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Histological changes and and serum lipid profile of selected rat tissues fed on *Cyperus esculentus* (tiger nut) tuber oil meal-based diet

[1]Bamishaiye E.I., [2]Muhammad N.O, [3]Bamishaiye O.M.*

[1] Nigerian Stored Products Research Institute, Kano. Nigeria
[2] University of Ilorin, Ilorin. Kwara State. Nigeria
[3] Federal College of Agricultural Produce Technology, Kan, Nigeria

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ABSTRACT

The effects of Cyperus esculentus (tiger nut) oil on lipoprotein composition, cholesterol and triglyceride levels and their histological changes were investigated in the brain, liver, kidney and heart of rats. Cyperus esculentus tuber oil was extracted from the tuber using a soxhlet extractor. Twenty, 3 weeks-old albino rats (Rattus norvegicus) with an initial average weight of 51.67±5.29g were grouped into two groups of ten (10) animals each. The first group was fed with soybean oil-based (control) diet; the second group on esculentus tuber oil meal-based diet for six (6) weeks. Histological examination of the selected tissues ((brain, liver, heart and kidney) showed no significant alteration in animals fed both the soybean oil and Tiger nut tuber oil fed animals. Generally, there was also no significant difference ($p>0.05$) in the serum lipid profile like concentration of Triglycerides, High Density Lipoprotein and Low Density Lipoprotein - cholesterol in rats fed on soybean based oil as compared to that of Tiger nut oil. However there was a significant decrease ($p < 0.05$) in the Total cholesterol of serum of rats fed with Tiger nut tuber oil-based meal. It was concluded that feeding cyperus esculentus tuber oil meal to rats might be safe on the lipid profile of rats.

Key words: Tiger nut, liver, kidney, soybean, oil

INTRODUCTION

Lipids are one of the main constituent of the diet. They are both a source of high energy and essential fatty acids (EFA) and are required for the absorption of fat-soluble vitamins [1]. Cardiovascular disease (CVD, is the leading cause of death in the world. Elevated concentrations of serum total cholesterol (TC), and LDL-cholesterol (LDL-C), have proved to be among the risk factors in the development of CVD [2]. Dietary fats play an important role in influencing blood lipid concentrations, thrombotic tendency and thus the onset of CVD [3]. Furthermore, high blood lipid levels, particularly total cholesterol (Tc) and low density lipoprotein cholesterol

(LDL-c) are usually related to promoting atherosclerotic syndrome. Hence interventions that lower these lipids in the blood can prevent the progression of the disease processes. Excess fat intake especially in the form of saturated fatty acids and cholesterol leads to the formation and deposition of lipids in various fatty tissues. Elevated serum concentrations of cholesterol in the total, LDL-C and VLDL-cholesterol as well as free fatty acids (FFA) have been shown to play a key role in contributing to the development of Coronary Heart Disease [4].

Tiger nut is cultivated in many countries of West Africa and a lot of people eat the tiger-nut without knowing the health benefits. Tiger nuts tuber have excellent nutritional qualities with a fat composition similar to olives and a rich mineral content, especially phosphorus and potassium. Tiger nuts are also gluten and cholesterol-free, and have very low sodium content [5]. The oil of the tuber was found to contain 18% saturated (palmitic acid and stearic acid) and 82% unsaturated (oleic acid and linoleic acid) fatty acids [6]. Research into lesser known plants is of very great importance as they can replace some of the beneficial expensive oil. Thus, the aim of this study was to assess the effect of tiger nut oil (an under-utilized crop) on cholesterol and triglyceride (TG) levels of the brain, kidney, liver and to reflect it on changes in histological data.

MATERIALS AND METHODS

Collection and preparation of seed sample: Dried tiger nut tubers used for this study was obtained from Hausa hawkers along Post Office in Ilorin, Kwara State, Nigeria and were identified as *Cyperus esculentus L* (tiger nut) by a taxonomist in the Department of Crop Science, Faculty of Agriculture, University of Ilorin, Nigeria. The seeds were screened to remove bad ones. The seeds were then dried to constant weight in an oven at 60 °C, milled using magic blender, SHB-515 model (made by Sorex company limited, seoul, Korea) put in air-tight containers and stored in desiccators for further analysis.

Oil Extraction

Lipid was extracted from the grounded Tiger nut using the soxhlet extractor as described by Folch *et al.* [7]. Chloroform: methanol (2:1 v/v) mixtures was employed for the extraction in order to extract an appreciable quantity of both the polar and non polar in the sample, this implies that all lipids are extracted with impurities and needs to be purified.

Purification of the extracted oil

The purification was done by employing the method of Folch *et al.* [7].

Feed Formulation: The purified tiger nut oil was used as a source of fat in the formulation of animal feed as shown in Table 1.

Animal Management: twenty (20) 3 weeks-old albino rats of both sexes with an initial average weight 51.67±5.29 g were randomly assigned into two (2) dietary treatment groups . Each treatment had two replicates with five animals per replicate. The animals were weighed prior and allowed to acclimatize to the laboratory environment for one week before the commencement of the feeding trial. The animals were supplied feed and water *ad libitum* and weighed (on weekly basis) for six (6) weeks. Group feeding was done to ensure animals in all group were subjected to the same conditions. Each group of rats was housed in a metal cage at room temperature. The rats were all fed their respective feds daily, weighed weekly and sacrificed at the end of the 6th week by anaesthesing them in a jar containing cotton wool soaked in diethyiether and disserting them quickly.

Table 1: Composition of diets g/kg

	Soybean oil	Tiger nut oil
Corn starch	476	476
Soybean	250	250
Rice bran	40	40
D-L methionine	4	4
Sucrose	100	100
Vitamin mix	50	50
Tiger nut oil	-	80
Soybean oil	80	-

Vitamin mix(per kg of diet): thiamin hydrochloride, 6mg; pyridoxine hydrochloride 7mg; nicotinic acid 30mg; calcium pantothenate 16mg; folic acid 2mg; biotin 0.02mg; cyanocobalamin 0.01mg, retinol palmitate 4,000u, cholecalciferol 1000u; tocopherol acetate 50u, menadione 0.05mg; choline chloride 2g.

They were weighed weekly and at the end of the experimental feeding period, were sacrificed by anaesthetizing with (cotton wool soaked in) chloroform. They were then quickly dissected to excise the brain, liver, kidney, and heart. The kidney was decapsulated. Serum samples were separated from the clot by centrifugation at 3000 g for 5 min using bench top centrifuge (MSE Minor, England). Serum samples were separated into sterile plain tubes and stored in the refrigerator for analyses. All analyses were completed within 24 h of sample collection.

Chemicals and Reagents

All reagents and chemicals that were used in this work were of analytical grade.

Analysis of Lipoproteins Determination of total and free cholesterol in the serum was by the method of Searcy and Bergquist [8]. Serum triglyceride (TG) was determined using the method of Tiez [9] after enzymatic hydrolysis by lipases LDL and HDL-cholesterol were determined according to the methods described by Friedwald *et al* [10].

Preparation of samples for histopathology

The brain (cerebral cortex), liver and kidney were quickly removed from sacrificed rats after which the kidney was decapsulated. They were cleansed of blood using 0.25M sucrose solution. They were then fixed separately in 10% formalin solution before being taken to the Chemical Pathology Department, University of Ilorin Teaching Hospital, Ilorin, Nigeria, where the histopathology was carried out.

Histological procedures

Fixed tissues were dehydrated through ascending grades of ethanol to absolute ethanol. They were cleaned in xylene, impregnated and embedded in paraffin wax (melting point 56^{o}C). Sections were cut at 5mm on a rotatory microtome. They were flattened on warm water and mounted onto albumerised slides and dried overnight. The sections were dewaxed in xylene and hydrated through descending grades of ethanol to water. They were initially stained in Harris haematoxylin and differentiated in acid alcohol and thereafter stained with methylene blue. They were then dehydrated in 95% alcohol, stained in 10% alcohol eosin, dehydrated in absolute alcohol, cleaned in xylene and mounted in Canada balsam. The resulting slides are then viewed under the light microscope. The photomicrographs were printed at a total magnification of x400.

Statistical analysis

Statistical analysis of data was determined with the use of Standard student's 't'-test method and $P < 0.05$ were regarded as significant. The group data are expressed as mean ± SD.

RESULTS AND DISCUSSION

Studies have shown that the amount of dietary fat intake positively correlate with serum total cholesterol value and morbidity from coronary heart disease [11]. However, a number of studies have demonstrated that the fatty acid composition of food was more associated with variations in the plasma total cholesterol concentration and development of atherosclerosis than the amount of fat consumed [12, 13]. The general picture is that saturated fatty acids increase plasma total cholesterol level and thus increase the risk of coronary heart disease, while unsaturated fatty acids have the opposite effect. Table 2 shows the effects of Tiger nut oil based diets and soybean oil based diet on the serum lipid level of rats fed with these diets for the period of six weeks. There was no significant difference ($p>0.05$) in the triacyglycerides, high density lipoprotein and low density lipoprotein cholesterol, but the numerical values were a bit higher in soybean based oil as compared to that of Tiger nut oil. However there was a significant decrease in the total cholesterol of serum of rats fed with Tiger nut tuber oil-based

Table 2: serum lipid profile (mmol/L) of rats placed on soybean oil-based and Tiger nut oil-based diets for 6 weeks

Parameter	Soybean oil	Tiger nut oil
Total cholesterol	2.55 ± 0.11^{a}	2.05 ± 0.06^{b}
Triacylglycerides	0.24 ± 0.03^{a}	0.18 ± 0.04^{a}
HDL-C	0.21 ± 0.01^{a}	0.18 ± 0.01^{a}
LDL-C	1.55 ± 0.07^{a}	1.58 ± 0.04^{a}

Each value is a mean of three determination ±SEM
Values with the same superscripts across the same row are not significantly different (P>0.05).

The non-significant difference in the triacyglycerides, high density lipoprotein and low density lipoprotein cholesterol with a decrease in the cholesterol level indicates that both seed oils did not support the build up of cholesterol that may cause plaques in blood vessels. This may not be unconnected with the fact that the animals were consuming a high amount of unsaturated fat (oleic acid in tiger nut oil) in their diet whose role is to maintain cellular integrity. This confirms the report of Osagie and Eka [14] that tiger nut reduces “bad” cholesterol (LDL-cholesterol) and increases the “good” one (HDL-cholesterol) and also reduces levels of triglycerides in blood. Cholesterol is an important part of the cell membranes and is important to structure and function of body cells [15].

Cholesterol is abundant in the liver, adrenal glands as well as the brain and the nervous system [16]. Liver can synthesize sufficient cholesterol for normal body functions and it is carried in the blood in form of lipoproteins [16]. High concentrations of all lipids, except the HDL-C are associated with an increased risk of artherosclerosis [17]. Triglycerides are the chemical form in which most fat exists in food as well as in the body. They're also present in blood plasma and, in association with cholesterol, form the plasma lipids.

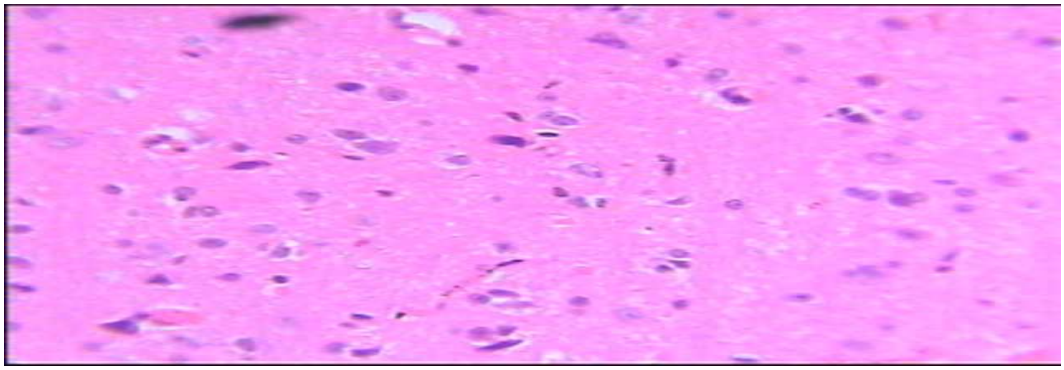

Plate 1: Photomicrograph (x400) shows the brain of rats placed on soybean oil based diets for 6 weeks (normal brain tissue)

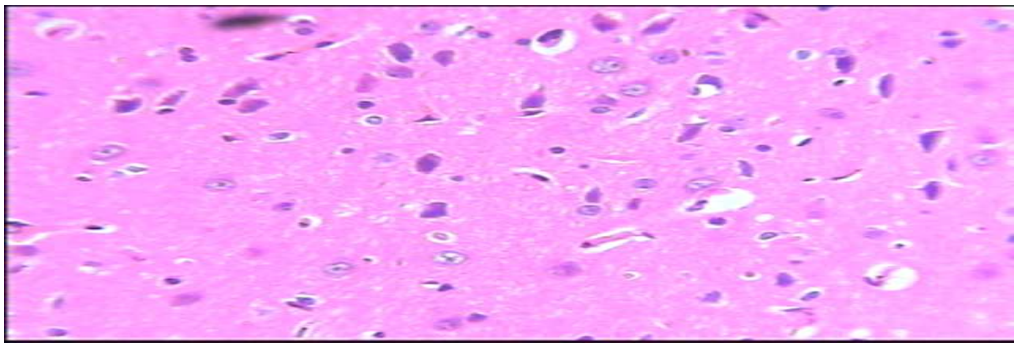

Plate 2: Photomicrograph (x400) show the brain of rats placed on *Cyperus esculentus* tuber oil based diets for 6 weeks (no visible microscopic change)

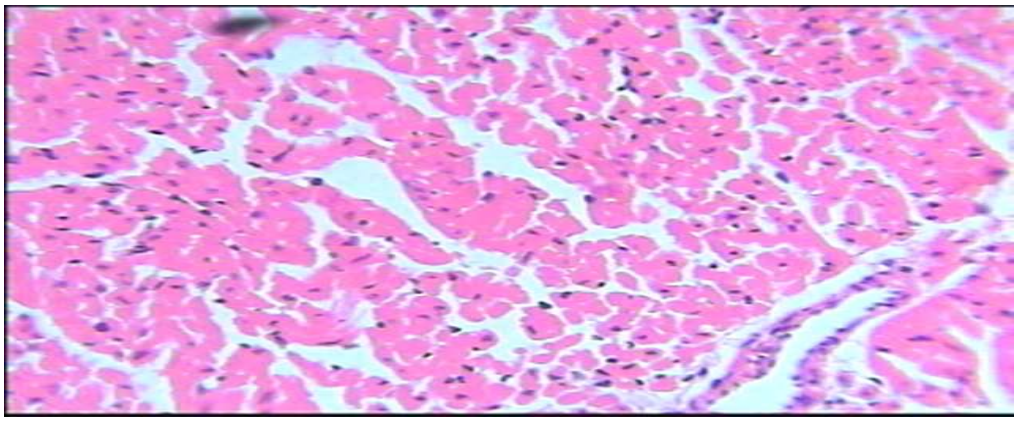

Plate 3: Photomicrograph (x400) show the heart of rats placed on soybean oil based diets for 6 weeks (Normal heart, normal cardiac muscle)

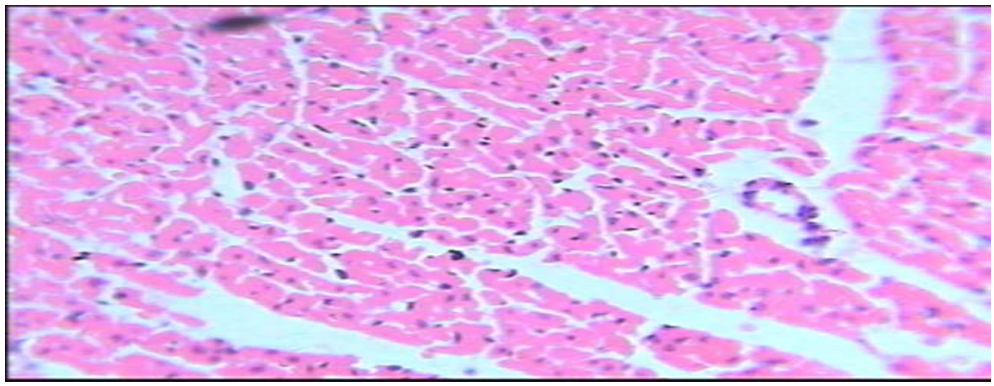

Plate 4: Photomicrograph (x400) show the heart of rats placed on *Cyperus esculentus* tuber oil based diets for 6 weeks (no visible difference from the control)

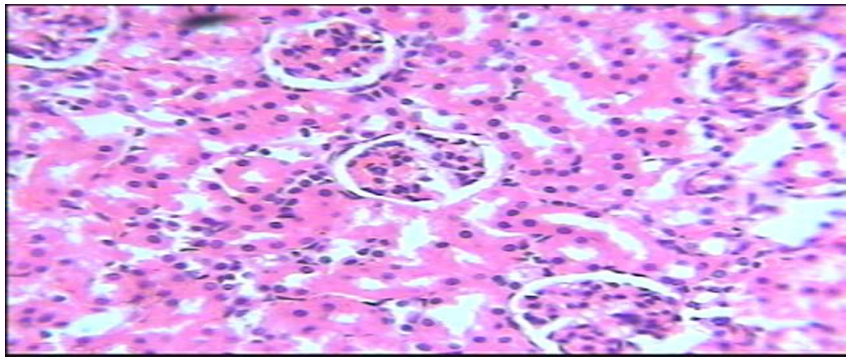

Plate 5: Photomicrograph (x400) show the kidney of rats placed on soybean oil based diets for 6 weeks (normal glomeruli and tubules)

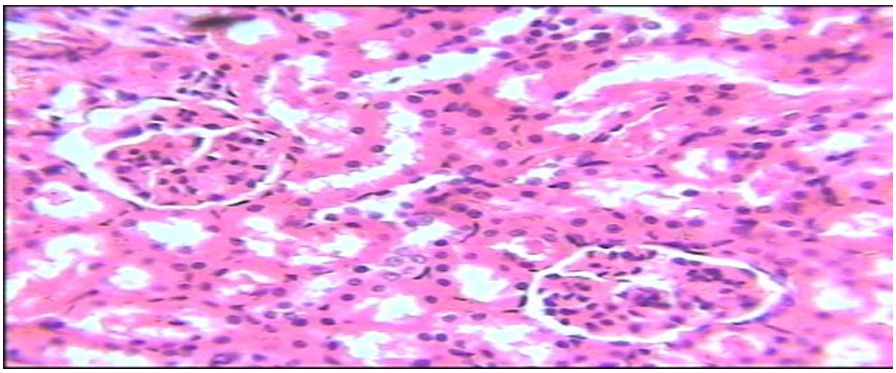

Plate 6: Photomicrograph (x400) show the kidney of rats placed on *Cyperus esculentus* tuber oil based diets for 6 weeks (no visible microscopic change)

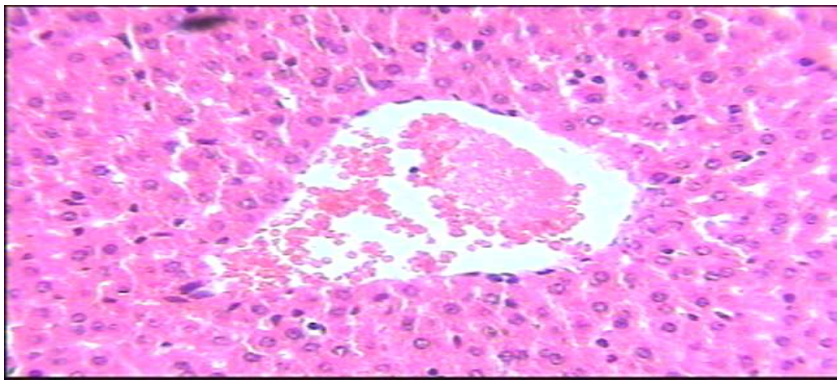

Plate 7: Photomicrograph (x400) show the kidney of rats placed on soybean oil based diets for 6 weeks {normal liver, normal central vein and radiating hepatic plates (cords)}.

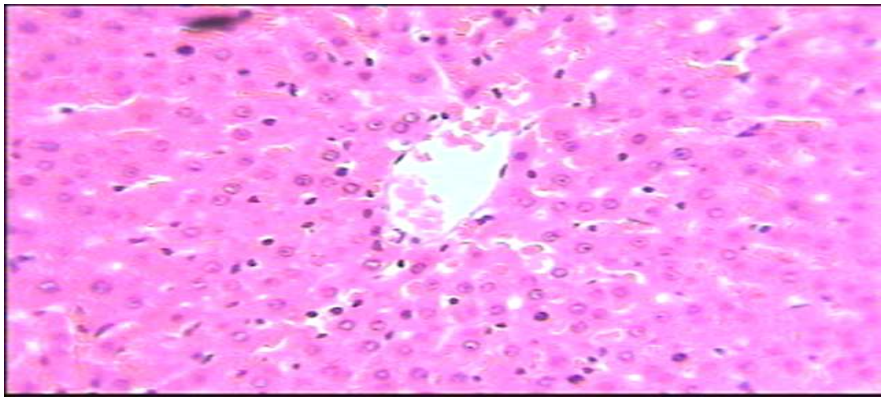

Plate 8: Photomicrograph (x400) show the liver of rats placed on *Cyperus esculentus* tuber oil based diets for 6 weeks (no visible abnormalities (normal liver architecture)

Conclusively, the use of tiger nut oil as edible oil as an oil especially in regions with high fat diets as staples is strongly recommended as it does not support the accumulation of toxic fats in vessels because of its high content of essential fatty acids and its positive health benefits on serum lipids.

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