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Effects Of Tetracarpidium Conophorum - 'Nigerian Walnuts' On Blood Lipids, Lipoproteins And Glucose Values In Adult Nigerians

Analike RA

Ahaneku JE

Njoku MC

Department of Chemical Pathology, Faculty of Medicine, College of Health Sciences, Nnamdi Azikiwe University, Nnewi campus, Nigeria

Ahaneku GI

Department of Medicine, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria

Ezeugwunne IP

Department of Human Biochemistry, Department of Human Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi campus, Nigeria

Ogbodo EC

Department of Medical Laboratory, Faculty of Human Biochemistry, College of Health Sciences, Nnamdi Azikiwe University, Nnewi campus, Nigeria

Abstract:

Objective: The study evaluates the beneficial effects or otherwise of Tetracarpidium conophorum (Nigerian walnuts) on plasma lipids, Lipoproteins and blood glucose.

Method: The study was conducted on twenty (20) apparently healthy subjects (10 males and 10 females), within the age range of 20-55years who were randomly recruited from NAUTH staff, Medical students and other subjects resident at Nnewi. The subjects were fed with 75g/day of cooked walnuts seeds for six weeks. Blood samples were collected at baseline and every week for six weeks and the biochemical parameters analyzed using standard laboratory methods.

Result: There were significant reduction in the plasma cholesterol (4.44±0.82mmol/l vs. 3.43±0.60mmol/l), plasma triglycerides (0.92±0.23mmol/l vs. 0.70±0.19mmol/l), plasma LDL-C (2.76±0.67mmol/l vs. 1.70±0.51mmol/l), and plasma LDL-C/ HDL-C ratio (2.24±0.54mmol/l vs. 0.61±0.21mmol/l) of the subjects compared with their baseline values; all P< 0.05. There was a significant increase in their HDL-C level (1.43±0.22mmol/l vs. 1.72±0.10mmol/l) compared with their baseline; P<0.05.

Conclusion: The result of this study shows that cooked Tetracarpidium conophorum seed has hypolipidemic effect on healthy subjects and this might be beneficial in preventing hyperlipidaemia and associated cardiovascular diseases.

Keywords: Tetracarpidium conophorum (Nigerian walnuts), Glucose, Lipids, Lipoprotein

I. INTRODUCTION

The use of Herbal remedies or food supplements has increasingly become attractive alternatives to prevent or treat hyperglycemia and hypercholesterolemia. Excellent safety profile, cost effectiveness and multiple beneficial effects aimed at improving the total quality of life of man, all contribute to the emerging trend of the increasing usage of dietary or herbal supplement around the world.

Medicinal plants have been known as first art of treatment to man and animals. Plants generally contain chemical compounds (such as saponins, tannins, oxalates, phytates, trypsin inhibitors, flavonoids and cyanogenic glycosides) known as secondary metabolites, which are biologically active. Secondary metabolites may be applied in nutrition and

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as pharmacologically active agents. Haniffa and Shanthi reported that the phytochemical screening of some medicinal plants revealed the presence of alkaloids, carbohydrates, flavonoids, saponnins and phenolic compound. They have antibacterial and anti parasitic properties. Plants are also known to have high amounts of essential nutrients, vitamins, minerals, fatty acids and fibre. An expanding body of evidence from epidemiological and laboratory studies have demonstrated that some edible plants as a whole or their identified ingredients with antioxidant properties have substantial protective effects on human carcinogenesis.

Nigerian walnut is botanically known as *Tetracarpidium conophorum* (Mull. Arg.) Hutch. & Dalziel. Its common name is African walnut and in Nigeria it is called Nigerian Walnuts. It is known as 'Okhue' or 'Okwe' in Edo State, 'Ekporo' by the Efiks and Ibibios of Cross River and Akwa Ibom states in Nigeria, commonly called 'ukpa', 'asala' and 'awusa' in the Eastern, Western and Northern Nigeria respectively and it is known in the littoral and the western Cameroon as 'kaso' or 'ngak'.

T. conophorum is a dry nut, enclosed between two semispherical hard shells joined together, and the fruit has a characteristic shape that resembles brains. *T. conophorum* plant is cultivated principally for the nuts which are cooked and consumed as snacks. Medicinal plants such as walnut leaf had been used to combat infection and alleviate stress with a potential for wound healing and antimicrobial effects in animals. It serves as an edible nut eaten between meals. A bitter taste is usually observed upon drinking water immediately after eating the nuts. This could be attributed to the presence of chemical substances such as alkaloids. Their white, slightly bitter flesh consists mainly of a blend of vegetable fats (60%), followed by a very respectable amount of protein (24%) and a lower amount of carbohydrates (10%). It is a climbing shrub 10-20ft long, and is found in the forest regions of Africa and India.

T. conophorum is used as a male-fertility agent [14]. Though *T. conophorum* nuts are generally eaten in Nigeria, very little work has been done on the proximate composition and heavy metal content of this nut. Edem et al. reported on the proximate composition, ascorbic acid and heavy metal contents of the nut. The presence of oxalates, phytates, tannins as well as proteins, fibres, oil and carbohydrate in *T. conophorum* has been reported. Bello et al. reported the presence of saponnins, tannins, alkaloids and cyanogenic glycosides in the Phytochemical screening of walnut leaves. These phytochemicals are natural plant based chemicals that have been identified as active compounds in disease prevention. Previous report showed that phytochemicals reduce LDL-cholesterol involved in depositing fat in the arteries and also prevent blood clotting which can reduce the risk of heart attack or stroke. Oyenuga reported on the amino acid and fatty acid components of the nut and on the use of its leaf juice for the treatment of prolonged and constant hiccups. Nwokolo also reported on the impact of traditional processing on the nutrient and sensory qualities of the nut. Okpero reported on the methods of processing the *T. conophorum* nuts while Okafor reported on the use of *T. conophorum* seeds and processing waste in livestock feed formulation.

Walnuts are rich in linoleic and linolenic acids and in other health-related compounds such as high-biological-value proteins (e.g. arginine) fibre, vitamins, tannins, folates and polyphenols which may provide additional antiatherogenic properties.

T. conophorum are rich in fiber, B vitamins, magnesium, and antioxidants such as Vitamin E. Medical studies have shown walnuts to improve several physical illnesses, promote weight loss and enhance overall health. These beneficial effects are probably linked to their high content in plant sterols and fat - but mostly Omega-3 polyunsaturated fats which is rare in our diet and are slowly and steadily disappearing from our diets but are absolutely essential for the good functioning of our bodies. Monounsaturated and polyunsaturated fats favorably affects cardio vascular disease (CVD) risk [24]. Polyunsaturated fatty acids may protect against cardio vascular disease (CVD) and may enhance tocopherol absorption.

Previous feeding studies with walnuts have reported a total and LDL cholesterol lowering effect following consumption of about 70–80 g/d of walnuts.

The present study is aimed at exploring the effects of Nigerian walnuts (*Tetracarpidium conophorum*) on blood lipids, lipoprotein and glucose in human subjects.

II. MATERIALS AND METHODS

A. SUBJECTS

A total of twenty (20) apparently healthy subjects (10 males and 10 females), within the age range of 20-55years were randomly recruited from NAUTH staff, Medical students and within Nnewi. Ethics Committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi approved the study design. Participants were informed about the study and only those who gave their consent were recruited for the study. The subjects fed on their normal diet with 75g/day of cooked walnuts daily for a period of six (6) weeks.

The mature husk of *Tetracarpidiumconophorum* nuts were purchased from Afor Nnobi market which is a local market close to Nnewi town and authenticated by Prof R.N. Okigbo of Botanyn Department of Nnamdi Azikiwe University, Awka. The nuts of were obtained by breaking the pod. The walnuts were boiled at 100^{o}C for 2 hours.

B. SAMPLE COLLECTION

Initial collection of 6mls of fasting Blood samples were obtained from all the participants. 2ml was dispensed into fluoride oxalate containers while 4ml was dispensed into EDTA containers and this serve as baseline samples. The subjects fed on their normal diet and 75g/day of cooked walnuts for six weeks and blood samples were similarly collected from the subjects once every week till the end of the six weeks feeding period. The blood samples were centrifuged at 4000 revolution per minutes and the plasma were separated into plain sample containers. The plasma obtained from the blood samples in fluoride oxalate containers were used for the determination of blood glucose while those obtained from the

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blood samples in EDTA containers were used for the determination of total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol. Height and weight of the subjects were taken at the baseline. Thereafter, weight was taken at the end of each week till the six week feeding period elapsed.

C. ANALYTICAL PROCEDURES

Plasma glucose was determined using glucose oxidase method [28]. Cholesterol was determined after enzymatic hydrolysis and oxidation. Triglycerides was determined following enzymatic hydrolysis with lipase. HDL determination was according to the method described by Assmann. LDL-cholesterol was determined after precipitation with heparin Body Mass Index was calculated using the relationship –

$$\text{Body Mass Index (Kg/M}^2) = \frac{\text{Weight in kg}}{(\text{Height in metre})^2}$$

C. STATISTICAL METHOD OF ANALYSIS

Results of parameters analyzed were expressed as mean ± SD. The mean differences were assessed using Analysis of variance and Paired Student-t test. The data were analyzed using Statistical Package for Social Sciences (SPSS) Version 19. Confidence limit was chosen at 95% ($P<0.05$). $P<0.05$= significant while $P>0.05$=Insignificant.

III. RESULTS

Table 1 shows that there was no significant difference observed in the body weight, body mass index and the blood glucose level of the subjects that consumed *Tetracarpidium conophorum* compared with their baseline values ($P>0.05$).

PARAMETERS	Baseline n=20	Week one n=20	Week two n=20	Week three n=19	Week four n=19	Week five n=17	Week six n=17
Weight (Kg)	72.90 ±6.05	72.85 ±6.12	72.75 ±6.03	71.00 ±5.95	70.45 ±5.77	70.05 ±5.74	68.60 ±4.90
Body Mass Index (Kg/m^2)	26.01 ±2.93	25.99 ±2.94	25.96 ±2.97	25.34 ±2.90	25.14 ±2.86	25.00 ±2.80	24.50 ±2.83

All values are expressed as mean ± SD
**= Significant*

Table 1: Comparison of the mean ± SD of the Anthropometric Parameters of subjects that consumed Nigerian walnuts (Tetracarpidium conophorum)

Table 2 shows that total cholesterol reduced at week five and six. The baseline value of triglyceride started to decline significantly only after six weeks of starting the study. The mean plasma High Density Lipoprotein Cholesterol increased at week three, four, five and six while the mean Plasma Low Density Lipoprotein Cholesterol reduced at week five and six. LDL-C/HDL-C were significantly reduced at week three, four, five and six, all at $P<0.05$. There was no significant difference when the mean values obtained for Blood Glucose in each week was compared ($P>0.05$).

PARAMETERS	Baseline n=20	Week one n=20	Week two n=20	Week Three n=19	Week four n=19	Week five n=17	Week six n=17
Glucose(mmol/L)	4.56 ±0.32	4.55 ±0.39	4.52 ±0.33	4.46 ±0.29	4.46 ±0.27	4.47 ±0.22	4.44 ±0.18
Tot.Chol(mmol/l)	4.44 ±0.82	4.24 ±0.81	4.18 ±0.78	4.07 ±0.68	3.93 ±0.66	3.65 ±0.62*	3.43 ±0.60*
Trigly (mmol/l)	0.92 ±0.23	0.93 ±0.19	0.85 ±0.16	0.81 ±0.18	0.77 ±0.19	0.75 ±0.18	0.70 ±0.19*
HDL-C (mmol/l)	1.24 ±0.12	1.29 ±0.11	1.34 ±0.14	1.38 ±0.11**	1.45 ±0.12**	1.49 ±0.13**	1.52 ±0.14**
LDL-C (mmol/l)	2.76 ±0.67	2.58 ±0.67	2.45 ±0.66	2.29 ±0.61	2.21 ±0.63	1.89 ±0.54*	1.70 ±0.51*
LDL-C / HDL-C	2.24 ±0.54	2.00 ±0.49	1.84 ±0.48	1.67 ±0.45*	1.53 ±0.44*	1.28 ±0.40*	0.61 ±0.21*

All values are expressed as mean ± SD
**= Significantly lower than baseline, $P<0.05$*
***= Significantly higher than baseline, $P<0.05$*

Table 2: Comparison of the mean ± SD of the Plasma Glucose and Lipid parameter values of subjects that were fed with Nigerian walnuts (T. conophorum)

Figure 1: Picture of Tetracarpidium conophorum (Nigerian walnuts)

IV. DISCUSSION

The findings in this study showed that after consumption of cooked Nigerian walnuts (*T. conophorum)* there were significant reductions in the plasma total cholesterol, triglycerides, LDL-C and LDL-C/HDL-C ratio and a significant increase in the level of Plasma HDL-C at the end of six weeks feeding period when compared with their baseline values. There was no significant difference observed in their blood glucose level, body weight and body mass index.

The finding of reduced plasma total cholesterol, triglycerides, LDL-C and LDL-C/HDL-C ratio in these subjects suggest that *T. conophorum* possess hypolipidemic effect and this is consistent with the finding of other authors who have reported a total and LDL cholesterol lowering effect following consumption of about 70–80 g/d of walnuts The reduction in LDL-C level observed is in line with the work of Anderson which showed that phytochemicals reduce LDL-cholesterol involved in depositing fat in the arteries, prevent blood clotting which can reduce the risk of heart attack or stroke. These reductions could be beneficial in preventing hyperlipidemia, hyperlipidemic complications as well as improving lipid metabolism.

This plant contain sterol and it has been established that plant sterols exert their hypocholesterolemic effect by inhibiting intestinal absorption of dietary as well as endogenous cholesterol. However, the molecular mechanisms for such inhibition are still not fully understood. It is generally accepted that the presence of increased quantities of plant sterols compete with cholesterol for micellar solubility, thereby lowering the amount of cholesterol available for absorption by intestinal mucosal cells. Plant sterols have been shown in clinical trials to block absorption sites in human thus helping to reduce cholesterol level in humans.

The water soluble fiber content of these plants has cholesterol lowering effect. It has been established that dietary fiber has many health benefits including decrease in serum LDL-cholesterol, improving triglycerides, and weight management. Currently, the mechanisms for cholesterol-lowering effect of water soluble fiber are not fully understood. One favorite hypothesis is that water soluble fiber binds to bile acids and decrease bile acid reabsorption in the intestine [41, 42]. Conversion of cholesterol into bile acids is one of major pathways for removing cholesterol from the body and is negatively regulated by bile acids through repressing cholesterol 7β-hydroxylase (CYP7A1), the rate-limiting enzyme for bile acid synthesis. Decreased reabsorption of bile acids thus favors cholesterol conversion into bile acids.

On the other hand, as biological detergents, bile acids are required for cholesterol solubilization and absorption in the intestine. Binding of bile acids to fiber decreases free bile acids for solublizing cholesterol, thus decreasing cholesterol absorption. The mechanism for insoluble fiber to exert hypocholesterolemic effect is largely unknown. A study shows that intake of insoluble fiber derived from cell wall significantly increases fecal excretion of both bile acids and neutral sterols. Such increased fecal excretion of bile acids may lead to hypocholesterolemic effect through similar mechanism as water soluble fiber.

The fiber content of *T. conophorum* provides bulk in the diet and this helps to enhance gastrointestinal function, prevents constipation and may thus reduce the incidence of metabolic diseases like maturity onset diabetes mellitus and hypercholesterolemia.

The plant which contains saponins and saponins have been shown to possess cholesterol lowering properties. Saponins is capable of causing hypocholestrolaemia because of its ability to bind cholesterol making it unavailable for absorption. Saponins reduce cholesterol by preventing its reabsorption after it has been excreted in the bile. Thus, the saponin content may have contributed to the significant reduction observed in the subjects.

In conclusion, since the consumption of cooked Nigerian walnuts *(T. conophorum)* reduces plasma cholesterol, triglyceride, LDL-C levels and LDL-C/HDL-C ratio, it may be of immense benefit in the prevention of hyperlipidemia and hyperlipidemic complications as well as improving lipid metabolism. This outcome might stimulate much interest in their consumption and this will obviously be of immense benefit to our people, who, though plagued with several ailments, lack the technological and economic power to tackle them with conventional / orthodox medicine.

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