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Biochemical Study of the Effect of African Walnut (*Tetracarpidium conophorum*) Seed Oil on Normal and Alloxan-Induced Diabetic Wistar Rats

Samuel Esosa Uhunmwangho*, Celestina Oluwaseun Olafusi and Onoriode Oyiborhoro

Department of Biochemistry, University of Medical Sciences, Ondo State, Nigeria

Correspondence should be addressed to Samuel Esosa Uhunmwangho, Department of Biochemistry, Faculty of Basic Medical Sciences, University of Medical Sciences, Ondo City, Ondo State, Nigeria

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ABSTRACT

Diabetes mellitus is a chronic disorder that is not only assuming pandemic proportions worldwide but also poised to affect the developing countries of the world much more than their developed counterparts. Therefore, this study was conducted to investigate the effect of feeding African walnut seed oil on blood glucose, lipid profile, liver function and hepatopathology of alloxan induced rats. The oil from the seed was extracted with n-hexane soxhlet apparatus and characterized by gas chromatography. Twenty rats were divided into four groups of five rats each and fed for 4 weeks this includes: Group A- animals were fed with water and feed no toxicant (positive control); Group B- alloxan and standard drug Diabetmin; Group C- alloxan and seed oil extract and Group D- toxicant only (negative control). The rats in groups B, C and D received intraperitoneal dose of alloxan (150 mg/kg body weight) after 30 days of feeding. Group C rats were fed with diet containing 10% of extracted *Tetracarpidium conophorum* seed oil throughout the period of experiment. Results indicated that the seed oil extract reduced blood glucose level significantly for rats in group C when compared with group D. *Tetracarpidium conophorum* seed oil extract regulated the concentration of biochemical parameters such as Cholesterol, LDL-C, HDL-C, AST, ALT and Triglyceride. Omega-3 and omega-6 long chain fatty acids was found to be highly incorporated in the liver biomembrane of the rats in group C. This work therefore showed that *Tetracarpidium conophorum* seed oil contains bioactive components that may oppose diabetes induced by alloxan.

KEYWORDS

Alloxan; Cholesterol; Diabetes; Triglycerides; Walnut

INTRODUCTION

A great deal of attention has been directed towards the relationship between regular nut consumption and diabetes risk. An inverse relation between frequent nut consumption and risk of incident diabetes has been reported from the previous analysis of the Nurses' Health study [1]. Studies have shown that a higher intake of MUFAs and PUFAs and a lower intake of saturated fat and trans-fat is associated with a reduced risk of diabetes [2]. Prior research also attests to the health benefits of consuming nuts high in polyunsaturated fats for individuals at risk for diabetes and or cardiovascular disease. African walnuts have a higher

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content of PUFAs including alpha- linolenic acid which may confer additional antiatherogenic influences [3].

African walnut (*Tetracarpidium conophorum*) is a well-known plant in West Africa. Walnut plant is cultivated principally for the nuts which are cooked and consumed as snacks, along with boiled corn [4]. It comprises of families such as *Juglandaceae*, *Euphorbiaceae* and *Olacaceae*. *Juglandaceae* is mostly found in the Southeast Europe to Japan, *Euphorbiaceae* is found in Nigeria and Cameroon while *Olacaceae* is found in Congo, Gabon and Liberia. Among members of the nut family, African walnut have been found to be particularly promising in terms of health benefits. Consumption of walnut has been shown to improve endothelial function in individuals with hypercholesterolemia and type 2 diabetes [5,6]. Efforts on how other components especially the oil content can be utilized to supplement the protective and nutritional needs of consumers have been largely ignored. This means there is need to investigate the biochemical properties of *Tetracarpidium conophorum* which influences anti-diabetic effects. In this study, we shall investigate the effect of feeding *Tetracarpidium conophorum* oil on alloxan induced wistar rats.

MATERIALS AND METHOD

The Study Location

Department of Biochemistry Laboratory, University of Medical Sciences, Ondo City, Ondo State, Nigeria.

Reagents/Chemicals

All reagents used were of analytical grade. Methanol (Sigma Chemicals Co, London), NaCl (BDH Chemicals Ltd., Eng.), Alloxan (Qualikems chemicals), AST, ALT, Cholesterol, HDL and TRIG kit (Random Lab. Ltd., United Kingdom).

Plant Material (Sample Collection)

Fresh *Tetracarpidium conophorum* fruits were obtained from farms in Ondo Town, Ondo State, Nigeria. The fruits were authenticated by a Taxonomist of the Botany Department, University of Medical Sciences, Ondo, Nigeria. At each harvest, 40 fruits will be collected randomly from three regions of the plant as follows, apical region - 10 fruits; middle region - 15 fruits; basal region - 15 fruits. The collected fruits were cleaned with a moist soft cotton wool and then the seeds carefully separated from the fruits and dried at 65°C for 4 hours. in an oven, crushed with a laboratory mortar and pestle and were kept in a well labeled airtight polythene bags or screw-capped bottles at 4°C for extraction.

Extraction of Oil from African Walnut

The Soxhlet extraction method according to AOAC (1996) will be employed. The sample (5.0 g) will be weighed into a weighed filter paper and folded neatly. This will be placed inside the pre-weighed thimble. The thimble with the sample will be inserted into the Soxhlet apparatus and extraction under reflux will be carried out with the n-hexane (40°C - 60°C boiling range) for 6 hours. At the end of extraction, the thimble will be dried in the oven for about 30 minutes at 100°C to evaporate off the solvent and cool in a desiccator and later weighed and kept in the refrigerator.

Feeding the Animals with Diet Containing Walnut Seed Oil

Wistar rats of both sexes (28 day old) were obtained from the Animal house of the University of Medical Science, Ondo, and were housed in metal cages in a well-ventilated room and they were allowed access to water and *ad libitum*. The experimental diet comprised of chickpea (51.4%), wheat (15.0%), groundnut cake (10.0%), skim milk powder (6.0%), mineral mixture (2.16%), vitamin mix (0.2%) and *Tetracarpidium conophorum* oil (15.0%). Overall, 20

Wistar rats were used. The animals were randomly divided into five major groups of 5 animals each. Group A animals were fed for 4 weeks with diet containing no *Tetracarpidium conophorum* oil and not injected with alloxan. Group B animals were fed for 4 weeks with normal diet, the animals injected with alloxan (150 mg/kg body weight) through intraperitoneal injection after 4weeks of feeding and treated with Diabetmin. Group C animals were fed for 4 weeks with diet containing *Tetracarpidium conophorum* oil, and given alloxan (150 mg/kg body weight) through intraperitoneal injection after 4 weeks of feeding. Group D were fed with diet containing no *Tetracarpidium conophorum* oil and given alloxan (150 mg/kg body weight) through intraperitoneal injection after four weeks of feeding and no standard drug was administered.

After injection of rats with alloxan, the blood glucose level was checked after a period of five days and rats with blood glucose level greater than 200 mg/dl were considered diabetic. Animals from each group were sacrificed after 4 weeks, and the serum and tissues collected for histopathological and biochemical analysis. Another portion of hepatic tissue was fixed in formalin (10%) for histopathological studies.

Sacrificing of Animals, Collection of Blood and Harvesting of Organs

To monitor glucose levels a weekly tail bleeding was done with a glucometer throughout the 4-weeks period. Blood was collected from the rats by cervical dislocation at the end of the experiment. Blood samples were collected into plain bottles and centrifuged to obtain serum for lipid profile and serum liver enzymes assay. From various groups of animal's liver tissues was removed carefully and thoroughly washed with ice cold saline. The wet liver tissues were weighed and homogenized in 0.1 M Tris-HCl buffer, pH 7.4 at 4°C. The homogenate was centrifuged at 2500 rpm for 10 minutes at 4°C using a refrigerated centrifuge.

Enzyme Assays

Assay of alanine aminotransferase (ALT) and aspartate aminotransferase were carried out using the procedure provided by the RANDOX KIT manufacturers (Crumlin, UK), according to the principle described by [7].

Estimation of total cholesterol

Cholesterol in serum was estimated by CHOD-PAP method using an enzymatic diagnostic kit from randox (Crumlin, UK). The absorbance of the sample and of the standard was measured against the reagent blank value at 546 mm.

$$\text{Total Cholesterol (mg/dl)} = \text{Absorbance of Total Cholesterol/Absorbance of Standard} \times 200$$

Estimation of triglycerides

In vitro quantitative determination of triglyceride (neutral fat) concentration in serum was done by using a diagnostic kit from randox. The absorbance of the test and standard was read against blank at 546 nm.

$$\text{Triglycerides} = \text{Absorbance of Test/Absorbance of Standard} \times 200$$

Estimation of HDL cholesterol

In vitro quantitative determination of the activity of HDL-Cholesterol in serum was estimated by CHOD-PAP method using an enzymatic diagnostic kit from randox.

Estimation of LDL cholesterol

VLDL Cholesterol was estimated by the standard formula and expressed as mg/dl, as follows.

$$\text{VLDL} = \text{Triglyceride}/5$$

$$\text{LDL cholesterol} = \text{Total cholesterol} - (\text{HDL cholesterol} + \text{VLDL cholesterol})$$

Histological Procedures

Hepatic tissues were fixed in 10% formalin immediately after harvesting. It was dehydrated in ethanol, purified in Xylene and subsequently embedded in paraffin wax. A microtome was used to cut the tissue into 0.05 mm - 0.15 mm sections, fixed on glass slides and further stained with Hematoxylin and Eosin (H and E). Slides were examined under light microscope at 100x magnification and micrographs taken with a digital camera.

Statistical Analysis

Statistical analyses were performed using both descriptive and inferential statistics using graph prism pad software. Results were expressed as mean ± Standard Deviation. P values of <0.05 was used to analyze the significance level.

RESULTS

Serum Glucose Levels

Figure 1 summarizes the effects of extracts of *Tetracarpidium conophorum* seed oil on alloxan induced diabetes. This was evaluated by determining the blood glucose levels of the diabetic Wistar rats. The seed oil extract was found to possess highest anti-diabetic activity in rats administered with seed oil extract (Group C). There was a significant decrease in the blood glucose levels of rats that were administered toxicant and oil extract (Group C) when compared to rats that were administered toxicant only (Group D) (P <0.05). There was a significant decrease in the blood glucose levels of rats that were administered toxicant and treated with oil extract (Group C) when compared with rats administered with toxicant and treated with standard drugs Diabetmin (Group B) (P <0.05).

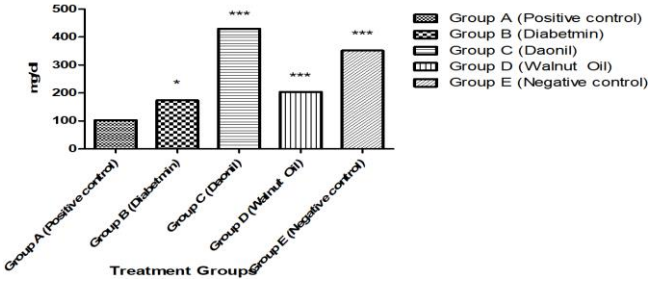

Figure 1: Mean (± SD) of serum glucose concentrations in experimental groups of rats at 28 days of treatment. Values are expressed as mean ± standard deviation of each group values.

Serum Metabolic Enzymes

AST and ALT concentrations followed same pattern in all groups. In seed oil extract treated group, slight lower enzyme level was observed in comparison with the Diabetmin tested diabetic group.

Serum aspartate aminotransferase (AST)

In the untreated diabetic rat group (Group D), AST concentration was highest and lowest in non-diabetic group (Group A). Treatment of diabetic rats with Diabetmin seed oil extract of Tetracarpidium conophorum reduced AST concentration to comparatively near normal values, whereas in the seed oil extract group AST activities was almost as high as in the untreated diabetic group (Figure 2).

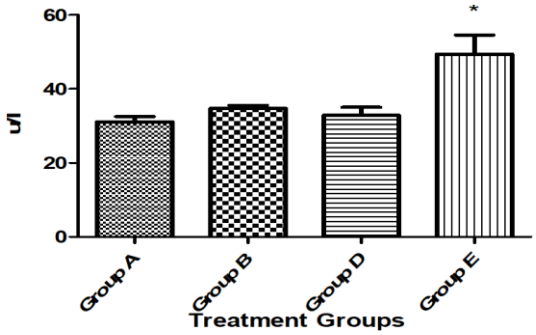

Figure 2: Serum aspartate aminotransferase (AST) activity (IU/L). Values are expressed as mean ± standard deviation.

Serum alanine aminotransferase (ALT)

Serum Alanine Aminotransferase concentration results showed the same pattern as AST. Mean ALT concentration was highest in the untreated diabetic control group but reduced to near normal by Diabetmin and almost to Diabetmin level by seed oil extract of *Tetracarpidium conophorum* (Figure 3).

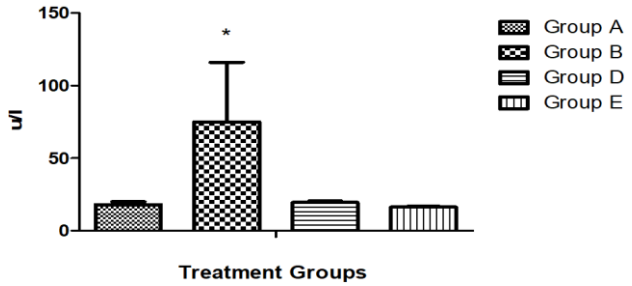

Figure 3: Serum alanine aminotransferase (ALT) concentrations IU/L). Values are expressed as mean ± standard.

Lipid Profile

Total cholesterol

Total cholesterol concentration was highest in the untreated diabetic group (200 mg/dl). Seed oil extract (180 mg/dl) showed efficiency at reducing total cholesterol to almost Diabetmin level (Figure 4).

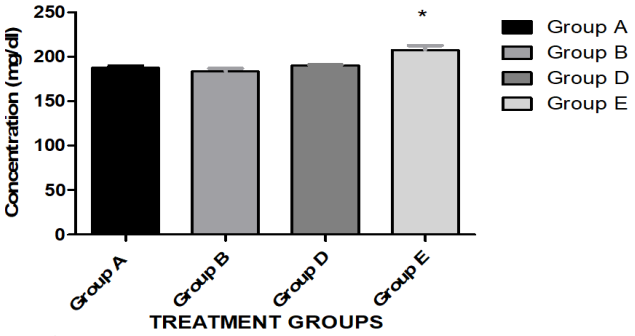

Figure 4: Serum total cholesterol concentrations (mg/dl). Values are expressed as mean ± standard deviation.

High density lipoprotein (HDL) cholesterol

Concentration of HDL-Cholesterol was highest in the non-diabetic normal rat group and also in seed oil extract treated group. Diabetmin recorded relatively lower concentration (Figure 5).

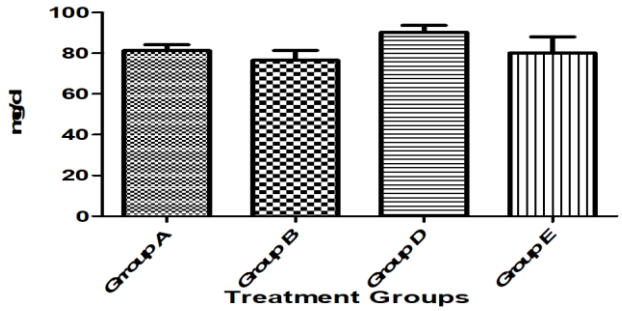

Figure 5: Serum HDL-cholesterol concentration (mg/dl). Values are expressed as mean ± standard deviation.

Low density lipoprotein (LDL) cholesterol

In untreated diabetic rat group, concentration of serum LDL cholesterol was high compared with the normal nondiabetic rat group. Diabetmin treated rat groups showed efficient reduction in LDL concentration, but seed oil extract treated rat group showed a low LDL concentration next to normal rat groups (Figure 6).

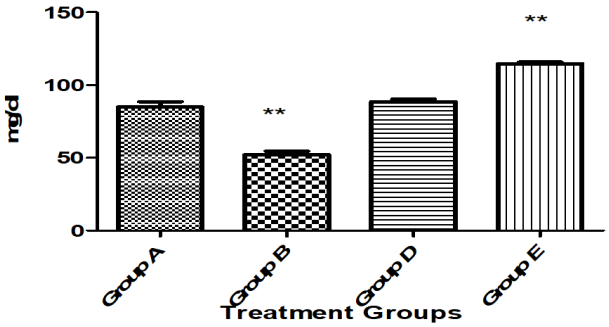

Figure 6: Serum LDL-cholesterol concentration (mg/dl). Values are expressed as mean ± standard deviation.

Serum triglycerides

Serum triglycerides was highest in untreated diabetes group but lowest in the seed oil extract treated group. Diabetmin treatment was minimally effective (Figure 7).

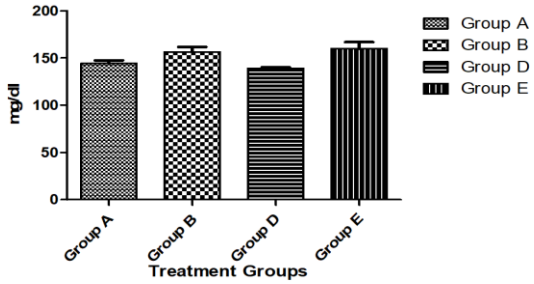

Figure 7: Serum triglycerides concentration (mg/dl). Values are expressed as mean ± standard deviation.

Histological Studies

Histological studies of the liver of alloxan induced diabetic rats

Normal liver histology was observed in non-diabetic control group and Diabetmin treated group except diabetic untreated group and seed oil extract treated group (Figure 8 - Figure 11).

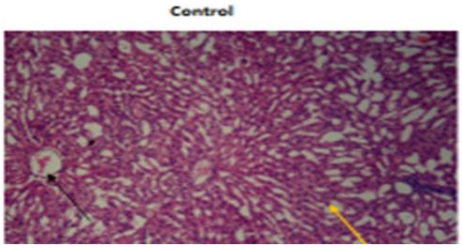

Figure 8: Photomicrograph of group A rats (100*) (liver).

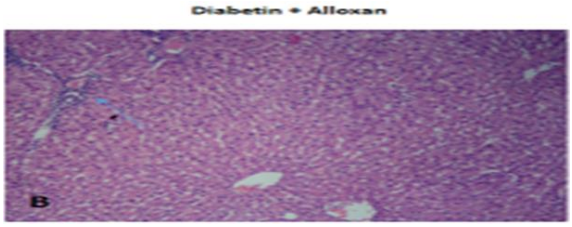

Figure 9: Photomicrograph of group B rats (100*) (liver).

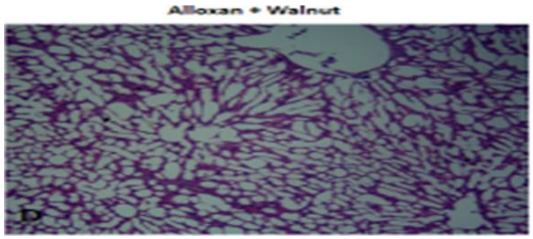

Figure 10: Photomicrograph of group C (100*) (liver).

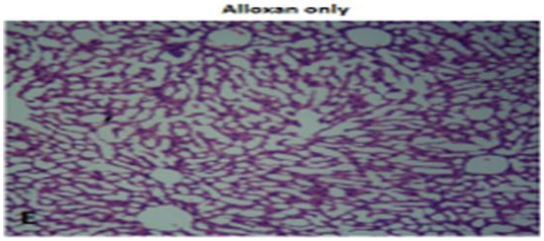

Figure 11: Photomicrograph of group D (100*) (liver).

DISCUSSION

In order to investigate the possible protective potentials of the seed oil extract of T.C in alloxan-induced diabetes rat model against diabetic liver damage, this study was conducted. The presence of phytochemicals and bioactive compounds such as alkaloids, flavonoids, tarpenoids and sterols in T.C seed oil marked it as a plant with beneficial therapeutic effects.

Alloxan induces selective partial destruction of beta cells of the pancreas [8]. This reduction of beta cells decreases insulin output resulting in hyperglycemia which causes a shift in reliance on fatty acid fuels, loss of weight and wasting of fat stores. T.C seed oil extract and diabetmin significantly increased the weight of diabetic rats in this study. Possible mechanism postulated to be responsible for gaining weight in diabetes is improved insulin sensitivity and lowering of blood glucose levels via the promotion of peripheral glucose uptake [9].

T.C seed oil extract significantly reduced blood sugar level as effective as reference drug diabetmin. This result is consistent with that obtained by [10]. This study has also revealed that diabetes causes serious abnormalities in the normal concentration of serum lipids (triglycerides, HDL and LDL) which were corrected by the seed oil extract of T.C. The findings of reduced plasma triglycerides, LDL and significant increase in the level of plasma HDL suggest that T.C seed oil extract possess hypolipidemic effect. The reduction in LDL level observed is in line with previous observations which suggest that phytochemicals reduce LDL involved in depositing fat in the arteries. T.C seed oil extract also contain sterol and it has been established that plant sterols exert their hypocholesterolemic effect by competing with cholesterol for micelle solubility, thereby lowering the amount of cholesterol available for absorption by intestinal mucosal cells [11]. The high HDL and low triglycerides level recorded in group C showed that T.C

seed oil extract is a good lipid lowering agent and can reduce the incidence of atherosclerosis.

Elevated serum levels of ALT and AST are markers of liver injury from induced oxidative stress [12]. The main pathway for the development of pathological changes in the affected organ is oxidative stress induced by hyperglycemia [13,14]. Alloxan induces the formation of ROS which mediate cellular damage [15]. The cellular damage may in addition induce autoimmune reactions against the beta cells. Administration of T.C seed oil extract and diabetmin significantly reduced the serum level of AST by mitigating the generation of ROS. Administration of T.C seed oil extract also significantly reduced the serum level of ALT in the alloxan induced rat models.

Furthermore, histopathological results showed group A to have intact cytoarchitecture with intact Islet of Langerhans and blood vessels. Group B treated with diabetmin showed mild insignificant histo-morphological distortions. Group C and Group D was characterized by loss of liver parenchyma, cell death, dilation of central vein, disorganization, presence of inflammatory red cells within and around the central vein with sinusoids as against result gotten by [16].

CONCLUSION

The African walnut (*Tetracarpidium conophorum*) is an important fruit of African origin that is widely cultivated and consumed for its economic and nutritional benefits. However, findings from this study, in consonance with previous observations, have also been able to highlight the medicinal properties and health benefits of *T. conophorum* seed oil, especially in the lowering of blood glucose levels through improvements in the sensitivity of cells and tissues to insulin and promotion of glucose uptake. These observations validate the use of *T. conophorum* for traditional management of hyperglycemia, with a potential for the application of its bioactive compounds for treatment of diabetes.

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