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RESEARCH ARTICLE

BIOCHEMICAL AND HISTOPATHOLOGICAL EFFECTS OF HYDROETHANOLIC EXTRACT OF *IRVINGIA WOMBOLU* KERNELS ON RAT LIVER AND KIDNEY

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Abstract

Objective: Many plants are consumed as food by humans for growth and survival, but a large number of these plants have not been tested for toxicity potential. Repeated consumption of such plants could lead to accumulation of toxic chemicals in the body and cause health-related problems. *Irvingia wombolu* kernel is widely consumed by many ethnic groups in Nigeria and some other African countries. The toxicity potentials of *Irvingia wombolu* kernel extract (IWKE) on the kidney and liver of rats was evaluated in this study.
Methods: Three groups of Wistar rats were fed orally with IWKE (50, 100, and 200 mg/kg b.w) daily for 28 days. The fourth group which is the control was treated with distilled water (10 ml/kg b.w) for the same period. The rats were sacrificed on the 29th day, and blood samples, kidney and liver were harvested for analyses. Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, albumin, bilirubin, creatinine, and urea were determined. Kidney and liver sections were examined for histopathological changes. Data were subjected to Student's t-test for statistical analysis.
Results: Serum levels of creatinine and urea were not significantly altered in the IWKE-treated rats compared with the control. At 200 mg/kg b.w, the extract significantly increased (p<0.05) serum levels of ALT, AST, total bilirubin, total protein, and albumin compared with the control. Significant distortions were observed in the liver sections of rats treated with 200 mg/kg b.w IWKE compared with control, but the structure of the kidney section of IWKE-treated rats was not significantly different from the control.
Conclusion: The results showed that repeated ingestion of *Irvingia wombolu* kernel at a dose of 200 mg/kg b.w for 28 days induced liver damage, but does not significantly affect renal function.
Keywords: Biochemical, histopathological, *Irvingia wombolu*, liver, toxicity.

INTRODUCTION

A large percentage of human diet is sourced from vegetation. Different parts of plants such as leaves, fruits, and seeds are prepared and consumed as food. Many of these plants have been reported to have nutritive values, but few have been subjected to safety tests, especially in rural areas[1]. The understanding is that since the plants have been consumed for many years, it is safe to eat. However, chemicals become toxic at certain doses, and those in the plants that we eat are no exemption[2]. Absence of signs of toxicity after consumption of a plant diet does not necessarily mean it is safe to eat in the amount normally consumed. Under certain conditions of exposure, it could still be risky to consume such diet. In determining the safety of a diet, consideration should be given to the amount consumed, length of exposure, and the toxicity of the plant[3]. It is true that many plants consumed as food in the usual amount contain less harmful constituents when compared with other species with high toxicity potentials. However, it is wrong to conclude that such plants are safe because regular consumption for a long duration may ultimately result

in health hazards[4]. Therefore it is important to carry out necessary toxicity tests for the safety of a plant diet to be established. Because of its importance in metabolism, the liver plays a major role in determining the pharmacokinetics of chemical elimination. It is one of the organs negatively affected by chemical toxicity.[5] Toxic influences of chemicals on the liver include hepatitis, cirrhosis, cholestasis, and liver failure. Liver toxicity has a wide-range of consequences because the liver is involved in such a diversity of body activities[6]. The kidney is the second major avenue for excretion of chemicals. The kidneys rapidly eliminate most of the products of liver metabolism that have been reabsorbed into the blood, but they also excrete many chemicals unchanged. Some of these chemicals are also toxic to the kidney and they can induce kidney disorders at certain doses and length of exposure[7]. *Irvingia wombolu* is a species of plant indigenous to Africa continent. The plant belongs to the family *Irvingiaceae*. There are six species of *Irvingia*, namely *I. gabonensis, I. grandifolia, I. smithii, I. wombolu, I. excelsa, I. robur*[8]. Among them, *I. gabonensis* and *I. wombolu* are used for thickening food[9]. The kernels are also used to prepare a popular Nigerian slimy soup known as 'Ogbono' and 'Apon' among Igbo and Yoruba tribes respectively[10]. For many households in Nigeria, no single day passes by without members consuming the soup. In spite of its widespread consumption, the safety of repeated ingestion of the plant has not been well-documented. In this study, the toxicity potentials of *I. wombolu* seed kernels on the liver and kidney was evaluated in rats.

MATERIALS AND METHODS

Preparation of the *I. wombolu* kernel extract

Fresh kernels of *I. wombolu* were obtained from LAUTECH Teaching and Research Farm, Ogbomoso, Nigeria. The kernels were identified in the Botany Department of Obafemi Awolowo University, Ile-Ife. The kernels were dried under shade in the Laboratory and the dry kernels were reduced to powder by a grinding machine. One kilogram of the powdered kernel was soaked in 1 liter of 80% ethanol for 48 hours and then filtered. The filtrate was allowed to evaporate to dryness, and the dry extract (IWKE) was stored in a refrigerator at 4^0C as stock material for the study.

Experimental animals

Twenty four healthy male Wistar rats weighing 140±20g were used for the study. They were kept in cages in a well-ventilated area in the Animal House of Department of Pharmacology and Therapeutics. They were allowed to get acclimatized with their new environment for 7 days before the study commenced. The rats were allowed to freely feed on standard animal feed and clean water.

The principle of Laboratory Animal Care guidelines and procedures were taken into consideration throughout the study[11]. Approval was also obtained from the Committee on Animal Use of Pharmacology Department, LAUTECH (Number: PT21/010).

Animal grouping

The rats were divided into four groups of six animals each. Group A served as the control and received distilled water (2 ml/kg body weight). Groups B, C, and D were treated per oral with 50, 100, and 200 mg/kg body weight of IWKE respectively. The animals were treated with the extract daily for 28 days.

Collection of samples

After the 28-day treatment, rats were made to fast for 12 hr after which they were sacrificed by cervical dislocation. Blood samples of rats in each group were collected in heparinized bottles. Plasma was separated from packed red blood cells into plain bottle after centrifugation at 5,000 rpm for 5 minutes. The liver and kidney tissues were harvested, rinsed in phosphate buffer, and fixed in 10% formalin solution. Plasma levels of biomarkers of kidney and liver functions, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), total bilirubin, total protein, albumin, creatinine, and urea were estimated using automated AU400 Chemistry Analyzer (Beckman Coulter)[12]. The kidney and liver samples were embedded in paraffin solution after fixation. Then tissue sections (5 µm thick) were cut by means of a microtome. The sections were subsequently incubated with hematoxylin and eosin for photomicroscopic evaluation[13].

Statistical analysis

Results from this study were expressed as mean ± SEM and subjected to Student t-test using GraphPad Prism version 5.0. Differences between groups were taken as statistically significant when p is less than 0.05.

RESULTS AND DISCUSSION

In this study, some biomarkers of hepatic function including ALT, AST, bilirubin, total protein, and albumin were determined in rats fed with kernel extract of *I. wombolu*. Urea and creatinine, which are biochemical indices of kidney function[14], were also determined in the IWKE-treated rats. Compared to control, no significant differences were observed in creatinine and urea levels in all the groups of IWKE-treated rats as shown in Figure 1. Serum levels of ALT, AST, and total bilirubin were significantly increased (p<0.05) in rats treated with 200 mg/kg b.w IWKE compared to the untreated control. ALT concentration increased from 33.08±4.69 to 39.82±3.15, while the concentration of AST increased from 14.15±1.89 to 19.37±2.59. The concentration of total bilirubin increased from 11.16±1.42 to 16.39±1.15. No significant differences in the levels of these parameters were observed at 50 and 100 mg/kg b.w. (Table 1). At 200 mg/kg b.w, the extract also increased total protein and albumin levels significantly (p<0.05) compared with the control. Total protein level was raised from 51.34±7.88 in the control group to 61.09±5.60 in treated rats. Serum level of albumin was significantly increased from 28.83 ± 4.24 to 39.11±4.52 (Figure 2).

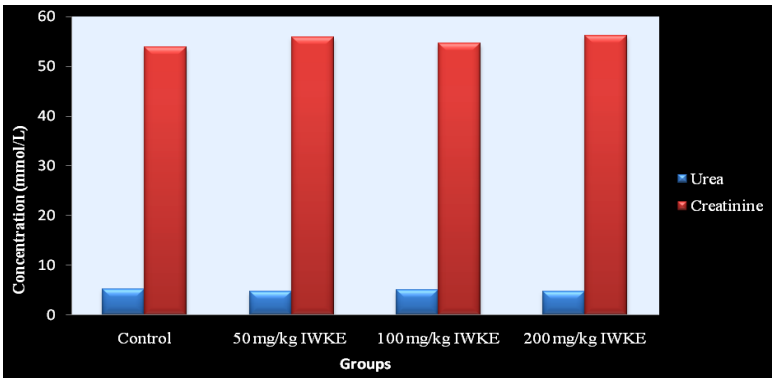

Figure 1: Effects of *I. wombolu* kernel extract on serum urea and creatinine in rats.

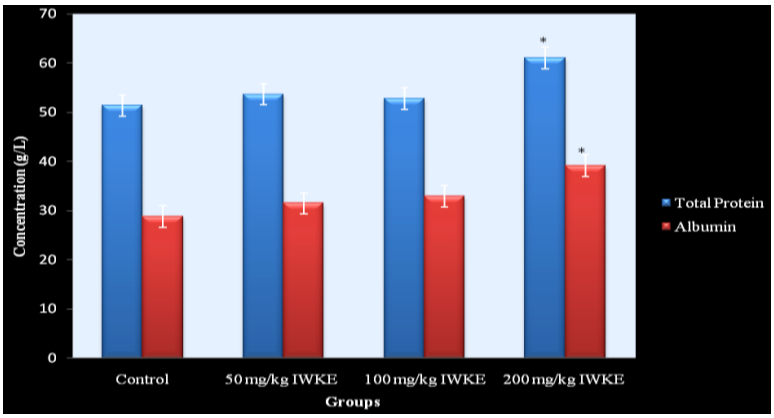

Figure 2: Effects of *I. wombolu* kernel extract on total protein and albumin in rats.

*$p<0.05$ compared with the control

These results indicate that IWKE did not induce damage of the renal tissues at the doses and duration of exposure. Significant increase in AST, ALT, bilirubin, albumin, and total protein levels at 200 mg/kg indicates cellular leakage and dwindling functional integrity of the cell membrane[15]. This suggests that IWKE could become toxic to the liver at this dose after prolonged use. It is noteworthy that the seed of *I. gabonensis*, a close family of *I. wombolu* has been reported to cause liver damage[16].

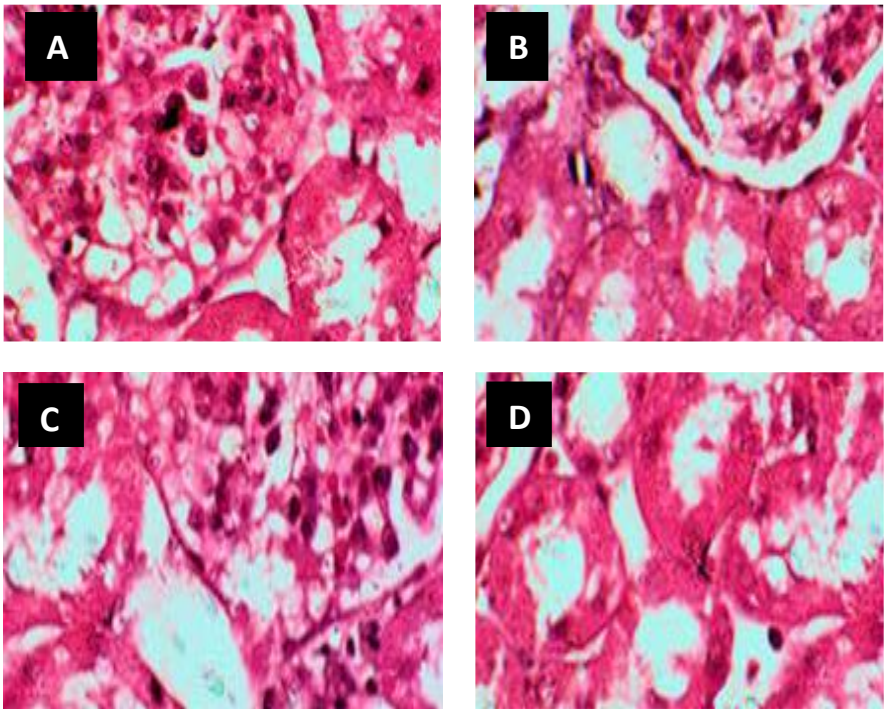

Figure 3: Kidney sections of rats treated with *I. wombolu* kernel extract.

(magnification x 200); A= Distilled water; B = 50 mg/kg b.w; C = 100 mg/kg b.w; D = 200 mg/kg b.w. All the sections appear normal; sections from treated rats are not significantly different from the control

Many of the xenobiotics that induce liver damage were reported to generate free radical during metabolism. Accumulation of such free radicals in the body ultimately results in liver toxicity and injury[17]. It is very likely *I. wombolu* kernels contain myristic acid and lauric acid, just like *I. gabonensis*[18]. Metabolism of these fatty acids could result in production of free radicals leading to hepatic disease[19].

Table 1: Effects of *I. wombolu* kernel extract on AST, ALT, and Total bilirubin in rats.

Group	ALT (IU/L)	AST (IU/L)	Total bilirubin (g/L)
Control	33.08±4.69	14.15±1.89	11.16±1.42
50 mg/kg b.w	33.52±4.70	15.55±3.01	11.50±2.21
100 mg/kg b.w	34.98±4.45	16.18±1.79	12.49±1.34
200 mg/kg b.w	39.82±3.15*	19.37±2.59*	16.39±1.15*

*$p<0.05$ compared with the control

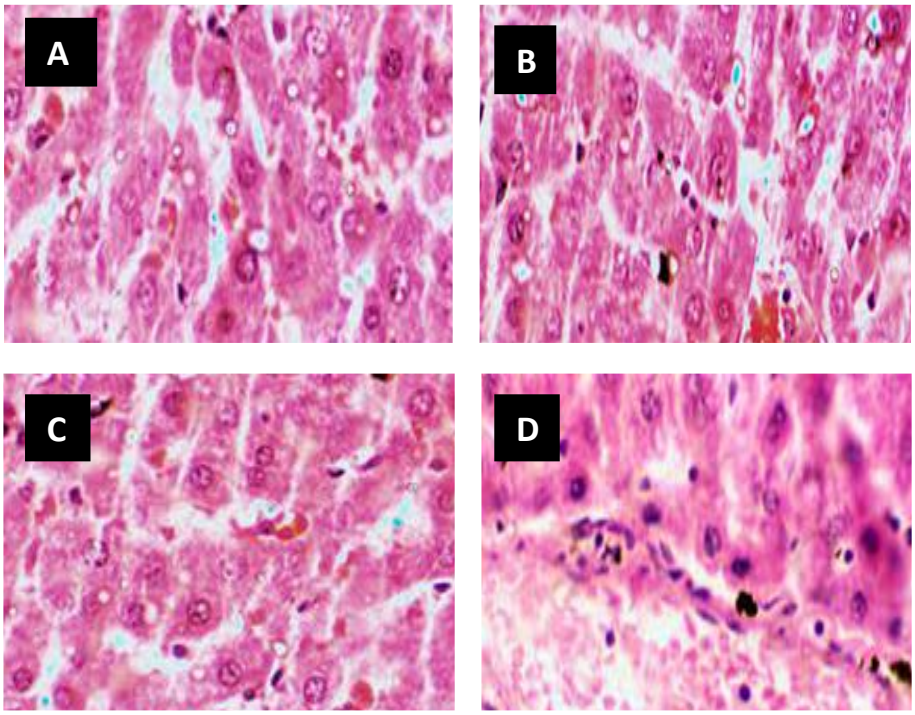

Figure 4: Liver sections of rats treated with *I. wombolu* kernel extract.
(magnification x 200); A=Distilled water; B=50 mg/kg b.w; C=100 mg/kg b.w; D=200 mg/kg b.w. Groups B and C are not significantly different from the control, but Group D showed hemorrhaging of the central vein, dilation of vessels, congestion, and inflamed hepatocytes

The results of histopathological assessment suggest that the structure of kidney sections of treated rats were not significantly different from those of the control (Figure 3), but distortions were observed in the architecture of liver sections of rats treated with 200 mg/kg b.w IWKE. Liver sections in this group of rats showed hemorrhaging of the central vein, dilation of vessels, congestion, and inflamed hepatocytes (Figure 4). These results support the data obtained from the biochemical tests.

CONCLUSION

Results from both biochemical and histopathological tests showed that repeated ingestion of *I. wombolu* kernel at a dose of 200 mg/kg b.w for 28 days did not induce significant adverse effects on renal function, but caused liver damage in rats. Caution should be exercised by those who are fond of food prepared from the plant.

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AUTHOR'S CONTRIBUTIONS

Kolawole OT: writing original draft, literature survey. **Ayankunle AA:** methodology, conceptualization. **Wakeel OK:** formal analysis, review. **Olofinnade AT:** investigation, data interpretation. **Olaniyi OS:** data curation, investigation. **Oluogun WA:** critical review, supervision. All authors revised the article and approved the final version.

DATA AVAILABILITY

The data supporting the findings of this study are not currently available in a public repository but can be made available upon request to the corresponding author.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this work.

REFERENCES

1. Adeneye AA. Subchronic and chronic toxicities of African medicinal plants. Toxicological Survey of African Medicinal Plants. Victor Kuete; Elsevier 2014:99-133. ISBN 9780128000182. *https://doi.org/10.1016/B978-0-12-800018-2.00006-6*
2. Thompson HJ. Improving human dietary choices through understanding of the tolerance and toxicity of Pulse crop constituents. Curr Opin Food Sci. 2019; 30: 93-97. *https://doi.org/10.1016/j.cofs.2019.01.001*
3. Ogori AF. Plant toxins. Am J Biomed Sci Res 2019; 4(3): 173-175. *https://doi.org/10.34297/AJBSR.2019.04.000793*
4. Petroski W, Minich DM. Is there such a thing as "anti-nutrients"? a narrative review of perceived problematic plant compounds. Nutrients 2020; 12(10): 2929. *https://doi.org/10.3390/nu12102929*
5. Fukushima H, Kubo Y, Matsugaki S, Sakemi R, Sakakibara S, Morimitsu Y. Liver injury caused by exposure to silica and 2, 2-dichloro-1,1,1-trifluoroethane (HCFC-123): a case report. Nihon Shokakibyo Gakkai Zasshi 2019; 116(11): 944-951. *https://doi.org/10.11405/nisshoshi.116.944*
6. Singh D, Cho WC, Upadhyay G. Drug-induced liver toxicity and prevention by herbal antioxidants: an overview. Front Physiol 2016; 6:363. *https://doi.org/10.3389/fphys.2015.00363*
7. Perazella MA. Pharmacology behind common drug nephrotoxicities. Clin J Am Soc Nephrol 2018; 13(2): 1897-1908. *https://doi.org/10.2215/CJN.00150118*
8. Gallois S, van Andel T, Heger, T, Sonke B, Henry AG. Comparing apples and pears: the hidden diversity of Central African Bush mangoes (Irvingiaceae). Econ Bot 2020; 74: 178–194. *https://doi.org/10.1007/s12231-020-09497-x*
9. Bamidele PO, Ojedokun OS, Fasogbon BM. Physico-chemical properties of instant ogbono (*Irvingia gabonensis*) mix powder. Food Sci Nut 2015; 3(4): 313–318. *https://doi.org/10.1002/fsn3.220*
10. Mateus-Reguengo L, Barbosa-Pereira L, Rembangouet W, *et al*. Food applications of *Irvingia gabonensis* (Aubry-Lecomte ex. O' Rorke) Baill., the 'bush mango': A review. Crit Rev Food Sci Nutr 2020; 60(14): 2446-2459. *https://doi.org/10.1080/10408398.2019.1646704*
11. National Institute of Health. Guide for the use of laboratory animals. DHHS, PHS, NIH Publication 1985; No. 85-23
12. Bailly AL, Correard F, Popov A, *et al*. *In vivo* evaluation of safety, biodistribution and pharmacokinetics of laser-synthesized gold nanoparticles. Sci Rep 2019; 9:12890. *https://doi.org/10.1038/s41598-019-48748-3*
13. Ahmadian S, Sheshpari S, Mahdipour M, *et al*. Toxic effects of VCD on kidneys and liver tissues: a histopathological and biochemical study. BMC Res Notes 2019; 12(1): 446. *https://doi.org/10.1186/s13104-019-4490-y*
14. Afsar T, Razak S, Aldisi D, *et al*. *Acacia hydaspica* R. Parker ethyl-acetate extract abrogates cisplatin-induced nephrotoxicity by targeting ROS and inflammatory cytokines. Sci Rep 2021; 11(1): 17248. *https://doi.org/10.1038/s41598-021-96509-y*
15. Jang M, Oh MS, Oh SC, Kang KS. Distribution of diseases causing liver function test abnormality in children and natural recovery time of the abnormal liver function. J Korean Med Sci 2016; 31(11): 1784-1789. *https://doi.org/10.3346/jkms.2016.31.11.1784*
16. Etta HE, Olisaeke CC, Iboh CI. Effect of *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) seeds on the liver and gonads of male albino rats. J Biol Agri Healthcare 2013; 2: 10-15. *https://doi.org/10.5580/223b*
17. Alkadi H. A Review on free radicals and antioxidants. Infect disord drug targets 2020; 20(1): 16-26. *https://doi.org/10.2174/1871526518666180628124323*
18. Okolo CO, Johnson PB, Abdurahman EM, Abdu-Aguye I, Hussaini IM. Analgesic effect of *Irvingia gabonensis* stems bark extract. J Ethnopharmacol 1995; 45(2): 125-9. *https://doi.org/10.1016/0378-8741(94)01199-a*
19. Das U. Essential fatty acids enhance free radical generation and lipid peroxidation to indice apoptosis of tumor cells. Clin Lipidol 2011; 6(4): 463-489. *https://doi.org/10.2217/clp.11.34*