Toxicological Assessment of Wistar rats fed with feed fortified with breadfruit.

ABSTRACT:

Background: The seed of breadfruit is a major food consumed in some parts of Nigeria. It has been identified as an important leguminous food that is highly rich in carbohydrates and protein with abundant minerals and vitamins and is known to contain important anti-nutrients. **Aim:** This study aims to assess the possible toxicological effect of feed fortified with cooked, parboiled, and grilled breadfruit on the liver and kidney function parameters of Wistar rats. **Method:** Kidney and liver function tests were carried out using standard diagnostic methods. **Results:** The results of the kidney function test carried out revealed that feed fortified with 30% cooked and parboiled breadfruit showed a significant (p<0.05) increase in the creatinine level while a significant increase (p<0.05) in the Urea level was observed in all the groups fed with the processed breadfruit with respect to the normal control group. The liver function test carried out showed an increase (p<0.05) in Alanine Transaminase (ALT) activity in the group fortified with 30% cooked breadfruit, a significant increase in Aspartate Transaminase (AST) activity (p<0.05) in all fortified groups with an exception to the group fortified with 30% cooked breadfruit. A significant increase (p<0.05) in Alkaline phosphatase (ALP) activity was seen in the group fortified with 50% grilled breadfruit.

Conclusion: The rise in the level of kidney and liver enzymes in the blood of the rats fed with fortified feeds may have occurred due to better nutritional status and muscle mass with the availability of more energy and protein in their diet but may also result in some tissue damage.

Comment [g1]: Increased Creatinine and urea levels are indices of kidney dysfunction

Comment [g2]: Rise in AST ,ALT and ALP indicates the oxidative damage at cellular level and they are the indicators of hepatic damage

Keywords: Breadfruit, Toxicity, kidney function, Liver function, cooked breadfruit, roasted breadfruit, grilled breadfruit.

INTRODUCTION

African breadfruit belonging to the family *Moraceae,* and genus *treculia* [1] is scientifically called *Treculia Africana* and locally known as Ukwa in Igbo and Afon in the Yoruba language and has been identified as an important leguminous food that is highly rich in protein [2] and amino acid needed for metabolic wellbeing of humans [3]. Based on literature studies, breadfruit is known to contain 52-72% carbohydrate [4] 17-22% protein [5,6] 7-9% Fat with abundance of vitamins and fiber [7].

The seeds of breadfruit are majorly consumed among the Igbos but also consumed by other Southern parts of Nigeria such as the Efiks, Kalabaris, Edos and the Ika Igbos in Delta State [8]. When processed properly, the extracted seed of breadfruit is said to be extremely healthy [9,10]. During processing, the seeds are fermented and then extracted from the fruit heads before it can

then be used for the preparation of delicious and nutritious diets. These extracted seeds could either be roasted, parboiled, dehulled, steamed or grilled [11].
According to Ezengige, [12], some nutritional and health benefits of breadfruit were summarized in the report. Chinedu *et al.* [3] reported that the seeds of breadfruit hold good promise for dietary control of type 2 Diabetes and other metabolic disease conditions. But like any other legumes, breadfruit is known to contain some anti-nutrients like protease inhibitors, tannins, saponins, oxalate, haemagglutinin, alkaloids, lectins, and hydrogen cyanide [9]. Breadfruit is highly rich in vitamins such as vitamin A, B_2, B_3, B_6, D, E and K and minerals such as sodium, copper, magnesium, and phosphorus [13].

However, the duration of cooking, temperature [2] and process of preparation, may greatly affect the nutritional content of the food [11,14,15]. The mineral and vitamins contentment of breadfruit differs when processed [13]. As a result of this, it has become very necessary for heat processing methods to be carefully selected and controlled to avoid damage to nutritive value, functionality, and reduce toxicity [2]. Medicinal plants are rich in phytochemicals and nutritional components that enhances its use in the treatment and management of certain ailments [16,17]. This study however tries to investigate the toxicological effect of bread fruit in Wistar rats fed with feeds fortified with different forms of processed breadfruit.

Methods

Sample Collection and Identification

The breadfruit used for this study was purchased from Orie market, Abagana in Njikoka Local Government Area, Anambra State, Nigeria. The sample was identified by a taxonomist in the Department of Botany, Nnamdi Azikiwe University, Awka. The voucher number as deposited in the herbarium of Nnamdi Azikiwe University, Awka is NAUH-77B.

Processing of Sample

The breadfruit was properly washed and mashed with water to remove its slippery nature and was then dried under room temperature for seven days. After the drying, the breadfruit was shared into three portions for processing.

Cooked Breadfruit

The first portion of the breadfruit was parboiled for 45 minutes. The pods were then separated from the chaffs with the help of a corona manual grinding machine. The breadfruit was then cooked using kerosene stove for a period of 2 hrs until it was soft and edible for consumption. Next, the cooked breadfruit was dried under room temperature and pulverized using corona manual grinding machine, and the dry powdered cooked breadfruit was stored inside a well labelled airtight plastic container until use.

Parboiled Breadfruit

The second portion of the breadfruit was parboiled by boiling it inside a pot containing water, on a stove for 45 minutes, till it was partly cooked. The pods were then separated from the chaffs with the help of a corona manual grinding machine, after which it was dried for one week under room temperature. Next, the pods were pulverized using corona manual grinding machine, and the now powdered parboiled breadfruit was stored inside a well labelled airtight plastic container until use.

Grilled Breadfruit

The third portion of breadfruit was grilled on a frying pan using a stove. The seeds were then separated from the chaffs of the pods and were pulverized using corona manual grinding machine. The now powdered grilled breadfruit was stored inside a well labelled airtight plastic container until use.

Composition of the Rat Feed

The standard feed used was a product of Novum Agric Industries. It was purchased from a Feed dealer in Awka. The ingredients used in the compounding of the standard feed include grains and cereals, vegetable, protein meals, vitamins, minerals, essential amino acids, anti-toxins, enzymes. The composition of the ingredients is as follows: Oil (6%), Protein (16%), Fibre (7%), Ash (10%), Calcium (0.95%) and Phosphorus (0.65%). The feed was fortified with the processed grilled, parboiled, and cooked breadfruit in the following percentages: Using an analytical weighing balance, the feed and respective breadfruits were each measured. To 70g of feed, 30g of grilled breadfruit was added; to 70g of feed, 30g of parbolied breadfruit was added; to 70g of feed, 30g of cooked breadfruit was added; also, to 50g of feed, 50g of grilled breadfruit was added; to 50g of feed, 50g of parboiled breadfruit was added; and to 50g of feed, 50g of cooked breadfruit was added. These formulations were repeated until enough feed was prepared which lasted for a period of one month.

Study Design

A total of 35 Wistar rats weighing between 120g-150g were purchased from Chris Experimental Animal Farm and Research Laboratory, Awka, Anambra State, and randomly divided into seven groups of five rats each and used for the study. They were maintained and housed in cages under standard environmental conditions (27°C ± 3°C, 12-hour light/dark cycle) in Chris Experimental Animal Farm and Research Laboratory, Awka. The rats were weighed, marked, and put into labelled cages. Their random blood glucose levels were also checked. The grouping are as follows:

Comment [g3]: Mention sex of animals maleor female

Group A – Normal Control
Group B – 70% Standard Feed fortified with 30% cooked breadfruit
Group C – 70% Standard Feed fortified with 30% parboiled breadfruit

Group D – 70% Standard Feed fortified with 30% grilled breadfruit
Group E – 50% Standard Feed fortified with 50% cooked breadfruit
Group F – 50% Standard Feed fortified with 50% parboiled breadfruit
Group G – 50% Standard Feed fortified with 50% grilled breadfruit

Feeding of the Experimental Animals
The experimental rats were fed accordingly using the feed prepared for each of the groups. The feeding was done for a period of four weeks after which the rats were fasted and anesthesized with chloroform before blood collection. Blood was collected by cardiac puncture and put in the EDTA bottles and plain bottles for hematological and biochemical analysis respectively. The carcasses were properly disposed by burying.

Liver Function Test
Serum biochemical indices routinely estimated for liver functions were analyzed. They include Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), direct and total bilirubin. The parameters were determined using Randox diagnostic test kits. The procedures used were according to the manufacturer's instruction.

Kidney Function Test
Serum biochemical indices routinely estimated for kidney functions were analyzed. They include Creatinine and urea. The parameters were determined using Randox diagnostic test kits. The procedures used were according to the manufacturer's instruction.

Data Analysis
Data obtained from the experiments were analyzed using the Statistical Package for Social Sciences software for windows version 23 (SPSS Inc., Chicago, Illinois, USA). All the data collected were expressed as Mean ± SEM. Statistical analysis of the results obtained were performed by using ANOVA Tests to determine if significant difference exists between the mean of the test and control groups. The limit of significance was set at $p<0.05$.

RESULTS
Result of Kidney Function Test
Table 1: Effect of feed fortified with cooked, parboiled and grilled Breadfruit seeds on kidney function parameters of Wistar rats expressed as mean ± SEM.

Groups	Creatinine (mg/dl)	Urea (mg/dl)
Normal Control	53.81±2.94	36.29±1.45
70% Std Feed + 30% cooked Breadfruit	62.00±2.19*a*	48.33±1.15*a*
70% Std Feed + 30% Parboiled Breadfruit	64.86±0.23*a*	46.48±2.33*a*
70% + Std Feed + 30% grilled Breadfruit	55.01±3.84	45.37±3.09*a*
50% Std Feed + 50% cooked Breadfruit	60.61±3.69	65.55±6.33*a*
50% Std Feed + 50% parboiled Breadfruit	59.87±218	59.63±3.37*a*
50% Std Feed + 50% grilled Breadfruit	61.23±2.42	62.04±1.58*a*

[a]Significant increase with respect to normal control.

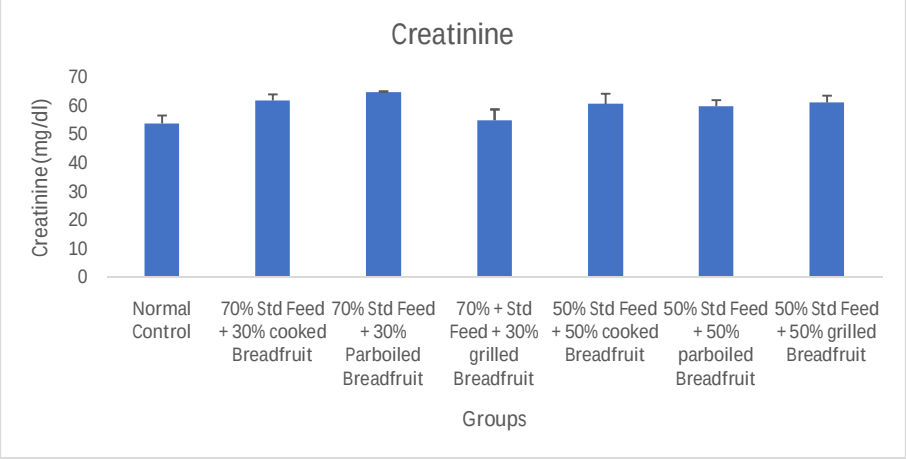

Figure 1: Effect of feed fortified with cooked, parboiled and grilled breadfruit on Creatinine levels of normal rats.

From figure 1 it is seen that the groups that were fed with feed fortified with 30% cooked and parboiled breadfruit showed a significant (p<0.05) increase in the creatinine level compared with

the normal control group which was fed with only the standard feed (figure 1). All other groups showed a non-significant (p>0.05) increase in the creatinine level compared with the normal control group.

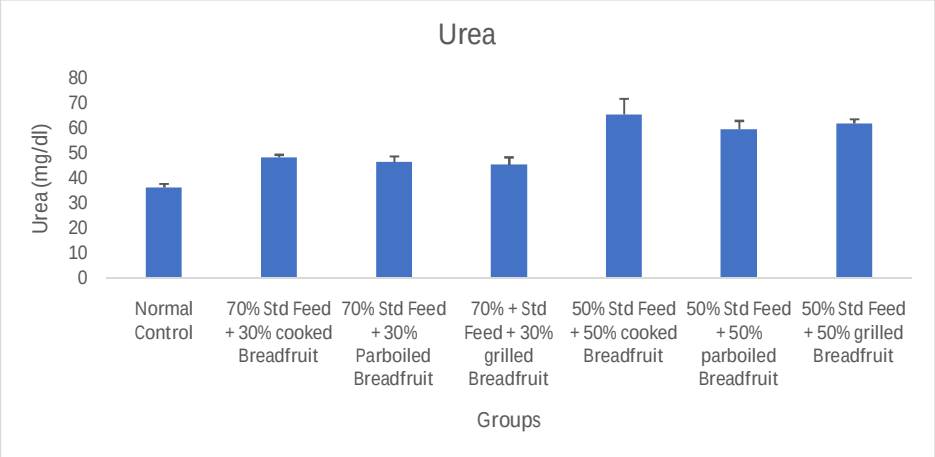

Figure 2: Effect of feed fortified with cooked, parboiled and grilled breadfruit on Urea levels of normal rats.

A significant increase (p<0.05) in the Urea level was observed in all the groups of rats fed with fortified feed when compared to the normal control (figure 2).

Result of Liver Function Test

Table 2: Effect of feed fortified with cooked, parboiled and grilled Breadfruit seeds on liver function parameters of Wistar rats expressed as mean ± SEM.

Groups	**ALT (IU/L)**	**AST (IU/L)**	**ALP (IU/L)**
Normal Control	8.00±0.00	26.00±1.00	264.96±3.90
70% Std Feed + 30% cooked Breadfruit	12.00±0.00*a*	*27.50±1.26*	269.74±3.10
70% Std Feed + 30% Parboiled Breadfruit	10.00±1.15	30.50±1.26*a*	271.17±10.35
70% + Std Feed + 30% grilled Breadfruit	8.00±0.00	35.25±2.17*a*	279.45±3.06

50% Std Feed + 50% cooked Breadfruit	8.00±0.00	33.25±1.03*a*	266.34±4.29
50% Std Feed + 50% parboiled Breadfruit	9.00±1.00	36.50±6.50*a*	258.06±4.29
50% Std Feed + 50% grilled Breadfruit	9.00±1.00	36.00±0.00*a*	296.01±9.18*a*

[a]Significant increase with respect to normal control.

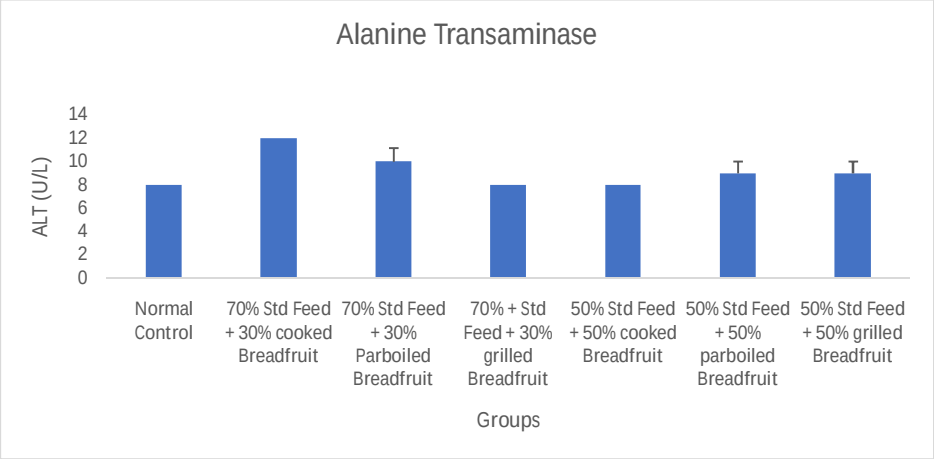

Figure 3: Effect of feed fortified with cooked, parboiled and grilled breadfruit on Alanine Transaminase activity of normal rats.

The Alanine Transaminase (ALT) activity remained constant in groups fortified with 30% grilled and 50% cooked breadfruit when compared with the normal control (figure 3). An increase in ALT activity was then observed in other fortified groups but significant increase ($p<0.05$) was only observed in the group fortified with 30% cooked breadfruit with respect to the normal control.

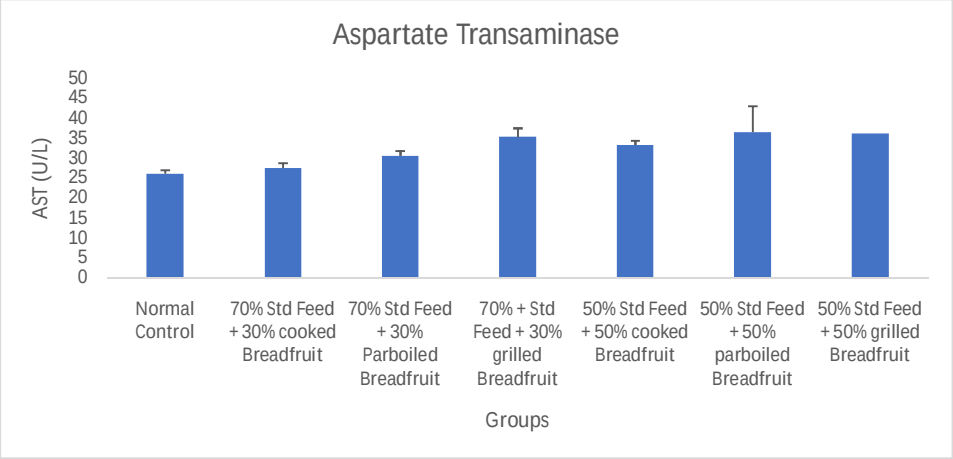

Figure 4: Effect of feed fortified with cooked, parboiled and grilled breadfruit on Aspartate Transaminase activity of normal rats.

From figure 4 it can be observed that the Aspartate Transaminase (AST) activity of the rats showed significant increase (p<0.05) in all fortified groups with an exception to the group fortified with 30% cooked breadfruit when compared with the normal control group.

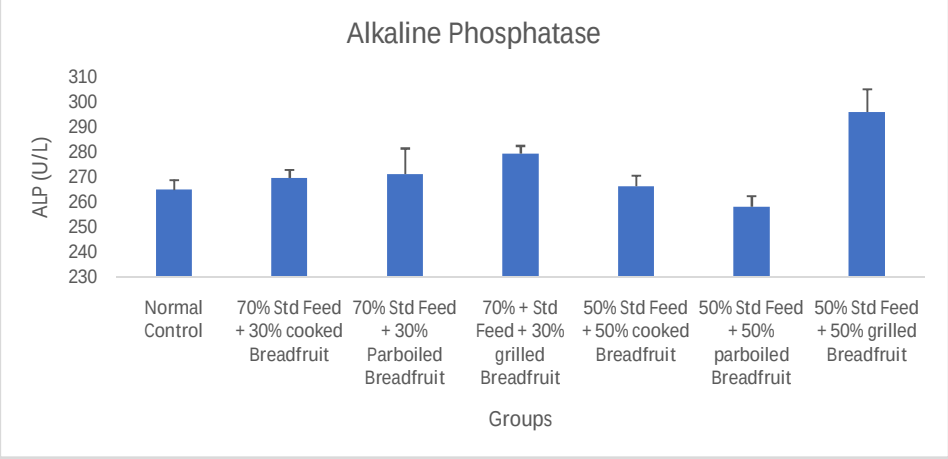

Figure 5: Effect of feed fortified with cooked, parboiled, and grilled breadfruit on Alkaline phosphatase activity of normal rats.

All fortified groups as seen in figure 5 showed an increase in Alkaline phosphatase (ALP) activity with an exception to the group fortified with 50% parboiled breadfruit when compared to

the normal control group. A significant increase (p<0.05) was only observed in the group fortified with 50% grilled breadfruit with respect to the normal control.

DISCUSSION

Creatinine derived from creatine is released into the plasma at a constant rate, and is then freely filtered by the glomerulus, but not reabsorbed or metabolized by the kidney [18]. Over time, Creatinine levels have been locally used to mirror the kidney function of an organism, but these levels are substantially affected by the nutritional status, muscle mass, age, and sex of the organism [19,20]. However, a few studies reveal the efficacy of medicinal plants in ameliorating diseases which can be partly attributed to its phytochemical constituents [21], and other important nutritional contents that protect the animals from adverse effects which can be seen from the biochemical indices [22,23].

From figure 1 it is observed that all fortified groups showed increased creatinine levels while the feed fortified with 30% cooked and parboiled breadfruit showed a significant (p<0.05) increase in the creatinine level. This increase in creatinine levels could be associated with increased survivability possibly due to better nutritional status and muscle mass which is in line with the research carried out by Storm *et al.* [19] and Fink *et al.* [24]. Medicinal plants improve the biochemical profiles of experimental animals immensely which could be because of its phytochemical, mineral or vitamin composition as revealed in previous studies [25-27]. Urea is a major nitrogenous waste product of metabolism that is generated from protein breakdown thus an increase in dietary protein results in increased urea production [28]. There was a significant increase (p<0.05) in the urea level of all the group of rats fed with feed fortified with breadfruit. As previously mentioned, breadfruit is highly rich in protein and can cause plasma/serum urea to rise.

Figure 4 explains that the Aspartate Transaminase activity of the rats showed a significant increase (p<0.05) in all fortified groups with the exception of the group fortified with 30% cooked breadfruit when compared with the normal control group. The higher AST values observed could be due to increase in muscular activity of the rats due to the availability of more energy and protein in their diet. A similar research article carried out by Unigwe *et al* [29] showed that a diet with 40% maize-replaced fermented cassava peels supplemented with maxigrain enzyme indicated significant increase when compared with the conventional maize-based diet in female pigs.

ALT activity showed significant increase (p<0.05) in the group fortified with 30% cooked breadfruit with respect to the normal control while ALP activity showed a significant increase (p<0.05) in the group fortified with 50% grilled breadfruit with respect to the normal control (figure 5). Literally, studies explains that the enzymatic activity of Aspartate Transaminase, Alkaline phosphatase and Alanine Transaminase are used to evaluate liver malfunctions as these liver enzymes levels are usually raised in acute hepatoxicity [30]. However, the increase in ALT and ALP level could either be a sign of an underlying condition affecting the liver or could be

due to increase in muscular activity of the rats due to availability of more energy and protein in their diet.

CONCLUSION

From the result of this research work, it can be inferred that fortification with cooked, parboiled and grilled breadfruit could provide better nutrition for the animal with the availability of more energy and protein in their diet but may also result to some tissue damage.

Comment [g4]: The results revealed that the supplementation of bread fruit increased the levels of AST,ALTand ALP which implies the liver damage was produced

Comment [g5]: Rise in BUN and creatinine indicates kidney dysfunction

ETHICAL APPROVAL

All experiments were supervised and approved by the Nnamdi Azikiwe University Animal Research Ethics Committee (NAU-AREC) in line with the principles of Animal Care and Use in Research, Education and Testing. The ethical approval number as issued by the NAU-AREC is NAU/AREC/2021/00040.

REFERENCES

1. Nuga OO, Ofodile. Potentials of *Treculia africana*, Decne. An endangered species of Southern Nigeria. J. Agric. Soc Sci. Res. 2010; 10:91-98.
2. Obiakor-Okeke PN, Nnadi CC. The effect of different processing methods on the Nutrient and Anti-Nutrient composition of African Breadfruit (*Treculia Africana*). International Journal of Nutrition and Food Sciences. 2014; 3(4):333-339.
3. Chinedu E. Sanni S, Theresa N, Ebere A. Effect of domestic cooking on the starch digestibility predicted glycemic indices, polyphenol content and alpha amylase inhibitory and properties of beans (phaseolis vilgaris) and breadfruit (Areculia africana). Intl. Journal Bio. Macromol. 2018;
4. Nwokeocha L. Ugbomoiko JO. Effect of Parboiling on the composition and Physicochemical properties of Treculia Africana Seed flours. Pakistan Journal of Nutrition. 2008; 7(2).
5. Fassasi OS, Eleyimmi AF, Fasasi AR, Karim OR. Chemical properties of raw and processed breadfruits (Treculia african) seed flour. J. Food. Agric. Env. 2003; 1459 – 1465.
6. Olapade AA, Umeonuora UC. Chemical and sensory evaluation of Africana breadfruit (*Treculia africana*) seeds processed with Alum, and Trona. Nig. Food. J. 2014. 32: 80-88.
7. Adumanya OC, Akuma TO, Onwusonye JC, Obi-Adumanya. Effects of Traditional Process methods on minerals content of African breadfruit (Treculia Africana) seeds. Intl Res. J. 2013; 4.:23-26.
8. Okonkwo EU, Ubani ON. Indigenous Technologies for the Dehulling, Storage and Utilization of Breadfruit Seeds Artocarpus Altilis (Park) Fosb (Treculia africana Decne) Family: Moraceae in Anambra state. Journal Research Agriculture. 2007; 4(1), 27-30.
9. Nwaigwe JO, Adejumo BA. Qualities of African Breadfruit (Treculia Africana) Seed Flour as Influenced by Thermal Processing Methods and Duration, International journal of technology enhancements and emerging engineering research. 2015. 3(4):102 -108.
10. Okafor IU. Development of Improved Method of Propagation Processing and Presention of Indigeneous Fruits or Vegetables in Nigeria Process Symposium in the Development of Indigenous Technology October 1988 Enugu Nigeria. 2008; pp 3-8.

11. Umezuruike AC, Nwabueze TU, Akobundu ENT, Ndife J. Effects of Processing on the Mineral Content of Roasted African Breadfruit (Trecula africana) Seeds, Nigerian Journal of Agriculture, Food and Environment. 2016; 12(4):18-22.
12. Ezengige G. African Breadfruit (*Treculia africana*) popularly known as Ukwa in Igbo is an Amazing Health Food. .Available.www.Healthbubbles.com/igb/2992. 2016;
13. Ezennaya CF, Ezeigwe OC. A comparative study of raw, boiled, and roasted *Treculia Africana* (African breadfruit). The Bioscientist Journal. 2023; 11(1):15-25.
14. Nwabueze TU, Iwe MO, Akobundu ENT. Unit Operations and analysis for African Breadfruit based spaghetti type product at extreme process combination, Journal of Food Technology. 2007; 5(1):42-45.
15. Fubara EP, Ekpo BO, Ekpete OA. Evaluation of the effects of processing on the mineral contents of maize (Zea mays) and groundnuts (Arachis hypogaea), Libyan Agricultural Centre International Journal. 2011; 52(3):133-137.
16. Enemor VHA, Ogbodo UC, Nworji OF, Ezeigwe OC, Okpala CO, Iheonunekwu GC. Evaluation of the Nutritional Status and Phytomedicinal Properties of Dried Rhizomes of Turmeric (*Curcuma longa*). Journal of Biosciences and Medicine. 2020; 8:163-179.
17. Eneh FU, Ezeigwe OC, Omeje M. (2020). Lipid peroxidation activity and phytochemical constituents of extract of groundnut peels. *Journal of Applied Life Sciences International*; 23(1):16-22.
18. Bagshaw SM, Gibney RT. Conventional markers of kidney function. Crit Care Med. 2008; 36:152-158.
19. Storm AC, Htike NL, Cohen DA, Benz RL. A surviving patient with record high creatinine. Open J Nephrol. 2013; 13:217-219.
20. Okechuku G, Upadhyay KK. Highest serum creatinine ever reported in a child. Hemodial Int. 2014; 18:849-850.
21. Enemchukwu BN, Ezeigwe OC, Orinya OF, Nwali UI, Chigbo CM, Iloanya EL. (2021). Phytochemical evaluation and Antimicrobial potential of methanol extracts of mistletoe (*Loranthus micranthus*) leaves grown on cola tree (*Cola nitida*) and oil bean tree (*Pentaclethra macrophylla*). *Journal of Medicinal Plants Studies*. **9**(2):28-32.
22. Onochie AU, Oli AH, Oli AN, Ezeigwe OC, Nwaka AC, Okani CO, Okam PC, Ihekwereme CP, Okoyeh JN. The Pharmacobiochemical Effects of Ethanol Extract of *Justicia secunda* Vahl Leaves in *Rattus Norvegicus*. *Journal of Experimental Pharmacology*. 2020; 12:423-437.
23. Onuabuchi NA, Ezeigwe OC, Agbo CA. Evaluation of Toxicity effects of Ethanol Extract of Fruit Rind of *Cucumis metuliferus* on the Biochemical Hematological and Histological Parameters of Albino Rats. *Journal of Complimentary and Alternative Medical Research*. 2022; 18(3): 15-27.
24. Fink JC, Burdick RA, Kurth SJ. Significance of serum creatinine values in new end-stage renal disease patients. Am J Kidney Dis. 1999; 1:694-701.
25. Ezeigwe OC, Okpala CO, Ogbodo UC, Anwuna CD, Ngwu OR, Chigbo CM. Effect of a combination of ethanol extract of *Ficus capensis* and *Cnidoscolus aconitifolius* on Liver and Kidney Function parameters of phenylhydrazine-induced anemic rats. *Asian Journal of Research in Biochemistry*. 2020; 6(2):1-6.

26. Ezeigwe OC, Oladejo AA, Iloanya EL. (2022). The impact of *Citrus aurantium* fruit juice on Bodyweight and Haematological Parameters of Wistar Rats. International Journal of Research and Reports in Haematology. 2022; 5(2):56-67.
27. Enemchukwu BN, Onwuatuegwu JTC, Ofomatah AC, Ezeigwe OC, Orinya OF, Iloanya EL, Okeke CM, Onyedinma EL. Biochemical and Haematological Effects of *Telfairia occidentalis* and *Amaranthus viridis* extracts on Anaemic Rats. Journal of Applied Sciences. 2022; 22(3):144-151.
28. Chris H. Urea and the clinical value of measuring blood urea concentration 2016.
29. Unigwe, CR, Balogun, FA, Odeyemi TA. Liver enzymes and histo-morphology of pigs fed fermented and enzyme-supplemented cassava peels meal-based diets. *Nigerian J. Anim. Sci.* 2018, 20(3):106-116.
30. Obi E, Orisakwe OE, Asomugha LA, Udemezue OO. The hepatotoxic effect of halofantrine in guinea pigs. *Indian Journal of Pharmaceutical Sciences.* 2004; 36(5):303-305.