EFFECTS OF ROASTED *TRECULIA AFRICANA* AND *ARACHIS HYPOGEA* SEEDS ON THE LIPID PROFILE OF ADULT ALBINO RATS

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Abstract- The study investigated the effects of roasted Treculia africana and Arachis hypogea seeds on the triglyceride, total cholesterol, high density and low density lipoproteins of adult female albino rats. This was with a view to determining the lipid lowering activity of the nutritional plants Twenty (20) rats weighing between 140 – 200 g were divided into five (5) groups of four (4) animals each. Group 1 served as control and received growers' mash only. Groups 2, 3 and 4 received a combination of growers' mash, Treculia africana and Arachis hypogea seeds while group 5 was administered Arachis hypogea seeds only. Each of the rats received 10 g of the supplements once daily for 28-days. The animals were bled and their sera obtained for triglyceride, total cholesterol, high density and low density lipoproteins assay. The result showed that roasted Treculia africana seeds had no significant reduction in the serum concentrations of triglyceride, total cholesterol, high density and low density lipoproteins of tested animals. However, Arachis hypogea seeds demonstrated a significant ($P < 0.05$) decrease in the level of serum triglycerides in the tested animals. It had no significant effects on the serum concentrations of total cholesterol, high density and low density lipoproteins. The study, therefore, concluded that roasted Arachis hypogea possesses significant serum hypotriglyceridaemic activity, but lacks activity against total, low and high density lipoprotein cholesterol. Treculia africana on the other hand had no hypolipoproteinaemic activity.

Index Terms: Treculia africana, Arachis hypogea, shelled peanut, unshelled peanut, growers' mash and albino rat.

I. INTRODUCTION

Treculia africana, the African breadfruit (NRCS, 2015) is a tree species in the genus Treculia. It is an edible plant consumed in most African societies, especially in Nigeria. T. Africana is the fruit tree of riverine forest in tropical Africa. The fruits are hard and fibrous, and can be the size of a volleyball and weigh up to 8.5 kg. Chimpanzees have been observed to use tools to break the fruits into small pieces that they can eat (Walker, 2009). The fruits contain polyphenols (Lawal, 1992). T. africana is a species of tree known as the African breadfruit. Several names are given to this species by different tribal groups viz. Afon (Yoruba), Barafuta (Hausa), Ize (Bini), Eyo (Igala), Ediang (Efik) (Irvine, 1981), but the most common is Ukwa (Igbo) (Nuga and Ofodile, 2010). The geographical distribution of T. africana extends through West, East and Central Africa. The species can grow below altitudes of 1,500 m (Nuga and Ofodile, 2010). T. africana is a large tree and is part of the family Moraceae. It grows in wet areas and forests. The species can grow up to a height of 30 m and flowers between October and February (Salami, 2002). The girth of the stem can attain 6 m. The bark is grey and discharges a cream latex that later turns red. The leaves are large and dark green above and lighter below. *T. africana* is evergreen and starts fruiting after four (4) years. The seeds of *T. africana* are used for cooking and are highly nutritious (Okafor,

1974, 1990; Onyekwelu, 2007). The seeds have an excellent dietetic potential whose biological value surpasses that of soybeans. It is a cheap source of vitamins, minerals, proteins, carbohydrates and fats (Okafor *et al.*, 1974; Makinde *et al.*, 1985). The seeds are cooked as porridge or roasted and sold with palm kernel as roadside snacks for commuters in most parts of Southeastern Nigeria. In ethnomedicine, *T. africana* is used as vermifuge, febrifuge, galactogue and laxative (Irvine, 1981). The seeds of *T. africana* may not be an efficient source of dietary fibre which is important in the lowering of blood cholesterol levels to minimise the risks of cardiovascular diseases caused by elevated serum cholesterol (Umoh, 1998).

Arachis hypogea (Groundnut, Peanut or Earthnut) is among the major oil seeds in the world. China, India and the USA are the main producers of groundnuts to the rest of the world (Campos-mondragon *et al.*, 2009). *Arachis hypogaea* Linn. is a native to a region in eastern South America (Weiss, 1983). Groundnut is now grown worldwide in the tropical and temperate zones primarily as an oil seed crop (Bansal *et al.*, 1993). The fat content in groundnut has been largely studied. In general, groundnuts contain 50-55 % fat of which approximately 30 % is linoleic acid and 45 % is oleic acid. High-oleic groundnuts rather than normal groundnuts have increased self life and thus improve the oxidative stability of peanut products (Isleib *et al.*, 2006). Groundnut seed contain 44-56 % oil and 22-30 % protein on a dry seed basis and is a rich source of minerals (phosphorus, calcium, magnesium and potassium) and vitamins (E, K and B group) (Savage and Keenan, 1994). The chemical composition of groundnut has been evaluated in relation to protein level (Young and Hammons, 1973), amino acid composition (Young *et al.*, 1974) and fatty acid composition (Grosso and Guznam, 1993) in some cultivars. The nutritive value is high as the groundnut is affordable and serves as good source of oil and protein (Atasie *et al.*, 2009). Peanut consumption is believed to lower the risk of cardiovascular diseases by decreasing serum low density lipoprotein cholesterol, and thus reduces the risk of development of Type II Diabetes mellitus (Fraser *et al.*, 1992). The health benefits of peanuts have been attributed to the presence of minerals and vitamins, fatty acids, fibre and bioactive compounds (Griel *et al.*, 2004). Peanut has a manifold of uses in the Traditional African Medicine (McDonald, 1998). The extrac is taken as a galactogogue and used as an eye drop to treat conjunctivitis (Steinman, 1996). The maceration of peeled seeds (taken in the form of liquor) is used to treat gonorrhea; maceration of seed coats against syphilis; maceration of seed coats and shells are used for eye disorders. The sap of ground leaves and seeds is used as ear drops against ear discharges. The leaf macerations taken as liquor has diuretic potential, just as leaf infusions taken as liquor is employed in female infertility, and as eye drops for eye injuries and cataract (Wayne, 1991). The plant ash in combination with salt is used for dental caries. The pod extract and young plants are known for their aphrodisiac property. *Arachis hypogea* is used as an emolient, relief of cough, and treatment of colitis and dysuria (Stalker, 1997). Peanut oil is edible, a skin softner and used in the treatment of cystitis (Indian Material Medica, 2000).

II. MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant seeds

The shelled and unshelled seeds of *Arachis hypogea* (Peanut, Groundnut or Earthnut) and *Treculia africana* (Breadfruit) were purchased from a local market, Eke Agbani (a sub-urban town) in Nkanu West Local Government Area of Enugu State, Southeastern Nigeria. The seeds were identified and authenticated by Mr. C.N. Okoli, a taxonomist with the Department of Biological Sciences, Renaissance University, Ugbo-Awka, Enugu State, Nigeria.

2.1.2 Chemical reagents

These assay kits were used in the study; total cholesterol kit (Reckon Diagnostics Private Ltd., Gonwa, Vadodara, Gujarat, India), triglyceride kit (Reckon Diagnostics Private Ltd. Gonwa, Vadodara, Gujarat, India), HDL-cholesterol kit (Reckon Diagnostics Private Ltd., Gonwa, Vadodara, Gujarat, India), LDL-cholesterol kit (Reckon Diagnostics Private Ltd., Gonwa, Vadodara, Gujarat, India.), distilled water (sourced from laboratory distiller).

2.1.3 Equipment

Digital photocolorimeter (EI Products, India Model No. 312), centrifuge (Medfield Equipment and Scientific Ltd., China, Model No. 80-2B), test tubes, syringes, haematocrit tubes, plain tubes, coagulant tubes, water bath (Jiangsu Zhengji Instruments Company Ltd., China, Model No. DK-8A), gulf electric balance (Gulf Scale, Sharjah, United Arab Emirate, Model No. AF-600).

2.2 Methods

2.2.1 Plant seed preparation

The shelled and the unshelled peanut (*A. hypogea*), as well as the bread fruit (*T. africana*) used in the study were processed by grinding mechanically until they became paste-like. They were weighed and stored in a clean and properly labeled containers. They were then transferred to the refrigerator until needed for experiment. Each animal received 10 g of the peanut (shelled or unshelled) or 10 g of the breadfruit paste (or a combination of 10 g each of both seeds), which were added to the animal feed (growers' mash).

2.2.2 Acclimatisation of animals

Twenty (20) adult female albino rats (Wistar strain) weighing between 140 – 200 g were used for the study. The animals were purchased from the animal facility, Department of Physiology, College of Medicine, University of Nigeria, Enugu Campus, Enugu State, Nigeria. The animals were housed in galvanised cages under good environmental conditions of 12/12 h light/dark cycle in cross ventilated rooms, with the home cages regularly cleaned. The animals were fed with pelletised commercial diet (Top growers' mash of the Premier Feeds Company Ltd., Enugu, Nigeria) purchased from Ogbette market, Enugu, Southeastern Nigeria. The peanuts and the breadfruit were incorporated into the growers' mash and water was administered *ad libitum*. The guidelines for the care and use of animals for research was strictly adhered to (NIH, 1991; NRC, 1996).

2.2.3 Experimental designs

Mature healthy female albino rats (Wistar strain) will be distributed into five (5) groups (1,2,3,4 and 5) with four (4) animals per cage. They will be acclimatised for seven (7) days in the laboratory and weighed individually prior to the study. The duration of the supplements will be 28-days. Group one (1), the control will receive growers' mash only. Groups 2,3,4 and 5 will be respectively administered growers' mash plus unshelled peanut (10 g), growers' mash plus shelled peanut (10 g), growers' mash plus roasted breadfruit (10 g) and unshelled peanut only (10 g) for 28-days. All the groups received growers' mash except group four (4). After the 28^{th} day of the experiment, the serum lipids (triglycerides − TG, total cholesterol – T-CH, high density lipoprotein – HDL and low density lipoprotein − LDL) assay will be performed to determine the blood lipid status of the individual animal in the various test groups.

2.2.4 Collection of blood samples

The blood samples of the animals were collected through a retro-orbital bleeding while observing caution to avoid haemolysis. The samples were then put into a non-coagulant tubes and spun at 1000 rpm for 10 min in order to separate the red cells from the serum.

2.2.5 Protocol for retro-orbital bleeding

i. Application of digital (right thumb) pressure to the external jugular vein (ejv) caudal to the mandible while gently elevating the upper eyelid with the index finger of the same hand.

ii. Insertion of the edge of a broken haematocrit tube into the conjunctiva of the mid-dorsal globe of the eye.

iii. Direction of the broken haematocrit tube into the caudo-medial (infero-medial) aspect of the mid-dorsal globe of the eye until blood begins to ooze.

iv. As soon as the required volume of blood is obtained, digital pressure on the ejv is released and the broken haematocrit tube removed.

2.2.6 Principles of Lipid Profile Tests

2.2.6.1 Triglyceride Assay: Glycerol-3-Phosphate Oxidase Method

$$\text{Triglycerides} + 3H_2O \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{Fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow[\text{Glycerokinase}]{} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow[\text{Glycerophosphate oxidase}]{} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-aminophenazone} + \text{4-chlorophenol} \xrightarrow{\text{Peroxidase}} \text{4-(p-benzoquinone-monoimino)-phenazone} + 2H_2O + HCl.$$

Assay Protocol-The estimation of serum TGs activity will be performed using the TG test kit (Reckon Diagnostics Private Ltd., Vadodara, Gorwa, Gujara, India) which will be purchased from Ogbette market, Enugu, Enugu State, Nigeria. The method earlier described by Fossati *et al.* (1982) and McGowan *et al.* (1983) will be employed in the study. Serum triglycerides will be enzymatically evaluated through a cascade of coupled reactions whereby triglycerides are cleaved hydrolytically to produce glycerol and fatty acids. The glycerol will be oxidised using glycerol oxidase, and hydrogen peroxide (H_2O_2), one of the reaction products, will be measured for cholesterol. Absorbance (Wavelength) reading will be measured at 505 nm.

Absorbance (Abs) = $\frac{\text{Abs of Test}}{\text{Abs of Std.}}$ x Conc. of Std. x Diluting factor

2.2.6.2 Total Cholesterol Assay: The Enzymatic Method

$$\text{Cholesteryl ester} + H_2O \xrightarrow{\text{Cholesteryl ester hydrolase}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholest-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{4-(p-benzoquinone-monoimino)-phenazone} + 4\,H_2O$$

Assay Protocol-The estimation of serum T-CH activity will be performed using the T-CH test kit (Reckon Diagnostics Private Ltd., Vadodara, Gorwa, Gujara, India) which will be purchased from Ogbette market, Enugu, Enugu State, Nigeria. The method previously described by Roeschlau *et al.* (1974) will used in the study. In this procedure, cholesterol will be evaluated enzymatically in the serum through a cascade of coupled reactions that hydrolytically split cholesteryl esters and oxidise the 3-OH group of cholesterol. Hydrogen peroxide (H_2O_2), a by-product of the enzymatic reaction will be measured quantitatively in a peroxidase catalysed reaction that generates a color. Absorbance will be measured at 505 nm. The intensity of the color that will be generated is directly proportional to the serum total cholesterol concentration.

2.2.6.3 High Density Lipoprotein Cholesterol Assay: Phosphotungstic Acid (PTA) Method.

Assay Protocol-The estimation of serum HDL-CH activity will be performed using the HDL test kit (Reckon Diagnostics Private Ltd., Vadodara, Gorwa, Gujara, India) which will be purchased from Ogbette market, Enugu, Southeastern Nigeria. The protocol previously described by Burstein *et al*. (1970) will be used in the study. High density lipoprotein can be separated from other lipoprotein fractions by using poly-ionic substances together with bivalent metal ion. HDL cholesterol will be separated from other lipoprotein fractions by the treatment of serum with a polysaccharide (PTA − phosphotungstic acid) and magnesium chloride. HDL cholesterol remains in solution (supernatant) while the rest of the lipoprotein fractions will be precipitated. Absorbance will be read at 505 nm wavelength.

$$\text{Serum} + \text{PTA reagent} \longrightarrow \text{HDL (Supernatant)} + \text{Other lipoprotein fractions (Precipitates)}$$

2.2.6.4 Low Density Lipoprotein Cholesterol Assay

The value of LDL-cholesterol will be determined mathematically using the Friedewald's equation provided the value of the TGs is known (Burstein *et al*., 1970).

LDL-CH = T-CH – [(HDL-CH) + (TG/5)]

The equation is valid only if the TGs value is normal or not very high (Burstein *et al*., 1970), and invalid if the LDL-CH is > 400 mg/dl (Contois, 2012).

2.2.7 Statistical Analysis

All data were expressed as mean±standard deviation (Mean±S.D.). Analysis of data was performed using the Student T-test. The level of statistical significance was accepted at 5 % ($P < 0.05$).

III. RESULTS

Table 1. Serum Levels of the Different Lipids Assay in the Unshelled Peanut Seed

Treatment group (Serum lipoprotein)	Growers' mash (Control)	Unshelled peanut (10 g)
TG	179.50±2.61	170.31±6.40*
TCH	162.58±1.44	162.83±0.46
HDL	116.50±2.69	117.45±1.68
LDL	10.17±3.95	11.31±2.26

Table 2. Serum Levels of the Different Lipids Assay in the Shelled Peanut Seed

Treatment group (Serum lipoprotein)	Growers' mash (Control)	Shelled peanut (10 g)
TG	179.50±2.61	185.32±1.39*
TCH	162.58±1.44	164.99±1.84
HDL	116.50±2.69	117.89±1.30
LDL	10.17±3.95	10.02±1.65

Table 3. Serum Levels of the Different Lipids Assay in the Roasted Breadfruit Seed

Treatment group (Serum lipoprotein)	Growers' mash (Control)	Roasted breadfruit seed (10 g)
TG	179.50±2.61	176.55±2.72
TCH	162.58±1.44	164.48±1.59
HDL	116.50±2.69	116.74±1.55
LDL	10.17±3.95	12.41±0.76

Table 4. Serum Levels of the Different Lipids Assay in the Unshelled Peanut Seed Alone

Treatment group (Serum lipoprotein)	Growers' mash (Control)	Unshelled peanut (10 g) Alone
TG	179.50±2.61	180.01±13.40
TCH	162.58±1.44	163.59±2.81
HDL	116.50±2.69	116.30±1.79
LDL	10.17±3.95	11.29±3.25

Table 5. Comparison of the Serum Levels of the Different Lipids Assay in Both the Unshelled Peanut and the Breadfruit Seeds.

Treatment group (Serum lipoprotein)	Growers' mash (Control)	Unshelled peanut (10 g)	Breadfruit seed (10 g)
TG	179.50±2.61	170.31±6.40*	176.55±2.72
TCH	162.58±1.44	162.83±0.46	164.48±1.59
HDL	116.50±2.69	117.45±1.68	116.74±1.55
LDL	10.17±3.95	11.31±2.26	12.41±0.76

IV. DISCUSSION

The study investigated the effects of roasted *T. africana* (bread fruit) and *A. hypogea* (peanut) seeds on the lipid profile status of mature adult female albino rats (Wistar strain). Lipid profile (lipid panel) is an aggregate term used when referring to the assessment of total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol, very low density lipoprotein cholesterol and the triglycerides. It is employed clinically in the identification of individuals with hyperlipoproteinaemia. Lipoprotein is an important marker for the development of cardiovascular diseases. The clinical management of dyslipidaemia (abnormally elevated

fats/lipids in the blood) is cardinal to the prevention of atherosclerosis and its manifestations *viz* coronary heart disease (CHD), ischaemic cerebrovascular disease and peripheral vascular disease (Mahley and Bersot, 2006; Gasko *et al.,* 2011). The measurement of LDL cholesterol and HDL cholesterol is pivotal to cardiovascular (CVS) disease risk assessment (Contois, 2012). There is a linear relationship between serum LDL cholesterol and the incidence of CHD. In a similar vein, there is an inverse correlation between HDL cholesterol and CHD. Prompt intervention with a manifold of medicines, e.g., HMG CoA reductase inhibitors (statins), fibric acid dervatives (fibrates), anion-exchange resins (bile acid sequestrants), and niacin (nicotinic acid) will decrease LDL cholesterol and ultimately lower the risk of CHD.

In this study, roasted *T. africana* and *A. hypogea* seeds did not significantly decrease the levels of LDL, HDL and T-CH in the experimental animals. However, a significant decrease in the level of the TGs was observed in the animals administered the unshelled peanut, thus suggesting that shelling of the roasted peanut probably increases the level of the triglycerides in test rats. In broad terms, these nutritional plants do not seem to possess any potential beneficial effects on reducing the risk of CVS diseases, since the T-CH, LDL and HDL levels were not altered significantly. Previous study on *T. Africana* for its hypolipidaemic activity by Ogbonnia *et al.* (2008) showed a decrease in the triglycerides, low density lipoprotein and increase in the high density lipoprotein. This was not demonstrated in this study. *T. africana* has been reported to have hypocholesterolaemic property because of its high content of saponin, which is believed to be hypocholesterolaemic (Ijeh, 2004). The seed extract of *T. Africana* has also been reported to significantly decrease the TGs and LDL cholesterol levels (Chukwu *et al.,* 1994; Akubor and Badifu, 2004). All these were not replicated in this study.

Arachis hypogea seeds have earlier been reported to provide a cheap source of high quality dietary lipids which are rich in mono- and polyunsaturated fats, and hence low in cholesterol thereby reducing the risk of CHD (Atasie, 2009). Experimental groups of animals whose feeds were replaced with 20 % peanut for at least five (5) weeks demonstrated a decrease in serum TGs, T-CH and LDL levels (Joan, 2010). *A. hypogea* was reported to be rich in plant protein, which helps to reduce the risk of CVS diseases and lower blood pressure (Joan, 2010). Fraser *et al*. (1992) suggested that peanut consumption reduces the risk of heart diseases by decreasing the serum LDL cholesterol level, thereby minimising the risk of development of Type II Diabetes mellitus. The assertions by these previous workers were not demonstrated in this study. *Arachis hypogea* significantly decreased the serum level of the triglycerides only, and thus did not produce any significant effect in the T-CH, LDL and HDL cholesterol levels.

V. CONCLUSION

The study, therefore, concluded that roasted *Arachis hypogea* possesses significant serum hypotriglyceridaemic activity, but lacks activity against total, low and high density lipoprotein cholesterol. *Treculia africana* on the other hand had no hypolipoproteinaemic activity.

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