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Investigations of Safety of Aqueous Fractions derived from the Ash of Deseeded Fruit Head of the Oil Palm

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ABSTRACT

In this paper, investigation of the safety of two aqueous fractions from the African oil palm deseeded fruit head ash extract was done by assessing the acute and subacute effects of the two oil palm ash derived fractions, (Fractions A and C) in Wistar rats. A total of ninety-eight rats of both sexes were used for this study. Oral acute toxicity was determined by a modification of Lorke's method using 56 rats. For the subacute study, 42 rats were weighed and randomly assigned to 7 groups (A1-A3, C1-C3, and B) of 6 rats each. Rats were weighed and graded doses of 1, 10 and 100 milligrams per kilogram body weight (mg/kg bw) of fraction A were administered to groups A1, A2, and A3; fraction C to groups C1, C2 and C3, and 0.5 milliliters distilled water/kg bw to the control group (B) orally, once daily for 28 days. After the treatment period, blood samples were collected from all the experimental animals

in each group for biochemical and haematological assays using standard procedures. Following blood sampling, the rats were sacrificed under mild anesthesia and the liver, heart, kidney, and spleen harvested for the histological assessment using standard histological methods. From this study, the LD_{50} of fractions A and C was 2449.5 and 3872 mg/kg bw respectively. Statistically significant increase in percentage body weight was observed in fraction A treated group after 2 weeks of treatment with 10 and 100 mg/kg and after 4 weeks with 10 mg/kg ($p<0.05$ each) of fraction and in the fraction C treated group at 10 mg/kg bw after 4 weeks when compared with the control group. Red cell count was significantly higher in group A2 ($p<0.01$) and A3 ($p<0.05$), while Hb concentration was significantly higher ($p<0.05$) in group A2 only, when compared with the control group. AST, ALT, ALP, total bilirubin, and direct bilirubin mean values of the fraction treated groups were comparable with that of the control after 4 weeks and not statistically significant ($p>0.05$). The liver, kidney, heart, and spleen showed no histological alterations in the fraction treated and control groups. This work, therefore, suggests that the palm ash in its use as a condiment, food additives, and herbal therapies in small quantities is relatively safe.

Keywords: African oil palm, Deseeded fruit head, Palm ash, Safety, Acute, Subacute.

INTRODUCTION

The use of herbal remedies and other active ingredients of medicinal plants even in the health care systems is on the increase worldwide [1,2]. Herbal prescriptions and remedies due to their availability, acceptability, and adaptability to the community are commonly utilized for healthcare in developing countries [3]. Based on inherited knowledge and long term usage for the treatment of various ailments over the centuries, medicinal plants are considered natural and therefore safer than conventional synthetic pharmaceuticals [4]. Traditional herbal therapies therefore have the potential to improve the healthcare system in many African countries [5] and the world at large. Unfortunately, there is limited scientific evidence regarding safety and efficacy to back up the continued therapeutic application of these remedies. With the upsurge in the use of herbal medicines, a thorough scientific investigation of these plants will go a long way in validating their folkloric usage [6].

The African oil palm (*Elaeis guineensis jacq)* is a single-stemmed cylindrical monoecious tree [7] and originates from equatorial tropical rain forest region of Africa, along the Gulf of Guinea in West Africa [8]. It is a valuable agro crop useful as food, feed for livestock, fuel, building materials, and industrial raw materials for cosmetics, pharmaceutical, and brewery industries [9]. The various parts of the tree are used traditionally for various medicinal purposes. Palm oil extracted from the mesocarp of the fruit is used traditionally for the treatment of headaches, pains, rheumatism, cardiovascular diseases, arterial thrombosis, and atherosclerosis. Studies show that the leaves may be beneficial in the treatment of cancer, cardiovascular diseases, kidney diseases, and wound healing. The sap is rich in phytochemicals that can be used to treat various diseases.

Soaps prepared with the ash from the deseeded fruit head of the oil palm (DFHOP) also known as empty fruit bunch are used in treating skin infections and filtrates from the ash are used locally in place of potash as food additives and tenderizer to prepare local soups, sauces and other dishes in many parts of Nigeria and neighboring countries like Cameroon, Chad, Ghana, and Niger [10]. Chemical analysis of the ash from the deseeded fruit head of the oil palm (DFHOP) reveals that it is alkaline (pH 10.9), contains metals such as Cr, Zinc, Ca, K, Na, and Mg; and anions such as Cl, PO^{3-}_4, NO^{3-}_4 and SO^{3-}_4 [11-16]. Many of these

ions play key roles in various biological processes. Despite the continuous use of this palm ash or its derivatives as food condiments and or traditional remedy, there is little information on the effects of ash from DFHOP or its components in biological systems, particularly the investigation of its safety.

This study, therefore, aims to investigate the acute and subacute effects of fractions obtained from the ash of DFHOP on heamatological, biochemical, and histological parameters in rat models. This study will help provide assurance of the safety of the preparations produced using the ash of DFHOP or its derivatives especially those used in preparing local dishes and remedies and provide a further understanding of the possible effects of these agents in health.

MATERIALS AND METHODS

Plant materials

Preparation of ash filtrate: The empty fruit bunches or deseeded fruit heads of the oil palm (DFHOP) were obtained In Nkanu East Local Government Area of Enugu State. The filtrate of the ash of DFHOP (DFHOPf) was prepared as described in the patent US7, 445,732B2 [11]. Briefly, the DFHOP was ashed in the open air and allowed to cool. The ash was collected, placed in a clean container, and mixed with an appropriate amount of distilled water (0.5kg ash/L), and the mixture allowed to stand overnight with intermittent stirring. Following solubilization, the mixture was filtered using two layers of thick white cotton cloth and, the filtrate recovered. The recovered filtrate was allowed to stand at room temperature overnight after which fluffy ashy sediment separated at the bottom leaving clean, brownish-gold colored supernatant on top. The sediment was separated from the brownish gold-colored liquid by decantation.

Fractional crystallization and separation of crystals: The recovered brownish-gold colored supernatant was heated to boil for two to three hours, and left to cool overnight. White crystalline salt, which precipitated out of the solution following the cooling, was collected by filtration using a fine mesh cheesecloth. The left-over solution was subjected to another cycle of heating and cooling to recover more crystals. The recovered crystal called DFHOPf-C (Fraction C) was dried by spreading on the Whatman filter paper at room temperature. Finally, the leftover supernatant was heated to dryness, resulting in the formation of a white anhydrous hard cake which was recovered and named DFHOPf-A (Fraction A).

Experimental animals and ethical issues

A total of ninety-eight (98) healthy adult Wistar rats of both sexes, aged 6 – 10 weeks were used for this study. They were procured from the Department of Veterinary Medicine, University of Nigeria, Nsukka. All the animals were kept in well ventilated and fly proof animal facility of the Department of Pharmacology and Therapeutics, University of Nigeria, Enugu Campus, Nigeria, and allowed to acclimatize for 7 days before the onset of the experiment. All animals were kept in polypropylene cages with stainless steel top grill (having separate compartments for food and water bottle) and maintained under standard laboratory conditions of humidity, temperature, and 12 hours light/dark cycle. A standard protocol was drawn up following the Good Laboratory Practice (GLP) regulations of the World Health Organization [17] and all investigations conducted following laid down procedures and guidelines issued by the National Research Council (NRC), [18] on proper care and use of laboratory animals and approved by the Institutional Animal Ethical Committee, University of Nigeria, Nsukka.

Experimental design

Acute oral toxicity study: The acute oral toxicity (LD_{50}) of fraction A and C was determined by a modification of Lorke's method [19] and it was done in two phases. A total of fifty-six rats were used for the acute toxicity study. In the first phase, (determination of toxic range) 28 rats, fasted overnight, were randomly divided into 7 groups of 4 rats (2 males and 2 females) per group. Graded doses (100 mg, 500 mg, and 1000 mg) of fraction A were administered to groups I, II, and III and fraction C to groups IV, V, and VI respectively per kilogram body weight by gavage. Group VII served as the control and was given distilled water orally. All the animals were then allowed free access to food and water and observed for clinical signs of acute toxicity (which included amongst others changes in eye color, fur appearance, stool consistency, food and water intake, and signs of lethargy) and mortality for the next forty-eight hours.

In the second phase, another set of 28 rats were assigned to 7 groups (I – VII) of 4 animals each, (1:1 male: female ratio). Groups I, II, III received 2000, 3000, and 5000 mg/kg bw of fraction A while group IV, V, and VI were administered 2000, 3000, and 5000 mg/kg bw of fraction C respectively. The control group (VII) was treated with orally administered distilled water, 0.5 milliliters (ml)/kg bw. The animals were observed for 24 hours and the number of deaths within this period recorded.

The LD_{50} was calculated after the experiment using the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_0 = Highest dose that gave no mortality,

D_{100} = Lowest dose that produced mortality

Subacute toxicity study: Forty-two rats were used for this study, 21 males and 21 females. Rats were weighed and randomly divided into 7 groups (A1, A2, A3, C1, C2, C3, and B) of 6 animals (3 males and 3 females) each. Group B served as the control while groups A1 - A3 and C1 - C3 the fraction A and fraction C treated test groups. After an overnight fast, graded doses, 1, 10 and 100 mg/kg bw, of fraction A were given to groups A1, A2, and A3 respectively; groups C1, C2 and C3 received 1, 10 and 100 mg/kg bw of fraction C and group B (control group) 0.5 ml/kg bw of distilled water correspondingly by gavage for 28 days.

Determination of percentage body weight: The body weight of each rat was measured once before the commencement of dosing (baseline weight), and fortnightly until the end of the study. The percentage change in weight from the baseline weight of each animal was calculated as follows:

$$\%\ \textbf{change in weight} = \frac{\textbf{body weight of animal} - \textbf{baseline weight}}{\textbf{body weight of animal}} \times \mathbf{100}$$

Sample collection

Blood samples: The animals were subjected to an overnight fast after 28 days and blood samples were taken through the retro-orbital plexus of the medial canthus under diethyl ether anesthesia using heparinized capillary tubes for haematological and biochemical assays. Two mls of blood was collected into dipotassium ethylene diamine tetra-acetic acid (K_2EDTA) bottle for complete blood count using an Abacus 380 haematological analyzer (Diatron). Another 3-5mls of blood was collected into clean plain bottles for biochemical tests, the blood was allowed to clot and the serum separated after centrifugation into clean bottles.

The sera were analyzed and the alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), total and direct bilirubin (T-Bil and D-bil), total protein, albumin, and globulin levels determined. ALP, ALT, AST, and albumin were determined using standard ready-to-use Randox reagent test kits (Randox Laboratories Ltd, County Antrim, United Kingdom) while T-Bil, D-Bil, and Total protein were estimated using Teco Diagnostic test kit (Anaheim, CA). Globulin was calculated from the difference between total protein and albumin.

Harvesting of tissues: At the end of blood collection, the animals were euthanized and the liver, kidney, heart, and spleen of each animal excised, rinsed in saline, and blotted dry. The harvested organs were examined grossly, weighed, and preserved in 10% buffered formalin for histological assay.

Statistical analysis

Data were expressed as mean±S.E and analyzed with one-way analysis of variance and followed where necessary, by Turkey multiple range tests using computer software IBM Statistical Package for Social Sciences (IBM SPSS) Statistics for Windows, version 24.0 (Armonk, New York: IBM Corp released 2016). Differences between means of control and treatment groups were considered significant at $p<0.05$.

RESULTS

Safety and pathophysiological effects

Table 1 shows the results of the acute toxicity in rats after 24 hours. No sign of toxicity was observed or death recorded in the first phase of the study in the control group and between 100-1000 mg/kg bw of fraction A and C. However, in phase 2 of the acute study, deaths were observed at 3000 mg/kg bw with fraction A while C registered no death. At 5000 mg/kg all the animals in group A died while 3 out of 4 (1 male and 2 females) died in group C in less than 24hours. It was observed that females were more affected than the males because the death occurred within 2 hours in all females that died while the time of death in males ranged from 4-12 hours with both fractions. In comparison, the control group showed no signs of toxicity.

Also, the changes observed after administration of fraction A included rapid breathing in the test animal at doses above 2000 mg/kg bw, with a reduction in activity and feeding, while with C same reaction occurred at 5,000 mg/kg. However, at doses in which the animal succumbed to death, signs of acute toxicity included, reduction in movement, food and water intake, rapid breathing in females, and death. All females that died in group A demonstrated partial paralysis of the right limb but this did not occur with their male counterparts. Oral LD50 was 2449.5 mg/kg b,w for fraction A and 3872 mg/kg b.w for fraction C in treated rats.

Table 1: Oral Acute Toxicity (LD_{50}) of Fraction A and C in Rats.

Phase 1		**Doses (mg/kg bw)**			**LD_{50}(mg/kg bw)**
Group	Sex	100	500	1000	
Fraction A $\frac{num.of\ death}{num.of\ survival}$	Males	0/2	0/2	0/2	-
	Females	0/2	0/2	0/2	-
Fraction C $\frac{num.of\ death}{num.of\ survival}$	Males	0/2	0/2	0/2	-
	Females	0/2	0/2	0/2	

Control $\frac{num.of\ death}{num.of\ survival}$	Males	0/2			-
	Females	0/2			-
Phase 2					
		2000	3000	5000	
Fraction A $\frac{num.of\ death}{num.of\ survival}$	Males	0/2	01-Feb	02-Feb	2449.5
	Females	0/2	01-Feb	02-Feb	2449.5
Fraction C $\frac{num.of\ death}{num.of\ survival}$	Males	0/2	0/2	½	3872
	Females	0/2	0/2	02-Feb	3872
Control $\frac{num.of\ death}{num.of\ survival}$	Males	0/2			-
	Females	0/2			-

Table 2 represents the effect of graded doses of Fraction A and Fraction C on percentage changes in body weight. There was a significant increase in weight with time in all the test and control groups. Weight gain was higher in the Fraction A treated group than their corresponding Fraction C counterparts at 2 and 4 weeks. One-way ANOVA revealed significant differences ($p<0.05$) in mean percent weight change between groups A1, A2, and A3 and the control group at 2 and 4 weeks, and between groups C1, C2, C3 and the control groups at 2 weeks. Multiple comparisons of means showed that significantly higher weight gain was observed at 2 weeks in 10 and 100 mg/kg bw ($p<0.05$) Fraction A, 1 mg/kg bw ($p<0.05$) Fraction C and at 4 weeks in 10 mg/kg bw Fraction A ($p<0.05$) when compared with their corresponding timed control.

Table 2: Percentage change in body weight of rats after Fraction A and Fraction C treatment.

Duration (wks)	**A1 1 mg/kg bw (n=6)**	**A2 10 mg/kg bw (n=6)**	**A3 100 mg/kg bw (n=6)**	**C1 1 mg/kg bw (n=6)**	**C2 10 mg/kg bw (n=6)**	**C3 100 mg/kg bw (n=6)**	**B Control (n=6)**
2	42.20 ±4.64	59.28 ±1.82*	45.12 ±4.96*	45.66 ±2.85*	32.811 ±4.57	36.28 ±3.83	26.12 ±5.80
4	90.83 ±8.74	103.68 ±12.93*	87.15 ±6.57	82.30 ±5.04	78.31 ±9.96	62.7 ±5.28	59.05 ±6.86
* $p<0.05$ (Compared with the Control Group)							

In Table 3 the effect of the palm ash fraction on haematological parameters of the rats after 4 weeks of administration is represented. One-way analysis of variance revealed statistically significant differences in total red cell count and haemoglobin concentration ($p<0.05$ for each) across the fraction A treated groups (A1, A2 and A3 corresponding to 1, 10 and 100 mg/kg bw respectively) and the control, group B. Post hoc analysis shows that red cell count was significantly higher in group A2 ($p<0.01$) and A3 ($p<0.05$) compared with the control group; while Hb concentration was significantly higher ($p<0.05$) in group A2 only when compared to the control group. However, no significant differences ($p>0.05$) were observed between fraction C at 1, 10, 100 mg/kg bw (C1, C2, C3) individually compared with the control group in all the parameters tested.

Table 3: Mean haematological values in the test and control groups after 4 weeks of administration.

Parameters	**A2 1 mg/kg bw (n=6)**	**A2 10 mg/kg bw (n=6)**	**A3 100 mg/kg bw (n=6)**	**C1 1 mg/kg bw (n=6)**	**C2 10 mg/kg bw (n=6)**	**C3 100 mg/kg bw (n=6)**	**B Control (n=6)**
RBC (10^{12}/l)	9.38 ± 0.19	9.76 ± 0.07**	9.68 ± 0.10*	8.79 ± 0.31	8.64 ± 0.65	9.09 ± 0.19	8.79 ± 0.31

HGB (g/dl)	13.43 ± 0.23	13.82 ± 0.28*	13.56 ± 0.21	12.8 ± 0.53	12.05 ± 0.82	12.88 ± 0.40	12.77 ± 0.33
HCT (%)	42.74 ± 0.87	43.39 ± 0.96	43.08 ± 0.77	40.27 ± 1.69	38.02 ± 2.65	40.06 ± 0.99	40.17 ± 1.17
MCV (fl)	45.5 ± 0.43	45.67 ± 0.67	45.00 ± 0.71	45.67 ± 0.62	46.17 ± 0.17	44.00 ± 0.73	45.33 ± 0.80
MCH (pg)	14.32 ± 0.18	14.52 ± 0.14	14.3 ± 0.21	14.55 ± 0.18	14.00 ± 0.34	14.05 ± 0.25	14.43 ± 0.35
MCHC (g/dl)	31.47 ± 0.15	31.88 ± 0.18	31.54 ± 0.09	31.82 ± 0.08	31.73 ± 0.26	31.85 ± 0.21	32.17 ± 0.37
RDWc (%)	20.72 ± 0.31	20.72 ± 0.36	21.02 ± 0.51	20.68 ± 0.53	21.67 ± 0.58	21.22 ± 0.51	21.00 ± 0.61
WBC (10^9/l)	14.53 ± 1.63	14.36 ± 1.61	14.62 ± 1.35	15.99 ± 1.74	14.28 ± 1.17	15.04 ± 1.03	16.19 ± 0.32
LYM (10^9/l)	10.01 ± 1.35	9.96 ± 1.27	10.13 ± 1.23	11.28 ± 1.09	10.06 ± 0.94	10.98 ± 1.05	10.38 ± 0.58
NEUT (10^9/l)	3.13 ± 0.19	3.26 ± 0.44	3.23 ± 0.41	3.14 ± 0.48	2.57 ± 0.31	2.65 ± 0.17	3.45 ± 0.37
MID (10^9/l)	1.39 ± 0.25	1.14 ± 0.17	1.26 ± 0.23	1.58 ± 0.25	1.66 ± 0.19	1.42 ± 0.1	1.82 ± 0.34
LYM (%)	67.9 ± 2.39	69.02 ± 2.08	68.68 ± 2.79	71.02 ± 1.59	70.27 ± 2.54	72.3 ± 2.19	65.6 ± 1.66
NEUT (%)	22.58 ± 2.07	23.03 ± 2.77	22.88 ± 3.52	19.37 ± 1.07	18.23 ± 2.41	18.17 ± 1.91	22.65 ± 2.65
MID (%)	9.52 ± 0.99	7.97 ± 0.89	8.42 ± 0.81	9.65 ± 0.71	11.5 ± 0.47	9.53 ± 0.64	11.78 ± 1.61
PLT (10^9/l)	666.67 ± 36.69	620.5 ± 42.28	606.2 ± 53.83	610 ± 55.60	668.83 ± 43.76	737.17 ± 43.47	596.67 ± 94.51
PCT (%)	0.47 ± 0.026	0.44 ± 0.034	0.41 ± 0.034	0.43 ± 0.04	0.48 ± 0.034	0.52 ± 0.033	0.45 ± 0.052
MPV (fl)	7.00 ± 0.052	7.02 ± 0.18	6.84 ± 0.15	6.98 ± 0.06	7.18 ± 0.079	7.08 ± 0.11	6.97 ± 0.11
PDWc (%)	35.78 ± 0.32	35.97 ± 0.58	35.2 ± 0.58	35.85 ± 0.19	35.93 ± 0.39	36.08 ± 0.35	35.7 ± 0.39
* $p<0.05$, ** $p<0.01$ (Compared with the Control Group)							

The effect of the fractions on AST, ALT, ALP, Total and direct bilirubin, total protein, globulin, and albumin is represented in Table 4. AST, ALT, ALP, total bilirubin, and direct bilirubin mean values of the fraction treated groups were comparable with that of the control after 4 weeks and statistically significant ($p>0.05$) for each parameter. Also, no significant differences were observed with changes in concentration in both fraction, A and C treated groups.

Table 4: Hepatic function of test and control groups after 4 weeks of treatment in rats.

Parameter	**A1 1 mg/kg bw (n=6)**	**A2 10 mg/kg bw (n=6)**	**A3 100 mg/kg bw (n=6)**	**C1 1 mg/kg bw (n=6)**	**C2 10 mg/kg bw (n=6)**	**C3 100 mg/kg bw (n=6)**	**B Control (n=6)**
AST (iu/L)	20.67 ± 1.8	18.67 ± 1.05	16.17 ± 1.85	17.67 ± 1.17	18.17 ± 1.14	19.83 ± 1.11	18.83 ± 1.4
ALT (iu/L)	17.83 ± 0.4	16.00 ± 1.21	15.00 ± 1.21	19.17 ± 6.47	21.5 ± 3.92	17.83 ± 1.22	17.00 ± 1.79
ALP (iu/L)	85.83 ± 9.29	82.67 ±17.64	82.5 ± 13.37	93.17 ± 15.01	75.83 ± 16.18	73.33 ± 15.81	70.83 ± 23.24
Tbil (µmol/L)	8.63 ± 0.69	8.68 ± 0.62	7.98 ± 0.74	7.48 ± 0.65	7.9 ± 0.36	8.92 ± 0.59	7.88 ± 0.47
Dbil (µmol/L)	4.7 ± 0.38	4.93 ± 0.42	4.53 ± 0.44	4.37 ± 0.48	4.23 ± 0.17	4.93 ± 0.42	4.47 ± 0.31
T.protein (g/L)	71.67 ± 1.12	73.00 ± 2.02	67.17 ± 1.58*	64.67 ± 1.43	68.67 ± 2.75	70.83 ± 2.14	72.33 ± 1.54
Albumin (g/L)	38.67 ± 0.84	37.67 ± 1.17	38.00 ± 0.78	36.5 ± 0.99	36.17 ± 1.49	38.5 ± 0.72	39.00 ± 0.86

Globulin (g/L)	33.00 ± 1.32	33.33 ± 2.28	31.17 ± 1.19	30.17 ± 0.91	32.5 ± 2.89	32.33 ± 1.89	33.33 ± 1.52
*p<0.05 (compared with the control group)							

Table 5 shows the effect of the fractions on the relative weights of the liver, kidney, spleen, and heart. At the end of the dosing period of 4 weeks, relative weighs of the liver, kidney, heart, and spleen did not show any statistically significant differences ($p>0.05$) in Fraction A (1, 10 and 100 mg/kg bw) and Fraction C (1, 10 and 100 mg/kg bw) treated groups compared with the control group. No significant variations in relative weights of all the organs tested were observed with changes in concentration in the test animals after the study periods.

Table 5: Effect of Fraction on Relative Organ Weights of Rats at 4 and 12 Weeks of Administration

Organ	**A1 (n=6)**	**A2 (n=6)**	**A3 (n=6)**	**C1 (n=6)**	**C2 (n=6)**	**C3 (n=6)**	**B (n=6)**
Liver (g)	4.28± 0.47	4.29 ± 0.20	4.00± 0.41	4.50 ± 0.38	5.65 ± 0.67	4.80 ± 0.56	5.30 ± 0.55
Kidney (g)	0.68 ± 0.04	0.64 ± 0.02	0.71 ± 0.04	0.74 ± 0.02	0.85 ± 0.09	0.77 ± 0.03	0.80 ± 0.05
Spleen (g)	0.33 ± 0.03	0.31 ± 0.03	0.36 ± 0.03	0.34 ± 0.04	0.36 ± 0.03	0.34 ± 0.03	0.38 ± 0.04
Heart (g)	0.44 ± 0.03	0.49 ± 0.03	0.51± 0.03	0.51 ± 0.03	0.48 ± 0.03	0.49 ± 0.05	0.51 ± 0.04

The heart, liver, kidney, and splenic sections showed normal histo architecture in all the experimental groups. 4 weeks of treatment did not show any histological lesions in the organs examined in all the test groups.

DISCUSSION

In many parts of Nigeria, the ash from the DFHOP or its filtrates are used in place of trona (potash) as a food additive, tenderizer [10], or as components of used for local remedies. Fraction A and C with their unique properties and mineral content are purer derivatives of the palm ash which possess the same properties as the palm ash and can, therefore, serve as a replacement for trona and palm ash. However, no work as regards its safety on ingestion has been reported. Acute oral toxicity (LD_{50}) showed no signs of toxicity or mortality in treated rats at doses below or equal to 2000 mg/kg of Fraction A and 3000 mg/kg bw for Fraction C. This indicates that both fractions may be safe for use as a food additive. At doses above 2000 mg/kg bw and 3000 mg/kg bw for fraction A and fraction C respectively, the animals exhibited some toxic changes which were more pronounced and manifested faster in the female rats. The quicker onset of signs observed in the females was most likely because females are more susceptible to systemic toxicities and are more sensitive to chemicals than their male counterparts.

Observed signs of toxicity include rapid breathing with reduced activity and food intake. At doses where the death occurred, paralysis of the right limb occurred in all the affected females seen as an extension of that limb and the animals were unable to use that part of the limb. The paralysis may be due to the high chloride level which may affect Ca^{2+} release in muscles. Studies have shown that the presence of excess chloride in the extensor *digitorum longus* of the rat and iliofibularis muscle of the toad myoplasm increases Ca^{2+} leakage from the sarcoplasmic reticulum due to the direct action on the ryanodine receptor/Ca^{2+} release channel [20,21]. Udeinya [20] recorded high chloride levels in DFHOP ash filtrate and from our preliminary study, the fractions were observed to have very high chloride contents. This increased chloride in the fractions may have possibly induced a potential across the sarcoplasmic reticulum (SR) which might reduce Ca^{2+} release via various mechanisms, thereby leading to partial paralysis or weakness of the limb.

Our study reveals that the subacute administration of Fraction A and Fraction C did not cause any haematotoxicity. Instead, Fraction A at 10 mg/kg bw caused a significant increase in red blood cell counts ($p<0.01$) and haemoglobin concentration ($p<0.05$) while 100 mg/kg caused an increase in RBC counts ($p<0.05$) only after 4 weeks of treatment. The fractions did not produce any significant changes in white cell and platelet parameters after the treatment period. Therefore, may have little or no effect on the white cell and platelet cell lines.

Body weights in the various groups of rats were assessed before the start of treatment and fortnightly throughout the experimental period. All treatment groups showed a time-dependent increase in percent (%) body weight including the control group. After 2 and 4 weeks, 10 mg/kg Fraction A group showed a significant increase in % bodyweight while a significant increase was observed after 2 weeks in percentage body weight in 100 mg/kg b.w Fraction A and 1 mg/kg b.w Fraction C treated groups compared to their corresponding timed control. The highest increase in weight was observed in the group with 10 mg/kg bw dose, with an increase of 59.28% to 103.62% which was more than one half to two times their original weight. Also, all the fraction A treated groups had a higher percentage weight gain than fraction C and the control group even though this not statistically significant ($p>0.05$). Results suggest that the fractions have no remarkable involvement in catabolic functions that can be reflected in body weight loss, muscle wasting, or processes that will affect the usual food and water intake in the animals.

At concentrations investigated, no untoward significant changes ($p>0.05$) in the activities of the hepatic enzymes, - aspartate transaminase, alanine transaminase and alkaline phosphatase (AST, ALT, and ALP) - of the fraction treated rats were observed compared to the untreated control groups after 4 weeks. Liver enzymes did not show any dose-dependent significant differences in mean levels in the same fraction treated groups. The aminotransferases (ALT and AST) are produced in the liver and are useful markers of hepatocellular damage [22]. Damage to the liver cells is characterized by an increase in plasma enzymes (ALT and AST). Usually, about 80% of AST is found in the mitochondria and the enzyme appears in higher concentrations in several tissues (liver, kidney, heart, and pancreas) whereas ALT is a purely cytosolic enzyme and localized primarily in the cytosol of hepatocytes [6]. AST within limits can provide a quantitative assessment on the degree of damage sustained by the liver [23] while ALT is a more sensitive marker of hepatocellular damage. Fraction A and fraction C did not appear to have any deleterious effect on liver function from this study. ALP, on the other hand, is found in several tissues, and elevated levels are commonly caused by liver diseases or bone disorder. From our findings, ALP levels were elevated at 4 weeks of treatment, in all fraction treated groups. 1 and 10 mg/kg bw fraction A treatment groups displayed the highest activity in ALP after 4 wks. However, this was not statistically significant when compared with the control group.

The enzymatic activity of ALP is usually raised in acute hepatotoxicity but tends to decrease with prolonged intoxication due to damage to the liver [10]. This is usually followed in most cases by hyperbilirubinemia since a rise in plasma ALP is usually a characteristic of cholestatic liver disease which will impair the hepatic capacity to excrete bilirubin. Bilirubin is a breakdown product of the haem component of the haemoglobin molecule. Total serum bilirubin is elevated in animals with hemolytic anemia (and this increase is caused mostly by an increase in the indirect bilirubin) or by congestion of the biliary duct or inability of the liver to excrete the newly formed bilirubin. From this study, no possible cholestasis occurred. This was confirmed by the non-significant differences, observed in total and direct bilirubin in the test animals compared with the control rats.

However, typically higher ALP levels are usually observed in children and adolescents because their bones are still growing. This probably explains the higher ALP values observed in all experimental animals at 4 weeks. The animals at the start of the experiment were 6-8 weeks old and considered as young rats. The growth rate was rapid as evidenced by their rapid

increase in weight especially between 2 and 4 weeks. The control group which had the lowest weight at 4 weeks also had the lowest ALP activity and the weights were not significantly different just like ALP values. Thus, by inference, the higher ALP values observed at 4 weeks rather than being due to liver toxicity or liver damage occurred most likely as a result of rapid growth observed in the test animals. Total protein, albumin, and globulin appeared normal in the experimental animals.

The effects of the fractions on the liver, kidney, heart, and spleen were further assessed through the determination of their relative weights and histoarchitecture. Administration of the palm ash fractions for 4 weeks did not lead to any significant change in relative weights of the organs assessed between the Fraction A and control or Fraction C and control treatment groups. No significant difference ($p>0.05$) was observed with changes in concentration of the fractions in rats given Fraction A or Fraction C. This serves as evidence that the changes in weight were not as a result of organ enlargement or damage or as a result of inflammatory conditions. Examination of the heart, liver, kidney, and spleen sections of the fractions treated groups, as well as the control group all, revealed normal histoarchitecture with no visible lesions. This is contrary to the report of Okoye et al., [15] who observed hepatotoxic effects in animals (rats) following administration of palm bunch ash after 21 days. Doses used in their research were over four times (480 and 900 mg/kg bw) the highest dose used in this study, and they suggested that the effects may be dose and duration dependent. Our purer and cleaner fractions did not show any cardiac or splenic toxicity as well as renal and hepatic injuries. This suggests the non-toxic (especially at low doses and short periods) nature of the palm ash derived fractions, as evidenced by the non-significant changes in cardiac, splenic, hepatic, and renal function, as well as relative organ weights.

CONCLUSION

The results from this study have demonstrated that subacute doses of the palm ash derived fractions does not appear to have any deleterious effect on haematological parameters, hepatic, renal function and integrity as we ll as cardiac and splenic histoarchitecture in experimental rats at doses up to a 100 mg/kg body weight. Therefore it may appear safe for potential use as a food additive and as a phytomedicine in small quantities. Further studies should be carried out to ascertain the long term toxicities of the palm ash derived fractions.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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