

Original Research Article

## Evaluating bioactive constituents and toxicological effects of aqueous extract of fermented *Pentaclethra macrophylla* seeds in rats

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### Abstract

**Objective:** This study aimed at evaluating the bioactive constituents and the toxicological profile of the aqueous fermented seed extract of *P. macrophylla*.

**Material and Methods:** The chemical constituents of fermented *P. macrophylla* were assessed using GC-MS. For acute toxicity study, one-time doses of up to 5000 mg/kg of the extract were orally administered to male and female rats whereas 200, 400 and 800 mg/kg of the *P. macrophylla* extract were orally administered daily for 14 days in sub-acute toxicity investigation. Biochemical, haematological and lipid profiles were assessed following standard methods.

**Results:** Bioactive compounds such as citronellol and oxirane, tetradecyl- (hexadecylene oxide) were identified in the extract. In acute toxicity test, no death or sign of toxicity was identified. For sub-acute study, ALT decreased significantly ( $p < 0.05$ ) while HDL-C had dose-dependent increases. No effect ( $p < 0.05$ ) on haematological parameters except on platelets was found. No histopathological changes were observed.

**Conclusion:** Our results demonstrated that the extract of fermented *P. macrophylla* caused no toxic effects in the rats at the tested doses. Therefore, they may be termed safe for consumption and therapeutic uses.

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### Introduction

*Pentaclethra macrophylla*, an oil bean, is a perennial leguminous tree native to tropical Africa, employed for traditional alternative medicine (TAM) when treating different forms of ailments. Anti-

inflammatory, anti-helminthic, abortifacient and analgesic activities of the leaves, stem bark, seeds and fruit pulp extracts have been reported (Akindahunsi, 2004). The medicinal properties are most likely a direct consequence of the bioactive

agents in the different plant parts (Akindahunsi, 2004; Fungo *et al.*, 2015). Several studies on the nutritional and physicochemical properties of *P. macrophylla* have already been performed which demonstrated the presence of proteins, amino acids, carbohydrates, fat, fibre, thiamine, riboflavin and niacin (Onwuliri *et al.*, 2004; Odoemelam, 2005; Ogueke *et al.*, 2010; Osagie-Eweka and Alaiya, 2010; Eze *et al.*, 2014). However, its bioactive constituents have not been studied. Fermented and unfermented *P. macrophylla* seeds contain essentially similar nutrient levels except for carbohydrates which increased slightly in fermented *P. macrophylla* seeds, suggesting that fermentation has no significant effect on nutrient content (Enujiugha, 2003; Monago *et al.*, 2004). The level of vitamins in *P. macrophylla* may be elevated when they are fermented, particularly thiamine, riboflavin and niacin (Achinewhu, 1983). Due to the high protein content (Achinewhu, 1983), *P. macrophylla* is considered an important and affordable protein source for communities whose staple foods lack proteins. However, a more recent work documented lowering of *P. macrophylla* protein content during fermentation with a little elevation in carbohydrates and oil concentration (Akindahunsi, 2004).

In Southern rain forest zone of West Africa, particularly in the Eastern region of Nigeria, about 50 million people depend on different parts of *P. macrophylla* tree for food, and charcoal making. The seed pulp of *P. macrophylla* is consumed boiled or roasted, and more frequently undergo fermentation prior to their consumption. Among the Igbos of Nigeria, its commonest folklore culinary application is the fermented seed product popularly called *Ugba*, which has a meaty taste and is served both as a delicacy and a soup flavoring agent (Mbajunwa *et al.*, 1998; Enujiugha and Akanbi, 2005). There have not been any established cases of acute or chronic poisoning due to *Ugba* consumption over the years. Interestingly, several health

benefits have been reported, including lowering of plasma cholesterol (Monago *et al.*, 2004), and reducing the risks of ulcer (Ugbogu *et al.*, 2017), cancer and some tobacco-related diseases (Chidozie, 2006). However, the presence of saponins (Achinewhu, 1983), a growth depressant, caffeoyl-putrescine (Ugbogu *et al.*, 2017) and a poisonous alkaloid, paucine (Isu and Njoku, 1997; Chidozie, 2006; Ugbogu *et al.*, 2017), which are toxic and with no nutritive value has been reported. It is assumed that fermentation of *P. macrophylla* seeds significantly reduces the content of the poisonous alkaloid such as paucine via leaching and degradation during the harsh thermal processing and fermentation processes (Onwuliri *et al.*, 2004). Akindahunsi (2004) reported no changes in tannin levels, and a marked increase in phytates, after extensive processing of *P. macrophylla*.

The proteolytic fermentation, which is not primed with a starter culture, occurs naturally under alkaline conditions mainly through the activity of species of the genus *Bacillus* (Isu and Njoku, 1997; Ogueke and Aririatu, 2004; Okwulehie, 2004; Kabuo *et al.*, 2007). However, the presence of *Escherichia coli* and *Staphylococcus aureus* (Ogueke and Aririatu, 2004), *Aspergillus flavus*, *Aspergillus niger*, *Penicillium chrysogenum* and *Fusarium spp* (Azubuine and Isu, 2006; Ogueke *et al.*, 2010) as well as *Candida tropicalis* and *Geotrichum candidum* (Ejiofor *et al.*, 1987; Ogueke *et al.*, 2010) has been reported. This poses potential health risks to consumers, but the subsequent heat treatment during cooking when *Ugba* is used as soup condiments, may eliminate these microorganisms.

No detailed and definite toxicological study has elucidated the safety of *Ugba* consumption. To lay these assumptions to rest, this paper therefore seeks to explore the possible toxicity of aqueous extracts of fermented *P. macrophylla* by analyzing blood, hepatic and renal profiles in rats. The histomorphological changes in the liver and

kidneys were also evaluated to corroborate the biochemical parameters.

### Materials and Methods

#### Sampling of *P. macrophylla*

*P. macrophylla* seeds were sourced from Eke Okigwe, Okigwe Local Government Area, Imo State, Nigeria. They were carefully sorted to remove contaminants. *P. macrophylla* seeds were authenticated at University of Nigeria Nsukka and deposited in the herbarium (No. UNN/138a).

#### Sample processing

For seed processing, the method of Isu and Ofuya (2000) was adopted with some modifications. The seed coats were softened by prolonged boiling (12 hr) and the released embryos were boiled for another 4 hr after fine slicing. Thereafter, the seed particles were washed thoroughly for 4 to 5 times with distilled water to reduce the bitter taste, wrapped in *Alchornea laxiflora* Benth leaves (okpokia leaves), packed in aerated bags. The wrapped sliced seeds were allowed to undergo fermentation at ambient temperatures and used after 3 days.

#### Preparation of *Ugba* seed extract

Exactly 200 g of the fermentation product (*Ugba*) was placed in 500 ml of distilled H<sub>2</sub>O for extraction at 25°C for 18 hr. Then, the solids were removed by filtration. The filtrate was immediately used for analytical purposes or further experiments.

#### Identification of the bioactive compounds of *P. macrophylla*

To identify the chemical constituents of the aqueous extract of fermented *P. macrophylla* (*Ugba*), GC-MS analysis (Agilent 7890A-5975C GC-MS system) with column, HP5 (30 m x 0.25 mm x 0.25 µm); and carrier gas, ultra-pure helium at a flow rate of 1.0 ml/min and a linear velocity of 37 cm/s was used (Ugbogu et al., 2019). The temperature of the injector was 250°C; oven temperature, was initially 110°C and increased at 10 °C/min until 280°C (7 min at each increment). The injection volume

was 0.5 µl in the splitless mode with a split ratio of 10:1; mass spectrometer and 70 eV electron ionization mode. The retention times as well as mass spectral data and fragmentation pattern were directly compared with those available in the National Institute of Standards and Technology (NIST) database for identification of the chemical constituents (Ugbogu et al., 2019).

#### Animal procurement and housing

The study was performed in rats weighing from 140 to 180 g. The animals were sourced from the University of Nigeria. Animals were acclimatized for a period of two weeks and fed on grower mash and allowed free access to water in rooms that were well ventilated at ambient temperature with 12/12 hr light/dark condition (Hajihosseini and Setorki, 2017; Ugbogu et al., 2018; Ugbogu et al., 2019). The animal studies were performed according to the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (NIH, 1985).

#### Acute toxicity study

Assessment of acute toxicity of *Ugba* aqueous extract was performed following the guideline described by Organization for Economic Cooperation and Development (OECD) (OECD, 2001) guideline 423 with minor modifications. The rats were randomly divided into six groups of 12; each group consisted of 6 male and 6 female rats. Post overnight fasting, one-time doses of 500, 1,000, 2,000, and 5,000 mg/kg of *Ugba* extracts were orally administered (gavage) while the control group received 0.25 ml/kg of distilled water. Behavioral changes and mortality of the animals were observed for 24 hr and thereafter, for a period of 14 days.

#### Sub-acute toxicity study

Twenty-four rats were randomly placed into four groups of six each. Group I served as a control which did not receive aqueous extract of *Ugba*. Group II, III and IV were

administered with 200, 400 and 800 mg/kg *Ugba* extract, respectively. Extract administration was performed daily by oral gavage over a period of 14 days. The time and doses used in this study were selected based on previous studies (Traesel *et al.*, 2016; Ugbogu *et al.*, 2018; Ugbogu *et al.*, 2019) and the results of the present acute toxicity study. Thereafter, the body weight of the rats was determined. After overnight fasting, they were anesthetized and sacrificed. The spleen, heart, lungs, kidney and liver were excised, cleaned by blotting with filter paper and weighed to determine their relative weight. For histological examinations, the organs were fixed in 10% formalin and embedded in paraffin wax. Whole blood was collected by cardiac puncture using sterile needles and kept in sterile plain and EDTA bottles for analyses.

#### Determination of biochemical, haematological and histopathological parameters

The enzymatic activities of the liver transaminases (aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP)) as well as triacylglycerol (TAG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were determined spectrophotometrically using the kits from Randox Laboratory Ltd., Co. Antrim (United Kingdom) according to the manufacturer's instruction. Urea, creatinine, Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup> and HCO<sub>3</sub><sup>-</sup> were spectrophotometrically determined using Teco test kits (Teco Diagnostics, USA). Haematological parameters were determined according to the method previously described by Afia and Momoh (2006) using the BC-3200 Auto-Haematology Analyzer. To study the histopathology of the liver and kidney, 5- $\mu$ m thick sections were stained with haematoxylin and eosin. The sections were viewed by an experienced pathologist using optical microscope (Fisher, 2002).

#### Statistical analysis

Analysis of data was performed by one-way analysis of variance (ANOVA) with Tukey *post-hoc* test. A p-value of  $\leq 0.05$  was considered statistically significant.

## Results

GC-MS analysis of an aqueous fermented seed extract of *P. macrophylla* revealed the presence of nine chemical compounds (Table 1).

Table 2 summarizes the result of the acute toxicity study of the *Ugba* extract in rats. Neither behavioural changes nor mortality was observed in the rats even at the highest dose of the *Ugba* extract (5000 mg/kg body weight). Furthermore, no significant change ( $p < 0.05$ ) in the relative weight of the spleen, heart, lung, kidney and liver even at the highest dose (800 mg/kg body weight) was observed when compared to the control group (Figure 1).

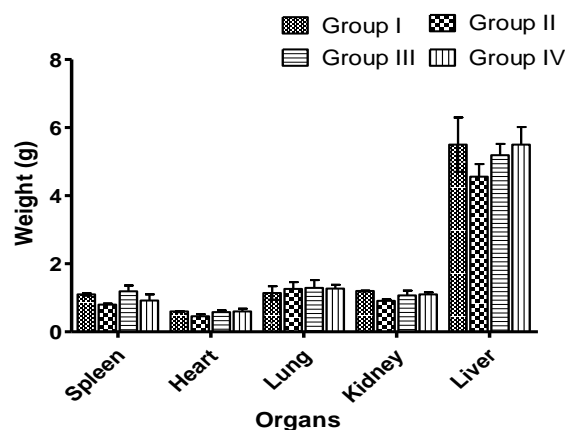
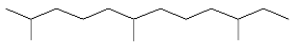
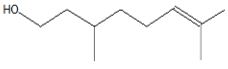
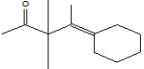
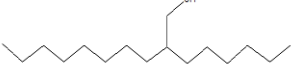
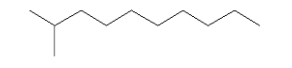
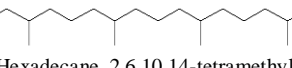
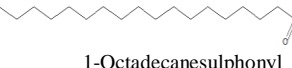
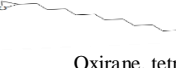


Figure 1. Effect of an aqueous *Ugba* extract on the relative organ weight. Rats were administered with 0.25 ml/kg of distilled water (Group I (Control)), aqueous fermented seed extract of *P. macrophylla* for 14 days at doses of 200 (Group II), 400 (Group III) and 800 (Group IV) mg/kg BW. The rats were sacrificed, and the organs were carefully removed and weighed. Values represent the mean  $\pm$  SD for  $n=6$ .

Daily administration of an *Ugba* extract to rats, resulted in significant reductions ( $p < 0.05$ ) in the ALT compared to the control group (Figure 2). ALP activity decreased in a dose-dependent manner, but the level of the decrease in the activity was

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not statistically significant ( $p>0.05$ ) (Figure

S/N	RT (min)	Structure/Name of the compound	Molecular formula	Molecular mass	Peak area %
1.	10.729		C <sub>15</sub> H <sub>32</sub>	212	8.78
2.	11.233	Dodecane, 2,6,10-trimethyl-	C <sub>19</sub> H <sub>40</sub>	268	29.68
3.	11.502	Pentadecane, 2,6,10,14-tetramethyl	C <sub>10</sub> H <sub>20</sub> O	156	3.00
4.	12.148	 Citronellol	C <sub>15</sub> H <sub>26</sub> O	222	1.67
5.	13.750	 2-Pentanone, 4-cyclohexylidene-3,3-diethyl-	C <sub>16</sub> H <sub>34</sub> O	242	1.25
6.	14.820	 1-Decanol, 2-hexyl-	C <sub>11</sub> H <sub>24</sub>	156	19.33
7.	15.501	 Decane, 2-methyl-	C <sub>20</sub> H <sub>42</sub>	282	26.81
8.	16.714	 Hexadecane, 2,6,10,14-tetramethyl-	C <sub>18</sub> H <sub>37</sub> ClO <sub>2</sub> S	352	6.62
9.	17.533	 1-Octadecanesulphonyl chloride	C <sub>16</sub> H <sub>32</sub> O	240	2.22
		 Oxirane, tetradecyl-			

2).

Table 1. GC-MS spectral analysis of *Ugba* extract.

Table 2. Acute toxicity results of *Ugba* extract.

Group	Dose (mg/kg)	D/T	Signs of toxicity
I	0.25 ml (H <sub>2</sub> O)	0/12	No observable toxic sign
II	500	0/12	No observable toxic sign
III	1000	0/12	No observable toxic sign
IV	2000	0/12	No observable toxic sign
V	5000	0/12	No observable toxic sign

Rats were administered aqueous fermented seed extract of *P. macrophylla* for 14 days at doses of 500, 1000 and 2000 and 5000 mg/kg BW and assessed for toxicological symptoms. D/T: Number of rat deaths/Total number of rats included in this group.

Furthermore, creatinine and urea concentrations decreased significantly in all test groups ( $p<0.05$ ) (Figure 3). In addition, no significant variations ( $p<0.05$ ) in the serum levels of serum electrolytes (Na<sup>+</sup>, and K<sup>+</sup>) were observed (Figure 3).

However, a significant decrease ( $p<0.05$ ) in the serum level of HCO<sub>3</sub><sup>-</sup> was observed when compared to the control group (Figure 3).

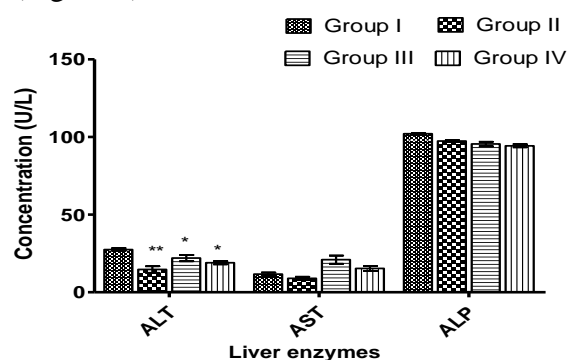
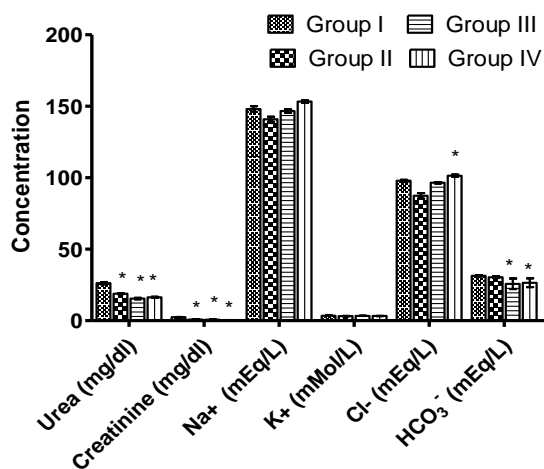


Figure 2. Effects of *Ugba* extract on hepatic enzymes. Rats were administered with 0.25 ml/kg of distilled water (Group I (Control)), aqueous fermented seed extract of *P. macrophylla* for 14 days at doses of 200 (Group II), 400 (Group III) and 800 (Group IV) mg/kg BW. The rats were sacrificed, and

the hepatic enzymes were determined in the blood serum. Values represent the mean±SD for n=6. Asterisk (\*) indicates significant difference at p<0.05 and \*\* indicates significance at p<0.01 difference compared to control.



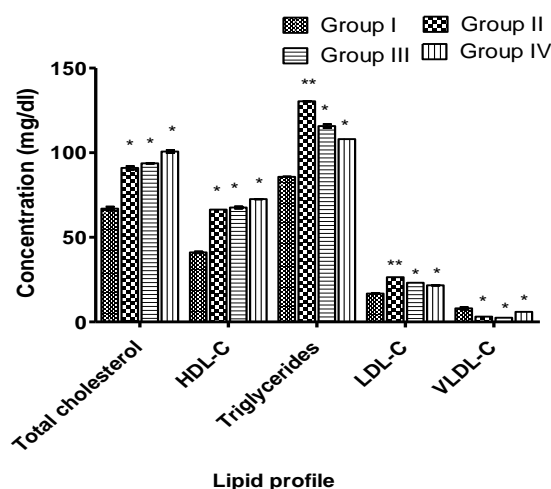
**Kidney function parameters**

Figure 3. Effects of *Ugba* extracts on kidney function parameters. Rats were administered with 0.25 ml/kg of distilled water (Group I (Control)), aqueous fermented seed extract of *P. macrophylla* for 14 days at doses of 200 (Group II), 400 (Group III) and 800 (Group IV) mg/kg BW. The rats were sacrificed, and the kidney function parameters were determined in the blood serum. Values represent the mean±SD for n=6. Asterisk (\*) indicates significant difference at p<0.05 compared to control.

Serum total cholesterol (TC), high-density lipoprotein (HDL), triglyceride and low-density lipoprotein (LDL) significantly increased (p<0.05) after 14 day oral administration of the extract in a dose-dependent manner (Figure 4). The serum level of very low-density lipoprotein (VLDL) decreased significantly (p<0.05) when compared to the control group.

Daily administration of *Ugba* extract to rats resulted in a non-statistically

significant (p>0.05) reduction in PCV, Hb and RBC (Table 4).



**Lipid profile**

Figure 4. Effect of *Ugba* extract on the lipid profile. Rats were administered with 0.25 ml/kg of distilled water (Group I (Control)), aqueous fermented seed extract of *P. macrophylla* for 14 days at doses of 200 (Group II), 400 (Group III) and 800 (Group IV) mg/kg BW. The rats were sacrificed, and the lipid profile assessed in the blood serum. Values represent the mean±SD for n=6. Asterisk (\*) indicates significant difference at p<0.05 and \*\* indicates significance at p<0.01 compared to control.

Increases in the level of platelets, lymphocytes and eosinophils were observed whereas a statistically non-significant (p>0.05) decrease in the levels of total WBC and neutrophils was found.

The effect of *Ugba* extract administration on the histopathological parameters of the liver and kidney of rats are given in Figures 5 and 6. The micrographs of the liver (Figure 5) and kidney (Figure 6) did not reveal any hepatotoxic or nephrotoxic effects after the 14 day period.

Table 3. The effect of *Ugba* extract on body weight of rats after 14 days of administration.

Parameter	Group I (Control)	Group II (200 mg/kg)	Group III (400 mg/kg)	Group IV (800 mg/kg)
Weight at day 1	156.00±4.54	161.67±3.31	160.33±4.50	144.40±1.53
Weight at day 14	180.20±13.58	178.33±7.64	178.60±2.34	171.67±3.71
Weight gain (g)	24.20	16.66	18.33	27.27

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Rats were weighed prior to the initial administration of aqueous fermented seed extract of *P. macrophylla* at doses of 200, 400 and 800 mg/kg BW for 14 days and weighed again before scarification. Values represent the mean  $\pm$  SD for n=6.

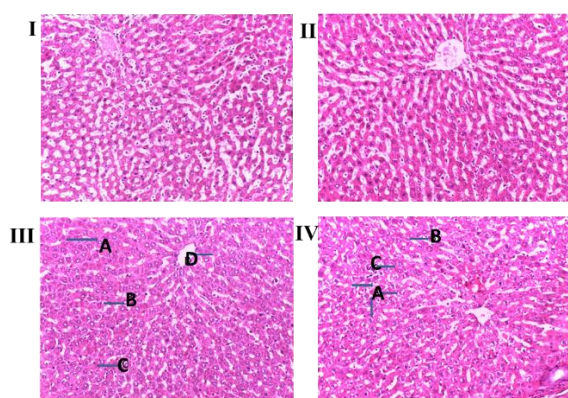


Figure 5. Micrographs of the liver sections obtained from untreated (control) and *Ugba* extract-treated Wistar rats. Rats were administered with 0.25 ml/kg of distilled water (Group I (Control)), aqueous fermented seed extract of *P. macrophylla* for 14 days at doses of 200 (Group II), 400 (Group III) and 800 (Group IV) mg/kg BW. The rats were sacrificed, and the liver sections were stained with haematoxylin and eosin staining (H&E) solution and observed under an Olympus light microscope equipped with a camera, at a magnification (40 $\times$ ). Alphabets A to D indicate points that appeared to have slightly dispersed histoarchitecture in the liver micrograph.

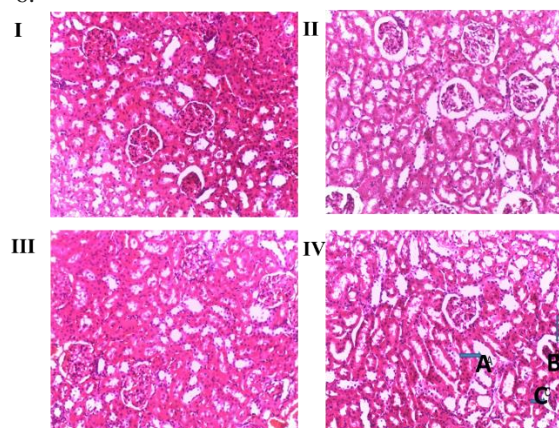


Figure 6. Micrographs of the kidney sections obtained from untreated (control) and *Ugba* extract-treated Wistar rats. Rats were administered with 0.25 ml/kg of distilled water (Group I (Control)), aqueous fermented seed extract of *P. macrophylla* for 14 days at doses of 200 (Group II), 400 (Group III) and 800 (Group IV) mg/kg BW. The rats were sacrificed, and the kidney sections were stained with haematoxylin and eosin staining (H&E) solution and observed under an Olympus light microscope fixed with camera, at a magnification (40 $\times$ ). Alphabets A to C indicate points that appeared to have slightly dispersed histoarchitecture in the kidney micrograph.

Table 4. Effects of *Ugba* extract on haematological parameters.

Parameter	Group I (control)	Group II (200 mg/kg)	Group III (400mg/kg)	Group IV (800 mg/kg)
PCV (%)	50.00 $\pm$ 2.00	45.03 $\pm$ 1.50	46.57 $\pm$ 1.16	48.47 $\pm$ 1.17
Hb (g/dl)	14.50 $\pm$ 1.10	12.40 $\pm$ 0.44	13.03 $\pm$ 0.15	14.03 $\pm$ 1.76
RBC ( $\times 10^{12}/l$ )	8.70 $\pm$ 0.20	7.55 $\pm$ 0.40	7.22 $\pm$ 0.18	7.68 $\pm$ 0.48
MCV (fl)	60.00 $\pm$ 0.30	58.50 $\pm$ 1.42	66.53 $\pm$ 1.19	66.90 $\pm$ 1.25
MCH (pg)	16.50 $\pm$ 0.50	16.16 $\pm$ 0.37	18.28 $\pm$ 0.15	17.80 $\pm$ 0.43
MCHC (g/l)	278.00 $\pm$ 5.00	275.33 $\pm$ 5.80	273.33 $\pm$ 2.52	273.33 $\pm$ 8.62
WBC ( $\times 10^9/l$ )	17.50 $\pm$ 2.30	14.15 $\pm$ 0.18	17.40 $\pm$ 1.11	14.83 $\pm$ 0.47
Lymphocyte (%)	43.30 $\pm$ 2.70	48.67 $\pm$ 1.15	46.00 $\pm$ 3.00	51.33 $\pm$ 1.53
Neutrophil (%)	49.00 $\pm$ 2.10	47.00 $\pm$ 3.61	46.00 $\pm$ 1.00	41.33 $\pm$ 1.53
Monocyte (%)	5.50 $\pm$ 1.00	4.33 $\pm$ 2.08	4.33 $\pm$ 1.53	4.67 $\pm$ 0.58
Eosinophil (%)	1.60 $\pm$ 0.10	1.67 $\pm$ 1.15	2.67 $\pm$ 2.08	3.00 $\pm$ 1.00
Basophil (%)	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
Platelet ( $\times 10^9/L$ )	560.00 $\pm$ 40.23	660.33 $\pm$ 64.94*	592.33 $\pm$ 113.58	686.33 $\pm$ 36.50*

Rats were administered with aqueous fermented seed extract of *P. macrophylla* for 14 days at doses of 200, 400 and 800 mg/kg BW. The rats were sacrificed, and the haematological parameters were assessed. Values represent the mean $\pm$ SD for n=6. Significant differences compared to control at p<0.05, are indicated with asterisk (\*).

## Discussion

*Ugba*, a traditional snack with a meaty taste is used for delicacy and soup flavoring

in mostly Southern Nigeria (Mbajunwa *et al.*, 1998; Enujiugha and Akanbi, 2005). Due to the non-uniform and non-standardized procedures (Njoku and Okemadu, 1989), the production process as well as the microbial population (NIH, 1985; OECD 2001; Afia and Momoh, 2006) involved in the fermentation process raises questions on product safety, quality and inconsistency. The present study was performed to increase our knowledge about the safety of *Ugba* for human consumption.

Two of the nine chemical compounds identified in *Ugba*, were reported to have pharmacological activities. Citronellol possesses anti-cancer, anti-inflammatory, anti-oxidant and wound healing potential (Zhuang *et al.*, 2009) and oxirane, and tetradecyl- have been shown to possess anti-microbial properties in a previous study (Manilal *et al.*, 2011).

Acute toxicological assessment is often employed as a pilot test when investigating the toxicity of substances and aims at deciphering the threshold of tolerance in an animal model (Ashafa *et al.*, 2012). *Ugba* induced neither mortality nor behavioral changes or signs of malaise even when rats were fed with 5000 mg/kg body weight. Adverse effects such as restlessness and anorexia were not observed even at 5000 mg/kg. Following the guidance document for testing acute oral toxicity by Organization for Economic Corporation and Development (OECD, 2001), compounds with LD<sub>50</sub> values of <2g/kg BW are generally considered relatively safe and doses >5g/kg BW are not considered dose-related. From the available indications, *Ugba* extract is tolerated well up to about 5 g/kg BW and therefore, dosages up to 800 mg/kg BW were chosen for a sub-acute toxicity study.

Organ weight provides a sensitive measure of toxicological assessment (Ugbogu *et al.*, 2016). After a 14 day period of *Ugba* treatment, weight of body of the rats and that of the organs exhibited a normal incremental pattern without marked differences between treated and control

groups (Table 3 and Figure 1). However, a critical analysis showed that the increase in body weight over time was dose-dependent, whereby the 800 mg/kg rats group gained the highest weight (27.27 g). Thus, treatment with *Ugba* extract did not result in a loss of appetite. Another plausible inference is the lipogenic potential of the *Ugba* which agrees with the observations from the lipid profile assessments in the current study (Figure 4). Although the mechanism is not confirmed, the hyperlipidemic effects of *Ugba* may be due to the high content of fatty acid of *P. macrophylla* which already was reported (Jones *et al.*, 1987; Udosen and Ifon, 1990). Thus, there are strong indications that these extracts portend risks of hypercholesterolemia at high doses, increasing the risks of atherosclerosis.

Assay of biochemical parameters was conducted to evaluate the health of the liver and the kidneys (Figure 2 and 3). The presence of normally tissue-specific enzymes and/or metabolites in the extracellular fluid provides clinical diagnostic insights into tissue injury (usually cell membrane disruption) and pathology. Hitherto compartmentalized enzymes exude from the tissue into the blood leading to increased serum level (Akanji *et al.*, 2008). The liver which is responsible for energy metabolism and system detoxification is delicate. The liver function tests (ALT, AST and ALP assays) revealed a toxin-triggered siege on the liver's integrity, albeit normal functioning of the liver. ALT and ALP displayed decreasing trends suggesting a hepatoprotective potential for the extract. Although significant dose-dependent increases in serum AST levels in extract-treated animals indicated hepatic impairment, an increasing ALT level is considered a relevant endpoint to determine hepatic damage (Ozer *et al.*, 2008). Since plasma AST and ALT concentrations increase in the events of inflammation of both cardiac and hepatic tissues (Wasan *et al.*, 2001; Witthawasku *et al.*, 2003),



myocardial infarction is not very likely to occur. The renal profile parameters such as urea, creatinine,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$  and  $\text{HCO}_3^-$  demonstrated reduced values in serum after administration of the aqueous *Ugba* extract for 2 weeks. All the reductions were significant, except for  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$ . Changes in blood urea,  $\text{HCO}_3^-$  and creatinine may be used as indices of glomerular filtration rate (GFR). Therefore, the observed decrease suggests improved kidney function (Ravanbakhsh et al., 2016). This suggests that *P. macrophylla* fermented seed extract may possess reno-protective properties.

This study did not demonstrate notable alterations in investigated haematological indices (Table 4). Usually, haematotoxicity results from changes beyond the reference ranges of these parameters (Dioka et al., 2002). The alternating reductions and elevations in the measured blood parameters could imply selective systemic toxicity (Ashafa et al., 2012), perhaps occasioned by variations in cell surface receptors or ion-gated channels. The total RBC count, PCV and Hb levels of extract-treated rats, compared to controls, as well as the MCV, MCH and MCHC (which are individual RBC indices used in anemia classification), were marginally altered, thus validating the fact that the extract does not affect erythropoiesis but protects erythrocytes from oxidative damage (Tarkang et al., 2012). White blood cells are known to rise in response to toxic environments (Agbor et al., 2005). At face value, the significant depletion of WBCs generally suggests immunotoxicity, but the equally significant boost in lymphocyte count, the main effector cells of the immune system (Odeghe et al., 2013), implies the apparent mounting of an immune response by the extract (WHO, 2003). Neutrophils and monocytes perform phagocytic functions against foreign invaders (Odeghe et al., 2013, WHO, 2003); their reduced number in circulation, though insignificant, lend credence to the fact that the fermentative microorganisms found in

*Ugba* are non-virulent, and may even be part of the body's microbiota (Isu and Njoku, 1997; Ogueke and Aririatu, 2004; Okwulehie, 2004; Kabuo et al., 2007). Eosinophils and basophils are allergy-inducible lymphocytes (Stone et al., 2010, He et al., 2013), so the unchanging status of basophils and elevation in eosinophil numbers are proof that the extracts contain negligible immunogenic allergens. Platelets protect blood vessels from endothelial damage and mediate blood clotting to repair damaged vessels. Very significant increases in platelet numbers further justify the use of the ripe fruits in wound healing (Akindahunsi, 2004). It has been posited that hematological alterations in animal studies may be employed in the prediction for translation to human toxicity (Olson et al., 2000; Adebayo et al., 2005). Thus, it can be inferred that the extracts possess antioxidant, immuno-modulatory and vaso-protective characteristics.

The outcomes of the haematological and biochemical assays were further confirmed by the homeostatic organs (kidney and liver) histopathological examination. The liver (Figure 5) and kidney (Figure 6) micrograph did not reveal any hepatotoxic or nephrotoxic effects 14 days post-administration of the extracts. A fatty liver is a common toxicant-induced complication; however, the hepatocytes can reverse the associated injury (Treinen-Moslen, 2001). Interestingly, despite the fattening property of the extract (as noted earlier), the liver did not show any fatty changes or vacuolations. However, the liver was characterized by a slightly dispersed histoarchitecture. The nephrons were visibly intact, with increased extract dosage widening the Bowman's capsule/glomerular diameter and tubular lumens, all positive factors for kidney function. These observations were confirmed by the relatively stable hepatic and renal profile parameters.

Given the results obtained in the present study, it can be holistically deduced that the aqueous extract of fermented *P.*

*macrophylla* did not cause any apparent *in vivo* toxicity in the rats at the tested doses. Therefore, they are not likely to have severe toxicological risks for humans. Because of its likely hypercholesterolaemic effects, balanced consumption of *Ugba* should be encouraged pending further research. Moreover, a comprehensive chronic toxicity of fermented *Ugba* extract is essential.

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### Conflict of interest

Authors declare that they do not have any conflict of interest and that they are accountable for the entire manuscript content.

### References

- Achinewhu SC. 1983. Protein quality of the African oil bean seed (*Pentaclethra macrophylla*). *J Food Sci*, 48: 1374-1375.
- Adebayo JO, Adesokan AA, Olatunji LA, Buoro DO Soladoye AO. 2005. Effect of Ethanolic extract of *Bougainvillea spectabilis* leaves on haematological and serum lipid variables in rats. *Biokemistri*, 17: 45-50.
- Afia ES, Momoh JA. 2006. Standard operating procedure. Unpublished guideline for the chemical pathology laboratory, National Hospital, Abuja.
- Agbor GA, Oben JE, Ngogang JY. 2005. Haematinic activity of *Hibiscus cannabinus*. *Afr J Biotechnol*, 4(8): 833-837.
- Akanji MA, Nafiu MO, Yakubu MT. 2008. Enzyme activities and histopathology of selected tissues in rats treated with potassium bromate. *Afr J Biomed Res*, 11:87-95.
- Akindahunsi AA. 2004. Physicochemical studies on African oil bean (*Pentaclethra macrophylla* Benth.) seed. *J Food Agric Environ*, 2: 14-17.
- Ashafa AOT, Orekoya LO, Yakubu MT. 2012. Toxicity profile of ethanolic extract of *Azadirachta indica* stem bark in male Wistar rats. *Asian Pac J Trop Biomed*, 2: 811-817.
- Azubuine CE, Isu NR. 2006. Fungal contamination of fermenting African oil seeds ugba. *Nig J Microbiol*, 20: 931-936.
- Chidozie JI. 2006. African oil bean seed as an essential food supplement in the control of cancer and tobacco related diseases. Proceedings from the UICC World Cancer Congress, July 8-12, Washington DC.
- Dioka C, Orisakwe OE, Afonne OJ, Agbasi PU, Akumka DD, Okonkwo CJ, Ilondu N. 2002. Investigation into the haematologic and hepatotoxic effects of rinba-cin in rats. *J Health Sci*, 48: 393-398.
- Ejiofor MAN, Oti E, Okafor JC. 1987. Studies on the fermentation of seeds of the African oil bean tree (*Pentaclethra macrophylla*). *Int Tree Crops J*, 4: 135-144.
- Enujiugha VN. 2003. Nutrient changes during the fermentation of African oil bean (*Pentaclethra macrophylla* Benth.) seeds. *Pak J Nutr*, 2003, 2: 320-323.
- Enujiugha VN, Akanbi CT. 2005. Compositional changes in African oil bean (*Pentaclethra macrophylla* Benth.) seeds during thermal processing. *Pak J Nutr*, 4: 27-31.
- Eze VC, Onwuakor CE, Ukeka E. 2014. Proximate composition, biochemical and microbiological changes associated with fermenting African oil bean (*Pentaclethra macrophylla* Benth) Seeds. *Am J Microbiol Res*, 2: 138-142.
- Fisher DI, Safrany ST, Strike P, McLennan AG, Cartwright JL. 2002. Nudix hydrolases that degrade dinucleoside and diphosphoinositol polyphosphates also have 5-phosphoribosyl 1-pyrophosphate (PRPP) pyrophosphatase activity that generates the glycolytic activator ribose 1, 5-bisphosphate. *J Biol Chem*, 277: 47313-47317.

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- Fungo R, Muyonga J, Kaaya A, Okia C, Tieguhong JC, Biadu-Forson JJ. 2015. Nutrients and bioactive compounds content of *Baillonella toxisperma*, *Trichoscypha abut* and *Pentaclethra macrophylla* from Cameroon. *Food Sci Nutr*, 3: 292-301.
- Hajihosseini S, Setorki M. 2017. The antioxidant activity of *Beta vulgaris* leaf extract in improving scopolamine-induced spatial memory disorders in rats. *Avicenna J Phytomed*, 7: 417-425.
- He S, Zhang H, Zeng X, Chen D, Yang P. 2013. Mast cells and basophils are essential for allergies: mechanisms of allergic inflammation and a proposed procedure for diagnosis. *Acta Pharmacol Sinica*, 34: 1270-1283.
- Isu N, Njoku H. 1997. An Evaluation of the microflora associated with fermented African oil bean (*Pentaclethra Macrophylla* Benth) seeds during Ugba production. *Plant Foods Hum Nutr*, 51: 145-157.
- Isu NR, Ofuya CO. 2000. Improvement of the traditional processing and fermentation of African oil bean (*Pentaclethra macrophylla* Benth) into a food snack-ugba. *Int J Food Microbiol*, 59: 235-239.
- Jones AC, Robinson JM, Southwell KH. 1987. Investigation into *Pentaclethra macrophylla* seed oil: identification of hexacosanoic (C26:0) and octacosanoic (C28:0) fatty acids. *J Sci Food Agric*, 40: 189-194.
- Kabuo NO, Uzuegbu JO, Ubbaonu CN, Oyeka EU. 2007. Effect of chemical compounds produced by microorganisms on organoleptic properties of ugba (*Pentaclethra macrophylla* Benth) during fermentation. *Proceedings of the Annual Conference of Nigerian Institute of Food Science and Technology*, Oct. 22-25, Abuja, Nigeria, 175-176.
- Manilal A, Sujith S, Sabarathnam B, Kiran G, Selvin J, Shakir C, Lipton A. 2011. Biological activity of the red alga *Laurencia brandenii*. *Acta Bot Croat*, 70: 81-90.
- Mbajunwa OK, Akingbala JO, Mulongoy K, Oguntimein G. 1998. Starter culture evaluation for the production of ugba from African oil bean seed (*Pentaclethra macrophylla*) [J]. *J Sci Food Agric*, 77: 127-132.
- Monago CC, Ogbomeh PA, Joshua PE. 2004. Effect of African oil bean seed *Pentaclethra macrophylla* Benth on blood cholesterol level in rats. *Global J Pure Applied Sci*, 10: 165-168.
- NIH. 1985. Care and Use of Laboratory Animals, National Institute of Health Publication, 85-123.
- Njoku HO, Okemadu CP. 1989. Biochemical changes during the natural fermentation of the African oil bean (*Pentaclethra macrophylla*) for the production of Ugba. *J Sci Food Agric*, 49: 457-465.
- Odeghe OB, Uwakwe AA, Monago CC. 2012. Some biochemical and haematological studies on the methanolic extract of *Anthocleista grandiflora* stem bark. *Int J Appl Sci Technol*, 2: 58-65.
- Odoemelam SA. 2005. Proximate composition and selected physicochemical properties of the seeds of African oil bean (*Pentaclethra macrophylla*). *Pak J Nutr*, 4: 382-383.
- OECD. 2001. The OECD guidance document on acute oral toxicity testing; organization for economic co-operation and development: Paris, France, pp. 1-14.
- Ogueke CC, Aririatu LE. 2004. Microbial and organoleptic changes associated with ugba stored at ambient temperature. *Niger Food J*, 22:133-140.
- Ogueke CC, Nwosu JN, Owuamanam CI, Iwouno JN. 2010. Ugba, the fermented African oil bean seeds; its production, chemical composition, preservation, safety and health benefits. *Pak J Biol Sci*, 13: 489-496.
- Okwulehie IC. 2004. Insect pests and mycoflora of oil bean (*Pentaclethra macrophylla* Benth.) pods and seeds in southeastern parts of Nigeria. *Fruits*, 59: 25-30.
- Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, Lilly P, Sanders J, Sipes G, Bracken W, Dorato M, Van Deun K, Smith P, Berger B, Heller A. 2000. Concordance of toxicity of pharmaceuticals in humans and in animals. *Regul Toxicol Pharmacol*, 32: 56-67.
- Onwuliri VA, Attah I, Nwankwo JO. 2004. Anti-nutritional factors, essential and non-essential fatty acids composition of Ugba (*Pentaclethra macrophylla*) seeds at different stages of processing and fermentation. *J Biol Sci*, 4: 671-675.
- Osagie-Eweka ESD, Alaiya TH. 2013. Effects of fermentation and heating on the functional properties of processed flour

- from African oil bean (*Pentaclethra macrophylla* benth) seeds. *Afr J Food Agric Nutr Develop*, 13: 8249-8257.
- Ozer J, Ratner M, Shaw M, Bailey W, Schomaker S. 2008. The current state of serum biomarkers of hepatotoxicity. *Toxicol*, 245: 194-205.
- Ravanbakhsh A, Mahdavi M, Jalilzade-Amin G, Javadi S, Maham M, Mohammadnejad D, Rashidi MR. 2016. Acute and subchronic toxicity study of the median septum of *Juglans regia* in Wistar rats. *Adv Pharmaceut Bull*, 6: 541-549.
- Stone KD, Prussin C, Metcalfe DD. 2010. IgE, mast cells, basophils, and eosinophils. *J Allergy Clin Immunol*, 125:73-80.
- Tarkang PA, Agbor GA, Tchamgoue DA, Yamthe TLR, David K, Ngadena YSM. 2012. Acute and chronic toxicity studies of the aqueous and ethanol leaf extracts of *Carica papaya* Linn. In wistar rats. *J Nat Prod Plant Res*, 2: 617-627.
- Traesel GK, Menegati, SELT, dos Santos AC, Souza RIC, Boas GRV, Justi PN, Kassuya CAL, Argandoña EJS, Oesterreich SA. 2016. Oral acute and subchronic toxicity studies of the oil extracted from pequi (*Caryocar brasiliense*, Camb.) pulp in rats. *Food Chem Toxicol*, 97: 224-231.
- Treinen-Moslen M. 2001. Toxic responses of the liver. In: Klaassen, C.D. (ed.). *Casarett and Doull's Toxicology: The Basic Sciences of Poisons*. New York: McGraw-Hill, pp. 471-489.
- Udosen EO, Ifon ET. 1990. Fatty acid and amino acid composition of African oil beans (*Pentaclethra macrophylla*). *Food Chem*, 36: 155-160.
- Ugbogu EA, Akubugwo IE, Ude VC, Gilbert J, Ekeanyanwu B. 2019. Toxicological evaluation of phytochemical characterized aqueous extract of wild dried *Lentinus squarrosulus* (Mont.) mushroom in rats. *Toxicol Res*, 35: 181-190.
- Ugbogu AE, Arunsi UO, Uche-Ikonne OO, Ude VC, Okezie E. 2017. Antiulcerogenic potentials of fermented aqueous extract of *Pentaclethra macrophylla* (Benth) seeds. *Am J Biomed Res*, 5: 57-64.
- Ugbogu AE, Okezie E, Uche-Ikonne C, Duru M, Atasi OC. 2016. Toxicity evaluation of the aqueous stem extracts of *Senna alata* in Wistar Rats. *Am J Biomed Res*, 4: 80-86.
- Ugbogu EA, Ude VC, Elekwa I, Arunsi UO, Uche-Ikonne C, Nwakanma C. 2018. Toxicological profile of the aqueous-fermented extract of *Musa paradisiaca* in rats. *Avicenna J Phytomed*, 8: 478-487.
- Wasan KM, Najafi S, Wong J, Kwong M. 2001. Assessing plasma lipid levels, body weight, and hepatic and renal toxicity following chronic oral administration of a water soluble phytosterol compound, FM-VP4, to gerbils. *J Pharm Pharm Sci*, 4: 228-34.
- WHO. 2003. WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants. World Health Organization, Geneva.
- Witthawasku P, Ampai P, Kanjanapothi D, Taesothikula T, Lertprasertsuke N. 2003. Acute and sub-acute toxicities of saponin mixture isolated from *Schefflera leucantha* Viguier. *J Ethnopharmacol*, 89: 115-121.
- Zhuang SR, Chen SL, Tsai JH, Huang CC, Wu TC, Liu WS, Tseng HC, Lee HS, Huang MC, Shane GT, Yang CH, Shen YC, Yan YY, Wang CK. 2009. Effect of citronellol and the Chinese medical herb complex on cellular immunity of cancer patients receiving chemotherapy/radiotherapy. *Phytother Res*, 23: 785-790.