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The Effects of the Aqueous Extracts of *Elaeis guineensis* Fruits on the Lipid Profile and Kidney Function Indices of Male Wistar Albino Rats

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Abstract

Increasing the intake rate of aqueous extracts of palm fruits by local consumers for their nutraceutical benefits has necessitated the scientific investigations of the properties of these extracts to ascertain their safety levels. This study investigates the effects of fresh and fermented aqueous extracts of palm fruits (*Elaeis guineensis*) on lipid profile and kidney function indices of male Wistar albino rats. In this study, forty-five male Wistar albino rats were randomly divided into five groups, with group one having five rats while groups 2 – 5 had ten rats each. Group 1 received normal saline 2 mL/kg body weight (b.wt) orally for twenty-eight days. Five rats each in groups 2 – 5 received 100, 200, 400, and 600 mg/kg b. wt. of fresh and fermented aqueous extracts of palm fruits respectively for twenty-eight days. Blood samples were collected on the 29th day, rats were sacrificed and kidneys removed for histopathological examinations. Data collected were statistically analysed using one analysis of variance. Total cholesterol and low density lipoprotein cholesterol concentrations in the palm fruit aqueous extract-treated groups were significantly ($p < 0.05$) higher than those of normal rats in a dose-dependent manner. Triacylglycerol and high density lipoprotein cholesterol concentrations in the rats treated with the palm fruit aqueous extract were not significantly higher ($p > 0.05$) when compared with the normal control except for group 5 of the rats treated with a high dose of fermented palm fruit aqueous extracts. Serum urea, creatinine and uric acid concentrations of rats treated with aqueous extracts of palm fruits showed a significant increase ($p < 0.05$) when compared with the normal control rats. The aqueous extracts treated rats also showed a significant increase ($p < 0.05$) in the serum electrolytes (Na^+, K^+, Cl^-, and HCO_3^-) concentrations relative to the normal control rats. Histopathological studies showed tubular degeneration and regeneration, tubular necrosis and mild multifocal vacuolar degeneration of renal tubular epithelial cells of the rats treated with the aqueous extracts when compared with the normal control rats. Results of the present study suggest that the aqueous extracts of palm fruits could adversely affect the lipid profile, damage and impair renal functions as depicted by the kidney histomorphologies and serum electrolytes concentrations.

Keywords: Elaeis guineensis; Kidney histomorphology; Serum electrolytes; Lipid profile; Urea; Creatinine.

1. Introduction

There is increasing number of kidney diseases and cardiovascular disorders including heart attacks and strokes which result not only from genetic factors but also from unhealthy lifestyles, drug toxicity, and unhealthy diets (Santoshkumar *et al*., 2013). Prevention remains the best option for surviving kidney and cardiovascular disorders till date, as they have limited treatment options coupled with huge emotional and financial burdens (Helin and Winberg, 2008). Lipid profile gives the breakdown of one's fats in the blood which includes total cholesterol, high density lipoprotein-cholesterol, low density lipo-protein-cholesterol, and triacylglycerol. In a situation where there are high serum levels of total cholesterol, low density lipoprotein-cholesterol, triacylglycerol, and low serum levels of high density lipoprotein-cholesterol, it is generally referred to as negative lipid profile or abnormal lipid profile (Jain *et al*., 2010; Luqman *et al*., 2012; Sivaiah Reddy, 2012). These have increased the rate of lipid profile associated disorders such as hyperlipidaemia, diabetes mellitus, atherosclerosis and chronic kidney disease (Santoshkumar *et al*., 2013). Consumption of high fat diets could lead to elevated serum levels of one or more of total cholesterol, low-density lipoprotein cholesterol, triacylglycerol, or both total cholesterol and triacylglycerol as in case of combined hyperlipidaemia (Luqman *et al*., 2012; Sivaiah Reddy, 2012).

Kidneys are the major excretory organs in humans which play vital roles in the formation and excretion of urine, formation and secretion of erythropoietin that controls the rate of red blood cell formation, and rennin that regulates blood pressure (Mona *et al*., 2013). In addition, kidneys are highly involved in the removal of waste products of cell metabolism, excess water, and salts

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from the blood keeping blood pH within physiological range (Santoshkumar *et al*., 2013; Ubon *et al*., 2017). It has been observed that failure in kidney functions is the main reason behind the increasing cases of chronic kidney diseases globally, which has independently increased the risk factor for cardiovascular diseases (Anawat *et al*., 2017). There is an increasing number of death resulting from cardiovascular diseases worldwide, although cardiovascular diseases are preventable largely by managing or controlling modifiable risk factors (Zemdegs *et al*., 2011). Cardiovascular risk factors such as smoking, inactive lifestyle, poor dietary habits, hypertension, abnormal lipid profile and obesity can be effectively managed while other risk factors such as genetic makeup of an individual, sex, and age are difficult to control (Zemdegs *et al*., 2011). The fact that many cardiovascular risk factors are controllable makes it necessary to identify manageable risk factors that could aid in the prevention and reduction of cardiovascular diseases. It has been reported that improved dietary habits, physical activity, and lifestyle or behavioural changes could significantly lead to the reduction of cardiovascular diseases (Hunter *et al*., 2010).

The aqueous extracts of palm fruits (*Elaeis guineensis*) are rich in antioxidant components and possess a significant antioxidant activity capable of ameliorating oxidative stress associated with the activities of reactive oxygen species (Uroko *et al*., 2017). High fatty acids, low proteins and carbohydrates found in the aqueous extracts of palm fruits could negatively alter the lipid profile especially if the aqueous extracts are richer in saturated fats and low density lipoprotein than the high density lipoprotein and unsaturated fats (Akinsorotan, 2013; Uroko *et al*., 2017). It has been shown that the aqueous extracts of palm fruits contain high mineral contents such as sodium, potassium, magnesium and calcium, which could adversely affect the serum electrolyte balance thereby impairing consumers' health (Ohimain *et al*., 2012). The increase in the rate of consumption of "ofe akwu" and use of aqueous extracts from palm fruits to substitute water in the production of palm oil have increased the rate of consumption of the aqueous extracts of palm fruits by humans. There has been no comprehensive study of the effects of the consumption of aqueous extracts from palm fruits on human health. The extracts may be rich in constituents that could negatively alter lipid profile thereby increasing the risk of cardiovascular diseases and other associated health consequences. In addition, the extracts of palm fruits may be nephrotoxic, adversely impair renal functions, and compromise the health of innocent consumers. Thus, this study is designed to investigate the effects of aqueous extracts of palm fruits on lipid profile and kidney function indices of male Wistar albino rats.

2. Materials and Methods

2.1. Preparation of Aqueous Extract of Palm Fruits

Palm fruit bunches were collected from the Obeakpu palm oil milling site in Njaba, Imo State and were threshed manually using axe to remove the fruits from the bunches. Loose fruits were handpicked, washed, and boiled in high temperature wet-heat treatment (120-140°C) for two hours, and crushed with the aid of a mortar and pestle. Palm oil was extracted with water and the kernels including other solid wastes were removed, leaving behind the aqueous portion. A volume of twenty litres of freshly prepared aqueous extract of palm fruit was obtained, and first filtered with a mesh cloth to remove suspended solids, and then filtered with Whatman No. 1 filter paper. The filtrate was divided into two equal volumes, one portion was stored in a refrigerator, and the other portion was kept out of the refrigerator to ferment for twenty-one days. This was done to mimic the situations where people consume the aqueous extracts freshly through "ofe akwu or banga soup" and the second situation where it is used as water substitute to produce palm oil irrespective of its age. The two extracts were concentrated to dryness in a water bath at 50 °C and were used for the study.

2.2. Collection of Animals for the Study

Animals used in this study were obtained from the Animal House of the Department of Zoology, University of Nigeria, Nsukka. The animals were acclimatized at the Animal House of the Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike for seven days under a twelve-hour cycle of light and dark with free access to standard animal feed and water.

2.3. Experimental Design

Forty five male Wistar albino rats were used for this study. The rats were divided into five groups with group one having five rats and serving as the normal control. Groups two – five had ten rats each with five rats in each group receiving fresh and fermented aqueous extracts respectively for twenty-eight days, after which the rats were sacrificed on the 29th day and blood samples were collected for lipid profile and kidney function indices analyses. Kidney was collected from the normal control group, and groups two – five treated with the fresh and fermented aqueous extract respectively for the histo-pathological examination. The acute toxicity testing of the extracts in earlier studies had shown that the extracts are not acutely toxic at the highest dose tested. Thus, the acute toxicity testing of the extracts were not carried out again in this study.

Group 1: Orally administered normal saline 2 mL/kg b. wt. for 28 days.

Group 2: Five rats each were administered 100 mg/kg b. wt. of the fresh and fermented aqueous extracts of *E. guineensis* fruits for 28 days respectively.

Group 3: Five rats each were administered 200 mg/kg b. wt. of the fresh and fermented aqueous extracts of *E. guineensis* fruits for 28 days respectively.

Group 4: Five rats each were administered 400 mg/kg b. wt. of the fresh and fermented aqueous extracts of *E. guineensis* fruits for 28 days respectively.

Group 5: Five rats each were administered 600 mg/kg b. wt. of the fresh and fermented aqueous extract of *E. guineensis* fruits for 28 days respectively.

2.4. Determination of Total Cholesterol (T. chol)

The total cholesterol concentration was determined according to the method described by Allain *et al.*, 1974. Cholesterol is determined by the enzymatic hydrolysis of

cholesterol ester by cholesterol esterase that generates cholesterol which, reacts with oxygen in the presence of cholesterol oxidase to liberate hydrogen peroxide. The hydrogen peroxide liberated reacts with phenol and 4-amino antipyrine in the presence of peroxidase to give quinonemine. Cholesterol is quantified by measuring the absorbance of the quinonemine formed at 546 nm which is proportional to the cholesterol concentration.

2.5. Determination of High Density Lipoprotein

The high density lipoprotein (HDL) concentration was determined according to the method described by Albers *et al*. 1978. The principle of this method is based on the removal of low density lipoproteins (LDL and VLDL) and chylomicron fractions by quantitative lipoprotein precipitation by the addition of phosphotungstic acid in the presence of magnesium ions. The high density lipoprotein (HDL) fraction which remains in the supernatant is determined.

2.6. Determination of Low Density Lipoprotein (LDL)

The low density lipoprotein concentration was determined by the method of Assmann *et al*., 1984. Low density lipoprotein-cholesterol (LDL-Cholesterol) is determined as the difference between the total cholesterol and cholesterol content of the supernatant after precipitation of the LDL fraction by polyvinyl sulphate (PVS) in the presence of polyethylene glycol monomethyl ether.

2.7. Determination of Triacylglycerol Concentration (TAG)

Triacylglycerol (TAG) concentration was determined according to the method described by Tietz, 1990. The triacylglycerol concentration is determined by the enzymatic hydrolysis of triacylglycerol and a couple of reactions that generate quinoneimine as an indicator. The absorbance of the quinoneimine generated at 546 nm is directly proportional to the triacylglycerol concentration.

2.8. Determination of Serum Urea Concentration

Serum urea concentration was determined according to the method of Varley and Alan, 1984. Urea in serum is hydrolyzed to ammonia in the presence of urease which can be measured photometrically by Berthelot's reaction.

2.9. Determination of Creatinine Concentration

Determination of serum creatinine concentration was carried out as described by Peters, 1942. Creatinine in alkaline solution reacts with picric acid to form a coloured complex. The amount of the complex formed is directly proportional to the creatinine concentration.

2.10. Determination of Sodium Ion (Na+) Concentration

The sodium ion concentration in serum was determined using the method of Terri and Sesin, 1958. Sodium is precipitated as the triple salt of sodium-magnesium uranyl acetate with the excess uranium, which reacts with ferrocyanide, producing a chromophore whose absorbance varies inversely as the concentration of sodium in the test specimen.

2.11. Determination of Chloride Ion (Cl-) Concentration

Chloride ion concentration in the serum was determined according to the method of Skeggs and Hochstrasser, 1964. Chloride ions form a soluble non-ionized compound with mercuric ions and displace thiocyanate ions from non-ionized mercuric thiocyanate. The released thiocyanate ions react with ferric ions to form a colour complex that absorbs light at 480 nm. The intensity of the colour produced is directly proportional to the chloride concentration.

2.12. Determination of Potassium Ion (K+) Concentration

The potassium ion concentration in the serum was determined according to the method of Terri and Sesin, 1958. The potassium ion concentration in the serum is determined by using sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension, the turbidity of which is proportional to the potassium ion concentration in the range of 2 – 7 mEq/L.

2.13. Determination of Bicarbonate Ion Concentrations

The serum bicarbonate ion was determined using the enzyme spectrophotometric procedures as described by Forrester *et al*., 1976. Phosphoenolpyruvate carboxylase (PEPC) catalyzes the reaction between phosphoenol-pyruvate and bicarbonate to form oxaloacetate and phosphate ion. Oxaloacetate is reduced to malate with simultaneous oxidation of an equimolar amount of reduced nicotinamide adenine dinucleotide (NADH) to NAD; the reaction is catalysed by malate dehydrogenase. This results in a decrease in absorbance at 380 nm that is directly proportional to the bicarbonate concentration in the serum.

2.14. Determination of Uric Acid Concentration

Uric acid concentration was determined according to the method of Trinder as contained in Biotrust assay kit, 1969. Uric acid is oxidized to allantoin and hydrogen peroxide in the presence of uricase. The liberated hydrogen peroxide is detected by a chromogenic oxygen acceptor in the presence of peroxidase. The red quinine formed is proportional to the amount of uric acid present in the sample.

2.15. Tissue Preparation for Histopathological Analysis

The experimental animals were humanely sacrificed at the end of the study. Gross lesions were recorded as observed during the post mortem examination. Sections of the kidney were collected for histopathological examination. The collected samples were fixed in 10 % phosphate buffered formalin for a minimum of forty-eight hours. The tissues were subsequently trimmed, dehydrated in four grades of alcohol (70 %, 80 %, 90 % and absolute alcohol), cleared in three grades of xylene and embedded in molten wax. On solidifying, the blocks were sectioned, 5µm thick with a rotary microtome, floated in a water bathe and incubated at 60˚C for thirty minutes. The 5µm thick-sectioned tissues were subsequently cleared in three grades of xylene and rehydrated in three grades of alcohol (90 %, 80 % and 70 %). The sections were then stained with Hematoxylin for fifteen minutes. Blueing was done with ammonium chloride. Differentiation was done with 1 % acid alcohol before counterstaining with Eosin. Permanent mounts were made on degreased glass slides using a mountant; DPX.

2.16. Slide Examination

The prepared slides were examined with a Motic™ compound light microscope using x4, x10 and x40

objective lenses. The photomicrographs were taken using a Motic™ 9.0 mega pixels microscope camera at x100 and x400 magnifications'

2.17. Statistical Analysis

The data obtained from the experiment were analysed statistically using one-way analysis of variance (ANOVA) to get the grouped mean which was used to determine the significant difference between the group means at 95 % confidence level (P <0.05). Statistical Products and Service solutions (SPSS) software was used for this analysis (IBM Corporation, 2011).

3. Results

The data in the figure 1 show the total cholesterol concentration in the male Wistar albino rats which were administered graded doses of the fresh and fermented aqueous extracts of palm fruits respectively. The rats in groups two – three that were administered the fresh aqueous extract showed no significant increase ($P > 0.05$) in the total cholesterol concentrations when compared with the normal control. Similarly, the rats in group five which were administered the fermented aqueous extract showed no significant increase ($P > 0.05$) in the total cholesterol concentration when compared with the normal control. However, rats in groups four – five that were administered the fresh aqueous extract and rats of groups two – four that were administered the fermented aqueous extract of palm fruits showed a significant increase ($P < 0.05$) in the total cholesterol concentrations when compared with the normal control.

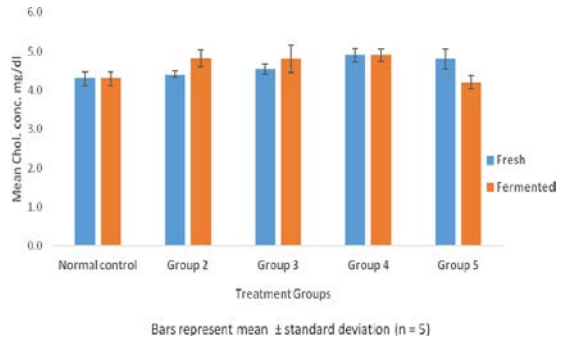

Figure 1. Effect of the aqueous extracts of palm fruits on serum total cholesterol concentrations of male Wistar albino rats

The data in figure 2 show the high density lipoprotein (HDL) concentrations of the normal rats and the rats administered graded doses of fresh and fermented aqueous extracts of palm fruits respectively. All the groups of rats administered fresh and fermented aqueous extracts respectively showed no significant increase ($P < 0.05$) in HDL concentration except the rats of group 4 that were administered fresh aqueous extract and rats of group 5 that were administered fermented aqueous extract that showed no significant decrease ($P < 0.05$) in HDL concentration when compared with HDL concentration of the normal control rats.

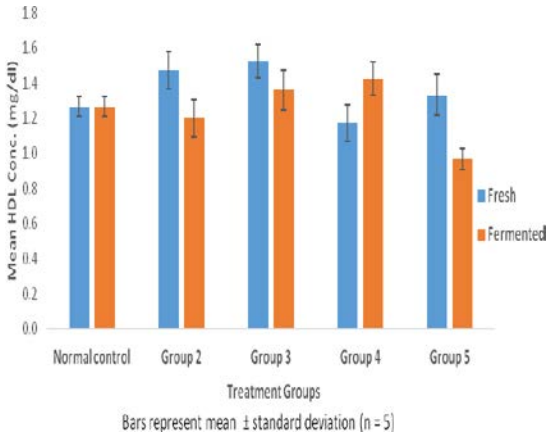

Figure 2. Effect of the aqueous extracts of palm fruits on serum high density lipoprotein concentrations of male Wistar albino rats

Figure 3 show the low density lipoprotein (LDL) concentration of normal rats and the rats administered graded doses of fresh and fermented aqueous extracts of palm fruits respectively. The rats of groups 2 and 3 that were administered fresh aqueous extract of palm fruits showed no significant ($P > 0.05$) decrease in LDL concentrations when compared with the LDL concentration of the normal control rats. Groups 4 and 5 rats that were administered the same fresh aqueous extract at higher concentrations showed no significant increase ($P > 0.05$) in LDL concentrations relative to the LDL concentrations of the normal control rats. However, groups 2 and 4 that were administered the fermented aqueous extract of palm fruits showed a significant increase ($P < 0.05$) in the LDL concentrations relative to the normal control rats. In addition, groups 2 and 5 rats administered the fermented aqueous extract showed no significant increase ($P > 0.05$) in LDL concentrations when compared with the LDL concentrations of the normal control rats.

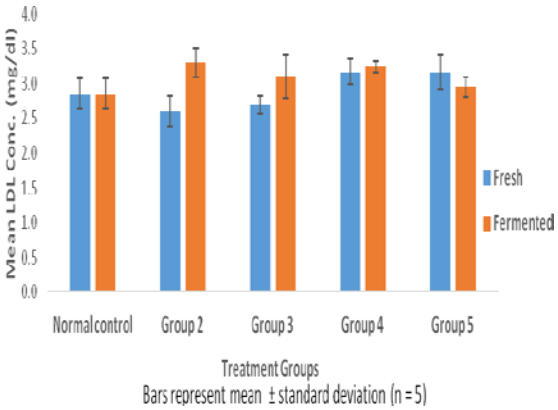

Figure 3. Effect of the aqueous extracts of palm fruits on serum low density lipoprotein concentrations of male Wistar albino rats

The data in figure 4 show the triacylglycerol (TAG) concentration of normal control rats administered distilled water and the rats administered graded doses of fresh and fermented aqueous extracts of palm fruits respectively. There were no significant ($P > 0.05$) increase in triacylglycerol concentrations observed in the rats of groups 2 and 3 that were administered the fresh aqueous extract of palm fruits when compared with the normal control. In addition, groups 2 and 3 rats administered

equivalent doses of fermented aqueous extract of palm fruits showed no significant ($P > 0.05$) increase in the TAG concentrations when compared with normal control. However, group's 4 – 5 rats administered equivalent doses of fresh and fermented aqueous extracts of palm fruits respectively showed no significant ($P > 0.05$) decrease in the TAG concentrations when compared with the TAG concentration of the normal control rats.

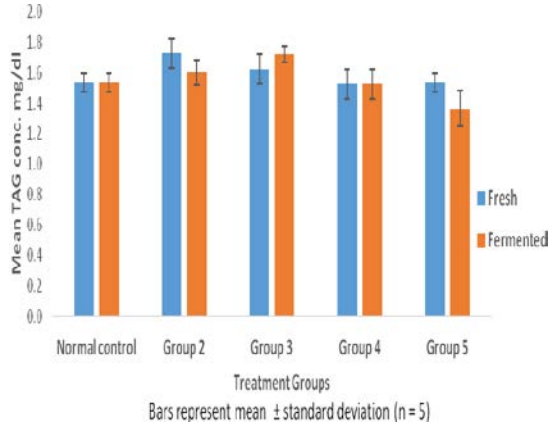

Figure 4. Effect of the aqueous extracts of palm fruits on the triacylglycerol concentrations of male Wistar albino rats

The data in figure 5 show the urea concentration of normal rats and the rats administered graded doses of fresh and fermented aqueous extracts of palm fruits respectively. Group 2 rats that were administered a low dose of the fresh aqueous extract showed no significant decrease ($P > 0.05$) in urea concentration when compared with the normal control. However, the rats in groups 3 – 5 administered the fresh aqueous extract of palm fruits and those in groups 2 – 5 administered the fermented aqueous extract of palm fruits showed a significant increase ($P < 0.05$) in the urea concentrations when compared with the urea concentrations of the normal control rats.

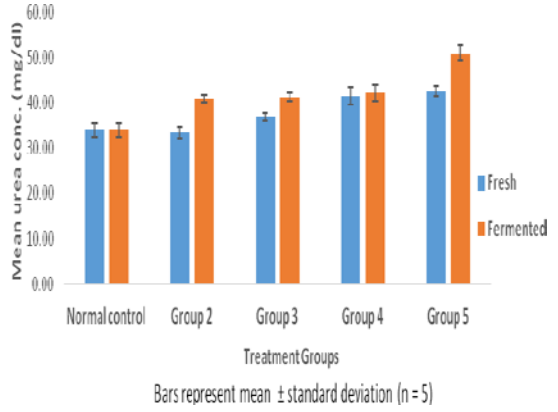

Figure 5. Effect of the aqueous extracts of palm fruits on serum urea concentrations of male Wistar albino rats

The data in figure 6 show the creatinine concentration of the normal control rats and the rats administered graded doses of fresh and fermented aqueous extracts of palm fruits respectively. All the rats administered the fresh and fermented aqueous extracts respectively showed a significant increase ($P < 0.05$) in the creatinine concentration respectively when compared with the creatinine concentration of the normal control rats.

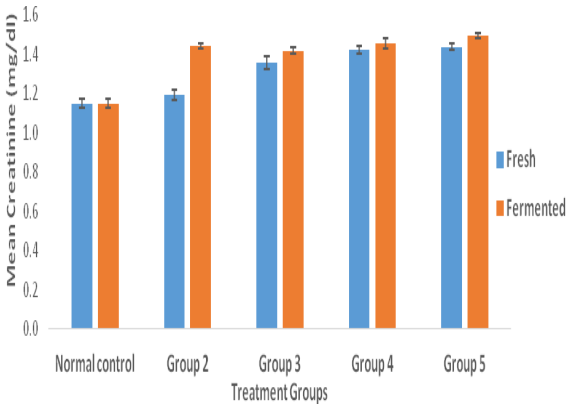

Figure 6. Effect of aqueous extracts of palm fruits on serum creatinine concentrations of male Wistar albino rats

Figure 7 shows the sodium electrolyte concentrations of the normal control rats and the rats administered fresh and fermented aqueous extracts of palm fruits respectively. All the groups of rats that were administered equivalent doses of the fresh and fermented aqueous extracts of palm fruits respectively showed a significant increase ($P < 0.05$) in the sodium electrolyte concentrations when compared with the sodium electrolyte concentrations of the normal control rats.

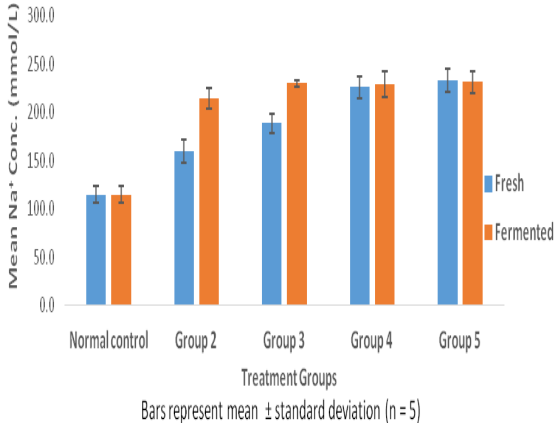

Figure 7. Effect of the aqueous extracts of palm fruits on serum sodium ion concentrations of male Wistar albino rats

Figure 8 shows the chloride ion concentration in the serum of normal control rats and the rats administered graded doses of fresh and fermented aqueous extracts of palm fruits respectively. Rats in groups 2 – 4 and rats in groups 6 – 5 that were administered fresh and fermented aqueous extracts of palm fruits respectively showed no significant increase ($P > 0.05$) in chloride ion concentrations when compared with the chloride ion concentrations of the normal control rats. In addition, group 5 rats that were administered the fresh aqueous extract of palm fruits and group's 2 – 3 rats that were administered the fermented aqueous extract showed no significant decrease ($P > 0.05$) in the chloride ion concentrations relative to the chloride ion concentration of the normal control rats.

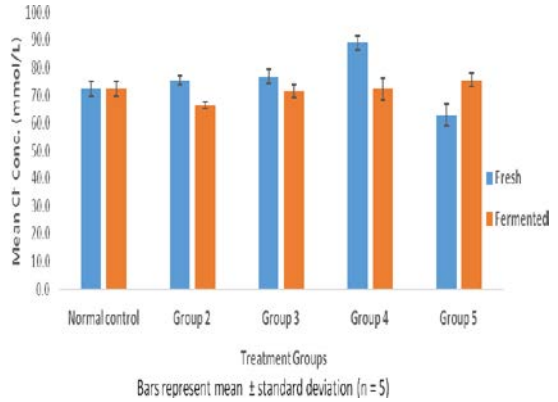

Figure 8. Effect of the aqueous extracts of palm fruits on serum Chloride ion concentrations of male Wistar albino rats

The data in figure 9 represents the serum potassium ion concentration of the normal control rats and the rats administered fresh and fermented aqueous extracts of palm fruits respectively. Group's 2 – 5 rats that were administered graded doses of the fresh extract of palm fruits showed a significant increase ($P < 0.05$) in the potassium ion concentration when compared with the potassium ion concentrations of normal control rats. Groups 2 and 4 that were administered the fermented aqueous extract showed no significant decrease ($P > 0.05$) in the potassium ion concentration when compared with the potassium concentration of the normal control rats. However, rats of group's 3 and 5 that were administered the same fermented aqueous extract of palm fruit showed an increase in the potassium ion concentrations, which is significantly high ($P > 0.05$) when compared with the normal control rats.

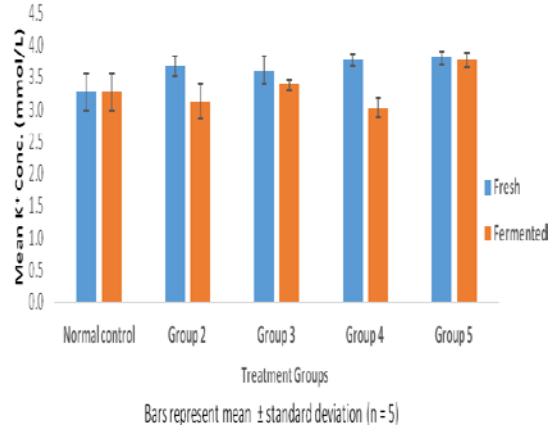

Figure 9. Effect of the aqueous extracts of palm fruits on serum potassium ion concentrations of male Wistar albino rats

The data in figure 10 show the hydrogen trioxocarbonate ion (HCO_3^-) concentrations of the normal control rats and the rats administered fresh and fermented aqueous extracts of palm fruits respectively. Rats of groups 2 and 3 that were administered the fresh aqueous extract of palm fruits showed a significant decrease ($P < 0.05$) in HCO_3^- concentrations when compared with the HCO_3^- concentrations of the normal control rats. Group-five rats that were administered equal doses of the fresh and fermented aqueous extracts of palm fruits respectively, showed a significant increase ($P < 0.05$) in the HCO_3^- concentrations relative to the HCO_3^- concentrations of the normal control rats. The group -three rats administered fresh extract showed no significant decrease ($P > 0.05$) in the HCO_3^- concentrations when compared with the HCO_3^- concentrations of the normal control rats. In addition, group-four rats administered the fresh aqueous extract and the rats of groups 3– 4 administered the fermented aqueous extract showed no significant increase ($P > 0.05$) in HCO_3^- concentrations when compared with the HCO_3^- concentrations of the normal control rats.

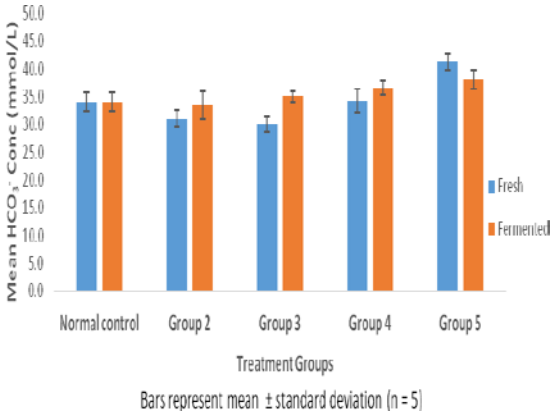

Figure 10. Effect of the aqueous extracts of palm fruits on serum bicarbonate ion concentrations of male Wistar albino rats

The data in figure 11 represent the uric acid concentrations of the normal control rats and the rats administered the aqueous extracts of palm fruits. Groups 2 – 4 that were administered the fresh aqueous extract of palm fruits showed a significant increase ($P < 0.05$) in the uric acid concentration when compared with the oral control rats. Group-six rats administered a high dose of the fresh aqueous extract showed no significant decrease ($P > 0.05$) in the uric acid concentration compared to the normal control. In addition, all the groups that were administered graded doses of the fermented aqueous extract of palm fruits showed no significant increase ($P > 0.05$) in uric acid concentration when compared with the uric acid concentration of the normal control rats.

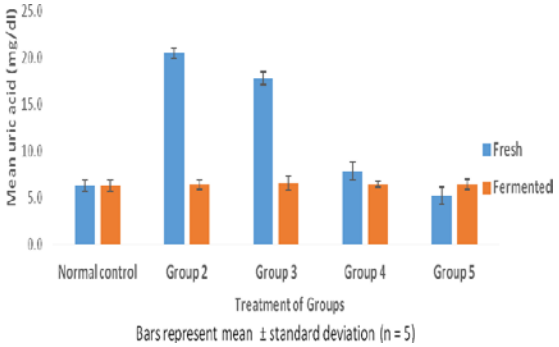

Figure 11. Effect of the aqueous extracts of palm fruits on serum uric acid concentrations of male Wistar albino rats

3.1. Histomorphological Features of Kidneys of Rats Administered Fresh and Fermented Aqueous Extracts of Palm Fruits Respectively

3.1.1. Group 1 (Normal Control Administered Normal Saline)

Sections of the kidney collected from the animals in this group showed the normal renal histo-architecture of laboratory rats. Normal Glomeruli (G) in normal Bowman's capsules (white arrow) was observed. The

Glomeruli were surrounded by a sea of normal renal tubules (proximal convoluted tubules, Pars recta, distal convoluted tubules and collecting ducts) both in the cortex and in the medulla. The renal interstitium were normal consisting of thin connective tissues and blood vessels (B). Renal tubules (arrow). H&Ex100; 400.

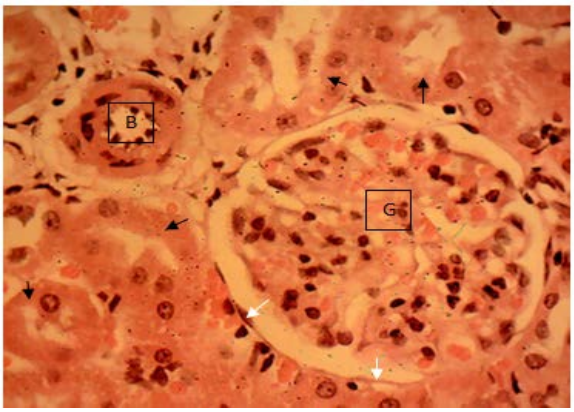

Figure 12. Kidney histomorphology of a normal control rat administered normal saline (2ml/kg b. wt.)

3.1.2. Group 2a (Administered Fresh Aqueous Extract)

Sections of the kidney collected from the animals in this group showed multifocal areas of tubular degeneration (black arrow) and regeneration (white arrow). The degenerating tubules showed tubular lining epithelial cells with cloudy, vacuolated cytoplasm and relatively normal to hyperchromatic nuclear, while the regenerating cells showed thin (barely visible) pale eosinophillic cytoplasm with large hypochromatic nuclei. Glomeruli (G). H&Ex100; 400.

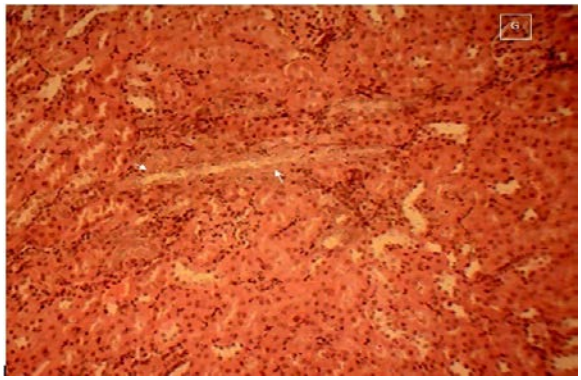

Figure 13. Kidney histomorphology of a rat administered fresh aqueous extract of palm fruits (100 mg/kg b. wt.)

3.1.3. Group 2b (Administered Fermented Aqueous Extract)

Sections of the kidney collected from the animals in this group showed the normal renal histo-architecture of laboratory rats. Normal Glomeruli (G) in normal Bowman's capsules (white arrow) was observed. The Glomeruli were surrounded by a sea of normal renal tubules (proximal convoluted tubules, Pars recta, distal convoluted tubules and collecting ducts) both in the cortex and in the medulla. The renal interstitium were normal consisting of thin connective tissues and blood vessels (B). Renal tubules (arrow). H&Ex100; 400

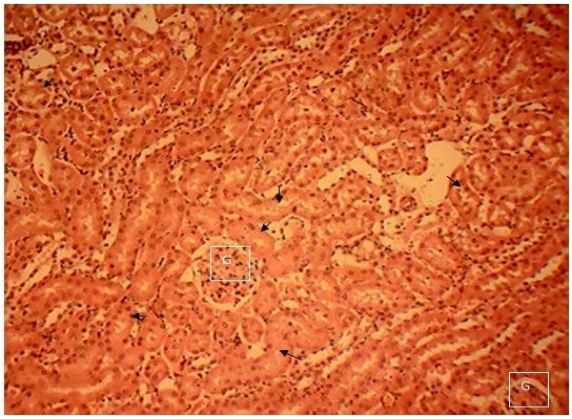

Figure 14. Kidney histomorphology of a rat administered fermented aqueous extract of palm fruits (100 mg/kg b. wt.)

3.1.4. Group 3a (Administered Fresh Aqueous Extract)

Sections of the kidney collected from the animals in this group showed a mild multifocal vacuolar degeneration of the renal tubular epithelial cells (arrow). The affected tubules showed epithelial lining cells with numerous minute clear cytoplasmic vacuoles and normal or pyknotic nuclei (blue arrow). Glomerulus (G). H&Ex400

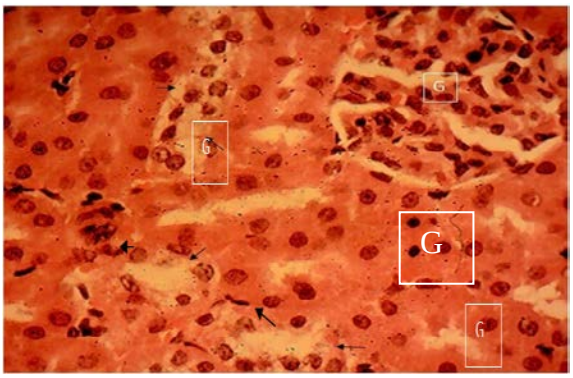

Figure 15. Kidney histomorphology of a rat administered the fresh aqueous extract of palm fruits (200 mg/kg b. wt.)

3.1.5. Group 3b (Administered Fermented Aqueous Extract)

Sections of the kidney collected from the animals in this group showed a mild multifocal vacuolar degeneration of the renal tubular epithelial cells (arrow). The affected tubules showed epithelial lining cells with numerous minute clear cytoplasmic vacuoles and normal nuclei. H&Ex400

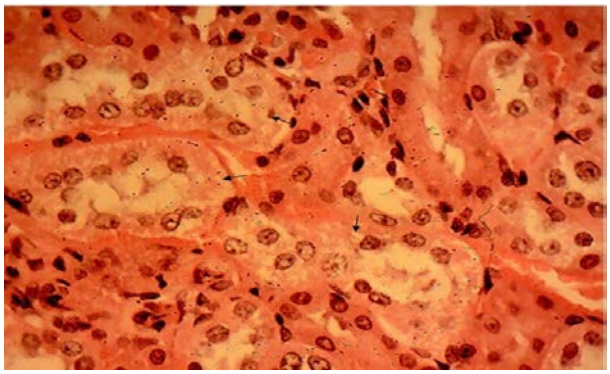

Figure 16. Kidney histomorphology of a rat administered the fermented aqueous extract of palm fruits (200 mg/kg b. wt.)

3.1.6. Group 4a (Administered Fresh Aqueous Extract)

Sections of the kidney collected from the animals in this group showed the normal renal histo-architecture of laboratory rats. Normal Glomeruli (G) in normal Bowman's capsules (white arrow) was observed. The Glomeruli were surrounded by a sea of normal renal tubules (proximal convoluted tubules, Pars recta, distal convoluted tubules and collecting ducts) both in the cortex and in the medulla. The renal interstitium were normal consisting of thin connective tissues and blood vessels (B). Renal tubules (arrow). H&Ex400.

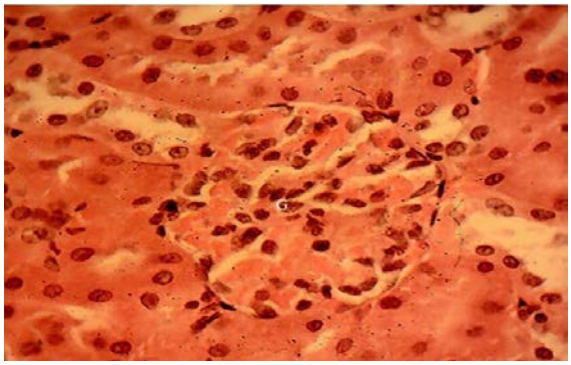

Figure 17. Kidney histomorphology of a rat administered the fresh aqueous extract of palm fruits (400 mg/kg b. wt.)

3.1.7. GROUP 4b (Administered Fermented Aqueous Extract)

Section of the kidney collected from this group showed focal area of tubular necrosis (black arrow) with mild to moderate inflammatory cellular infiltration (white arrow) of the renal interstitium. H&Ex100; 400.

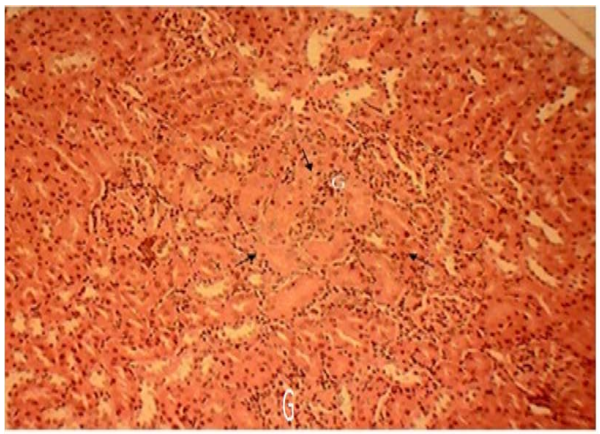

Figure 18. Kidney histomorphology of a rat administered the fermented aqueous extract of palm fruits (400 mg/kg b. wt.)

3.1.8. Group 5a (Administered Fresh Aqueous Extract)

Sections of the kidney collected from the animals in this group showed the normal renal histo-architecture of laboratory rats. Normal Glomeruli (G) in normal Bowman's capsules (white arrow) was observed. The Glomeruli were surrounded by a sea of normal renal tubules (proximal convoluted tubules, Pars recta, distal convoluted tubules and collecting ducts) both in the cortex and in the medulla. The renal interstitium were normal consisting of thin connective tissues and blood vessels (B). Renal tubules (arrow). H&Ex400.

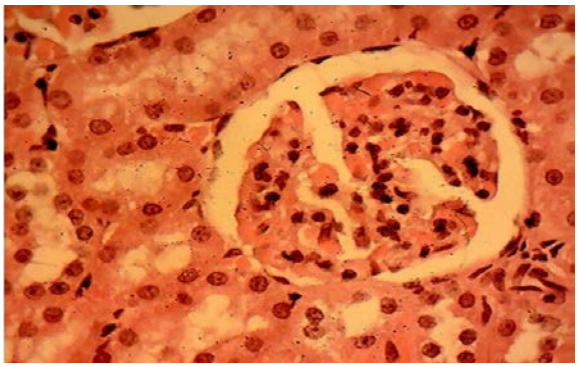

Figure 19. Kidney histomorphology of a rat administered the fresh aqueous extract of palm fruits (600 mg/kg b. wt.)

3.1.9. Group 5b (Administered Fermented Aqueous Extract)

Sections of the kidney collected from the animals in this group showed a mild multifocal vacuolar degeneration of the renal tubular epithelial cells (arrow). The affected tubules showed epithelial lining cells with numerous minute clear cytoplasmic vacuoles and relatively normal to pyknotic (blue arrow) nuclei. Compare with normal renal tubule (white arrow). Glomerulus (G). H&Ex400

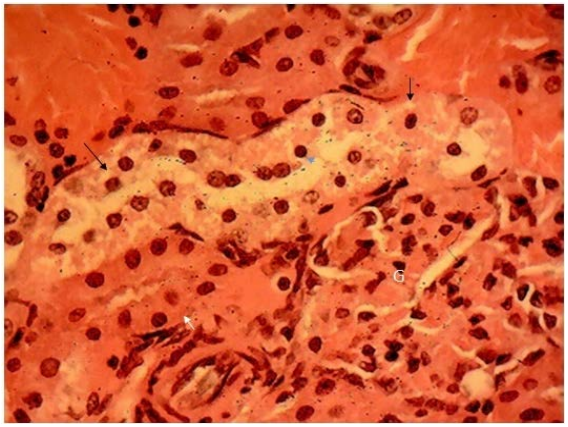

Figure 20. Kidney histomorphology of a rat administered the fermented aqueous extract of palm fruits (600 mg/kg b. wt.)

4. Discussion

This study investigates the effects of fresh and fermented aqueous extracts of *Elaeis guineensis* (palm fruits) on lipid profile and kidney function indices of male Wistar albino rats. The main objective is to understand any possible health implications of the consumption of fresh and fermented aqueous extracts of palm fruits on the consumers. Lipid profile is the breakdown of the components of fats found in one's blood at any given time. Such fats include triacylglycerol, total cholesterol, LDL - cholesterol and HDL – cholesterol which when present in an abnormal proportion increases the risk for cardiovascular diseases. Abnormal lipid profile has been greatly associated with heart attacks and strokes due to atheroma (atherosclerosis) caused by the deposition of cholesterol along the walls of arteries (Morris and Ferdinand, 2009).

The no significant increase ($P > 0.05$) in the total cholesterol concentration observed in the rats that were administered lower doses of fresh aqueous extracts of palm fruits showed that the increase in cholesterol concentrations observed in this study are likely a product of chance and may not be attributed to any effects by the aqueous extracts. Total cholesterol concentrations in blood are not constant but fluctuate based on the prevailing physiological conditions. However, higher cholesterol concentrations observed in groups 4 and 5 of the rats administered the fresh aqueous extract could be attributed to the ability of the fresh aqueous extract of palm fruits to increase cholesterol concentrations at higher doses. The significant increase observed in the total cholesterol concentrations in groups 2, 3 and 4 of the rats administered graded doses of the fermented aqueous extract of palm fruits could be attributed to the cholesterol promoting constituents (such as palmitic acid, lauric acid, stearic acid and glycidyl fatty ester) that may be present in the fermented aqueous extract (Kromhout *et al*., 1995; Sambanthamurthi *et al*., 2000). The constituents of the aqueous extract which could not promote an increase in the total cholesterol concentrations when equivalent doses of the fresh aqueous extract were administered may have been fermented or metabolised into more potent cholesterogenic metabolites that effectively promoted the increase in the total cholesterol concentrations in the rats. Additionally, the significant decrease observed in the total cholesterol concentration in the group-five rats that were administered the highest dose of the fermented aqueous extract of palm fruits suggests that the extract contains low concentration of anti-cholesterogenic constituents. The increased dose of the extract administered to group 5 of the rats may contain enough anti-cholesterogenic agents that drastically caused a decrease in the total cholesterol levels in the rats. The ability of the fermented aqueous extract to increase the total cholesterol concentration at low doses makes it bad for consumption as it could easily lead to an abnormal lipid profile, and aggressively endanger the lives of its consumers. Although, cholesterol plays vital roles in the body such as being an insulator, in addition to the maintenance of the membrane stability, the formation of steroid hormones among others, high concentrations of cholesterol in the blood increases the risk of heart diseases. Sparling *et al*. (1999) have reported that high concentration of serum cholesterol is one of the main controllable risk factors for cardiovascular diseases. Thus, care should be taken to avoid endangering one's health due to the unhealthy eating.

High density lipoprotein (HDL) cholesterol is a component of lipid profile generally considered as good cholesterol. HDL – cholesterol when present in higher concentrations regulates the amount of LDL – cholesterol in the blood. It achieves this maintenance of LDL – cholesterol level in the blood by transporting it from the arteries to the liver where it will be eliminated from the body (Hunter *et al*., 2010). The no significant increase in the HDL – cholesterol concentrations observed in this study could be ascribed to the normal changes in the physiological conditions rather than to the effects of the aqueous extracts, as its concentrations are not static, but fluctuates within a range of values. Similarly, the no significant decrease in the HDL – cholesterol concentrations observed in the rats of group 5 which were administered a higher dose of the fermented aqueous extract suggests by increasing the dose of the fermented extract administered, a significant decrease in the HDL – cholesterol concentration may result. This could negatively affect the health of an individual especially when there is a high concentration of LDL – cholesterol in the blood circulation. The abnormal lipid profile has been largely attributed to the increasing cases of cardiovascular diseases, which in turn is very relevant to the high mortality indices (Lewington *et al*., 2007; McQueen *et al*., 2008). Food items that significantly increase the HDL – cholesterol level at the expense of LDL – cholesterol is nutritionally preferred to those that decrease the HDL – cholesterol concentration and increase the LDL – cholesterol concentration. This is attributed to the health-promoting benefits associated with HDL – cholesterol as it helps remove LDL – cholesterol from the body (Morris and Ferdinand, 2009).

A healthy heart is maintained when there is low concentrations of LDL-cholesterol and high concentrations of HDL – cholesterol in the blood. This ratio reduces the risk of atherosclerosis and heart diseases. Higher concentrations of LDL–cholesterol and low concentrations of HDL – cholesterol in the blood lead to the narrowing of the arteries due to the deposition of LDL–cholesterol on the arterial walls, hindering the transport of blood to the brain and other vital organs (Nazawi and El-Bahr, 2012). The no significant differences observed in the HDL – cholesterol concentrations in the rats administered graded doses of fresh aqueous extract of palm fruits maybe attributed to the fresh extract having low saturated fats and a substantial amount of unsaturated fats that lower the LDL – cholesterol concentrations and promote a healthy heart. On the other hand, there were significant increases in the HDL – cholesterol levels of the rats in groups 2 and 4 that were administered the fermented aqueous extract of palm fruits which indicates that the extract may be rich in saturated fats and other cholesterogenic constituents that promote the HDL – cholesterol formation. Thus, the fermented aqueous extract of palm fruits could have deleterious effects on individuals who consume it regularly. The use of these aqueous extracts in the production of palm oil as a water substitute and in the preparation of local soup "ofe akwu" should be

discouraged. Although, the abnormal lipid profile is promoted by a genetic factor, the lack of physical activity and other related diseases, adhering to the principles of healthy eating remains the best way to maintaining a balanced lipid profile and reducing risk of strokes and heart diseases (Zemdegs *et al*., 2011).

Triacylglycerol is a major energy store in the body and its concentrations are greatly influenced by genetic factors, physical activities and the food intake. Low serum triacylglycerol concentrations promote a good health and heart functions while high triacylglycerol concentrations increases the risk of heart diseases. The no significant increases and decreases observed in the rats administered graded doses of the fresh and fermented aqueous extracts of palm fruits respectively relative to the normal control suggest that the extracts lack potentials to increase the blood triacylglycerol levels to the extent that will make their consumers vulnerable to heart diseases (Zemdegs *et al*., 2011). The observed change in the triacylglycerol concentrations might be attributed to the extracts. The fluctuations in triacylglycerol levels may be a result of some changes in the normal physiological conditions.

The dose-dependent significant increase observed in the creatinine concentrations of the rats administered fresh and fermented aqueous extracts of palm fruits respectively relative to the normal control could be attributed to some adverse effects of the aqueous extracts of palm fruits on kidney functions which led to the impairment of glomeruli filtration rate and increased the creatinine concentrations in the blood. Creatinine, which is a product of creatine phosphate breakdown released from skeletal muscles at a steady rate, serves as a more sensitive and specific test for renal functions because the changes in its concentration give a better assessment of the renal status. An increased serum creatinine level signifies impaired renal function in most cases (Nwodo *et al*., 2015). However, the high intake of dietary protein, oral creatine intake, crush injury and some drugs such as cimetidine and trimethoprim have been shown to raise the serum creatinine level even in the absence of any renal damage or renal malfunctioning. The significant creatinine concentrations observed in this study signify a renal impairment in rats. The serum concentrations together with the changes observed in the histomorphology of the kidney agree with the findings of Spanaus *et al*., (2010) and Dalton, (2010) who had independently reported that the serum creatinine concentration is a very reliable indicator of the glomerular filtration rate which is largely the most sensitive serum biomarker for detecting any little or early changes in the glomerular filtration rate in individuals. It is also in line with the earlier findings by Anjaneyulu *et al*., (2004) which state that increase in urea and the serum creatinine concentrations in rats indicates a progressive renal damage. The observed renal impairment in rats calls for a critical investigation of the effects of the aqueous extracts on the renal function indices of humans to prevent any associated adverse health effects.

Urea is a metabolic product of ammonia resulting from the breakdown of proteins and other amino group containing compounds in the liver. It is relatively non-toxic and serves as one of the markers of renal function though less specific. Increase in the blood urea concentration is associated with an impaired renal function. The dose-dependent increase in the serum blood urea concentrations observed in this study are probably due to the toxic effects of these extracts on the kidney that impaired the ability of renal glomeruli to filter urea from the blood (Nwodo *et al*., 2015). Measuring blood urea concentration is a less specific and sensitive method for ascertaining the renal function of an individual as its blood concentration can be increased by stress, high protein diets, and upper gastrointestinal (GI) bleeding (Wijeysundera *et al*., 2007). It has been shown that an increase in the urea concentration commonly occurs when there is impairment of the renal function and in combination with the observed increase in the serum creatinine concentration which indicates a progressive renal damage as observed in this study (Anjaneyulu *et al*., 2004, Shrestha *et al*., 2008)

Serum electrolytes concentrations affect the body functions including the regulation of blood acidity, water balance, muscle functions, nerve conduction, blood clotting and body fluids for the sustenance of life (Chernecky and Berger, 2013). Electrolyte imbalance frequently occurs due to the kidney failure, dehydration, fever and vomiting, thereby disturbing normal cellular functions (Husain *et al*., 2009). The high serum electrolytes concentrations observed in this study could be attributed to the effect of the extracts on kidney functions and partly to the richness of the extracts in micronutrients such as potassium, sodium, calcium and magnesium (Uroko and Njoku, 2014). Coupled with creatinine and urea, serum electrolyte concentrations give a better measure of kidney functions and a guide for choosing the treatment option. The excessive serum potassium concentration as seen in this study commonly occurs because of renal malfunction associated with the inability of the kidney to excrete potassium ions and the increased amount of potassium released from the damaged cells. An abnormal high potassium level could trigger a cardiac arrest, and may be effectively treated with insulin and glucose administration to get potassium into cells before addressing the main cause of the increase in the serum potassium concentrations (National Institute for Health and Care Excellence, 2013).

Uric acid is a metabolite of purine catabolism that is filtered by the glomeruli and both reabsorbed and secreted by the renal tubules (Rodwell, 2003). Blood uric acid concentration usually increases with renal damage or renal impairment and has been used as an indicator of renal functions. However, the increase in the blood uric acid concentration is not specific to renal impairment because many conditions can lead to increase in the blood uric acid concentration under normal renal functions. Severe haemolytic anaemia, lead poisoning, burns, a crush injury, tumour lysis and diuretic drugs are the most established causes of an increased blood uric acid concentration apart from renal impairment. The increased blood uric acid concentration observed in the rats administered low doses of the fresh aqueous extract show that the extract possesses chemical constituents capable of impairing renal functions possibly by causing severe haemolytic anaemia, or interfering with the glomeruli filtration rate, reabsorption, and secretion of uric acid by the renal tubules. Although many researchers have suggested that hyperuricemia only indicates renal dysfunction rather than a risk factor for its

progression, it is generally considered as an independent risk factor for the development and progression of coronary artery diseases (Madsen *et al*., 2005; Roncal *et al*., 2007). Thus, the consumption of fresh aqueous extract of palm fruits is of great health concern. None of the rats administered the fermented aqueous extract showed an increase in blood uric acid concentration suggesting that fermentation might have detoxified nephrotoxic constituents responsible for the observed increase in the blood uric acid concentration of the rats administered the fresh aqueous extract into non-toxic metabolites. Thus, the fermented aqueous extract of palm fruits may be said to lack the potential of impairing renal functions.

5. Conclusion

The findings of this study suggest that the fermented aqueous extracts of palm fruits have a high potential of negatively altering the lipid profile and increasing the health risks associated with a poor lipid profile. In addition, both the fresh and fermented aqueous extracts of palm fruits possess potentials that can impair renal functions if consumed in excess or for a long period as demonstrated in this study. At present, there is no scientific data on the effects of these aqueous extracts on humans, thus, the consumption of aqueous extracts of palm fruits through local dishes such as "ofe akwu" should be minimized, and their use as a water substitute for the production of palm oils in various palm oil mills should be discouraged.

Ethical Approval

A written ethical approval has been collected and preserved by the author(s) concerning the international standards or university standards.

Competing Interests

The authors declare that no competing interests exist.

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