

Liver Enzymes Functions Effect of Raphia Hookeri Fruit Pulp (Mesocarp) Extract in Male Wistar Rat's Model

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Abstract

This study is aimed at determining selected liver enzymes functions effect of Raphia Hookeri fruits pulp extract in male wistar rats. A total of 32 apparently healthy rats of weights between 130g and 200g were involved. The rats were segregated into 4 groups, 3 groups administered orally with doses of extract at 500mg/kg, 1000mg/kg and 2000mg/kg body weight while control group fed with feed and water for 28 days. The animals were sacrificed, samples taken, laboratory test done for liver enzymes functions. SPSS version 21.0 (ANOVA) followed by Post-Hoc multiple comparison test used and $p < 0.05$, values were expressed as SEM for statistical analysis. Results showed AST level of groups 3 and 4 had significant decreases when compared to control and 500mg/kg group respectively. AST level of group 4 was significantly lower compared to that of group 3. ALT level indicated non-significant changes in treated groups when compared to both control and test groups. The ALP levels in groups 2 and 3 showed significantly raised levels of ALP when compared to that of control had significantly reduced level of ALP compared to that of group 2. Increased AST an indication to measure serum ALT to determine whether the increased AST is from liver Willard & twedt, 2012. So the decreased level AST has demonstrated ameliorative effect of the raphia hookeri fruit pulp on hepatic disease and any other disease that causes an increase AST in the body. Regular consumption of this fruit is recommended for those with liver issues.

Keywords: Liver enzymes, Raphia Hookeri, Fruit Pulp Extract, liver function effect, Male Wistar Rats.

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INTRODUCTION

The Raphia hookeri plant belongs to the family of Raphia palm trees and are found in abundance in the south-eastern southern part of Nigeria, especially in the southern part, Rivers state, Abua/Odual LGA, Emoh Community (Egbono *et al.*, 2023). The fruit whose pulp is considered edible in some parts of Nigeria like Rivers state, Abua/Odual LGA, Emoh Community and not edible in other parts which made its consumption rate low or none in such parts (Egbono *et al.*, 2023). The boiled fruit pulp is commonly called 'Ogbusi' by the Abua people and mostly eaten with tapioca (processed cassava) commonly known as 'Ataka' by the Abua people of Rivers state in Nigeria (Egbono *et al.*, 2023). Raphia Hookeri fruit pulp is a good source of phytochemicals and some micronutrients and is locally consumed as a snack (Tatianan *et al.*, 2023). In fact, the

inhabitants of Emoh village in Abua and the people of Abua/Odual LGA hypothesized that the Ogbusi boost immunity, inhibit plasma glucose, reduce blood pressure, ameliorate fat and boost hematopoiesis, etc (Egbono *et al.*, 2023). Its fruit is large, cone-shaped with a single hard nut having an outer layer of overlapping reddish brown scales and in-between the outer layer of scales and the hard seed is a yellow, mealy, oil-bearing mesocarp or pulp (Mbaka *et al.*, 2012). Similarly, Ndon, 2003 described raphia hookeri fruit as large, cone-shaped with a hard nut having an outer layer of rhomboid-triangular and overlapping reddish-brown scales. Between the outer layer and the seed, is a yellow, oil-bearing mesocarp or pulp (Ndon, 2003). The pulp extract of Raphia hookeri was shown to contain vitamins C and E, carotenes, niacin, alkaloid, saponins, flavonoids and phenols which explains its

antioxidant activity (Edem *et al.*, 1984; Akpan and Usoh, 2004; Dada *et al.*, 2017). Flavonoids and tannins as phenolic compounds in plants are a major group of compounds that act as primary antioxidants by scavenging free radicals (Polterait, 1997).

The pulp has been reported to contain useful and therapeutic nutrients and chemicals. It is hard and often boiled before consumption. The oil processed from this plant is used for cooking and making margarine while the pulp is usually consumed with boiled cassava Mbaka, *et al.*, 2012. Given its hard and relatively dry nature attributed to its high fiber content, it could be conveniently processed into flour, as an alternative form for consumption or added to pastries that are less diversified in nutrients. The pulp is known by locals as an appetizer and aphrodisiac (Mphoweh *et al.*, 2009). Many uses it for medicinal purposes and it has been reported to contain phytochemicals with antimicrobial properties (Ogbuagu, 2008). The investigation carried out by Ogbuagu, 2008 showed that the pulp has higher concentrations of vitamin E (1.04 mg·100 g⁻¹), niacin (0.2 mg·100 g⁻¹), alkaloid (5 g·kg⁻¹), saponins (3.6 g·kg⁻¹), flavonoids (4 g·kg⁻¹) and phenols (4.1 g·kg⁻¹) than the seed, but the seed has higher values of vitamin A (0.16 mg·100g⁻¹), thiamine (0.07 mg·100 g⁻¹), riboflavin (0.07 mg·100 g⁻¹), nitrates (3.05 mg·100 g⁻¹) and nitrites (0.29 mg·100 g⁻¹), and of the toxic elements: lead (0.03 µg·g⁻¹), mercury (0.04 µg·g⁻¹), arsenic (0.23 µg·g⁻¹) and cadmium (0.04 µg·g⁻¹) than the pulp and the pulp and seed of *R. hookeri* are non-toxic and can serve as food as well as in medicine.

Investigations carried out by Edem *et al.*, 1984 to determine the chemical composition of the fruit of the raffia palm (*Raphia hookeri*: Family, Palmaceae or Palmae) and the peel and pulp (edible portion) were analysed. The effect of boiling on the chemical composition of the pulp was also investigated, it revealed that the peel contained more moisture (62.4%) than the pulp (38.0%) in terms of wet weight. Again, the protein and ether extract contents of the peel were found to be 3.2% and 1.8% of dry material, respectively. The ash content was 5.5%. Crude fiber gave a very high value of 70.3% for the peel, but the carbohydrate content was low (19.3%). There were decreases in the values of some nutrients after boiling the edible pulp of the fruit. Protein content decreased from 6.1% to 4.4% upon boiling. Ether extract and carbohydrate contents decreased from 11.8% to 11.3% and from 61.4% to 58.8%, respectively. Boiling increased the crude fiber and ash contents of the pulp from 17.7% and 3.0% to 21.2% and 4.3%, respectively. The calorific value decreased from 380.5 kcal to 354.7 kcal. Also revealed that tannin content was highest of all the toxic substances evaluated, there was a decrease from 597 to 360mg100g on boiling. The peel contained 234mg100g tannins and 24.3mg100g hydrocyanic acid. Boiling the pulp resulted in reduction

of the HCN from 12.4 to 9.2mg100g, phytic acid from 1.0 to 0.4mg100g, and oxalate from 26.4 to 17.6mg100g. The peel had more oxalate (39.6mg100g) and cyanide (24.3mg100g) but less phytic acid (0.6mg100g) than the pulp (Edem *et al.*, 1984). Also, ascorbic acid and carotene contents decreased upon cooking the pulp from 63.0mg100g and 33.4 µg100g to 28.3mg100g and 10.6 µg100g, respectively. The peel had an ascorbic acid content of 37.2mg100g and carotene content of 8.6 µg100g. Also Calcium, potassium, sodium and phosphorus decreased with cooking, while magnesium, zinc and iron contents were increased. Potassium had the highest level followed by calcium. The pulp had (mg/100 g): K, 1075; Ca, 875; Mg, 315; Zn, 9.6; P, 76.8; and Na, 16. The peel had (mg/100 g): Ca, 250; Mg, 450; K, 700; Na, 8; Zn, 3.5; and P, 37.7. Copper, chromium and cobalt were not detected in the fruit (Edem *et al.*, 1984).

An enzyme is a type of protein in a cell that acts as a catalyst and allows certain bodily processes to happen. High alkaline phosphatase levels in the liver may indicate the following conditions: Cholestasis of pregnancy, this is a common liver disease that can develop late in pregnancy. Cirrhosis of the liver, cirrhosis is a late-stage liver disease in which healthy liver tissue is replaced with scar tissue and the liver is permanently damaged. Hepatitis, hepatitis is inflammation of the liver. There are many types of hepatitis. Biliary atresia, this is a rare condition that happens when there's a blockage in the tubes (ducts) that carry bile from your liver to your gallbladder. It occurs in infants. Biliary stricture, this condition happens when the bile duct (the tube that takes bile from the liver to the small bowel) gets smaller or narrower. Biliary obstruction due to cancer, certain cancers, including cholangiocarcinoma and pancreatic head adenocarcinoma, can block your bile ducts or pancreatic ducts. Mononucleosis ("mono"), this is a contagious infection that can sometimes cause swelling in your liver (Cleveland Clinic medical professional 2021). Due to little or lack of available scientific investigation about the medicinal benefits of consuming fruits of this plant and the high rate of consumption of this fruit by people of Emoh community, Abua/Odual LGA of Rivers State, Niger Delta region, southern Nigeria prompted the lead researcher who is from Emoh community to carry out this research work to ascertain among other aspects the effect of the aqueous extract of raphia hookeri fruit pulp (mesocarp) on some selected liver enzymes after a regular consumption by wistar rats.

MATERIALS AND METHOD

Materials

Syringe, Surgical gloves, Weighing balance, Cotton wool, Dissecting board, Dissecting blade, Beaker, Permanent marker, Local bottle, Slides, Wistar rats. Chloroform, etc.

Animal Preparation

A total of Thirty-Two (32) healthy male wistar rats of weight ranging from 130g to 200g were used for this study. These rats were all housed in the preclinical animal house, Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria. The animals were maintained in a well-ventilated animal house under optimum condition of humidity, temperature and natural light-dark cycle were allowed free access to food and water. The experiment protocols and procedures used conform to the international guidelines of the care and use of animals in research and teaching American physiological society, 2002.

Acclimatization of the Animals

After identification, the animals were weighed using a weighing balance and housed in a clean plastic cage with 12 hours light-darkness cycle, for four weeks so as to acclimatized to the environmental condition of the University of Port Harcourt, the study was generally conducted in accordance with recommendation from the 1983 declaration of Helsinki on guiding principles in the care and use of animals.

Experimental Extract

The Extract of *Raphia Hookeri* fruit pulp was used for the experiment.

Preparation of Extract *Raphia Hookeri* Fruit Pulp (Mesocarp)

Maceration method was used, the fruit pulp were air-dried in order not to kill the active ingredients, then it was finally crushed and soaked in a maceration jar about 1000gram of the extract was dissolved in 2000ml of water and allowed to stand for 72 hours with a continuous agitation to enable a good yield after which it was filtered and the filtrate was mounted on a water bath to evaporate the liquid content at temperature of 65 degrees Celsius, after evaporation the weight of the extract was taken and it was stored for use.

Study design

A total of thirty-two (32) healthy male Wistar rats of weight ranging from 130gram -200grams were used for this study. The animals were divided into two groups:

Control group and Dose dependent group

The dose dependent groups were further divided into three (3) subgroup two (2), three (3) and four (4), each of the subgroups contains eight animals in each cage compartment.

Mode of Administration of Extract

In the course of oral administration of the extract to the animals the following doses were administered for each group except the control group for twenty-eight (28) days. The Lethal dose (LD 50) of

the aqueous extract of *Raphia Hookeri* fruit was calculated using Lorke's method, 5000mg/kg body weight of wistar rats was attained, therefore the male wistar rats were not given extract beyond 5000mg/kg body weight:

Group 1(control): Were given animal feed and water

Group 2: Were given 500mg/kg body weight of the extract.

Group 3: Were given 1000mg/kg body weight of the extract.

Group 4: Were given 2000mg/kg body weight of extract.

Extract Administration

Raphia Hookeri Aqueous extract was administered orally daily for 28 days at a dose of 500mg/kg body weight of the animal to dose dependent group 2, 1000mg/kg body weight to dose dependent group 3, and 2000mg/kg body weight to dose dependent group 4.

Collection of Sample

The Aqueous extract which was gotten from *Raphia Hookeri* fruits was purchased from Emoh community in Abua/Odual LGA in Rivers State, southern Nigeria and the rats were sacrificed after 28 days of treatment. The rats were anaesthetized with chloroform one at a time. They were then sacrificed while still under anaesthesia. Each rat was dissected and the liver of each animal was excised and blood samples were collected from each of them through cardiac puncture for liver enzymes evaluation.

Liver Enzymes Laboratory Analysis

AST/ALT (Reitman and Frankel method)

Principle: (AST) X – oxoglutarate L – aspartate GOT L – glutamate oxalacetate. Ast was measured by monitoring the concentration of oxaloacetate hydrazones formed with 2,4 dinitrophenyl-hydrazine.

Procedure:

ALT and AST are the same.

The test tube was labelled as sample blank and sample.

Pipette 0.5ml of reagent (R1) into all the test tubes.

0.1ml of the sample was added to the tubes labelled sample.

The sample was mixed and incubated for exactly 30mins of 37°C

Pipette 0.5ml of reagent 2 (R²) to all the test tube.

0.1ml of the sample was added to the tube labelled sample blank.

The sample was mixed and allowed to stand for exactly 20mins at 25°C

Pipette 5.0ml of NaOH to all the test tubes.

The absorbance of the sample was mixed and read against the sample and read against the sample blank after 5mins at 540nm until u/L

ALP (Kochmar, J.F and MOSS, D.W. Method) until u/L.

Principle: Alkaline phosphatase (ALP) acts upon buffer sodium thymolphthalein monophosphate. The addition of an alkaline reagent stops enzymes activity and simultaneously develops a blue photometrically.

Procedure: The test tubes were labelled as standard, blank and test.

Pipette 0.5ml of Alp substrate into all the test tubes and incubate at 37°C for 3mins.

50ul of the standard sample and d/w was added appropriately

The sample was incubated for exactly 10mm at 37°C.

Pipette 2.5ml of Alp colour developer into all the test tubes and was well mixed.

Zero the spectrophotometer at 590um with the blank.

The absorbance was read and recorded.

STATISTICAL ANALYSIS

The data obtained from the present study were subjected to statistical analyses using the Statistical Package for Social Sciences (SPSS) version 21.0. Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Post-Hoc multiple comparison test and $p < 0.05$ was considered statistically significant. The values were expressed as mean \pm standard error of mean (SEM).

RESULT

Table 1: Effect of administration of fruit extract of *Raphia hookeri* (FERH) on liver enzymes level in male Wistar rats

Group and Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Group 1: Control Group	29.00 \pm 2.00	16.50 \pm 4.50	15.00 \pm 0.00
Group 2: Low Dose treated (500mg/kg b.w FERH)	27.00 \pm 0.00	14.50 \pm 1.44	23.75 \pm 1.65 ^a
Group 3: Medium Dose treated (1000mg/kg b.w FERH)	15.33 \pm 4.63 ^{a, b}	12.00 \pm 0.00	22.00 \pm 1.73 ^a
Group 4: High Dose treated (2000mg/kg b.w FERH)	7.75 \pm 0.75 ^{a, b, c}	12.00 \pm 0.00	18.75 \pm 1.10 ^b

Values represent mean \pm SEM, n=5; ^a Significant at $p < 0.05$ compared to Group 1; ^b Significant at $p < 0.05$ when compared to group 2; ^c Significant at $p < 0.05$ when compared to group

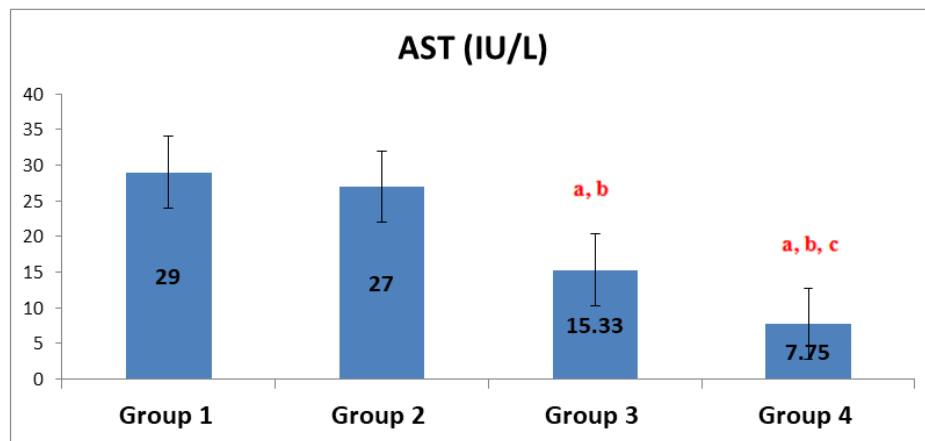


Figure 1: Effect of administration of fruit extract of *Raphia hookeri* (FERH) on Aspartate Transaminase (AST) level in male Wistar rats

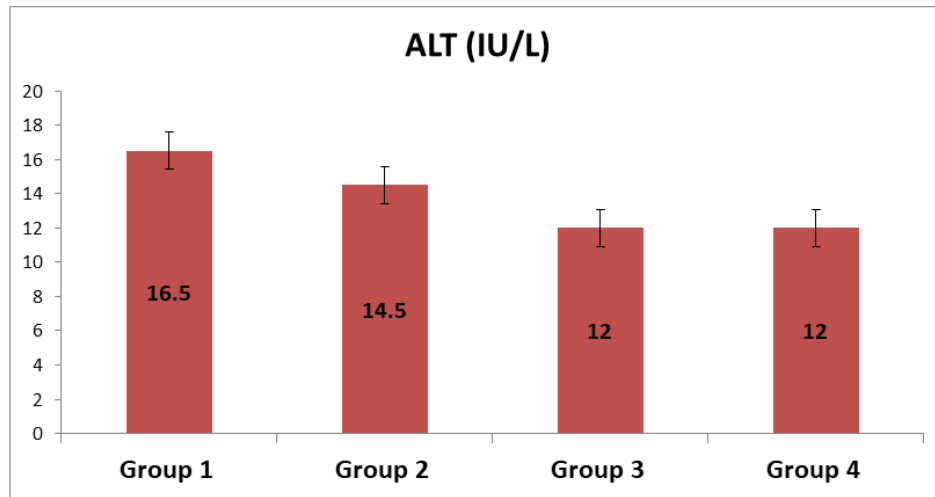


Figure 2: Effect of administration of fruit extract of *Raphia hookeri* (FERH) on Alanine Transaminase (ALT) level in male Wistar rats

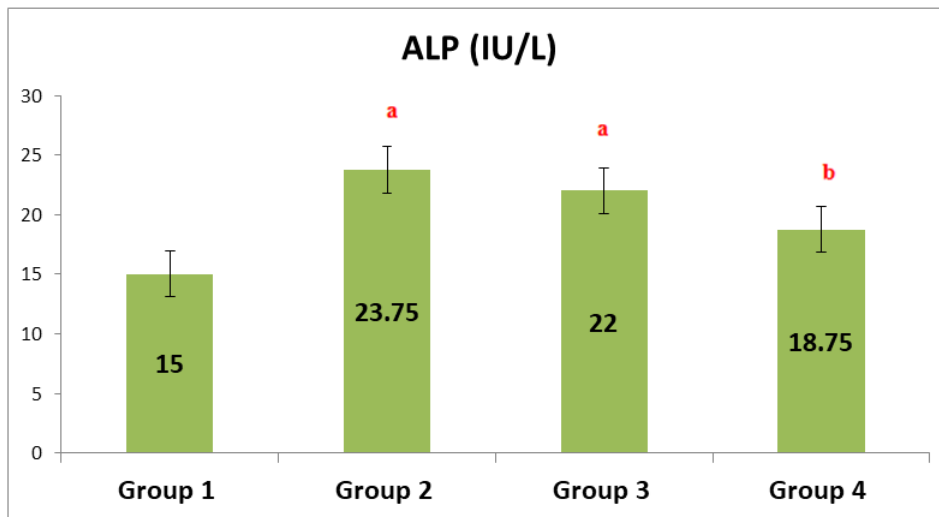


Figure 3: Effect of administration of fruit extract of *Raphia hookeri* (FERH) on Alkaline Phosphatase (ALP) level in male Wistar rats

DISCUSSION OF FINDINGS

The results of the laboratory analysis carried out to investigate the effect of administration of fruit extract of *Raphia hookeri* (AFERH) on some liver enzymes level in male Wistar rats revealed that the AST level of groups 3 and 4 treated with 1000mg/kg body weight and 2000mg/kg body weight FERH respectively had significant decreases ($p < 0.05$) when compared to those of Groups 1 (control) and 2 (500mg/kg body weight FERH treated group) respectively. More so, the AST level of group 4 was seen to be even significantly lower compared to that of group 3 rats. The outcome of the plant extract treatments on the ALT level indicated generally non-significant ($p > 0.05$) changes in all treated groups when compared to both the control and amongst themselves. Aspartate aminotransferase (AST), which functions to transaminate aspartate, has been commonly used as a biomarker for hepatic injury Gwaltney-Brant (2021). High levels of AST in the blood may indicate hepatitis, cirrhosis, mononucleosis, or other liver

diseases. High AST levels can also indicate heart problems or pancreatitis (Tenner *et al.*, 1994). Increased serum ALT reflects cell membrane damage and leakage; significant AST increases tend to reflect more serious hepatocyte damage because the mitochondria are not damaged as readily as is the cell membrane. AST is, however, present in significant quantities in many other tissues, including muscle and RBCs; therefore increased AST is not as specific for hepatic injury as is increased ALT. Exercise and intramuscular (IM) injections may increase serum AST, the most common causes of increased AST include hepatic disease, muscle disease (inflammation or necrosis), or hemolysis (spontaneous or artifactual). Increased AST is an indication to measure serum ALT to determine whether the increased AST is from the liver (significant increases in both ALT and AST suggest that AST increases are of hepatic origin), Willard & twedt, 2012. So the decreased level AST shows an ameliorating effect of the *raphia hookeri* extract on hepatic disease

and any other disease that cause an increase AST in the body. Considering the outcome of the extract treatment on ALP levels of the study animals, the rats in groups 2 and 3 showed significantly raised levels of ALP when compared to that of group 1. Further, the rats in group had significantly reduced level of ALP compared to that of group 2. Alkaline phosphatase (ALP) is an enzyme that's found throughout your body. An enzyme is a type of protein in a cell that acts as a catalyst and allows certain bodily processes to happen. High alkaline phosphatase levels in the liver may indicate the following conditions such as cholestasis cirrhosis, hepatitis of the liver (Cleveland Clinic medical professional 2021). In as much as these enzymes play critical physiologic roles to ensure homeostasis in the body, there is need for effective regulation of their levels and activities to avert liver damages in the human body. The finding of this work has revealed a natural remedy to liver damages that arise as a result of a rise in levels of some of these enzymes in the body, hence the recommendation of regular consumption of raphia hookeri fruit pulp as stable food commonly called Ogbusi by Abua people in Rivers state, Nigeria (Egbono *et al.*, 2023).

CONCLUSION

The outcome of this investigation has been able to reveal that extract of raphia hookeri fruit pulp (mesocarp) when frequently consumed has ameliorative effect on the liver by causing a decrease in the levels of some liver enzymes in the body. Consumption of boiled ripe *Raphia Hookeri* fruit pulp popularly known as Ogbusi by the Abua as staple food (Egbono *et al.*, 2023) has been proven to have medicinal benefits in this study and therefore recommended for those have with liver problems to consume the fruit pulp (mesocarp) of this plant regularly as natural remedy.

RECOMMENDATION

Its therefore recommended that the fruit pulp of raphia hookeri plant should be consumed intermittently as staple food and widely cultivated in the society because of its nutrients and medicinal benefits to avoid the extinction of the this plant.

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