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Biochemical and Histopathological Studies on the Effect of Raw and Cooked *Tetracarpidium conophorum* on Male Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SAM designed the study, author PNU wrote the protocol, and author VOA wrote the first draft of the manuscript. Author VOA managed the literature searches, analyses of the study, author EGA performed the spectroscopy analysis and author COA managed the experimental process and author SIE identified the species of plant. Author CVA performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study is mainly aimed at investigating the study the toxic effects of raw and cooked *Tetracarpidium conophorum* nut taken as snacks in Nigeria.
Study Design: Both biochemical and histopathological effects the raw (RTC) and cooked (CTC) *Tetracarpidium conophorum* of different diet formulation were carried out following 30 days period of oral feeding.
Methodology: Rats were divided into six groups of five rats each. Each feed and walnut was weighed and mixed in the ratio of 1:1 before administration. Group A: Normal animal feed, Group B: Mixture of animal feed and cooked nut (ratio of 1:1). Group C: Mixture of animal feed and the

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raw nut (ratio of 1:1), Group D: 100% of the cooked nut, Group E: 100% of the raw nut while Group F: Mixture of raw nut and cooked (ratio of 1:1).

Results: Result showed that the aspartate amino transferase activity of group E and F (100% RTC and 50% RTC+50%CTC) respectively were significantly higher ($P<0.05$) than that of the control group (Group A). Alanine amino transferase activity of group B, E and F (50% feed + 50% CTC, 100% RTC and 50% CTC + 50% RTC respectively) were significantly higher ($p<0.05$) than that of the group A (normal group). The ALP levels of group D (100% CTC) recorded a significant increase ($p<0.05$) when compared with that of the control. The urea concentration of group F was significantly higher ($p<0.05$) than that of the control group while there was no significant difference ($p>0.05$) between the creatinine concentration of that of test groups and the control. Histopathological results showed mild to severe changes in the liver of all the test groups. However, no such changes were observed in the kidney of the test groups.

Conclusion: The results seem to suggest evidence of toxicity especially for RTC, indicating that cooking of the nut might have reduced the level of toxicity.

Keywords: Tetracarpidium conophorum; lobular architecture; inflammation; Histopathology; Creatinine; Alkaline phosphatase.

1. INTRODUCTION

Toxicity is the extent at which a compound causes harm to animal and plant tissue or organs. It can be acute, sub chronic, or chronic. There is a wide distribution of biologically-active constituents throughout the plant kingdom, particularly in plants used as animal feeding stuff and in human nutrition [1]. The knowledge that these compounds elicit both toxic and advantageous biological responses has given rise to several investigations in recent times as to their possible physiological implications in various biological systems [1]. Toxicity is expressed generally as a dose-response relationship, involving the quantity of substance to which the organism is exposed to [2]. Furthermore, it is well known that many plant products contain some toxic substances which when consumed could accumulate gradually in the body and later cause some damages to cells, tissue or organs [3]. *T. conophorum* (Mull. Arg.) Hutch & Dalz (Euphorbiaceae), known as conophor (English), ukpa (Igbo-Eastern Nigeria), awusa or asala (Yoruba-Western Nigeria), is a perennial climbing shrub of 3–6 m long. Walnut is a member of the family of Juglandaceae (English walnut), Euhporbiaceae (African walnut) and Olacaceae (African walnut). Euphorbiaceae family is mainly found in Nigeria with its peculiar characteristics [4]. The plant is cultivated principally from the nuts which are cooked or consumed raw as snacks [5]. A bitter taste could be felt upon drinking water immediately after eating the nut. This bitterness could be attributed to the presence of chemical substances such as alkaloid [6]. The presence of oxalate, phytates and tannins in the raw *Tetracarpidium conophorum* nut have been reported [4]. The proximate composition, ascorbic acid and heavy metal contents of the nut have also been reported [7]. In Nigerian folk medicine, the leaves are used as male fertility active principle in the treatment of dysentery in southern Nigeria. The amino acid and fatty acid components of the nut and the use of its leaf juice for treatment of prolonged and constant hiccups have been reported [8]. Oladiji et al. [9] addressed the toxicological consequences of consuming the oil-based *Tetracarpidium conophorum* on rats. There are several reports on the content of the nut but there is paucity of information on biochemical and histopathological effect of cooked and raw *T. conorphorum* nut. This study is aimed at addressing toxic effect of consuming raw and cooked *Tetracarpidium conophorum* nut.

2. MATERIALS AND METHODS

2.1 Plant Materials

Fresh *Tetracarpidium conophorum* nut was obtained from its tree at Olido village, Igbo-Eze North L.G.A. of Enugu State, Nigeria and was identified by Mr. Ozioko Alfred, a taxonomist at Biodiversity and Resouces centre, Nsukka.

2.2 Experimental Animals

A total of 30 male albino rats weighing120-150g was obtained from the animal house of the Faculty of Biological Sciences, University of Nigeria, Nsukka. They were acclimatized for one week under standard environmental conditions and maintained on a regular feed and water *ad libitum.*

2.3 Preparation of Walnut Meal

The method described by Malu et al. [10] was adopted for formulation of the meal. The nut was de-shelled and sun-dried for seven days and ground with the aid of an electric blender. Cooked and raw seed were ground separately into fine powder. They were kept in air-tight container and stored in a refrigerator.

2.4 Animal Grouping and Feed Formulation

Rats were divided into six groups of five rats each. Animal feed and walnut were weighed in the ratio of 1:1 before administration. The rats were fed *ad libitium* for period of 30days. The animals were handled according to the guidelines of the ethical committee on the use and care of experimental animals of the University of Nigeria, Nsukka.

Group A: Commercial rat pellet, Group B: Mixture of commercial rat pellet and cooked nut (ratio of 1:1). Group C: Mixture of commercial rat pellet and the raw nut (ratio of 1:1), Group D: 100% of the cooked nut, Group E: 100% of the raw nut and Group F: Mixture of raw nut and cooked (ratio of 1:1).

2.5 Determination of Biochemical Parameters

The activities of aspartate aminotransferase and alanine aminotransferase were assayed using the procedures described by Reitman and Frankel [11] while the method of Wright et al. [12] was used for the assay of ALP activity. The concentrations of urea and creatinine were determined using the method of Tietz [13] as contained in Randox test kit. Histopathology was also carried out on the liver and kidney tissues by the methods of Bora [14].

2.6 Statistical Analysis

Statistical analysis of the data for multiple comparisons was performed by one-way analysis of variance (ANOVA) followed by Duncan's test used for post-hoc. $P<0.05$ was considered statistically significant. (SPSS 17.0 software package). All values are presented as mean ± S.D.

3. Results

3.1 Alanine Aminotransferases

The groups of rats fed with 50% feed + 50% CTC (group B), 100% CTC (group D)), 100% raw (group E), 50% CTC + 50% RTC (group F) diets had significantly high ($p<0.05$) serum ALT activities when compared with that of the control group (group A).

3.2 Aspartate Aminotransferases (AST)

The rats fed with 100% raw (group E) and 50% cooked + 50% raw (group F) diet were significantly higher ($p<0.05$) serum ALT activities

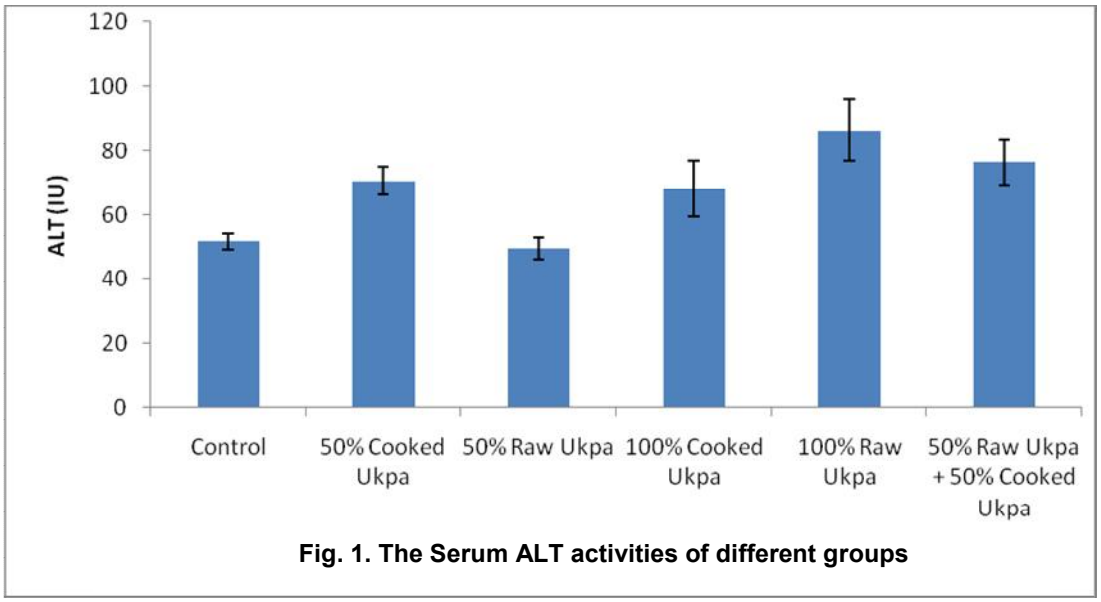


Fig. 1. The Serum ALT activities of different groups

than those fed with 100% CTC (group D), 50% feed + 50% RTC (group C) and those of the control group (group A).

3.3 Alkaline Phosphatase (ALP)

Rats fed with 50% feed+ 50% CTC (group B) and 100% (group D) had significant increase ($p<0.05$) on the activity of serum alkaline phosphatase while raw diets seemed to have no significant ($p>0.05$) effect on the enzyme when compared with that of the normal group (group A).

3.3.1 Urea

Group C and E (50% feed + 50% RTC and 100% raw respectively) had significant increase ($p<0.05$) in the concentration of urea. Raw nut diets seemed to have significantly ($p<0.05$) increased the concentration of serum urea, suggesting possible effects on kidney cells.

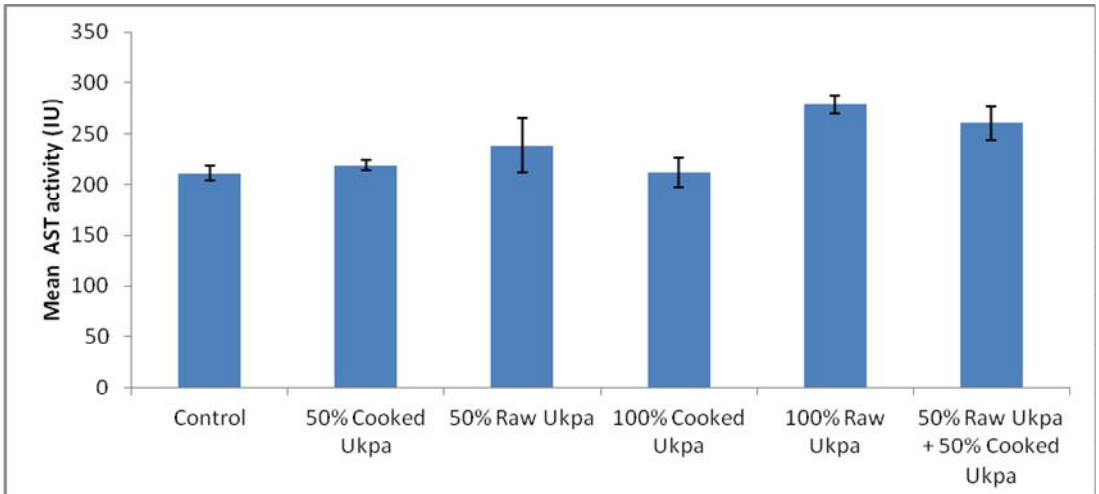


Fig. 2. Effect of cooked and raw *T. conophorum* on AST activity in different groups

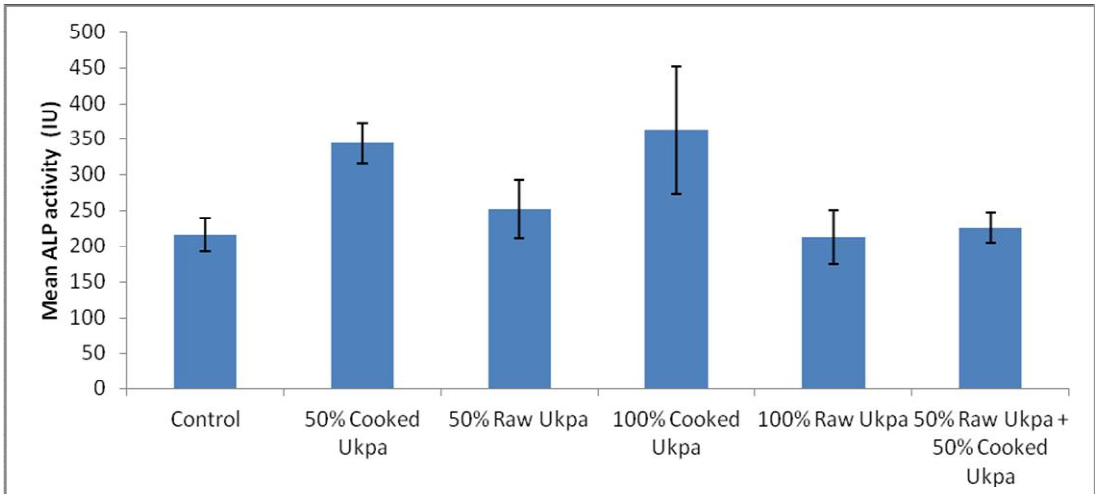


Fig. 3. Effect of cooked and raw *T. conophorum* on ALP activity in different groups

3.3.2 Creatinine

The effect of both raw and cooked nuts diets seemed to have no significant ($p>0.05$) difference on the kidney tissues (Fig. 5). These two results could suggest that the nut when eaten raw could have mild effect on the creatinine concentrations but these effects could be reduced when the nut is cooked.

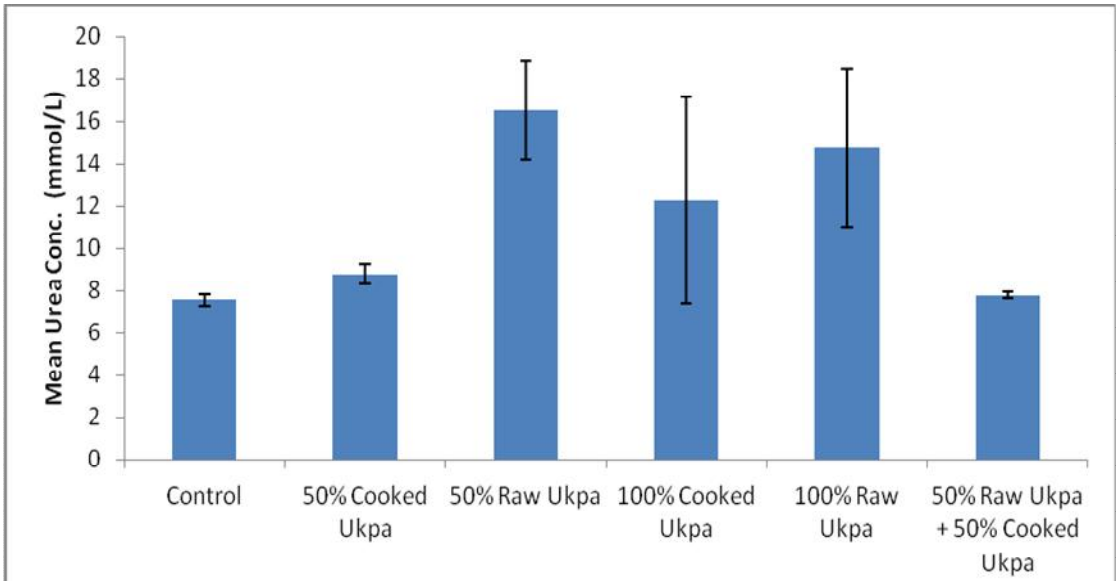


Fig. 4. Effect of cooked and raw *T. conophorum* on urea concentration in different groups

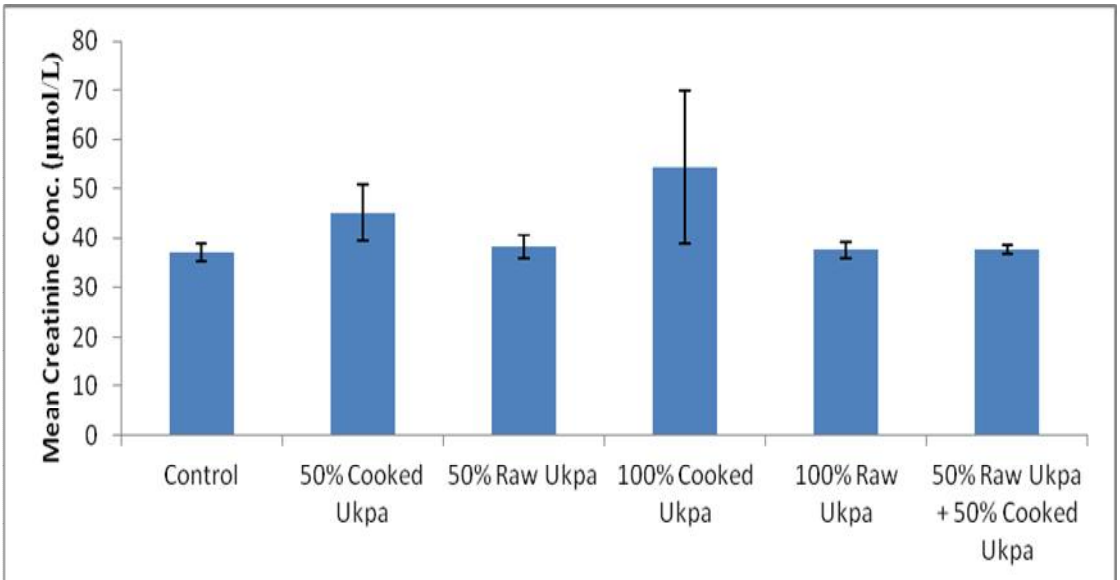
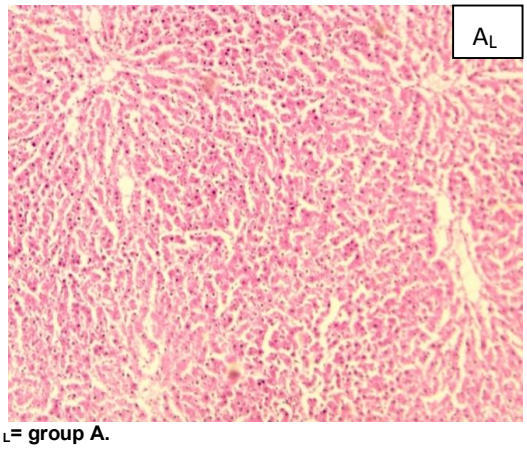
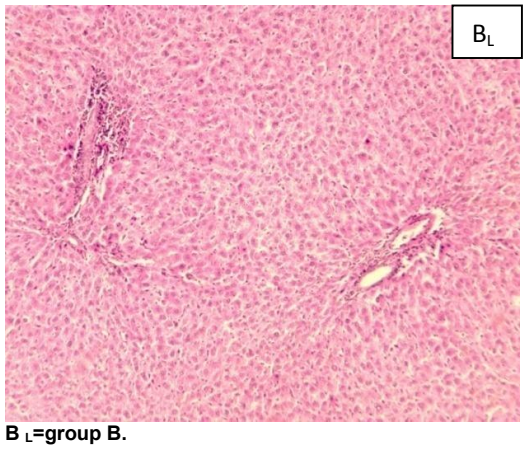


Fig. 5. Effect of cooked and raw *T. conophorum* on creatinine concentrations of different groups of rats

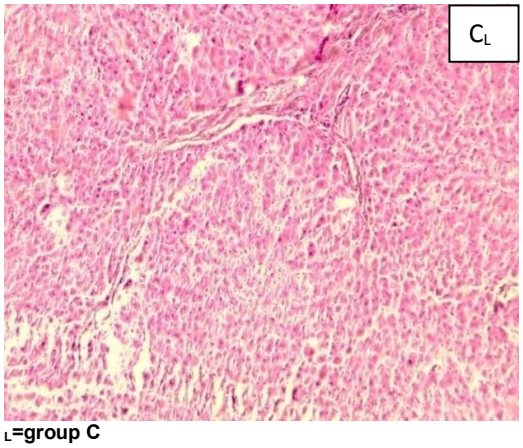
3.4 Histological Examination of the Rat Liver of Different Groups



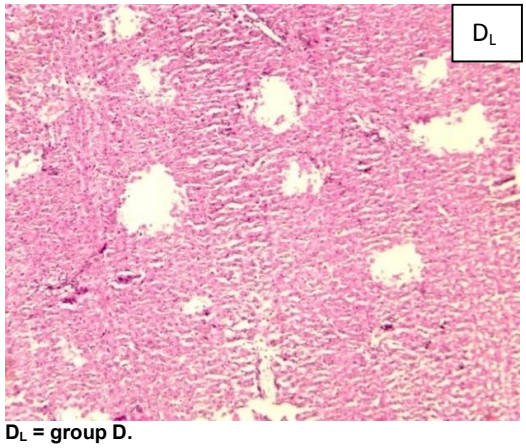
A_L= group A.



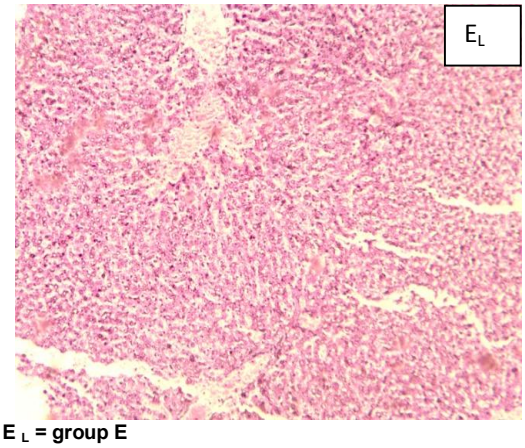
B_L=group B.



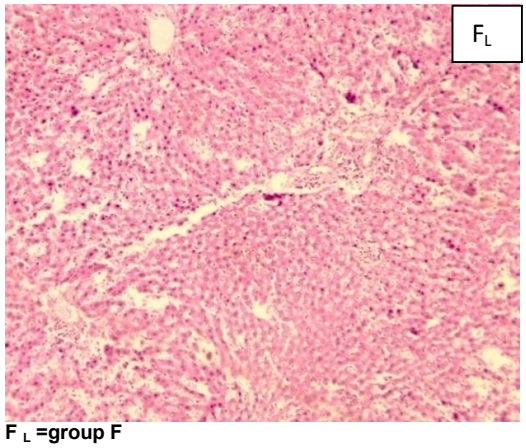
C_L=group C



D_L = group D.



E_L = group E



F_L =group F

Plate A_L (Conrol): shows no inflammation, sinusoids are normal with preserved hepatocytes. Plate B_L: shows mild periportal lymphocytic infiltrates with lobular architecture largely spared when compared with control which has the hepatocytes preserved. Plate C_L: shows severe distortion of the lobular architecture with extensive haemorrhage, lymphocytic infiltration is normal when compared with the control. Plate D_L: shows bridging fibrosis with portal inflammation when compared with the control. Plate E_L: shows portal inflammation but lobular architecture is preserved when compared with the control. Plate F_L: shows focal portal fibrosis and inflammation with occasional necrosis of hepatocytes when compared with control which has the hepatocytes preserved with no inflammation. These distortions suggest unpleasant effects on the liver in all the plates except the control.

3.5 Histological Examination of Rat Kidney of Different Groups

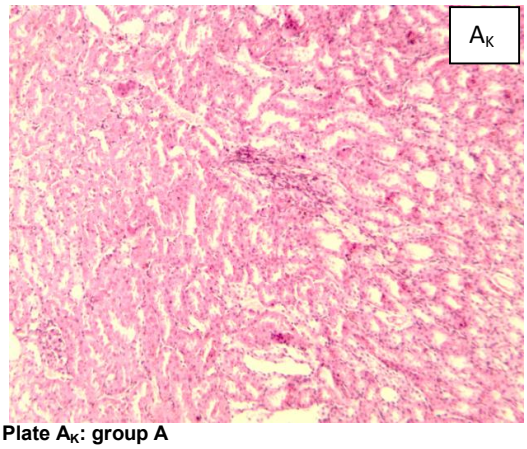


Plate A_K: group A

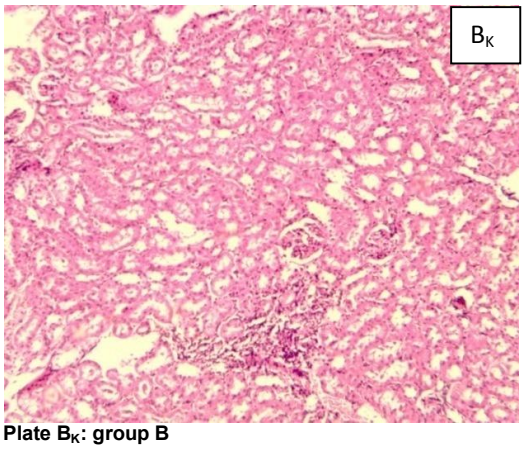


Plate B_K: group B

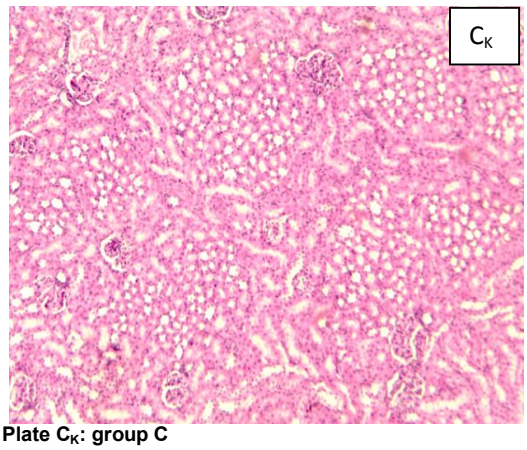


Plate C_K: group C

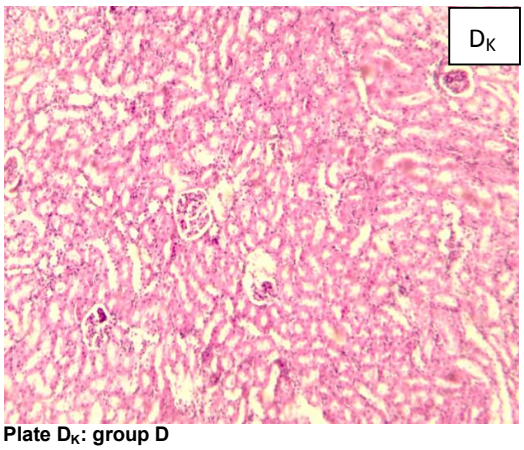


Plate D_K: group D

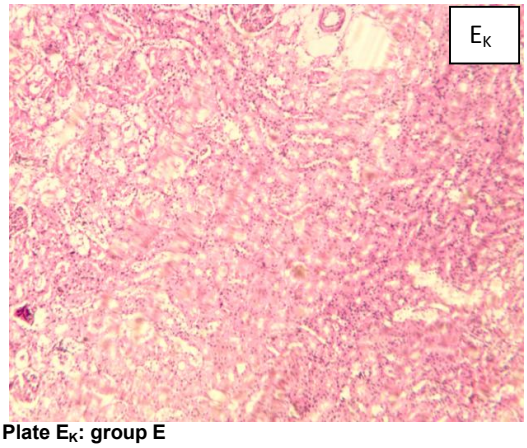


Plate E_K: group E

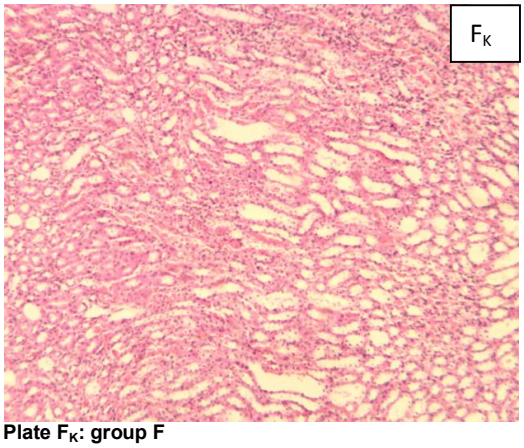


Plate F_K: group F

Plate A_K (Control): shows no tubular necrosis, vascular engorgement with no inflammatory cells. Tubules are essentially normal. Plate B_K: Tubules show mild lymphocytic infiltrates with glomeruli essentially normal. Plate C_K: shows vascular congestion with intratubular haemorrhage. Plate D_K: Renal capsules are normal with no obvious inflammation. Plate E_K: Tubules and glomeruli are essentially normal. Plate F_K: Tubules are preserved, no obvious damage with glomeruli normal.

These suggest that the nut has no obvious effect on the kidney when compared with that of the control.

4. DISCUSSION

This study evaluated the effects cooked *Tetracarpidium conophorum* (CTC) and raw *Tetracarpidium conophorum* (RTC) could exert on the liver and kidney of male albino rats. The function of the liver was evaluated by measuring serum Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and serum alkaline phosphatases (ALP) activities coupled with the histopathology of the liver cells. Kidney function was also evaluated by measuring serum urea and creatinine concentrations as well as histopathology also carried out on the kidney cells. Aminotransferases (AST and ALT) considered in this study are useful biomarkers because it reflects level the toxicity of liver especially when cytolysis has taken place [15,16]. The ALT and AST of rats in group E (100% RTC) and group F (50% RTC + 50% CTC) were significantly high ($P<0.05$) compared with that of the control (Figs. 1 & 2). The ALT activity of group B (50% rat feed + 50% CTC) and group D (100% CTC) were also significantly high ($P<0.05$) compared to that of the control. Due to increase in intake of CTC as observed during the experiment (especially group B and group D), there is a probable increase in amino acid metabolism which could bring about increases in both AST and ALT of group B (50% rat feed + 50% CTC) and group D (100% CTC) to control amino acid metabolic challenge. Food intake is influenced by palatability as well as source of amino acid profiles [17]. Enujiugha [2] had earlier noted that moderate thermal treatment in humid conditions (such as cooking) enhances digestibility due to partial protein unfolding and destruction of some secondary metabolites such as trypsin inhibitors. It appears that cooking enhanced digestion of *Tetracarpidium conophorum* protein much more than the RTC. The increase in ALP activity of rats in group B and group D as shown in (Fig. 3) could be attributed to the accumulation of some metabolites during the long term *T. conophorum* consumption and protein catabolism as contained in the proximate analyses [18]. These factors could have contributed mainly to the liver damage [19,20]. Although the result of the kidney function test (urea) suggested mild kidney damages, that of aspartate amino transferase suggested that the liver was strongly affected by the raw nut as compared to the cooked nut. Normally, enzymes will not always be found in the serum except there is disruption of the ordered lipid bilayer of the plasma membrane, as obtained in plate D_L of histological studies, there was bridging fibrosis with portal inflammation. Thus, the increase in the serum ALP activity of rats in group B and group D fed with 100% CTC suggests altered permeability of the plasma membrane, which could have resulted in the leakage of the enzyme into the serum [16, 21]. It was also observed that rats fed with RTC diets especially group E (100% RTC) showed some signs like bloody stool and swollen heads, the rats in group E even recorded some mortality. It is possible that *T. conophorum* contain some toxic peptides that may be responsible for these signs and symptoms. This result contradicted the earlier report that the nut can be taken raw, roasted or boiled [22]. The interactive effects of the cooked and raw *Tetracarpidium conophorum* nut on the liver and kidney could not be correctly interpreted. Nevertheless the result seems to suggest that raw nut increases hepatotoxicity more than the cooked nut as indicated in the histopathology results (plate B_L- F_L). Also liver obtained various pathological changes in comparison with that of the control in plate A_L. There was severe congestion of central veins and sinusoids and also necrosis of hepatocytes in plate C_L & F_L. Inflammatory area around portal tract was also observed in plate C_L, E_L, F_L. The hepatocellular damage was evidenced by the elevated liver enzymes (ALT & AST), which was attributed to the dilatation and congestion of hepatic vessels and sinusoids and are all direct effect of the toxins [23]. There could also be an impairment of cellular respiration that would lead to tissue necrosis especially of the brain, liver and heart [24]. Hence the hepatocellular damage could be attributed to the presence of both raw and cooked *Tetracarpidium conophorum* nut meal. The nitrogen component of urea (blood urea nitrogen – BUN) is the end product of protein degradation [17]. Rats in group C fed with 50% rat feed + 50% RTC and E fed with 100% raw displayed significantly higher ($p<0.05$) urea concentration (Fig. 4) than that of the control. This could be due to increased catabolism of amino acids that resulted to increase in concentration of nitrogen component of urea as protein catabolism increases. Blood urea nitrogen is elevated in kidney damage, excessive protein intake and low fluid intake [25]. The concentration of urea of group C fed with 50% rat feed + 50% RTC and group E fed with 100%, supported our earlier suggestion that the RTC-containing diet increases toxicity, though there was no clear evidence of nephrotoxicity as shown in histopathological studies (plate A_k-F_k). The non significant increase in creatinine and knowing that urea be influenced by protein or

fluid intake [25] suggests that there could be normal urea and creatinine excretion; this suggests that the kidney function may not have been affected by the diet formulation, since urea and creatinine levels are basically used to determine functions of kidney.

5. CONCLUSION

The result of this study suggests that the raw *Tetracarpidium conophorum* nut could inflict some hepatocellular damage. However, cumulative effect of long term consumption of the cooked nut could not have been exonerated in causing some kidney or liver damage.

CONSENT

Not applicable.

ETHICAL APPROVAL

The animals were handled according to the guidelines of the Ethical Committee on the use and care of experimental animals of the Department of Biochemistry, University of Nigeria, Nsukka.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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