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Nutritional and Toxicological Implications of Drinking Heat-treated Fresh *Raphia hookeri* Sap in Rats

***Ibegbulem, C.O.[1], Eyong, E.U.[2] and Essien, E.U.[2]**

[1]Department of Biochemistry, Federal University of Technology, Owerri, Nigeria.
[2]Department of Biochemistry, University of Calabar, Calabar, Nigeria.

ABSTRACT: Nutritional and toxicological implications of drinking heat-treated fresh *Raphia hookeri* sap (HTF*Rh*S) in place of water were studied in rats. Seven to eight weeks old healthy albino *Rattus norvegicus* of the Wistar strain of both sexes (n = 8 per group) that weighed 57.46±0.91 g were used. Test rats drank HTF*Rh*S (40.10 mg/ ml, pH 6.36±0.00 at 29.37±1.0^{o}C) for 35 days in place of water after heating it to 85^{o}C (to halt fermentation and distill off pre-formed ethanol) and cooled. Control rats drank only tap water. Body weights, feed and fluid intakes, organ (liver, kidneys, spleen and heart) weights, haematological parameters (like haemoglobin, Hb; packed cell volume, PCV; mean corpuscular haemoglobin concentration, MCHC; white blood cells, WBC), liver function indices and serum lipid profile were evaluated at the end of the study. HTF*Rh*S did not contain ethanol. When the HTF*Rh*S was administered to the rats, there was no significant difference ($p>0.05$) between the HTF*Rh*S-treated rats and control rats for feed conversion ratio (FCR), haematological parameters and organ weights. Serum AST and ALT activities however increased significantly ($p<0.05$) on consumption of HTF*Rh*S. Serum AST/ALT ratios showed that HTF*Rh*S was more hepato-toxic to the male rats (ratio: 0.98±0.05) and more cardio-toxic to the female rats (ratio: 1.05±0.04). Serum albumin/ globulin ratios of HTF*Rh*S-treated rats increased significantly ($p<0.05$) presenting a symptom of xanthomatosis. Serum [LDL-cholesterol] of HTF*Rh*S-treated rats increased while their serum [HDL-cholesterol] decreased. Their serum total cholesterol/HDL-cholesterol and LDL-cholesterol/HDL-cholesterol ratios indicated atherogenicity; with the female rats being more ($p<0.05$) susceptible. In conclusion, the heat-treated fresh palm sap was not safe when drunk in place of water.

KEYWORDS: Heat-treatment, nutritional, *Raphia hookeri* sap, rats, toxicological, water

1.0 Introduction

Raphia **(**Raffia palm) is a genus of twenty species of palms native to tropical regions of Africa, Madagascar, Central and South America that grow up to 16 m tall with remarkable compound pinnate leaves which are the longest in the plant kingdom; leaves of *R. regalis* being up to 19.81 m long and 3 m wide (Uhl and Dransfield, 1987).). The African continent has a long tradition of palm wine production from *Elaeis guineensis* (African oil palm), *Hyphaene* spp (doum palm), *Raphia* species (raffia palm) as well as *Phoenix redinata* (Senegal date palm) (FAO, 1998a). In Nigeria, the trunk of African oil palm tree and the young flowering spathe of *Raphia* species (raphia palm) tree serve as the main sources of the palm saps.

Corresponding Author
Tel.: +2348037239349;
E-mail: ibemog@yahoo.com.

The sweeter palm exudates obtained from *Raphia* species (locally called *Ngwo* in Igbo) are popular than those got from *E. guineensis* (locally called *Nkwu* in Igbo) more because of the less expertise involved in its tapping than the availability of the palm tree. The sap from the trees makes a pleasant drink, and palm wine can be made from it by fermentation (Ukhun *et al*., 2005).

In Bangladesh, Burma, Cambodia and Indonesia, palm sap from mainly *Borassus flabellifer* (*Borassus* palms) are fed to pigs as prime means of providing of most of the energy needed in the diet, converting palm products to protein, and fat in the fattening process (Dalibard, 1999). Though the social and ergogenic roles of palm wine are greatly acknowledged, the concern about its consumption has mainly been due to its alcohol content. Problems of alcohol use are heart disease, certain forms (oesophageal, breast,

colon, pancreatic) of cancers, cirrhosis of the liver, foetal damage, among others (Wardlaw and Kessel, 2002).

The fermentation process increases the level of thiamin, riboflavin, pyridoxine and vitamin B_{12} (FAO, 1998b). The palm wine in West Africa is high in vitamins B_{12}, which is very important for people with low meat intake and who subsist primarily on a vegetarian diet (Ezeagu and Fafunso, 2003). Yeasts, lactic and acetic acid bacteria are all important in the fermentation of palm sap and influence the composition of the product (Amoa-Awua *et al*., 2007).

Heat treatment has been used as a method of preserving of palm wine (Ibegbulem, 2012a). Nwaiwu *et al*. (2005) had reported that yeast cells are damaged at temperatures of 80^{o}C and above. Since palm sap can be consumed to provide most of the energy needed in the diet as well as serve as a means of converting palm products to protein and fat, it can also increase dietary water. Reports on the consumption of this seemingly nutritious drink in place of water are scanty.

The aim of this study was to investigate the nutritional and toxicological implications of drinking heat-treated fresh *R. hookeri* sap (HTF*Rh*S) by evaluating its effects on haematological parameters, liver function indices, serum lipid profile and growth performance indices using rats as model.

2.0 Materials and Methods

2.1 Tapping of fresh palm sap

The trees were identified and authenticated by Dr. F.N. Mbagwu of the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria, using their leaf samples. Voucher samples were deposited in the herbarium of Imo State University, Owerri, with the number: IMSUH 305.

2.2 Animal feed

The grower's mash (guinea feed) used was produced by Bendel Feed and Flour Mill Limited, Sapele, Delta State, Nigeria.

2.3 Rats

A total of thirty-two weanling albino *Rattus norvegicus* (both sexes) of the Wistar strain were purchased from the animal colony of the Department of Biochemistry, University of Port Harcourt, Port Harcourt, Nigeria. The rats were between seven to eight weeks old.

2.4 Treatment of the fresh palm sap

The fresh palm sap that was used in the study was tapped overnight from *Raphia hookeri* G. Mann. and H. Wendl. palm trees by a palm wine tapper at Orodo, Mbaitoli Local Government Area of Imo State, Nigeria. The fresh palm sap was heated to 85^{o}C to form the HTF*Rh*S. The heat-treatment was intended to halt fermentation and distill off pre-formed ethanol (b.p 78^{o}C). The HTF*Rh*S was cooled and stored in a refrigerator at 4^{o}C, to arrest microbial activities and avoid freeze injuries, until required for use.

2.5 Detection of ethanol in palm sap

The presence of ethanol in palm sap was detected before and after heat-treatment using Jones Reagent (prepared by dissolving 2.67 g $NaCr_2O_4.2H_2O$ in 2.3 ml of concentrated H_2SO_4 then diluting to 10.0 ml with distilled water). Briefly, 1.0 ml of acetone was mixed with 0.5 ml of palm sap and 0.5 ml of Jones Reagent added and shaken; the disappearance of the orange colour of the Jones Reagent and the formation of greenish and blue-green precipitates indicated the presence of ethanol (Ibegbulem, 2012a).

2.6 Determination of pH

The pH of the HTF*Rh*S was determined by the electrometric method using a digital pH meter (Labtech, India) that had been standardized using buffer solutions of pH 4 and 8, respectively, as described by AOAC (1990). The glass electrode that was connected to the digital pH meter was blotted dry of the buffer solutions using a filter paper, inserted into the HTF*Rh*S and the pH and prevailing temperature readings recorded.

2.7 Determination of the concentration of the heat-treated palm sap

The concentration of the HTF*Rh*S was estimated by evaporating known volumes (100.0 ml each) to dryness in 200.0 ml capacity pre-weighed beakers using a heating mantle (Chikpas Genopack, Nigeria), cooling and re-weighing the pre-weighed beakers bearing the dry matter as described by AOAC (1990). The weight of the dry matter was determined and the concentration of the HTF*Rh*S expressed as the ratio of the weight of dry matter to volume of HTF*Rh*S evaporated.

2.8 Feeding study and the effects of the heat-treated palm sap

Rats were allotted to 4 groups of 8 rats each (2 groups for males and 2 groups for females). The groups, on sex bases, were equalized as nearly as possible on weight basis. They weighed 57.46±0.91 g. Each rat was housed in a wire-screened cage with provisions for feed and fluid. Acclimatization of the rats to their new environment (at the laboratory of the Department of Biochemistry, Federal University of Technology, Owerri, Nigeria) lasted for 4 days. The experiment was conducted for 35 days. All the rats were maintained under the same light and dark cycles (circadian rhythm) and ambient room temperature. Two groups of the test rats (of the respective sex) were respectively placed on the HTF*Rh*S in place of water for their respective rat groupings while tap water served as the only source of fluid for the control group. All the rats were maintained on grower's mash *ad libitum* as the only source of feed. The experimental procedures were approved by the institutional animal ethical committee for usage of animals in experiments.

2.9 Preparation of serum

On day 35 of the experiment, each rat was re-weighed before being anaesthetized in dichloromethane vapour. Incisions were then made into their thoracic cavities. Blood samples were collected by heart aorta puncture using a 10 ml hypodermic syringe. A quantity (1.0 ml) of the blood sample was quickly transferred into a sequestering bottle containing EDTA as anticoagulant and another 5.0 ml transferred into a test tube and allowed to clot for 10 min. Serum was collected by aspiration using Pasteur pipette.

2.10 Determination of biochemical parameters

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were evaluated using the methods described by the manufacturer of commercial Randox® diagonistic kit (product of Randox® Laboratories Ltd., Antrim, United Kingdom). Serum total cholesterol, high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), total bilirubin, total protein and albumin contents were evaluated using methods described by the manufacturer of commercial BioSystems® diagonistic kit (product of S.A Costa Brava of Barcelona, Spain). Briefly, ALT and AST activities were determined using the method of Reitman and Frankel (1957); total cholesterol content of the serum was determined using cholesterol oxidase and peroxidase as described by Allain *et al* (1974) and Melattini *et al* (1978); HDL-C was determined using the phosphotungstate/Mg-cholesterol oxidase and peroxidase method as described by Grove (1979) and Burstein *et al* (1980); LDL-C was determined by precipitating it with polyvinylsulphate and calculating its concentration as the difference between the serum total cholesterol and the cholesterol in the supernatant after centrifugation as described by Assmann *et al* (1984); total bilirubin concentration was determined using the method that employed the use of diazotized sulphanilic acid as described by Pearlman and Lee (1974); total protein concentration was determined by the Biuret method as described by Gornall *et al* (1949); albumin concentration was determined using the method that employed the use of bromocresol green as described by Doumas *et al* (1971) and globulin concentration was calculated as the difference between the serum total protein and albumin content as described by Ibegbulem (2012b). Haemoglobin (Hb) concentration was evaluated by the cyanmethaemoglobin method (Bain and Bates, 2002). Packed cell volume (PCV) was

determined by the microhaematocrit technique (Baker *et al*., 2001). Mean cell haemoglobin concentration (MCHC) was calculated as the ratio of the haemoglobin concentration to that of the PCV and expressed in percentage (Baker *et al*., 2001). The white blood cells (WBCs) were counted after lysing the red blood cells and staining the WBC nuclei in a haemocytometer using 2% acetic acid tinged with gentian violet, mounting the haematocytometer under a microscope and counting at x10 (Baker *et al*., 2001).

2.11 Determination of anthropometric data

The respective kidneys, spleen, heart and liver that was excised and dropped in the 10% formolsaline solution (prepared by mixing 10.0 ml normal saline with 90.0 ml formaline) was blotted using a filter paper and the weight determined using an analytical balance.Body weight gained was calculated as the difference between the final and the initial body weights. The growth rate of the rat was calculated as the ratio of the weight gained by the experimental animal to the number of days (35 days) constituting the experimental period. Feed conversion ratio was calculated as the ratio of the total feed consumed to that of the body weight gained during the number of days that constituted the experimental days.

2.12 Statistical analysis

Data were analysed using of the student's t-test of significance at $p \leq 0.05$. Comparisons were done between both sexes and groups.

3.0 Results

Ethanol was detected in the untreated fresh palm sap but was not detected in the HTF*Rh*S. The HTF*Rh*S was acidic (pH 6.36 at 29.37±1.0^{o}C) and had a concentration of 40.1 mg/ml.

The moisture and ash were its main and least constituents, respectively of HTF*Rh*S (Table 1).

The HTF*Rh*S did not significantly ($p>0.05$) affect the weight gained, growth rate, total feed consumed and feed conversion ratio and the sex of the rat did not significantly ($p>0.05$) affect the levels of these parameters (Table 2).

The HTF*Rh*S-treated rats consumed more ($p<0.05$) fluid than their control counterparts and this provided extra energy to the HTF*Rh*S-treated rats. The sex of the rat did not significantly ($p>0.05$) affect the levels of these measurements (Table 3).

The organ weights were not significantly ($p> 0.05$) affected when the HTF*Rh*S was taken in place of water. The sex of the rat did not also significantly ($p>0.05$) affect the weights of the organs (Table 4).

The haematological indices were not significantly ($p>0.05$) affected when the HTF*Rh*S was taken in place of water. The sex of the rat did not significantly ($p>0.05$) affect the levels of the parameters (Table 5).

Consuming the HTF*Rh*S significantly ($p<0.05$) increased serum AST and ALT activities. The AST/ALT ratios of the HTF*Rh*S-treated rats were significantly ($p<0.05$) higher than those of their controls. ALT activities of the male HTF*Rh*S-treated rats were higher ($p<0.05$) than those of their female counterparts. However, the sex of the rat did not significantly ($p>0.05$) affect the levels of the other parameters (Table 6).

The serum total protein and albumin contents were not significantly ($p>0.05$) altered by the HTF*Rh*S but their serum globulin contents were significantly ($p<0.05$) reduced, while their albumin/globulin ratio were significantly ($p<0.05$) increased. Sex of the rat did not significantly ($p>0.05$) affect the levels of the parameters (Table 7).

The serum total cholesterol did not increase significantly ($p>0.05$) upon consuming HTF*Rh*S. Serum HDL-cholesterol were reduced significantly ($p<0.05$) and serum TC/HDL-cholesterol ratios, LDL-cholesterol concentrations and LDL-cholesterol/HDL-cholesterol ratios were significantly ($p<0.05$) increased upon consuming HTF*Rh*S. The LDL-cholesterol/ HDL-cholesterol ratio of the female HTF*Rh*S-treated rats significantly ($p<0.05$) increased more than those of their male HTF*Rh*S-treated counterparts. However, the sex of the rats did not significantly ($p>0.05$) affect the levels of the other parameters (Table 8).

Table 1: Proximate composition of the heat-treated palm sap*

Moisture (%)	Crude protein (%)	Crude fat (%)	Ash (%)	Total Carbohydrate (%)	Energy content (Kcal/ 100ml)
93.99±0.04	2.77±0.04	0.52±0.03	0.19±0.15	2.53±0.02	25.88±0.03

*Results are given as mean ± SD of triplicate determinations.

Table 2: Effect of heat-treated palm sap on weight gained, growth rate, total feed consumed and feed conversion ratio*

	Weight gained (g)		Growth rate (g/ day)		Total feed consumed (g)		Feed conversion ratio	
Group	Male	Female	Male	Female	Male	Female	Male	Female
Palm Sap (8)	35.85 ± 4.02[a]	33.95 ± 4.33[a]	1.03 ± 0.11[c]	0.97 ± 0.12[c]	335.47 ± 3.54[e]	334.16 ± 4.81[e]	9.36 ± 1.30[g]	9.85 ± 1.11[g]
Control (8)	41.94 ± 9.14[a]	38.27±13.41[a]	1.21 ± 0.27[c]	1.09 ± 0.38[c]	335.74 ± 19.71[e]	338.02 ± 16.80[e]	8.01 ± 1.20[g]	8.83 ± 1.25[g]

* Results are given as mean ± SD of the number of observations indicated in the parentheses.
M = male rats, F = female rats, PS = palm sap, (8) = number of rats.
Values on the same row and column, against a parameter, bearing the same superscript letter are not significantly different (p>0.05).

Table 3: Fluid intake and energy contents of rats*

	Fluid intake (ml)		Extra energy content (kcal/ 470 ml)‡	
Group	Male	Female	Male	Female
Palm Sap	470.00 ± 0.01[a]	470.00 ± 0.02[a]	121.64 ± 0.01	121.64 ± 0.02
Control	405.00 ± 5.97[b]	401.25 ± 7.81[b]	0.00 ± 0.00	0.00 ± 0.00

* Values (8 ± SD) carrying the same superscript for each parameter across the row and down the column are not significantly different (p>0.05).
‡Calculated from Table 1.

Table 4 - Effects of the heat-treated palm sap on organ weights of test rat.*

	Kidney (g)		Spleen (g)		Heart (g)		Liver (g)	
Group	Male	Female	Male	Female	Male	Female	Male	Female
Palm Sap (8)	0.86 ± 0.18^{a}	0.88 ± 0.09^{a}	0.35 ± 0.13^{c}	0.35 ± 0.05^{c}	0.35 ± 0.04^{e}	0.32 ± 0.02^{e}	4.22 ± 0.86^{g}	4.01 ± 0.46^{g}
Control (8)	0.90 ± 0.06^{a}	0.83 ± 0.11^{a}	0.41 ± 0.06^{c}	0.33 ± 0.09^{c}	0.35 ± 0.03^{e}	0.33 ± 0.05^{e}	4.10 ± 0.27^{g}	4.04 ± 0.72^{g}

* Values (8 ± SD) carrying the same superscript for each parameter across the row and down the column are not significantly different (p>0.05).

Table 5 - Effects of the heat-treated palm sap on haematological indices of test rat*

	Hb (g/dl)		PCV (%)		MCHC		WBC (number/L)	
Group	M	F	M	F	M	F	M	F
PS (8)	10.80 ± 2.38^{a}	10.10 ± 1.24^{a}	34.75 ± 2.63^{c}	35.50 ± 3.32^{c}	0.31 ± 0.11^{e}	0.29 ± 0.13^{e}	4312.5 ± 908.64^{g}	3725 ± 1834.17^{g}
Control (8)	8.98 ± 0.46^{a}	8.05 ± 1.00^{a}	34.50 ± 1.73^{c}	31.75 ± 6.24^{c}	0.26 ± 0.14^{e}	0.25 ± 0.12^{e}	4175.00 ± 1074.32^{g}	5000 ± 1802.31^{g}

* Values (8 ± SD) carrying the same superscript for each parameter across the row and down the column are not significantly different (p>0.05).
Hb = haemoglobin, PCV = packed cell volume, MCHC = mean corpuscular haemoglobin concentration, WBC = white blood cells,

Table 6 - Effects of heat-treated palm sap on alanine aminotransferase and aspartate aminotransferase activities and total bilirubin of test rats*

	AST (U/I)		ALT (U/I)		AST: ALT		Total Bilirubin (mg/dl)	
Group	Male	Female	Male	Female	Male	Female	M	F
Palm Sap	34.45 ± 2.58^{a}	33.20 ± 2.56^{a}	35.00 ± 2.71^{e}	31.50 ± 1.73^{f}	0.98 ± 0.05^{k}	1.05 ± 0.04^{k}	1.27 ± 0.21^{m}	1.10 ± 0.18^{m}
Control	20.98 ± 0.45^{b}	20.75 ± 0.25^{b}	24.73 ± 2.36^{c}	24.48 ± 5.00^{c}	0.85 ± 0.07^{j}	0.85 ± 0.17^{j}	1.13 ± 1.06^{m}	1.16 ± 0.06^{m}

*Values (8 ± SD) carrying the same superscript for each parameter across the row and down the column are not significantly different (p>0.05).
AST = aspartate aminotransferase, ALT = alanine aminotransferase,

Table 7 - Effects of the heat-treated palm sap on serum proteins of test rats*

	Total Protein (g/l)		Albumin (g/l)		Globulin (g/l)		A/ G ratio	
Group	M	F	M	F	M	F	M	F
Palm Sap	56.00 ± 3.81^{x}	58.58 ± 4.95^{x}	40.10 ± 5.76^{q}	40.68 ± 7.42^{q}	15.90 ± 2.06^{s}	17.90 ± 2.50^{s}	2.52 ± 0.40^{w}	2.27 ± 0.10^{w}
Control	58.58 ± 4.15^{x}	61.05 ± 1.91^{x}	36.30 ± 1.81^{q}	36.30 ± 1.81^{q}	22.28 ± 2.30^{t}	24.25 ± 1.00^{t}	1.63 ± 0.90^{x}	1.47 ± 0.30^{x}

*Values (8 ± SD) carrying the same superscript for each parameter across the row and down the column are not significantly different (p>0.05).

Table 8 - Effects of the heat-treated palm sap on atherogenicity indices of test rat serum (mg/dl)*

	TC		HDL- C		TC/ HDL-C ratio		LDL- C		LDL-C/ HDL-C ratio	
Group	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Palm Sap	104.15 ± 8.34^{g}	103.63 ± 12.62^{g}	49.17 ± 5.17^{k}	45.84 ± 2.18^{k}	2.12 ± 0.06^{s}	2.26 ± 0.17^{s}	75.00 ± 3.21^{u}	80.56 ± 9.62^{u}	1.53 ± 0.10^{y}	1.76 ± 0.13^{m}
Control	100.00 ± 9.06^{g}	100.00 ± 15.70^{g}	59.42 ± 1.58^{j}	57.87 ± 1.20^{j}	1.64 ± 0.11^{t}	1.73 ± 0.24^{t}	63.89 ± 3.21^{v}	65.28 ± 2.78^{v}	1.08 ± 0.09^{z}	1.13 ± 0.03^{z}

*Values (8 ± SD) carrying the same superscript for each parameter across the row and down the column are not significantly different (p>0.05).
HDL-C = High density lipoprotein cholesterol, LDL-C = Low density lipoprotein cholesterol, TC = Total Cholesterol, M = male rats, F = female rats, PS = palm sap, Ctrl = control, (8) = number of rats.
Values on the same row and column, against a parameter, bearing the same superscript letter are not significantly different (p>0.05).

4.0 Discussion

The HTF*Rh*S used in the study was a water-based beverage (Table 1) which did not contain ethanol. However, proximate compositions of palm saps may vary according to the season and water content of the soil.

The nutritional implications of drinking the HTF*Rh*S in place of water indicated that the HTF*Rh*S-treated rats' abilities to convert feed mass into body mass (their FCR) were not altered (Table 2). However, digestion and assimilation of nutrients were disturbed by between 11.55% (for the female rats) and 16.85% (for the male rats). The taste of the HTF*Rh*S seemed to have influenced the higher fluid intakes of the HTF*Rh*S-treated rats (Table 3), and the extra energy it provided seemed to have been the reason for the reduced, though not significantly ($p>0.05$), feed intakes by the HTF*Rh*S-treated rats (Table 2).

The organs of the HTF*Rh*S-treated rats seemed not to have developed any pathological conditions (Table 4) and the levels of the normal blood constituents were not changed (Table 5). Organ weight evaluation is an essential part of the toxicologic and risk assessment of drugs, chemicals, biologic, food additives, and medical devices (Michael *et al*., 2007).

The increased serum AST and ALT activities upon consuming the HTF*Rh*S (Table 6) contrasted with the results presented by the organ weights in Table 4, which did not present any pathogenesis. This suggested that the cardiac muscles and/or hepatocytes may have been damaged. Even though the organs were not hypertrophic, the affected organ (heart or liver) may have had some lesions necessitating the supportive biochemical analyses which are more sensitive markers of organ damage. The AST/ALT ratios of the HTF*Rh*S-treated rats showed that the pathologic condition, if any, was more hepato-toxic to the male HTF*Rh*S-treated rats and more cardio-toxic to the female HTF*Rh*S-treated rats. Elevation of the ALT activities in the male HTF*Rh*S-treated rats over those of their female counterparts indicated that the livers of the male HTF*Rh*S-treated rats may have been more susceptible to the effects of the HTF*Rh*S. Though the pre-formed ethanol in the palm sap was distilled off, it may have been re-produced in the rats because alcohol is a natural product of microbial fermentation of sucrose in the gut. Even though blood levels of ethanol in the HTF*Rh*S-treated rats were not estimated, intestinal microorganisms have been reported to produce ethanol from glucose (Nelson and Cox, 2000) and this finds its way into the blood stream (Nelson and Cox, 2000; Wardlaw and Kessel, 2002). The HTF*Rh*S may have also been progressively fermented to by the ubiquitous air-borne microorganisms while it waited to be lapped up in the fluid trough. Ethanol was reported to have been re-produced in a similarly heat-treated palm wine upon bench storage in sterilized glass bottles that were plugged tightly with cotton wool (Ibegbulem, 2012a) indicating that some of the microorganism survived the heat treatment. Presumably, the ethanol that may have been re-produced caused the elevated serum AST and ALT activities. Serum total bilirubin levels in the HTF*Rh*S-treated rats (Tables 6) suggested that they were not jaundiced. The HTF*Rh*S-treated rats did not also exhibit hypoalbuminemia (and hyperalbuminemia) as well as hypoproteinemia (and hyperproteinemia) as their serum albumin and total protein levels compared favourably with values presented by the control rats (Table 7).

The serum total protein and albumin concentrations in the HTF*Rh*S-treated rats also indicated that their kidneys were not damaged. Damaged kidneys normally leak proteins into urine thereby reducing their serum levels (Shryock and Swartout, 1980; Malhotra, 1989). The decreases in serum globulin concentrations of the HTF*Rh*S-treated rats without concomitant decreases in the concentrations of their serum albumin led to increases in their albumin/ globulin (A/G) ratios (Table 7). This presented symptoms of both xanthomatosis and biliary cirrhosis which are characterized by increased serum LDL-cholesterol and A/G ratio (Malhotra, 1989). It may however be resolved in favour of xanthomatosis because the HTF*Rh*S also increased serum LDL-cholesterol concentrations of the HTF*Rh*S-treated rats (Table 8) but did not disturbed digestion and assimilation of nutrients, as presented by their feed conversion ratios (Table 2). On the other hand, biliary cirrhosis develops over several years and disturbs digestion as a symptom (Shryock and Swartout, 1980).

The atherogenic markers of the lipid profiles of the rats showed that the HTF*Rh*S was atherogenic to the HTF*Rh*S-treated rats; having elevated serum LDL-cholesterol and reduced serum HDL-cholesterol levels (Table 8). The risk of developing atherosclerosis is directly related to plasma LDL-cholesterol and inversely related to HDL-cholesterol levels (Nelson and Cox, 2000). The atherogenic markers suggested that there may have been an increase in the synthesis of LDL-cholesterol or a reduction in cellular LDL-cholesterol receptor activity. The increased serum total cholesterol/HDL-cholesterol and LDL-cholesterol/HDL-cholesterol ratios of the HTF*Rh*S-treated rats also suggested that these rats had higher concentrations of cholesterol than phospholipids in their sera. LDL structurally contains more cholesterol than phospholipids while HDL contains more phospholipids than cholesterol (Nelson and Cox, 2000). HDL also encourages the uptake of cholesterol from tissues, in a reverse tissue cholesterol transport, and transfers such to the liver for excretion as bile acids and bile salts (Nelson and Cox, 2000; Glew, 2006). The female HTF*Rh*S-treated rats were more susceptible to atherogenicity (Table 8) occasioned by their higher serum LDL-cholesterol/ HDL-cholesterol ratio. An organism's sex has been reported as a factor that influences toxicity due to different hormonal status (Zakrzewski, 1991).

In conclusion, the HTF*Rh*S did not affect growth, organ weights and haematological parameters but increased serum aspartate and alanine aminotransferase activities, and presented symptoms of atherosclerosis and xanthomatosis when consumed in place of water for the duration of the study. It was more hepato-toxic to the male rats and more cardio-toxic to the female rats; with the female rats also being more susceptible to atherogenicity.

Conflict of interest: Authors do not have any conflict of interest.

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