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Serum Lipid Profile and Organ Histology of Albino Rats Fed on Conophor (*Tetracarpidium conophorum*) Nut Oil-Based Diets

Justina Y. Talabi[1], Oladotun O. Oguntoyinbo[2] and Victor N. Enujiugha[3*]

[1]Department of Human Nutrition and Dietetics, Afe Babalola University, Ado-Ekiti, Nigeria.
[2]Department of Food Science and Technology, Lagos State University of Science and Technology, Ikorodu, Nigeria.
[3]Department of Food Science and Technology, Federal University of Technology, Akure, Nigeria.

*Correspondence: vnenujiugha@futa.edu.ng Tel.:+234(0)8034261870

Abstract

The effect of feeding Albino rats with diets composed of oils extracted from raw and processed conophor nuts on the blood serum lipid profile and organ histology were evaluated in this study. Freshly harvested conophor nuts were processed by cooking (at 100 ºC for 90 minutes) and toasting (at 145 ºC for 50 minutes). Oils extracted from the raw and processed nuts and commercially available soybean oil were fed to four groups of albino rats (each group consisted of five rats) over an experimental period of twenty-eight days after acclimatization. The serum lipid profile [Total cholesterol (TC), α-Lipoprotein (HDL-C), β-Lipoprotein (LDL-C) and Triglycerides (TG)] of the blood obtained from the animals upon sacrifice was determined. Organs (heart and liver) excised from the animals were subjected to histological assay. The serum lipid profile results showed that the parameters were within safe limits. Photomicrographs obtained from the histopathological assay showed that organs from the animals fed on oil from cooked conophor maintained normal histology. Oil from cooked conophor samples gave the best biochemical results compared to oils from both raw and toasted nuts with respect to the effects of consumption on the serum lipid biomarkers and organ histology.

Keywords: *Conophor nut oil, processing, animal assay, serum lipids, histopathology*

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Introduction

The conophor plant [*Tetracarpidium conophorum* Müll (Arg) *Euphorbiaceae*], commonly called the African walnut is a perennial climbing shrub found in the forest zones of sub-Saharan Africa (Enujiugha, 2003). The economic importance of the species lies in edibility of its oil-rich seeds, which are consumed by various populations in Nigeria, Sierra Leone and the lower Congo region (Enujiugha, 2008). The seed is made up of two cotyledons enclosed in a hard brown shell-like case within the pods (Nkwonta *et al*., 2010). The oil content of raw conophor nut is 49.18% (Enujiugha and Ayodele-Oni, 2003), although a previous study showed that the seed oil did not perform acceptably as a frying oil for potato, plantain and yam chips (Oyinloye and Enujiugha, 2017). Conophor nut oil has high iodine value making it good for soap making but less effective as a drying oil. However, Oladiji *et al*. (2010) reported that *Tetracarpidium conophorum* oil did not adversely affect growth performance and the feeding appetite of experimental rats.

Disposition of blood pressure and coronary heart disease has been found to be in string correlation with lipid profile particularly with blood cholesterol level (Cotran, 1999). Total lipid profile of an individual is a contributed Principe resulting from blood cholesterol along with its associated verities of lipoproteins i.e. high-density lipoproteins (HDL, or α-lipoproteins) low density lipoproteins (LDL, or pre-β-lipoproteins) and triglycerides (Iftekhar *et al*., 2006). Many investigators have pointed out that excessive intake of dietary saturated fat and especially cholesterol increases the serum cholesterol, thus leading to a high risk of cardiovascular diseases (Ye and Kwiterovich, 2000).

With the increased interest in the exploitation and evaluation of less-common oilseeds as potential solution to the problem of fat-related cardiovascular diseases, it is important to examine the physicochemical characteristics and in-vivo lipid profile of oil from raw and processed conophor nut in order to ascertain its potential health and commercial status using conclusions drawn from the concentrations of the polyunsaturated fatty acids, blood serum lipid [Total Cholesterol (TC), High Density Lipoprotein-Cholesterol (HDL-C), Triglycerides (TG), Low Density Lipoprotein-Cholesterol (LDL-C)] profile and concentrations of the sterols that may be present.

Materials and Methods

Serum Lipid Profile Determination

Chemicals

Kit reagents for total cholesterol, triglycerides and high density lipoprotein cholesterol were obtained from a chemical store in Idumota, Lagos State, Nigeria. Dissecting tools and anaesthesia (trichloromethane) were obtained from the Department of Biochemistry, Federal University of Technology, Akure, Nigeria.

Animals

Twenty albino rats weighing between 68.27 g and 115.13 g were obtained from a private rats weaning outfit near the University of Ibadan, Oyo State,

Nigeria and acclimatized for five days prior to the experiment. They were assigned to four groups and housed in netted wooden cages at room temperature 25±2 ºC. The animals received conophor oil based pastry and water *ad libitum* for an experimental period of twenty-eight days. The study received approval from the Ethics Committee (FUTA/SAAT/ETH/2018.13).

Experimental Diet Formulation
The experimental diets were prepared by modifying the standard methods described by Oyetayo (2006). The compositions of the diets are shown in Table 1. After a thorough homogenization of the ingredients, the mixture was baked in an oven at a temperature of about 110 ºC for 60 minutes.

Experimental Design
The animals were housed in groups of five rats each and divided into the cage cells using the method of Bobadoye *et al*. (2016). The first group (O) served as the control and was treated with soybean oil based pastry. Groups 'C', 'R' and 'T' consumed the Cooked, Raw and Toasted conophor oil based pastry respectively (a modification of the method suggested by El-Banna *et al*. (2009). The treatment extended for a period of twenty eight days.

Sample Collection
The animals were starved overnight for 12 hours before the blood was collected. Rats were anaesthetized with cotton wool treated with trichloromethane ($CHCl_3$), and venous blood samples were collected by direct heart puncture in sterilized vials and ejected into EDTA bottles. The blood samples were centrifuged at 4,000 rpm for 10 minutes at 25 ºC and serum was recovered.

Table 1: Composition of Experimental Diets

Ingredients	Diet groups			
	1	2	3	4
Casein (mg)	100	100	100	100
Oil (10ml)	O	C	R	T
Wheat flour (g)	60	60	60	60
Vitamin/mineral mix (mg)	50	50	50	50
Water (ml)	45	45	45	45

C, R and T= Oils from cooked, raw and toasted conophor nuts, respectively. O= oil from control.

Serum Lipid Assessment
The levels of serum Total Cholesterol (TC), High Density Lipoprotein-Cholesterol (HDL-C) and Triglycerides (TG) measurement were performed at the Food Chemistry Laboratory, according to GPO-PAD method, CHOD-PAP and Enzymatic end point methods respectively i.e. the manufacturer's procedures in the kit reagents. Low density Lipoprotein (LDL-C) was calculated according to the methods of Friedewald *et al.* (1972).

Determination of blood cholesterol
Reagent blank and standard were prepared by mixing 10 µl distilled water and 10 µl standard respectively each with 1000 µl of reagent. The three aliquots were separately mixed and incubated for 10 minutes at 25 ºC. The absorbance of the samples (A_{sample}) against the reagent blank was measured within 60 minutes at 500 nm-wavelenghth.
Conc. of cholesterol in sample = $(\underline{\Delta A_{sample}}/\Delta A_{standards})$ x (conc. of standard)
Or Conc. of cholesterol in samples = 553 xΔA (mgldl)

Determination of HDL-Cholesterol
Precipitation: About 500 µl of samples and standard each was separately mixed with precipitant. The mixtures were allowed to stand for 10 minutes at room temperature and then centrifuged for 10 minutes at 4,000 rpm. The clear supernatant was separated off within two hours and the cholesterol content was determined by the CHOD-PAP methods i.e. 100 µl each of reagent blank, standard and sample were separately mixed with 1000 µl of reagent. The three aliquots were incubated for 10 minutes at 25 ºC. The absorbance of the sample (A_{sample}) and standard ($A_{standard}$) were measured against the reagent blank within 60 minutes at 500 nm wavelength.

Concentration of HDL cholesterol = $(\underline{\Delta A_{sample}}/\Delta A_{standards})$ x (conc. of standard)
Or HDL-C = 180 x ΔA samples (mgldl)

Determination of triglycerides
Ten microliter (10 µl) each of standard and sample were separately mixed with 1000 µl of reagent. The mixtures were incubated for 10 minutes at 25^{O}C. The absorbance of the sample (Asample) and standard (Astandard) were measured against the reagent blank within 60 minutes at 500 nm wavelength
Triglyceride concentration = $(A_{sample}/A_{standards})$ x (conc. of standard)
Or Triglyceride concentration = A_{sample} x 1048 mgldl

Determination of LDL-C
The Low Density Lipoprotein-Cholesterol was calculated according to the method of Friedewald *et al*., (1972).
LDL-Cholesterol = Total cholesterol – Triglycerides – HDL-Cholesterol

Histological Examination of Experimental Animals' Organs
Histopathological assay of the organs (heart and liver) excised from the animals fed with raw and processed conophor oils was determined by reading the photomicrographs obtained from the slides prepared from the organs by a Pathologist at the Veterinary Anatomy Department, University of Ibadan, Nigeria using the standard methods described by Bancroft and Stevens (1990).

Procedure: The tissues pieces were fixed in a suitable fixature, typically formalin and embedded in melted paraffin wax. The wax block was then cut on a microtome to yield a thin slice of paraffin containing the tissue. The specimen slice was then applied to a microscope slide, air dried and heated to cause the specimen to adhere to the glass slide. Residual paraffin was then dissolved with usually followed by rising with an acid-alcohol followed by rinsing with water to remove the acid alcohol. The hematoxylin was blued by the bluing solution. The bluing solution was removed by rinsing with water. Other cytoplasmic elements were stained with an alcoholic solution of eosin Y, a red stain and a light green. The excess stain was removed by water in series of sequential washes in a dehydrating reagent. The slide was contacted with a chemical clearing agent (toluene, xylene or t-butanol) to remove residual dehydrating reagent remaining from the washing step. A cover slip mountant was applied to the slide after removing the slide from a chemical clearing agent. The clearing agent evaporates and the mountant hardens leaving a stained and mounted slide. Histopathological assessment and photomicrography of the prepared slides was done by a pathologist using an Olympus light microscope with attached Kodak digital camera.

Results and Discussion

Serum lipid profile of blood obtained from albino rats after 4-week consumption of experimental conophor oil diets
The results of the serum lipid profile (*in vivo*) are shown in Table 2. The values obtained for each of the parameters did not exceed the safe limits of US guidelines for serum lipid profile. Highest blood cholesterol level was recorded for the animals that fed on oil from cooked conophor nuts. Since the elevation of serum cholesterol, triglycerides and β-lipoprotein are of health significance, oil from raw conophor nuts would be better for consumption.
The total blood cholesterol (TC), Triglycerides (TG), High Density lipoprotein Cholesterol (HDL-C) and Low Density Lipoprotein Cholesterol (LDL-C) values ranged from 81.73-134.80, 24.76-187.76, 53.05-53.73mg/dl, respectively. The values conform and are within the <240 mg/dl (TC), <200 mg/dl (TG), ≥10 mg/dl (HDL-C) and <160 mg/dl (LDL-C) recommended for healthy individuals by the US guideline on health (mnuqol.com/blood-lipid-profile.htm); as such, none of the experimental animals have the tendency to develop hypercholesteromia and other serum-lipid induced/related health issues.

Table 2: Serum Lipid Profile of Albino rats after 4 week consumption of Experimental *T. conophorum* Oil Diets (mg/dl).

Parameters	Cooked	Toasted	Raw	Control	US guideline
Cholesterol	134.80±9.70^{a}	120.80±0.00^{ab}	92.80±9.70^{b}	81.73±19.63^{bc}	<240
Triglyceride	54.49±8.58^{a}	187.76±7.43^{b}	24.76±4.29^{c}	71.82±4.29^{a}	<200
HDL-C	53.17±0.13^{a}	53.17±0.07^{a}	53.73±0.07^{b}	53.05±0.15^{a}	≥ 10
LDL-C	70.61±8.96^{a}	29.81±1.08^{b}	34.11±10.23^{b}	14.19±20.14^{b}	<160

Values in the table are means of three determinations ±SD. Means in the same row with different superscripts were not significantly different (p<0.01).

From the effects of blood lipid profile on health standpoint, the results are better than those reported by Ighotsu and Tonukari (2010). The higher value obtained in the literature mentioned may possibly be as a result of consumption of various oils in diet. The values reported for the parameters compare favourably with 145.2-155.5 mg/dl (TC); 87.3-95.4 mg/dl (LDL-C); 37-41.9 mg/dl (HDL-C) and 80.2-112.2 mg/dl (TG) reported by Abubakar *et al.* (2009) on the Relation of Body Mass Index with Lipid Profile in Healthy female of Lower socioeconomic group in Kaduna, Northern Nigeria. On the

effect of Garlic consumption on Blood Lipid, results obtained compare well (but for cooked) with the results obtained by El-Banna *et al.* (2009). Yeo and Su (2000) reported that a statistically significant association occurs between elevated serum Total cholesterol and Low Density Lipoprotein Cholesterol (LDL-C) and the severity of Retinal hard exudation in patients with diabetic retinopathy. In the same study, Yeo and Su (2000) reported a literature support that oedema and hemorrhage may result from the incorporation of triglycerides into the cell membrane leading to changes in membrane fluidity and leakage of plasma constituents into the retina. Elevated levels of Triglyceride, Cholesterol and LDL-C are documented as risk factors for atherogenesis. LDL-C in its oxidized or acetylated form has been identified as a major atherogenic particle; as it not only load macrophages with cholesterol for the formation of foam cells but also because it is chemotactic for circulating monocytes, is cytotoxic and can adversely alter coagulation pathways (Abubakar *et al.*, 2009). The blood level of HDL-C in contrast bears an inverse relationship of the risk of atherosclerosis and coronary heart disease that is, higher the level, smaller the risk (Witztum and Stemberg, 1991; Palinski *et al.*, 1989). Literature reveals that correlations exist between Serum lipid profiles and sperm parameters. Vignon *et al.* (1989) found that increased triglyceride have deleterious effects on spermatogenesis. In like findings, Erqun *et al.* (2007) investigated the correlations of semen parameters with lipid concentrations among 18 infertile men. Their results showed that increase of very Low Density Lipoprotein (LDL-C) and Triglyceride were significantly correlated with decreased sperm motion characteristics. Mohammed *et al.* (2009) found out that triglyceride above normal level was related with abnormal sperm morphology and motility.

It is expected that the serum lipid profiles of the animals fed with oils from the toasted conophor nuts would be free of total serum cholesterol, but it was still present and this might be due to the presence of desmosterol which is a precursor of cholesterol (Bourre *et al*., 1990).

Histological assay of organs from albino rats after 4-week consumption of experimental *T. conophorum* oil diets

The results of histological examination of the organs excised from the albino rats that fed on samples of conophor oil experimental diets over a four-week period are shown on Figures 1 and 2. The summary of the conclusions drawn from the photomicrographs are shown in Table 2. The photomicrographs were magnified (x400) to show the conditions of the cell vacuoles and hepatocytes. Cooked conophor-related diets gave the safest results.

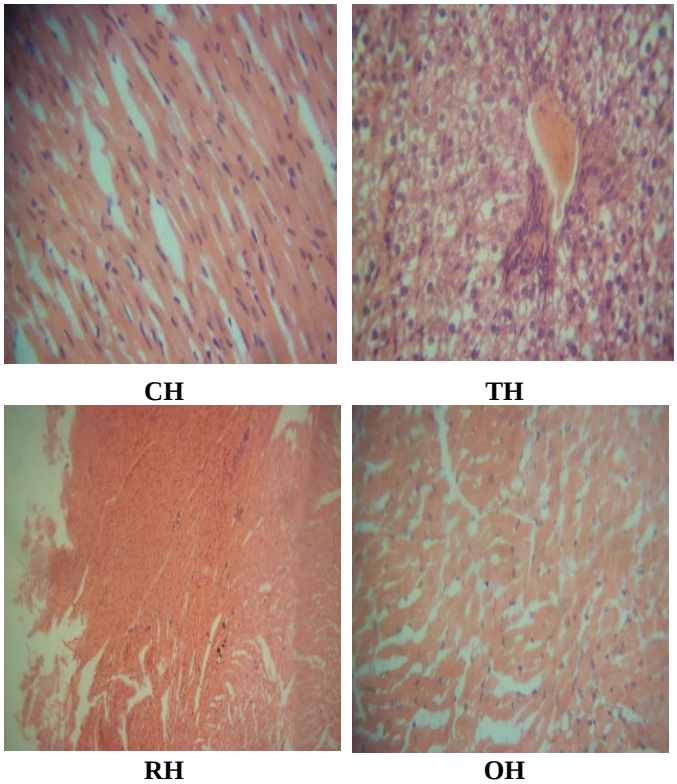

Figure 1: Histological Examination of Heart (photomicrographs) (x400 mag.).

The histopathological study showed that the hearts do not have any visible lesion (Figure 1). There were no inflammatory or degenerative conditions seen in the histological structure of organs excised from animals that fed on oil from cooked conophor. This is due to the fact that the oils do not contain toxic polycyclic aromatic hydrocarbons (PAHs) and dioxin (polychlorinated dibenzo-p-dioxin and polychlorodibenzo furans, a family of polychlorinated tricyclic aromatic compounds) which are documented for their mutagenic or carcinogenic potential (Shastry *et al.*, 2011). The results obtained during the heart histology also show that the PUFAs in the oils are not thermal stressed and so cytotoxic aldehyde products which could promote the induction, development and progression of cardiovascular diseases are not generated (Quiles *et al.* 2002).

From the summary of the histopathology (Table 3), only the liver obtained from the animals that fed on oil from cooked conophor (CL) maintained a normal liver with normal hepatic chord arrangement. The liver of animals that were fed on the toasted conophor oil sample (TL) showed a form of cell sickness as evidenced by the presence of numerous lipid vacuoles in the cytoplasm which is a result of the cells inability to metabolize and dispose lipids. There is also severe vacuolar degeneration. Like TL, OL also showed some vacuolar degeneration though milder than TL. No necrotic and fibrotic changes were seen in the liver tissue histology. There is a very severe vacuolar degeneration of hepatocytes in RL. This slide also shows hepatic necrosis (cell death) at the periportal area around the blood supply channels. The occurrence of hepatocytes necrosis as induced by the consumption of oil from the raw conophor is an evidence of hepatitis (Nwaopara *et al*., 2007). This evidence shows that the raw conophor seeds possess some chemical and pharmacological properties similar to the classes of drugs that are capable of inducing liver damage and thus explains their capability to effect the histological changes observed. The presence of 5-α cholestane in the oil may be related to the vacuolar degeneration. The severity of the degeneration correlates with the levels of 5-α cholestane in the oils. The higher the levels of 5-α cholestane, the higher the damage to the liver as noticed in the photomicrographs. There is established evidence that 5-α cholestane is harmful. Although there is a limited evidence of a carcinogenic effect, there is however a danger of serious damage to health by prolonged exposure through inhalation and if swallowed. Absence of vacuolar degeneration and the normal hepatic cord arrangement in the liver histology of animals fed on oil from cooked conophor may be due to the reduced levels of 5-α cholestane.

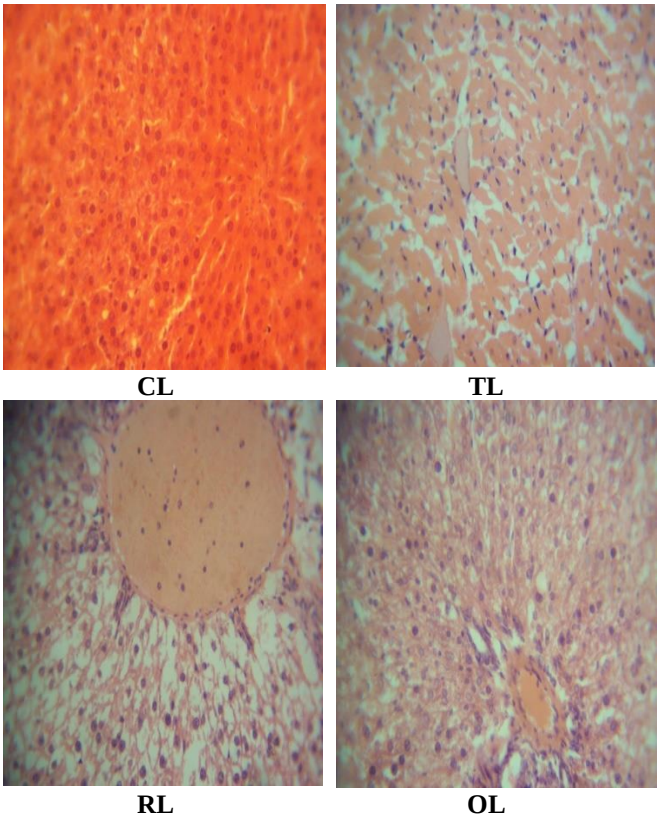

Figure 2: Histological Examination of Liver (photomicrographs) (x400 mag.).

Table 3: Summary of Histopathological Assay of Organs from Albino Rats after 4 Week Consumption of Experimental *T. conophorum* Oil Diets

Slides	Report
CH:	Cardiac (Heart) muscle: No visible lesion seen. There was no inflammatory or degenerative condition seen in the histological structures.
TH:	Cardiac (Heart) muscle: No visible lesion seen, there are traces of inflammation.
RH:	Cardiac (Heart) muscle: No visible lesion seen, there are traces of inflammation.
OH:	Cardiac (Heart) muscle: No visible lesion seen, there are traces of inflammation.
CL:	Liver: Normal liver with normal hepatic chord arrangement.
TL:	Liver: There is severe, diffuse hepatic vacuolar degeneration. The hepatocytes have a form of cell sickness characterized by inability of the cells to metabolize and disperse lipids. Thus there are numerous lipid vacuoles in the cytoplasm. This might further reduce the metabolic efficiency of the liver cell. It is reversible if the inciting cause is removed. Otherwise it may lead to cell death and inflammation of the hepatocytes later.
RL:	Liver: Very severe form of vacuolar degeneration of the hepatocytes. There are also fewer areas of hepatic necrosis (cell death) at the periportal area (around the blood supply channels).
OL	Liver: There is also vacuolar degeneration here. Periportal and extensive. Just a bit milder than **TL.**

Conclusion

This study examined the serum lipid profile and organ histology (heart and liver) of albino rats of Wistar strain fed on raw and processed (cooked and toasted) conophor nut oil-based diets. The findings reveal that oil from cooked conophor samples gave the best biochemical results with respect to the effects of consumption on the serum lipids and organ histology.

Declarations

Competing Interest

The authors declare no competing interest.

Authors' Contributions

All listed authors contributed equally to the literature writing, review, research process and editing of this article.

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