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Renal and electrolyte profiles of Wistar albino rats fed with *Irvingia wombolu* kernel powder and its oil extract

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Abstract

Irvingia gabonensis Kernel powder and the oil extracts with and without testa (±testa)were mixed with commercial animal feed (50:50)and fed to groups of animals. Animals in groups A and B were fed with the powder at an equivalent concentration of 67mg/kg body weight while those in groups C and D were fed with the equivalent4ml of the oil extract in the mixture. Animals in group E, the control, were fed on the commercial feed only. All the animals were fed in an aluminum cage for 21 days and sacrificed the following day. Blood from the jugular vein was collected in universal bottles and used for determination of creatinine, urea, sodium, potassium and bicarbonate. It was observed that all the treated groups and the control showed no significant difference (P≥0.05) in their Nak concentrations. There were significant differences(p≤ 0.05) in the K+ concentrations between groups A,B, E and groups C, D respectively .Significant statistical differences(P≤0.05) were also observed in the creatinine concentrations of all the treated groups with the exception of group C. There were higher urea concentrations in groups C and D than A, B and E(P≤0.05).Bicarbonate showed the least concentration in group E with relatively higher concentrations in groups C and D(P≤0.05)

Keywords: *Irvingia gabonensis*; Testa;Urea; Creatinine

1. Introduction

Irvingia is a non- timber forest tree comprising the stem, leaves, roots and fruits(1). Commonly referred to as wild bush mango and African bush mango, the tree is named after a Royal Navy surgeon and botanist, George Irving(2). It is of the order *Malpighiales,* family *Irvingianceae* and comprises seven species. In the family *Irvingiaceae, Irvingia gabonensis* and *Irvingia wombolu* are well known. *Irvingia* is native to Central and West African Countries. Specifically, the species *wombolu* and *gabonensis* span through the humid forest zones of West and Central Africa.

The flesh and the kernel of *I. gabonensis* var.*gabonensis* are edible while only the kernel of var. *wombolu* is edible. The former is for this reason called sweet bush mango and the latter bitter bush mango. Some local names for the kernels of African bush mango include: ogbono among the Ibos, dikanut among the Camerounians, oro –egili and oro –aikpele among the Igala people of Kogi State, Nigeria.

Indigenous African tribes have used the fruits in various ways including its usage as a thickening agent for traditional soups. When squeezed, the oil from the fruit can also be used for cooking. The western world, however, consider it a super fruit because of studies showing that extracts from its seeds and the fruit as a whole couldhelp in loss of weight, control of blood cholesterol as well as reduction in blood glucose (3).

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1.1. Justification For the Study

Irvingia gabonensis has been known to cause reduction in weight, waist circumference and systolic blood pressure. In addition, the presence of appreciable fibre content in *I. gabonensis* makes it a very good option in reduction of blood sugar and hence management of diabetes mellitus (3).

There are many literature reportson the medicinal use of *Irvingia gabonensis*stem bark extracts and kernelin management of some common ailments in Africa, such asweight and glucose control but the same can not be said of literatures on the renalprofile and some electrolyte changes that occur following administration of kernel fractions.In the rare publications on this subject matter that I have stumbled upon, reference is hardly made to the use the dehusked kernel(-testa) The current study is aimed at providingthis knowledge gap.

Aim and Objectives

The aim of the study was to determine the effect of *I. gabonensis* (var. *wombolu*) kernel powder and oil extracts with and without testa (±testa) on selected electrolyte concentration and renal profile of wistar albino rat.

The specific objectives of the study were to determine the mean values of sodium, potassium,bicarbonate ions, and the mean values of urea and creatininine concentrations in wistar rats fed with *Irvingia gabonensis* kernel powder with and without testa(±testa)

2. Materials and methods

2.1. Collection and Treatment of Kernel Samples

Mature *I.wombolu* fruits were plucked from the tree at Aiyolo Dekina Locol Government., Kogi State, Nigeria. The fruits were then split open with a stainless steel knife; the kernels were removed from the shell and then divided in to two equal portions. The testa of one of the portions was left intact (Iwkt) while the other was removed (Iwk).

Oil was extracted from part of Iwk and Iwkt by Soxhlet extraction method as described by Pearson(4). The oil samples so extracted were labelled Iwot and Iwo respectively. The Iwkt or Iwk kernel powder sample was undefatted in this study.

2.2. Purchase of Reference Commercial Feed

The commercial feed used in this study was pelletized growers mash procured from a commercial shop located opposite Grimard Hospital. Anyigba, Kogi State, Nigeria. The composition of the feed, according to the manufacturers was as follows ;crude protein (15%), fat,(7%), crude fibre (10%), calcium (1.0%), available phosphorus (0.35%) and metabolisable energy (2550 Kcal/Kg).

2.3. Oil Extraction

Oil was extracted using Soxhlet extraction method as described by Pearson(4}. Boiling flasks, 250 ml, were dried in oven at a temperature of 110 ^{0}C for thirty minutes and transferred into desiccators to cool. Respective samples (800 g) of Iwkt and Iwk were later weighed into labelled thimbles. Correspondingly labelled cooled boiling flasks were then weighed and filled with petroleum ether (40-60%). The extraction thimble was then plugged lightly with cotton wool, the Soxhlet apparatus assembled and allowed to reflux for six hours. The thimble was then removed carefully, the petroleum ether in the top container of the set-up collected and drained into a flask for re-use.

The flasks were later removed, when it was free of petroleum ether, and dried at 110 ^{0}C for 1 hr after which they were transferred into desiccators, allowed to cool and then weighed.

2.4. Experimental Animals

The experimental animals used were wistar albino rats with weight range of 120 – 180 g obtained from the animal house of Kogi State University, Anyigba. The animals were kept in standard aluminium cubicles at the animal house of Biochemistry Department, Kogi State University, Anyigba.

Twenty-five animals were grouped into five with each cubicle containing five rats. The cubicles were labelled A, B, C, D and E.

2.5. Treatment Protocol

Kernel powder and the oil extracts (±testa) were mixed with commercial animal feed (50:50) and fed to groups of animals. Animals in groups A and B were fed with the powder at equivalent concentration of 67 mg/Kg body weight while those in groups C and D were fed with the equivalent 4ml of the oil extract. Group E, the control, was fed only with commercial animal feed. They were also provided with potable water ad libitum.

The treatment lasted for a total of twenty-one days. On the twenty-second day , the animals were sacrificed using a sharp surgical blade; part of the blood was collected in in universal bottles.

Table 1 Protocol for Animal Treatment

Sample Types	Animal groups (5/ group)
(Iwkt)	A
(Iwt)	B
(Iwot)	C
(Iwo)	D
Control	

Iwkt = *Irvingia* Kernel Powder (+testa);Iwk = *Irvingia* Kernel Powder (-testa); Iwot = *Irvingia* Kernel Oil (+testa); Iwo= *Irvingia* Kernel Oil (-testa); Control = Commercial Animal feed

2.6. Determination of Serum Electrolytes

The serum level of sodium (Na+), potassium (K+), and bicarbonate (HCO3-) were determined according to the methods of Velapoldi et al. (5), using Randox and Teco kits purchased fromCrumlin, Antrim, UK. The blood for this purpose was first collected in universal bottle, spun in a centrifuge at 1500 rpm for 5 min after which the resulting supernatant was used for determination of the electrolytes according to manufacturer's guidelines.

2.7. Determination of Creatinine and Urea

The serum levels of creatinine and urea were determined according to the method described by Bartel and Bohmer (6), using Reflotron Plus biochemistry analyser, Roche Diagnostics GmbH, Germany.

Principle of reaction:

- Creatinine in alkaline solution reacts with picric acid to form a colored complex. The amount of the colored complex formed is directly proportional to the creatinine concentration.
- Urea: The diacetyl monoxime methodology for BUN determination is direct and measures the chromogen formed from the condensation of urea with diacetyl. Diacetylmonoxime is hydrolysed under acidic conditions to produce diacetyl which then condenses with urea to form a pink chromogen that is measured at 520nm.Thiosemicarbazide and ferric ions are employed to enhance the color development. Appropriate quantities of the supernatants (30 µl) were introduced into the test strip with the aid of the reflotron strip provided. The strip was inserted in the test chamber and, following the direction on the screen, the results were displayed and recorded.

3. Results

The following parameters were analyzed as presented below:

- Sodium: There were no significant differences ($P \geq 0.05$) among the groups including the control.
- Potassium: There were significant differences ($P \leq 0.05$) between groups A, B, E and groups C, D respectively. Group C and D had relatively higher concentration.
- Creatinine: The control group was significantly different from the treated samples ($p \leq 0.05$) except sample C.
- Urea: Higher levels of urea were found in samples C and D, than A, B and E ($p \leq 0.05$)
- Bicarbonate: The least bicarbonate concentration was observed in sample E while relatively higher concentration were obtained with sample C and D ($p \leq 0.05$)

Table 2 Some Electrolyte and Renal Profiles of Wistar Rats fed with *Irvingia gabonensis* var .*wombolu* kernel powder and Oil (± testa) on 22nd Day

Parameter **Powder (+t)**	**A** **Powder (+t)**	**B** **Oil (-t)**	**C** **Oil (+t)**	**D** **Oil (-t)**	**E** **Control**	**LSD**
Sodium (meq/l)	125.00±3.23^{a}	137.60±1.73^{a}	129.03±3.12^{a}	117.70±4.14^{a}	122.42±2.31^{a}	30.22
Potassium (meq/l)	3.66±0.62^{a}	3.62±0.30^{a}	7.02.00±0.64^{b}	9.12±1.40^{b}	3.60.01±1.84^{a}	3.14
Creatinine (mg/l)	1.70±0.10^{b}	1.63±0.06^{b}	1.12±0.04^{a}	1.60±0.10^{b}	1.20±0.12^{a}	0.23
Urea (mg/l)	40.80± 0.45^{a}	41.61±1.10^{a}	45.50±3.32^{b}	45.52±0.62^{b}	37.86±0.90^{a}	4.64
Bicarbonat(mmol/l)	36.67±1.50^{b}	36.37±1.22^{b}	40.28±0.42^{c}	41.46±0.41^{c}	29.62±0.58^{a}	2.62

Results are as expressed as mean ± standard error of mean for n = 3: A-D refer to animals treated with I.wkt, I.wk, I.wot and I.wo respectively and E is the control: Values with similar alphabet in a row show no significant difference at P ≥ 0.05

4. Discussion

In this study, powdered forms of *Irvingia gabonensis* var. *wombolu* and their oil extracts, with and without testa, were analysed for their effects on therenal protein profiles and selected electrolyte concentration of wistar rats, previously fed for 21 days. A commercial feed as stated in the methodology, was used as control (E) while a 50 : 50 mixture of the commercial feed and the processed *wombolu* kernel powder were fed to some groups (A and B) ; the equivalent amount of the oil replaced the kernel powder in the feeding of groups (C and D). The following were our observations:

4.1. Sodium

The result showed no significant difference between the treated groups and the control (P > 0.05), implying that the *Irvingia wombolu* kernel contained insignificant sodium content, with attendant positive effect on health.

4.2. Potassium

The observed relative hyperkalemia in C and D might be due to the correspondingly higher levels of glucose on day 22nd (table7), there being a direct relationship between uptake of glucose and potassium by cells ([7]).Potassium ion has an inverse relationship with intracellular sodium concentration in maintaining homeostasis in the body. The normal value is 3.5 – 5.5 meq /l .

The presence of myristc acid (49- 50%) in *Irvingia gabonensis* oil has been reported ([8]). This medium- chain fatty acid has a property similar to non- steroidal anti inflammatory drugs like aspirin (9). However, fat-soluble constituents of the kernel extracts could also account for the observed relative hyperkalemia in groups C and D. because impaired renal function could have reduced the kidney's ability to handle potassium, leading to hyperkalaemia (10) Groups C and D animalshad high urea values which were indicative of kidney damage and consequent hyperkalemia. Caution should, therefore, be exercised with frequent and direct application ofI. *wombolu* oil extract with other (food) products containing potassium.

4.3. Bicarbonate

The plasma value varies widely with age but at about 18 yrs of age, it stabilizes at 22 -29 mmol.The result showed significant statistical differences (p ≤ 0.05) between the control and all the treated groups, with the powdered groups showinglower values than the oil groups. It implied that variations in treatment had effects, with relatively higher solubility of phytochemicals such as polyphenolic substances in the oil extracts and that *Irvingia wonbolu* kernel powder is relatively safer than the oil at similar level of consumption.

4.4. Creatinine

Except group C, the creatinine levels were higher in all the treated groups and were significantly different (p≤ 0.05), implying early onset of kidney damage. Again the need for caution in direct application of the *I.wombolu* oil products, is hereby expressed, especially in patients diagnosed with renal pathology. Equally important is a call for restriction of this product in kidney failure patients

4.5. Urea

Urea is produced as a breakdown product of protein and filtered by the kidney. The normal urea level is 20 – 40 mg/dl. This study demonstrated significant differences (p≤ 0.05) between the oily samples C and D, and the others. This rise in urea levels in these groups –azotaemia – might be indicative of onset of renal damage. The presence of polyphenolic compounds in the kernels of Irvingia kernel would support the work of ([11]) who demonstrated the hepato-renal toxic effect of ethanolic seed and leaf extracts of *Irvingia gabonensis* on wistar albino rats ([12]) had reported toxic effects on the liver and gonads of albino rats treated with *Irvingia gabonensis* extracts. It was however, contradicted by the work of Ojo et al([13]) who had shown the nephro- protective effect of stem bark extract of the plant on wistar albino rats. The cautionary remarks on creatinine above also applies. However, in the African traditional meals where *Irvingia* kernel are used in soup – thickening, the quantity consumed per day is usually below laboratory conditions of study.

5. Conclusion

Irvingia gabonensis var. wombolu is a tree that grows in the wild in many countries of Africa. It has a wide range of economic, nutritional and medical benefits. This study examined the renal profile and selected electrolyte concentrations of wistar rats fed with kernel fractions of *Irvingia gabonensis* for a period of 21 days. It was observed that the sodium ion concentrations of the treated groups showed no significant statistical difference(P≥ 0.05), implying that the kernel is safe for consumption even in hypertensive cases. The observation also indicated that treatment given to kernel samples were of no effect. The statisticallysignificantly levels of potassium, bicarbonate, urea and creatininemeans that consumption of *Irvingia gabonensis* fractions should be done with caution in many disease conditions especially in renal disease conditions. Excessive or monotonous consumption of this product should be avoided in all situations. Those will renal compromise should avoid it completely.

Compliance with ethical standards

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Disclosure of conflict of interest

There was no conflict of interest.

Statement of ethical approval

Ethical approval was obtained from the ethical committee of the university prior to the study.

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