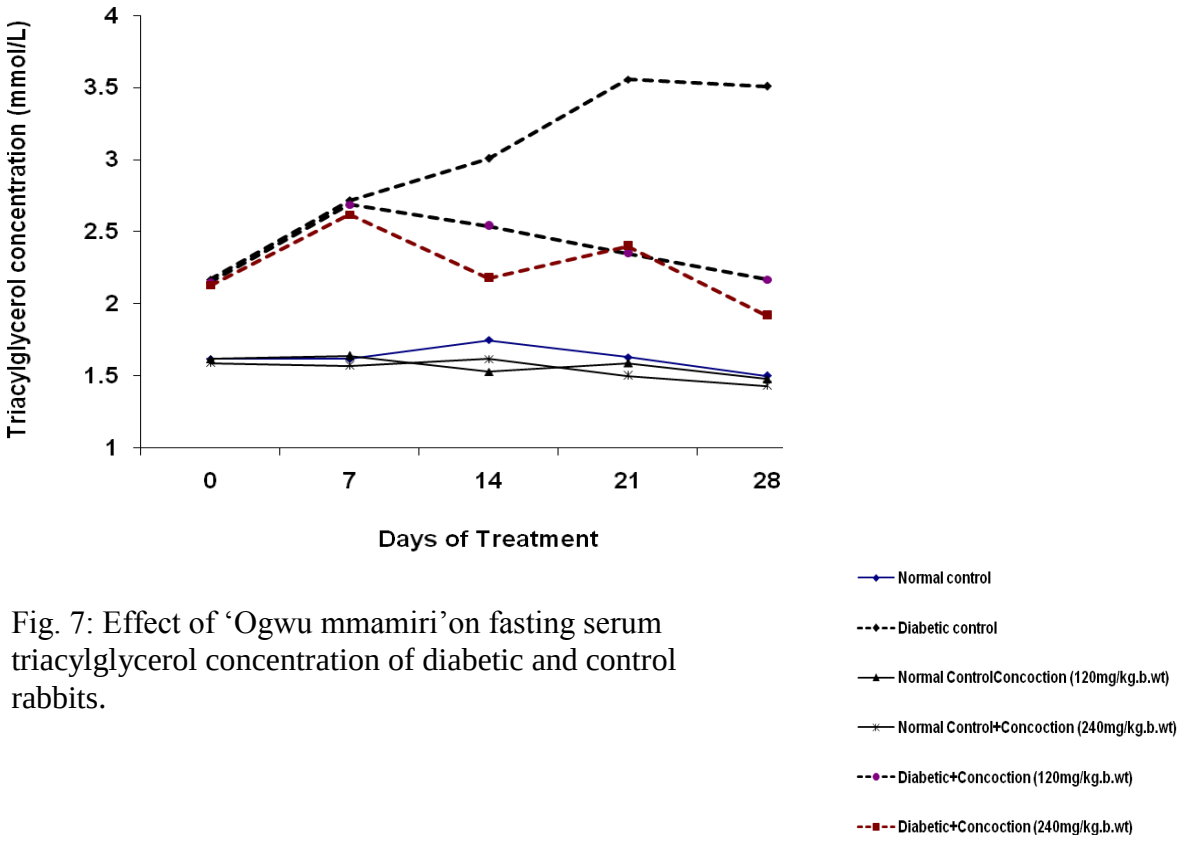

Fig. 7: Effect of 'Ogwu mmamiri'on fasting serum triacylglycerol concentration of diabetic and control rabbits.

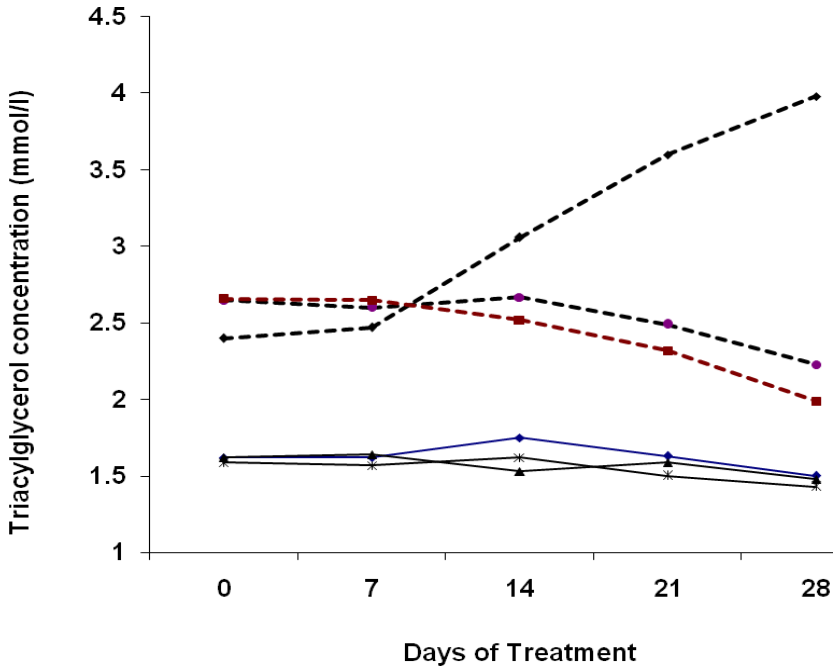

Fig. 8: Effect of 'Ogwu mmamiri'on fasting serum triacylglycerol concentration of liver-damaged and control rabbits.

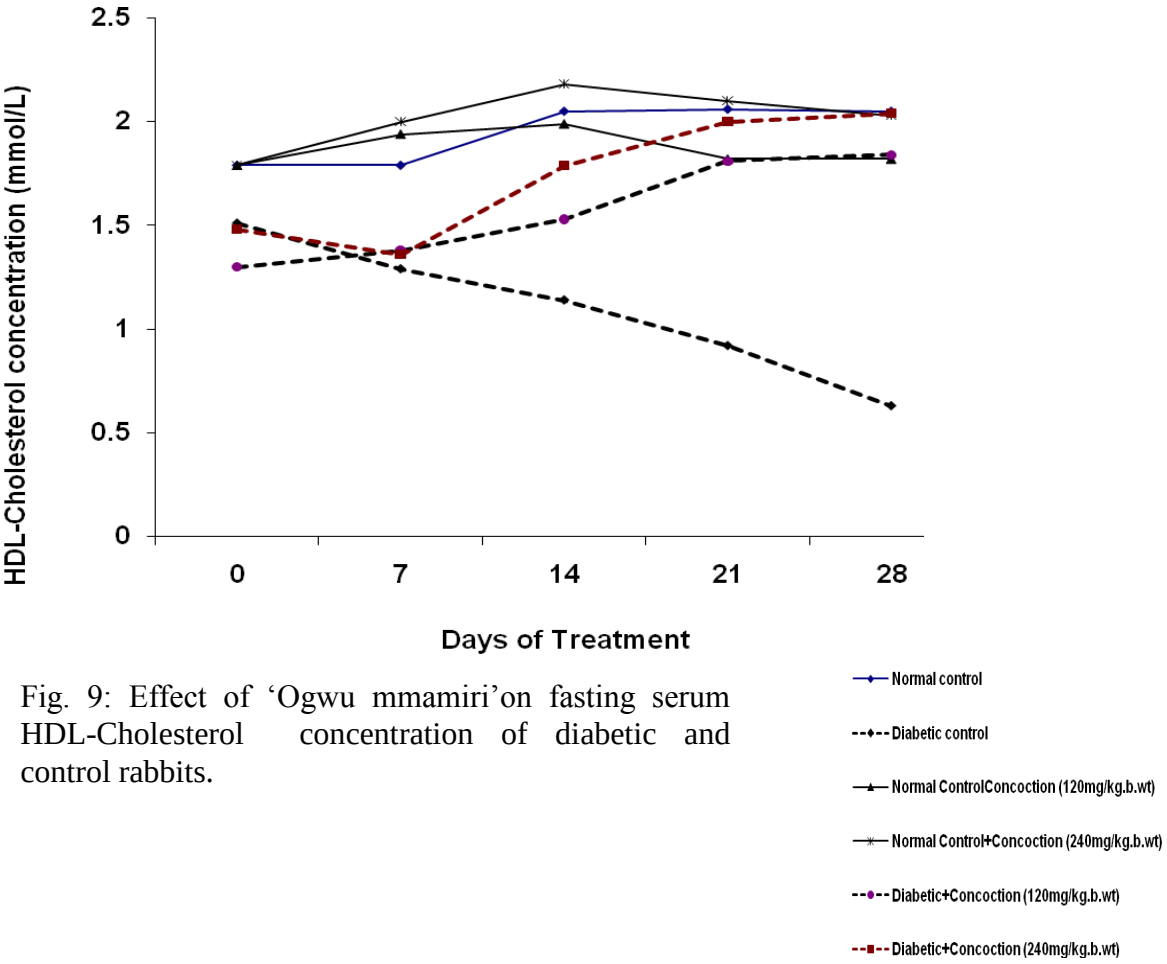

Fig. 9: Effect of 'Ogwu mmamiri'on fasting serum HDL-Cholesterol concentration of diabetic and control rabbits.

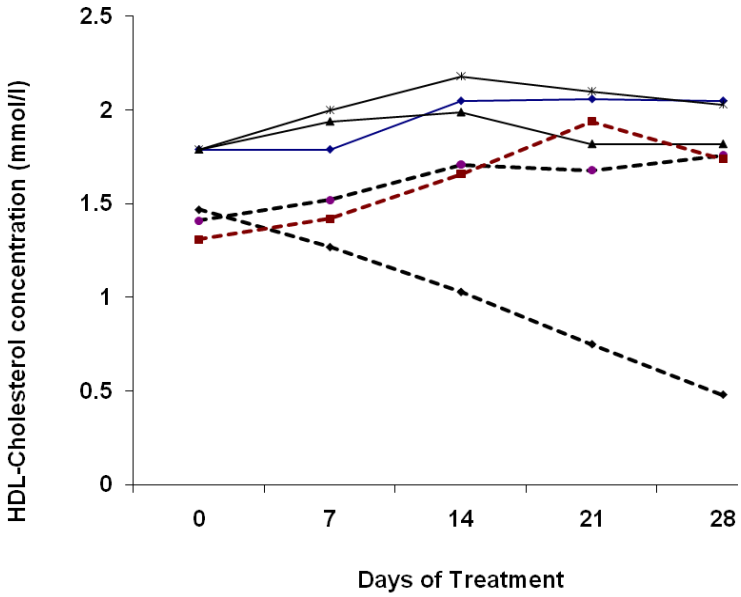

Fig. 10: Effect of 'Ogwu mmamiri'on fasting serum HDL-Cholesterol concentration of liver-damaged and control rabbits.

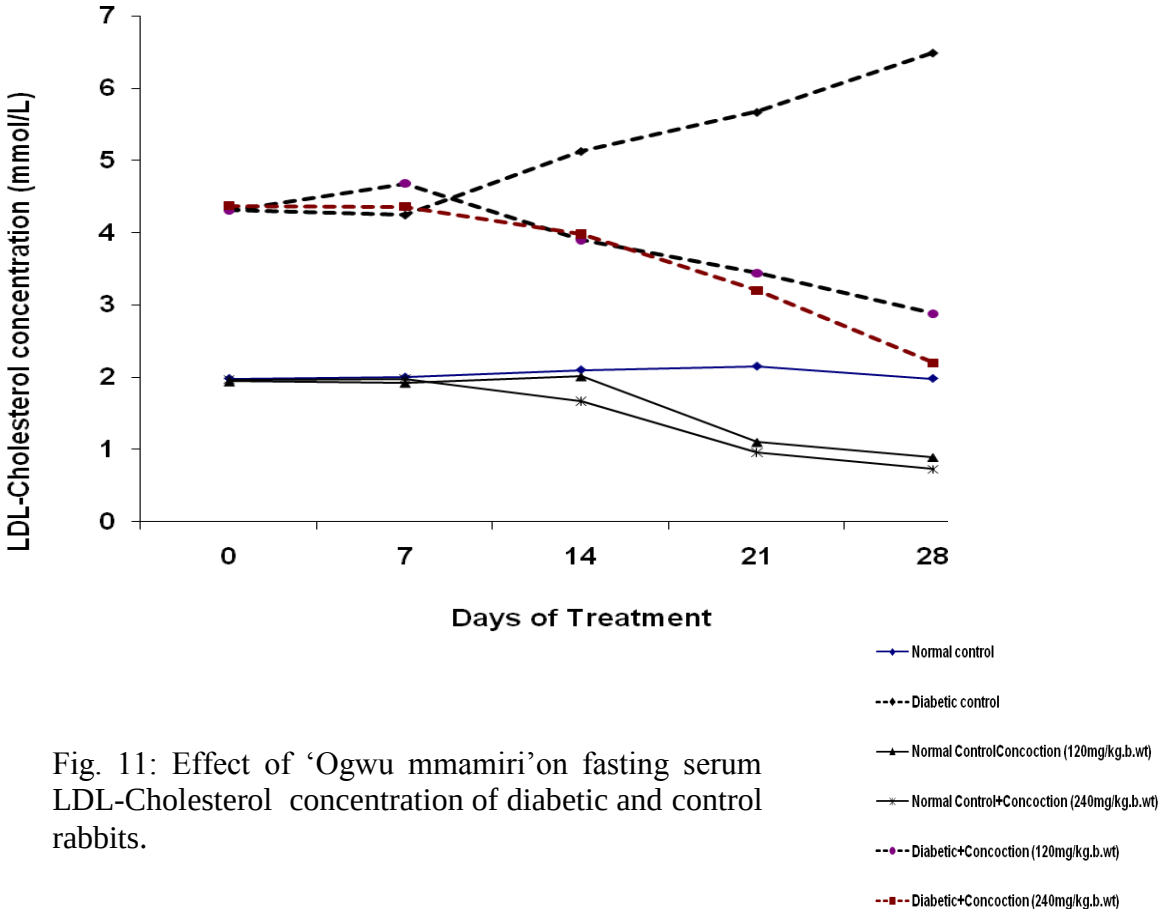

Fig. 11: Effect of 'Ogwu mmamiri'on fasting serum LDL-Cholesterol concentration of diabetic and control rabbits.

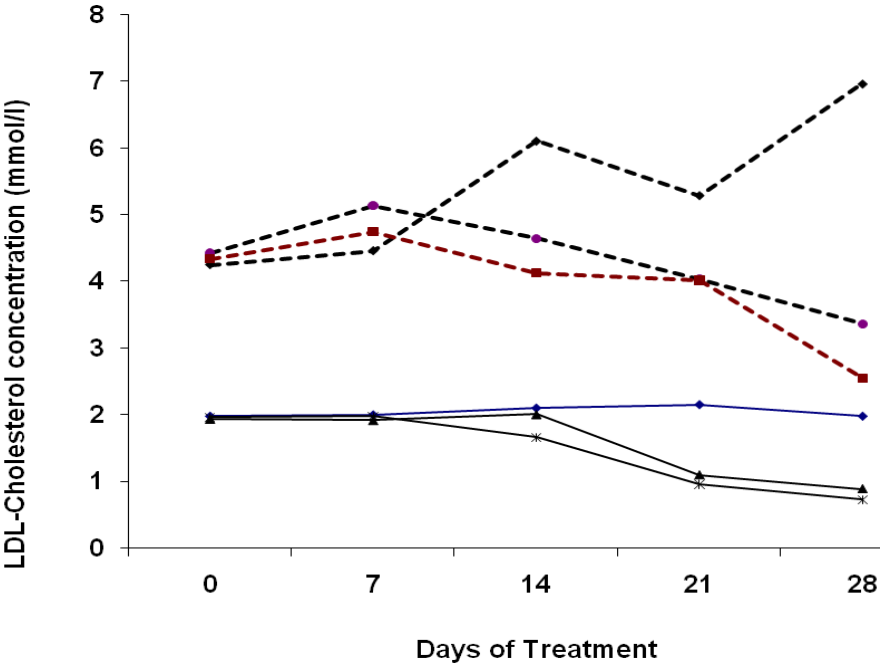

Fig. 12: Effect of 'Ogwu mmamiri'on fasting serum LDL-Cholesterol concentration of liver-damaged and control rabbits.

Figure 7 shows that the crude drug caused dose-dependent non-significant ($p>0.05$) reductions in serum triacylglycerol concentrations in both the normal (8.6% and 10.1% reductions respectively) and diabetic (0.9% and 9.9% reduction) rabbits when compared with their respective baseline values and even with those of the normal control animals. Similarly, reductions of 15.8% and 25.2% in serum triacylglycerol concentrations were recorded for liver-damaged rabbits at 120mg and 240mg/kg respectively, compared to their baseline values (Figure 8).

Figures 9 and 10 show that the induction of diabetes and liver damage caused significant ($p<0.05$) reductions (58.3% and 67.3% respectively) in serum HDL-cholesterol concentrations relative to the untreated animals. The reduction in HDL-cholesterol concentrations of diabetes-induced rabbits was, however, significantly ($p<0.05$) reversed by 41.5% and 37.8% margins upon the administration of the crude drug at 120mg and 240mg/kg respectively, using the animals' HDL-cholesterol concentrations on the zero day as baseline values (Figure 9). Similarly, the crude drugs significantly ($p<0.05$) increased the HDL-cholesterol concentrations of the liver- damaged animals by 24.8% and 32.8% respectively, relative to the baseline value (Figure 10).

The crude drug elicited dose-dependent significant ($p<0.05$) reductions in LDL-cholesterol concentrations of both the normal (54.1% and 62.9%) and diabetic (33.1% and 49.7%) animals at 120mg and 240mg/kg respectively (Figure 11). Similarly, significant ($p<0.05$) dose-dependent reductions of 23.9% and 41.2% in serum LDL-cholesterol concentrations were obtained for the liver-damaged rabbits that had been administered the two different doses of the crude drug (Figure 12). Meanwhile, induction of diabetes and liver-damage respectively caused 50.2% and 64.0% increases in LDL-cholesterol concentrations relative to the LDL-cholesterol levels of the normal control animals (Figures 11 and 12).

DISCUSSION:

Studies indicate that lowering triacylglycerol and/or lowering total cholesterol (TC) or low-density lipoprotein cholesterol (LDL-C) levels, or increasing high-density lipoprotein-cholesterol (HDL-C) levels may prevent, control, and even reverse adverse lipid metabolic outcomes. An increase in any of the parameters (except HDL-cholesterol) and a decrease in HDL-cholesterol is a significant and independent marker of possible coronary problems [25]. The actions exhibited by this polyherbal formula are in agreement with the foregoing. The drug reduced the LDL-C and increased the HDL-C levels significantly ($p<0.05$) in all experimental groups. The percentage reductions in the LDL-C (and percentage increases in HDL-C) by the drug were more pronounced in the diabetic animals than in the liver-damaged animals, even as the chloroform damage of the liver significantly ($p<0.05$) raised the levels of lipids (except HDL-C) in the chloroform-treated control group relative to the diabetic control group. HDL removes cholesterol from atheroma within arteries and transports the same back to the liver for excretion or re-utilization. Therefore, HDL-C protects against atherosclerosis and cardiovascular diseases [26]. Unlike HDL-C, LDL-C transports cholesterol through the arteries where it can be retained by arterial proteoglycans, thus initiating and sustaining plaque formation. LDL-C also increases the risk of cardiovascular disease when it invades the endothelium and becomes oxidized. LDL-C becomes atherogenic when it is modified by oxidative reaction. Since the oxidized form is easily

retained by the proteoglycans, increased levels of LDL-C has been associated with atherosclerosis, heart attack, stroke and peripheral vascular disease [27].

Chloroform, on its own, damages hepatocytes by forming trichloromethyl radicals which bind to the tissue macromolecules and induce peroxidative degradation of polyunsaturated fatty acids in membrane lipids of liver cells [28]. The drug therefore has desirable health outcomes, not only in increasing HDL-C and decreasing LDL-C, but also in being able to ameliorate hepatotoxicity induced by chloroform. Similar reports have been made for some other Nigerian crude drugs [14, 29].

The drug also significantly ($p<0.05$) reduced blood glucose concentrations in both normal and alloxan-induced diabetic animals, but not in the liver-damaged animals. This suggests that the crude drug may be acting on the pancreas with possible enhancement of insulin production and/or release. Alloxan monohydrate induces "chemical diabetes" in a wide variety of animal species by damaging the insulin-secreting pancreatic β-cells, resulting in decreased endogenous insulin release, which will in turn lead to decreased utilization of glucose by the tissues [30]**.** The drug significantly lowered the glucose level of the normal group, and also successfully countered the alloxan-induced hyperglycemia in the test groups. This further confirms its hypoglycemic activity and possible insulinotropic activity, a relationship that was reported by Igwe *et al*. [1]. The serum lipid-lowering effects of the drug may also be credited to this possible action on the pancreas since, apart from the regulation of carbohydrate metabolism, insulin is also known to play an important role in lipid metabolism. Specifically, it activates β-hydroxy-β-methylglutaryl-CoA reductase and lipoprotein lipase, enzymes required in the metabolism of cholesterol and triacylglycerol respectively [1].

Serum amylase activity was not significantly ($p>0.05$) different among the groups. This may be because alloxan is a cell-type specific toxin that damages only and specifically the β-cells of pancreas and even though amylase is synthesized and secreted mainly by the pancreas, the specific cells in the pancreas responsible for amylase synthesis may not have been affected by the action of alloxan. Furthermore, there is little to no known serum amylase activity contribution from the liver [31]. Therefore, the damaged or normal liver did not produce any significant difference in the activity of amylase, which is a known marker of pancreatic integrity [32].

CONCLUSION: the study showed that the crude drug is hypoglycemic, has beneficial health outcomes with regards to lipid parameters. It is not toxic to the pancreas and is even apparently hepatoprotective, considering its protective effects on normal liver and its reversal of liver damage as seen from liver function test parameters. The use of the crude drug '*Ọgwụ mmamiri'* (meaning 'drug to control urination') in South Eastern Nigerian ethnomedicine for the management and treatment of '*Ọria mmamiri'* (urine disease, i.e., diabetes) is based on ethnopharmacological experience. Its application in the management of diabetes is therefore scientifically sound and compares with findings for other functional foods [2] in diabetic mice, a model for human Type 2 diabetes mellitus.

Author's disclosure statement: We wish to confirm that no competing financial or non-financial interests exist between the Authors and any other people or institution.

Contribution of individual authors: KCA – Initiated and sponsored the research work.
OAO – Designed the research work and wrote up the manuscript. CUI – Partook in the research work analyzed the data and edited the manuscript.
WAN & CAO – Carried out the laboratory analyses.

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