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Research Article

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Effect of African Walnut (*Tetracarpidium Conophorum*) on Lipid Profile and Glucose Level in Albino Rats

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Abstract

This study was designed to investigate changes in lipid profile and glucose levels in twenty albino rats fed with African walnut. The twenty albino rats were divided into four groups with five animals in each group. Group 1 were placed on normal diet, group 2,3 and 4 were given 1% cholesterol,1%cholesterol and 1% African walnut seed powder, and 1% cholesterol and 5% walnut seed powder respectively. The level of serum lipid and plasma glucose was carried out by spectrophotometric method. The results were analyzed for statistical significance using analysis of variance ANOVA, p-value < 0.05 were considered statistical significant. The level of total cholesterol increased in group 2(164.60 ± 7.09g) when compared to control (160.20±2.28g) at p<0.05.While a significant increase was seen in mean value of group 2(220.00±33.918mg/dl) and group 3(120.00±36.74mg/dl) when compared to value of control (66.0±11.40mg/dl). Hence these observation indicate that supplementation of African walnut seed could be beneficial to patient with cardiovascular disease and diabetes.

Keywords: *Tetracarpidium conophorum*, Lipid Profile, Glucose, Rats

Introduction

Heart disease is one of the most leading risk factors of death in Nigeria and is predicted to be the main cause of death worldwide by 2020. Hypercholesterolemia is a major risk factor for coronary artery disease and thus for myocardial infarction and stroke [1]. High serum low density lipoprotein-cholesterol levels particularly pose a significantly increased risk for heart diseases that increase death rate of coronary artery patients. Hypercholesterolemia is more prevalent among industrials societies [2].

Hypercholesterolemia diets increase cholesterol, low density lipoprotein-cholesterol and triglycerides. On the other hand it reduces low density lipoprotein-receptor activities in liver elevating of serum high density lipoprotein-cholesterol can prevent development of hypercholesterolemia and cardiovascular disease (CVD). Previous studies showed that antioxidants may reduce the possibility of CVD occurrence due to hypercholesterolemia [3]. The use of antioxidants is the most preferred way to inhibit lipid oxidation [4]. Recently, some negative side effects of the commonly used synthetic antioxidants have been established. Evidences revealed that these compounds may be implicated in many health risks, including cancer and carcinogenesis. Therefore, there is a tendency towards the use of plant origin [5].

Walnut (TetracarpidiumConophorumor Plukenetiaconophora) belongs to the family Euphorbiaceae [6]. The walnut is generally referred to as the conoph or tree or conophor nut. The plant is popularly known as African walnut, black walnut and Nigerian walnut [7]. In Nigeria, among the Yoruba tribe, the walnut is known as awusa or asala, ukpa, or okeokpokirinya in Igbo and gawudibairi in Hausa; and it is known as okhue or ok we among the Bini tribe of Edo State.

In Sierra Leone, it is called musyabassa and in western Cameroon, it is known as kaso or ngak, among other local names. However, lack of storage facilities has hampered the market value of the walnut and the nuts must be consumed within 1–2 days when cooked or else they will become foul-smelling and unpleasant for sale and consumption [8]. The seeds are consumed as snacks and refreshments. During its season, hawkers relate their walnuts' quality to kola nut maturity (asala-ogbokoko bi obi). The buyers also shake each seed nut to ensure that the seed is intact in the hard coat, and sometimes the quality of the seed nut can be seen from the nut colour and size. It is a perennial cash crop and an economic tree that is widely grown for its edible seed nut it is also used as wood in the timber industry [9].

Heart disease is one of the most leading risk factors of death in Owerri Nigeria and is predicted to be the main cause of death worldwide. Hypercholesterolemia is a major risk factor for coronary artery disease and thus for myocardial infarction and stroke [10]. High serum low density lipoprotein-cholesterol levels particularly pose a significantly increased risk for heart disease that increase death rate of coronary artery patients. Walnut is a valuable crop being the nut very popular and largely consumed. Not only dry fruits (nuts) but also other components of walnuts such as green walnuts, shells, kernels, barks, green walnut husks (epicarp) and leaves have been used in both cosmetic and pharmaceutical industries [11].

Different studies have demonstrated the potential antioxidant effects of walnut products, especially fruits, leaves and liqueurs which produced by green fruit [12]. There are, however, little reports known about hypoglycemic and hypolipidemic effects of walnut leaf. Hence the need for this study which will help in bridging this gap in knowledge.

Materials and Methods

Plant Material

The seeds of African walnut were purchased within the city of Owerri, Imo state, Nigeria. The seed was identified by Herbarium Unit, Department of Plant science and biotechnology, Faculty of science, Imo state university Owerri, Imo state, Nigeria.

Seed Preparation

The seed was cut into bit and allowed to air dry for 2 weeks. The dried seed was grinded into powdered form using a grinding machine. Then 1g of the powdered walnut was mixed with 100g of feed to represent 1% weight concentration of walnut- food mixture.

Experimental Animals

Twenty mature male Wistar rats (160-225 g) obtained from an animal house in Imo state University, Owerri, Nigeria were acclimatized for two weeks in well ventilated animal house with a constant 12 hour light: 12 hour dark lighting schedule and comfortable temperature of 27 ± 2°C. The animals were housed in wire gauzed cage with wood chip beddings.

They were fed with growers' marsh pellet diet (obtained from Orchads Feeds Limited, Owerri, Imo State) and water was given ad *libitun*.

Experimental Design

Walnut seed powder was mixed with usual food in 1%and 5% weight concentration. Twenty albino rats (mean weight, 250-300g) were divided into following 4 groups with 5 animals in each group. The first group received normal daily diet, group 2 received 1% cholesterol only, and group 3 received 1% cholesterol and 1% walnut seed powder in daily diets for twenty-eight days, while group 4 received 1% cholesterol and 5% walnut seed powder in daily diets for twenty-eight days.

Sample Collection

Blood samples were collected with the aid of a syringe by cardiac puncture from the rats at the twenty-eight day. 5ml of the blood was placed in a plain container the serum was separated from blood sample in the plain container and stored in a freezer at -200C.

Laboratory Assays; The Serum lipide profile was determined by spectrophotometric method: total cholesterol (TC) total triglyceride (TG), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein [13].

Statistical Analysis

All data generated from this study were subjected to statistical analysis. Mean, standard deviation, student's t-test, and correlations were analyzed using SPSS. Results were expressed as Mean ± SD. The 5% (0.05) level of significance was adopted for significance.

Table 4.1: Result showing mean ± standard deviation of body weights of the four groups of rat in grams (g).

Weight(g)	Initial weight (g)	Final weight (g)	weight change (g)
Group1 (Control)	156.00±4.18	160.20±2.28	4.2±1.9
Group 2(Test)	164.00±5.48	164.60 ± 7.09*	0.6±.1.61
Group 3 (Test)	158.80 ± 5.45	162.00± 5.19	3.2 ± 0.26
Group 4 (Test)	157.80 ± 2.86	161.20± 5.63	3.4 ± 2.7

Key: * Statistically different ($p<0.05$) when the final body weight of each group was compared with the final body weight of the control group.

Table 4.2: Result showing mean ± standard deviation of Lipid profile (Total Cholesterol, Triglycerides, High density Lipoprotein, Low density Lipoprotein) on the effect of ethanol seed extract of walnut in cholestoremic albino rats

Parameters	Group1 (Control)	Group 2(Test)	Group3 (Test)	Group4 (Test)
Total Cholesterol (mg/dl)	151.20±6.30	265.00±29.158*	206.80±8.34*	155.6±9.29
Triglycerides (mg/dl)	86.00±10.86	139.00±20.12*	99.80±10.85	76.80±6.98
LDL (mg/dl)	76.60±9.18	127.20±21.38*	94.60±8.08*	70.72±9.83
HDL (mg/dl)	47.20±1.92	28.20±5.31*	37.60±3.65*	48.2±2.86

Key: $P<0.05$ = Significant
* Significant when compared with the control group at $p<0.05$

Table 4.3: Result showing mean ± standard deviation of blood glucose level on the effect of ethanol seed extract of walnut in albino rats

Parameters	Group1 (Control)	Group 2(Test)	Group3 (Test)	Group4 (Test)
Glucose (mg/dl)	66.00±11.40	220.00±33.918*	120.00±36.74*	82.00± 17.88

Key: P<0.05 = Significant
* Significantwhen compared with the control group at p<0.05

Discussion

Hypercholesterolemia is a major risk factor for coronary artery disease and thus for myocardial infarction and stroke [1]. Previous studies have shown that antioxidants may reduce the possibility of CVD occurrence due to hypercholesterolemia and cardiovascular disease.

The use of traditional medicine and medicinal plants in Africa and Nigeria specifically as a normal approach to the maintenance of health is an age-long approach and is gaining more awareness due to its efficacy and recent advances in research [14].

The result of the current study showed that there was statistically significant increase (p<0.05) in the mean value of total cholesterol, triglyceride, LDL and statistically significant decrease (p<0.05) in the mean value of HDL in group 2 rats when compared to rats in group 1. The increase is as a result of the administration of 1% cholesterol to the rats in group. The result is in agreement with the report by Pereira et al., ([12] in who observed that rats given increased dose of cholesterol experiences an increase in body weight and an increased chance of cardiovascular disease due to increase in total cholesterol and triglyceride level.

In this study, it showed that there was no statistically significant difference in the mean value total cholesterol, triglyceride, HDL, LDL-cholesterol in rats in group 4 administered with 1% cholesterol and 5% walnut , when compared with the control group, and a statistical difference in the rats in group 2 when compared with rats in group 4. The significant difference is as a result of the ameliorating effect of walnut seed extract. Walnut seed also consists of remarkably antioxidant components. These hypolipidemic effects found in this current study may be related to its antioxidant properties .Flavonoids are major antioxidants present in walnut leaf [12]. It also may distribute lipid profile properly and prevent lipid and cholesterol accommodation [15]. A study on diabetic rats demonstrated the reducing effects of walnut leaf ethanolic extract on triglycerides and cholesterol that again is in agreement with the results of [16]. In other study, Chlorogenic acid from walnut leaf indirectly interferes in cholesterol synthesis pathway and inhibit HMGcoA reductase enzyme, reduce cholesterol production, also increase its bile secretions [17]. There is other possible mechanism, walnut leaf is good sources of unsaturated fatty acids (monounsaturated fatty acids (MUFA) and PUFA) known for their favorable effects on blood lipids that reduce total cholesterol and LDL-C [18]. The possible mechanisms by which walnut leaf may improve lipid profiles may include the effects of these compounds, fiber, micronutrients such as vitamin E and C, folic acid, copper, magnesium, plant protein (e.g.,arginine), plant sterols, and phenolic components [19]. Fiber in walnut seed can interfere with lipid and cholesterol intake and metabolism thus has useful effect. It was reported by Divband et al., [20] that triglycerides and LDL-C were reduced in diabetic rats that treated with walnut leaf extracts which is consistent with the results obtained from the seeds. They also showed that HDL-C was increased by walnut leaf treatment.

In conclusion, the results obtained from this study suggest that *Tetracarpidiumconophorum* (walnut) nut had antihyperglycaemic effect. It significantly lowered blood glucose in rats that receive 5% of walnut compared with respective fasting blood glucose levels of group two that was induced with diabetes. The mechanism of action of antihyperglycemic effect of the extract was not known but some medicinal plants with hypoglycemic properties are known to increase circulating insulin level in normoglycemic rats. The observed antihyperglycemic activity was in agreement with the findings of Hegsted et al., [18] who reported that *Tetracarpidiumconophorum* nut decreased blood glucose level in diabetic rats; however, they did not explain the mechanism of action.

In conclusion, Walnut seed has hypocholesterolemic and hypoglycaemic, and may be used for reducing CVD risks and probable treatment of hypercholesterolemia and diabetes.

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