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Original Research Article

Investigation of Physiologic Effect of Prolonged Consumption of Raphia Hookeri Fruits Pulp Aqueous Extract on Renal Functions of Male Wistar Rats

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Abstract

The aim of this study is to investigate the physiologic effect of prolonged consumption of Raphia Hookeri fruits pulp aqueous extract on renal functions of male wistar rats. A total of 32 male wistar rats of weight ranging from 130gram to 200grams were used. The extract was administered orally for each 4 groups except control (group1) for twenty-eight (28) days. Group 1 rats were given animal feed and water only, group 2 were given 500 mg/kg body weight of the extract, group 3 were given 1000 mg/kg body weight of the extract, group 4 were given 2000 mg/kg body weight of extract. The statistical analysis done using mean and standard deviation, P value at ≤ 0.05 and results showed that the sodium ion levels in all the test groups were marginally raised when compared to group 1, potassium ion levels in all test groups had only non-significant variations when compared to both the control and test groups but in test groups were seen to be slightly reduced with respect to group 1. Groups 2, 3 and 4 indicated elevated bicarbonate ion but of all, it was most and significant in group 4 when compared to group 1. Chloride ion indicated non-uniform and non-significant changes when compared to both the control and test groups. Creatinine all indicates non-significant effects of the extract when compared to group 1. The increase observed in urea and creatinine indicates that kidney function would deteriorate as it prolongs which negatively alter the renal physiology of the male wistar rats.

Keywords: Prolong consumption, Raphia Hookeri, Fruit Pulp Extract, Renal Physiology, Wistar Rats.

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Introduction

The ripe boiled Raphia Hookeri fruits pulp locally called 'Ogbusi' by Abua people in Abua/Odual local government area of Rivers State, Niger Delta region, Southern Nigeria. The Ogbusi (Raphia Hookeri fruits pulp) is usually soaked in water or stored in refrigerator to maintain nutrients and commonly consumed with tapioca. 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. The Ogbusi (Raphia Hookeri fruits pulp) is frequently consumed in Abua because the plant is abundantly found there. Raphia Hookeri plant is also found in other West African

countries. This plant is classified under palm tree and belongs to the family of Palmae or Palmacea which originated from tropical West Africa where it extended to, raphia palms strive predominantly in swampy areas which are mostly hydromorphic (Imogie AE, 2008), (Mphoweh et al., 2009). The raphia palm grows in the form of a monocarpic crop with a terminal inflorescence that undergoes hepaxanthic flowering. At the vegetative stage, the crop is characterized by continuous stem elongation (Adeneyi AA, Akpabio UD, 2011), with an inflorescence which is the sink for the photosynthate which is tapped and referred to as "palm wine". Other parts of the raphia palm tree such as the leaves, roots, branches and seed are also exploited for craft work and traditional medicines (Mphoweh et al., 2009), (Obahiagbon FI, 2009), (Ezeagu et al., 2003) .The inflorescence emerges from the base of the fanlike leaves, and they bear the male and female flowers that will develop to form the fruits and the seeds.

All parts of the plant are well utilized by locals for various things ranging from building materials as twine, rope; personalized items like baskets, placemats, hats, shoes to consumables like oil, wine and food (Akinola et al., 2010; Afolayan et al., 2014). Raphia fruit pulp is a good source of phytochemicals and some micronutrients and is locally consumed as a snack (Tatianan 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 BA, 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 BA, 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. 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 MN, 2008). The Ogbusi (fruit pulp) of the raphia hookeri plant consumption is abysmally low and this may be due to little or lack of the knowledge of its medicinal benefits to the renal functions in spite of its reported high fiber, mineral. vitamins, and phytochemical contents (Ogbuagu MN, 2008). Hence, this present study is aim at evaluating the effect of raphia hookeri fruits pulp aqueous extract on renal functions of male wistar rats' model.

MATERIALS AND METHOD

Materials

Animals: Wistar rats Chloroform Syringe Surgical gloves Weighing balance Cotton wool
Dissecting board
Dissecting blade
Beaker
Permanent marker
Local bottle
Slides

ANIMAL PREPARATION

A total of Thirty-Two (32) healthy male wistar rats of weight ranging from 130gram to 200grams were used for this study. These rats where 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 Aqueous extract of Raphia Hookeri Mesocarp (fruit pulp) was used for the experiment.

Preparation of Aqueous Extract Raphia Hookeri Mesocarp (Fruit Pulp)

Maceration method was used, the Mesocarp (fruit pulp) were air- dried in other 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 aqueous 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: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, Niger Delta Region, 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 and kidney of each animal was excised and blood samples were collected from each of them through cardiac puncture for renal indices and electrolytes evaluation.

LABORATORY TESTS AND ANALYSIS

The following laboratory tests were carried out:

Sodium (Maruna and Trider method) mmol/L was used for the analyses.

Principle: The present method is based on reaction of sodium with a selective chromogen producing a chromophore whose absorbance varies directly as the concentration of sodium in the test sample.

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

Pipette 10ml of the reagent into all test tubes.

0.0ml of the samples was added into appropriate tubes The samples were mixed and incubated for 5 mins at 25c

The absorbance at 630nm was read and recorded.

Potassium (Tiets N.W. method) unit mmol/L was used for this analysis.

Principle: The amount of potassium is determined by the use of sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension. The turbidity of which is proportional to potassium concentration in the sample.

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

Pipette 1ml of the reagent was released into all the tubes.

10ml of the sample was added into appropriate tubes.

The samples were mixed and allowed for 3mins at 25c.

Zero the spectrophotometer using blank at 500nm

The absorbance was read and recorded.

Chloride: (Levinson S.S. Method) unit mmol/L was used.

Principle: The quantitative displacement of thiocyanate by chloride from mercuric thiocyanate any subsequent formation of a red ferric thiocyanate complex is measure calorimetrically.

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

Pipette 1.0ml of the reagent into the tubes.

10ul of the samples were added into the tubes appropriately.

The samples were mixed and incubated for 5mins at 25c

The absorbance at 480nm was read and recorded.

Bicarbonate (HCO³) (Back Titration Method)

Principle: Serum HCO³ was reacted with excess standard HCl. The remaining HCl was back titrates with standard NaOH using phenol red as indicator.

Procedure: 50ml conical flask was added to a CO² – free d/w 250ul, 200ul sample, 0.0/NHCL 1mml, was well mixed and 3 drops of phenol red was added. The flask was whirl to release the CO². The resultant solution with 0.0/N NaOH was titrated until the initial light yellow colour fades to a light purple at the endpoint. The remaining NaOH that does not take part in the reaction was read. The reading obtained was divided by + wo: This gives the concentration of HCO₃ in the sample unit mmol/L.

Creatinine (Direct End-Point Method) umol/L

Principle: Creatinine reacts with picric acid in alkaline solution to form a coloured complex. The amount of complex formed is directly proportional to the creatinine concentration.

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

Pipette 2.0ml of reagent, into all the tubes.

0.1ml of the sample, standard and d/w was added into the tubes respectively.

The sample was mixed and after 30seconds, the absorbance of the standard and sample was read. Exactly 2mins later, the absorbance of the standard and sample was read. A_2 of standard and sample $A_1 - A_2 = A$

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).

Ethical Considerations

This study was approved by the center for Research ethics.

RESULTS AND ANALYSES

The results of Effect of administration of aqueous fruit extract of *Raphia hookeri* on some electrolytes in male Wistar rats are shown below

Group and Treatment	Sodium ion (Na ⁺) (mmol/L)	Potassium ion (K ⁺) (mmol/L)	Bicarbonate ion (HCO-3) (mmol/L)	Chloride ion (Cl ⁻) (mmol/L)
Group 1: Control Group	99.00 ± 1.00	2.45 ± 1.75	35.00 ± 3.00	92.00 ± 17.00
Group 2: Low Dose treated (500mg/kg b.w AFERH)	168 ± 29.81	1.20 ± 0.25	52.75 ± 10.76	99.25 ± 5.81
Group 3: Medium Dose treated (1000mg/kg b.w AFERH)	105.67 ± 24.63	1.43 ± 0.98	40.00 ± 12.12	80.00 ± 2.08
Group 4: High Dose treated (2000mg/kg b.w AFERH)	167.50 ± 15.25	0.97 ± 0.35	67.00 ± 1.96 ^a	83.75 ± 9.17

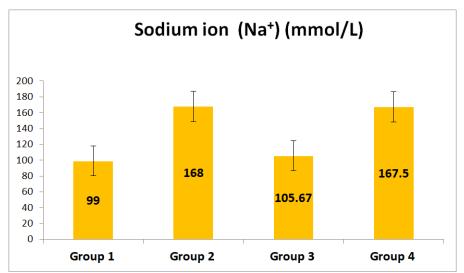
Values represent mean ± SEM, n=4; a Significant at p<0.05 compared to Group 1; Significant at p<0.05 when compared to group 2; Significant at p<0.05 when compared to group 3. AFERH = aqueous fruit extract of *Raphia hookeri*.

Effect of administration of aqueous fruit extract of *Raphia hookeri* on Creatinine and Urea Levels in male Wistar rats

Group and Treatment	Creatinine	Urea
	(umol/L)	(mmol/L)
Group 1: Control Group	219.00 ± 9.00	1.30 ± 0.10
Group 2: Low Dose treated (500mg/kg b.w AFERH)	219.50 ± 6.86	1.17 ± 0.22
Group 3: Medium Dose treated (1000mg/kg b.w AFERH)	216.67 ± 2.40	1.27 ± 0.12
Group 4: High Dose treated (2000mg/kg b.w AFERH)	219.75 ± 5.39	1.42 ± 0.02

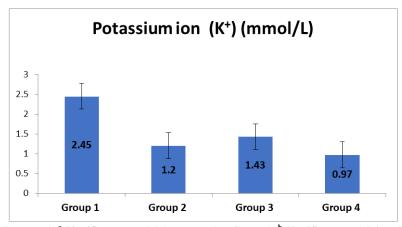
Values represent mean ± SEM, n=4; ^a Significant at p<0.05 compared to Group 1; ^b Significant at p<0.05 when compared to group 2; ^c Significant at p<0.05 when compared to group 3. AFERH = aqueous fruit extract of *Raphia hookeri*.

Effect of aqueous fruit extract of Raphia hookeri on sodium ion (Na+) in male Wistar rats



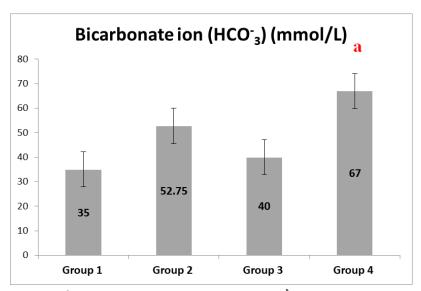
Values represent mean ± SEM, n=4; ^a Significant at p<0.05 compared to Group 1; ^b Significant at p<0.05 when compared to group 2; ^c Significant at p<0.05 when compared to group 3. AFERH = aqueous fruit extract of *Raphia hookeri*.

Effect of aqueous fruit extract of Raphia hookeri on potassium ion (K⁺) in male Wistar rats



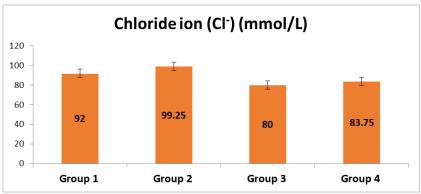
Values represent mean \pm SEM, n=4; ^a Significant at p<0.05 compared to Group 1; ^b Significant at p<0.05 when compared to group 2; ^c Significant at p<0.05 when compared to group 3. AFERH = aqueous fruit extract of *Raphia hookeri*.

Effect of aqueous fruit extract of Raphia hookeri on bicarbonate ion (HCO3) in male Wistar rats



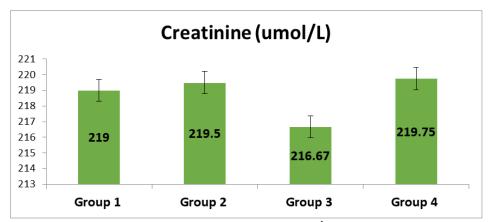
Values represent mean \pm SEM, n=4; ^a Significant at p<0.05 compared to Group 1; ^b Significant at p<0.05 when compared to group 2; ^c Significant at p<0.05 when compared to group 3. AFERH = aqueous fruit extract of *Raphia hookeri*.

Effect of aqueous fruit extract of Raphia hookeri on chloride ion (CI) in male Wistar rats



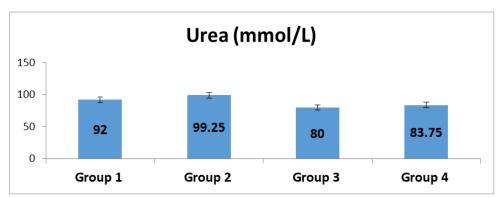
Values represent mean ± SEM, n=4; ^a Significant at p<0.05 compared to Group 1; ^b Significant at p<0.05 when compared to group 2; ^c Significant at p<0.05 when compared to group 3. AFERH = aqueous fruit extract of *Raphia hookeri*.

Effect of aqueous fruit extract of Raphia hookeri on creatinine in male Wistar rats



Values represent mean ± SEM, n=4; ^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 3. AFERH = aqueous fruit extract of *Raphia hookeri*.

Effect of aqueous fruit extract of Raphia hookeri on urea in male Wistar rats



Values represent mean ± SEM, n=4; ^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 3. AFERH = aqueous fruit extract of *Raphia hookeri*.

DISCUSSION OF RESULT

The results of the analysis of renal physiology revealed at p-value ≤ 0.05 a statistically significant decreased level of Potassium across all the groups treated with aqueous fruit extract of Raphia Hookeri compared to the control. Hypokalaemia is caused as a result of potassium depletion. Hypokalemia leads to several important disturbances of renal function. Potassium depletion causes tubulointerstitial fibrosis that is generally greatest in the outer medulla. Although usually reversible, it may result in renal failure. These include reduced medullary blood flow and increased renal vascular resistance that may predispose to hypertension, tubulointerstitial and cystic changes, alterations in acid-base balance, and impairment of concentrating mechanisms. Also revealed significant increase of sodium althrough the groups treated with aqueous fruit extract of Raphia Hookeri compared with the control. In renal epithelial cells, a rise in sodium uptake across the apical membrane increases intracellular sodium concentration, which in turn stimulates the turnover rate of Na+-K+-ATPase and thereby enhances sodium efflux across the

basolateral membrane, condition called Hypernatremia (High Level of Sodium in the Blood) A prolonged increase in sodium (Hypernatremia) causes dramatic hypertrophy and hyperplasia and a rise in the quantity of Na+-K+-ATPase in the basolateral membrane..The result is in agreement with Armstrong et al., (2002) that stated that increase of sodium induces acute diuretic effect. It displayed a decrease in chloride in groups 3 and 4 and an increase in group2, a prolonged decrease of chloride causes hypochloremia, a condition of an electrolyte imbalance that occurs when there's a low amount of chloride in the body. Metabolic alkalosis is directly associated with hypochloremia as sodium bicarbonate reabsorption in the proximal convoluted tubule increases in hypovolemic settings with increased levels of angiotensin II Akoum, et al., 2021. Also revealed an increase of bicarbonate although groups treated with aqueous fruit extract of Raphia Hookeri. Metabolic alkalosis there is excess of bicarbonate in the body fluids. It can occur in a variety of conditions. It may be due to digestive issues, like repeated vomiting, that disrupt the blood's acid-base balance. It can also be due to complications of

conditions affecting the heart, liver and kidneys (Cleveland Clinic, 2023).

CONCLUSION

Prolonged consumption of Raphia Hookeri fruit pulp has effect on the renal physiology of the wistar rats. The kidneys eliminate, among other products, urea, uric acid, and creatinine, in addition to metabolizing and eliminating drugs and toxins Baynes, et al., 2006. Therefore, a decrease in urinary volume causes an increase in the passive reabsorption of urea and a decrease in its elimination, which depends on protein intake and catabolism (Castaño-Bilbao et al., 2009). The results statistically showed a significant increase Creatinine levels in groups 2 and 4 and a significant decrease in group 3 when compared with the control group and a decrease urea levels in groups 2 and 3, with a significant increase in group 4. The increase observed in urea and creatinine indicates that kidney function would deteriorate as it prolongs which negatively alter the renal physiology of the male wistar rats. Hence, the finding of this study has further confirm the effects aqueous fruit extract of Raphia Hookeri on renal functions of wistar rats.

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