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COMPARATIVE EVALUATION OF THE EFFECTS OF PALM BUNCH ASH AND TRONA ON THE LIVER OF ALBINO RATS

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ABSTRACT

Aim: To evaluate the haematological, biochemical and histological effects of palm bunch ash (PBA) and trona when used as tenderizers.

Methods: Thirty male albino rats were randomly grouped into five designated 1(control), 2, 3, 4 and 5, and were orally given 2 ml of distilled water, 486 mg/kg of trona, 972 mg/kg of Trona, 486 mg/kg of PBA, 972 mg/kg of PBA, respectively for 21 days.

Results: The study showed decreased body and liver weights (195.00 ± 20.50 to 152.80 ± 27.81{$p<0.05$} and 9.13 ± 1.28 to 7.38 ± 1.59 {$p>0.05$}, respectively) and higher relative weight (4.68 ± 0.98 to 4.83 ± 0.34 {$p>0.05$}) and serum levels of alkaline phosphatase (165.83 ± 16.73 to 349.67 ± 201.99 {$p>0.05$}). Significant differences in lactate dehydrogenase activity ($p<0.05$) and insignificant differences in the estimated haemoglobin, packed cell volume, serum amylase and transaminases were also observed ($p>0.05$). Liver microscopy in the test groups revealed evidences of hepatitis and cholestasis.

Conclusion: this study suggests that both trona and PBA are more toxic at 972 mg/kg but less toxic at 486 mg/kg body weight. Thus, lower concentrations are advised when used as tenderizing agents.

Key words: Trona, Palm bunch ash, Tenderizer, enzymes

INTRODUCTION

Trona, also known as cooking potash, is traditionally known as kaun among the Yoruba, kanwa among the Hausa and akanwu among the Igbos and Okanwa among Igalas and Ikoro among Egbira people of Nigeria. It is found in the Northern parts of Nigeria, particularly Kano and Maiduguri States and neighboring countries such as Chad and Niger (Ajiboye et al., 2013). The popularity of trona is such that in some parts of the world, it is ranked next in importance to the common table salt (Makanjuola and Beetlestone, 1975). It is a local earthy material used widely in West Africa and particularly in Cameroon, Ghana and Nigeria, as a cooking ingredient in vegetable soups (Ekosse, 2010). Edijala (1980) reported that trona increases the green colour and texture of vegetables as well as reduces the cooking time of legumes. It is used in some concoction for curing cough and ameliorating toothache, stomach pains, and constipation. More so, it is administered to women postpartum to enhance maternal quality and quantity of breast milk (Davidson et al., 1974). Palm bunch ash (PBA), traditionally known as Ngu in eastern Nigeria, is used in place of trona as food additive and tenderizer. It is also believed to be a non purgative substance in preparing crude palm oil and African salad popularly known as Abacha. PBA produced by burning or ashing, which constitutes about 6.5% by weight of the empty fruit bunch, contains 30–40% K_2O (Lim and Zaharah, 2000) and could thus be used as source

of potassium fertilizer. PBA has high pH and contains varying amounts of other nutrients such as calcium (Ca), phosphorus (P), and magnesium (Mg). Studies have it that PBA is an effective fertilizer and liming material for increasing soil fertility, pH, and nutrient uptake by crops such as maize and cassava (Ojeniyi et al., 2009), but no study have demonstrated its effect in the body following its use as food tenderizer. Thus, the continuous and indiscriminate use of PBA as food additive in many African communities following the reported deleterious effect of trona precipitated this study. Hence, this study aimed to evaluate the health complications associated with palm bunch ash and trona consumption by way of comparative analyses.

MATERIALS AND METHODS

Study Area

The study was carried out at the animal facility in the Department of Medical Laboratory Science, Faculty of Health Sciences, Madonna University Elele, Rivers state, Nigeria. The study area is located in the tropics (with the mean daily temperature of 29 ºC) at the southern part of Nigeria; latitude 5 27-5 31N and longitude 6 55-7 85E (Gobo, 1988). It is bordered by four neighboring communities namely Isiokpo, Umuagwo, Ahoda, and Omoku.

Test Samples Collection

The trona sample was obtained at the Elele market, Rivers state, Nigeria and was identified at the Geology department, grounded into fine powder using mortar and pestle. Empty palm bunches were obtained at the palm oil mil at Elele, Rivers State. They were ashed in an oven at 100^{o}C and allowed to cool. The ash was collected and sieved to remove particles which were not properly burnt. The potash powder and palm bunch ash powder were weighed accurately using an electric balance, mixed with an accurate amount of water (486 mg per kg/1 ml) and stored in dry plastic containers.

Experimental Animals and Ethical Issues

Thirty adult male Albino rats of age 8 weeks, weighing 130 to 200g were used for this study. They were bred in the animal farm of the department of Medical Laboratory Science, Madonna University. Before the experiment commenced, the rats were allowed to acclimatize for a period of two weeks, all the animals were allowed fed with grower's mash and water ad libitum. They were kept under uniform laboratory conditions and exposed to 12 hrs of light and 12 hrs of darkness throughout the duration of the experiment. The experiment was conducted in accordance with the Guidelines of the U.S. National Institute of Health (NIH, 1985) on the care and use of laboratory animals, principles of good laboratory procedure (WHO, 1998) and the 1964 declaration of Helsinki.

Experimental Design

The thirty rats with a mean weight of 162g were randomly divided into five groups, with each group consisting of six rats. Animals in groups 2, 3, 4 and 5 served as treatment groups while those in group 1 served as the control. The selected or fixed doses of test samples were derived from the study carried out by Ebadan et al. (2014), where 3000 g/kg body weight were administered to experimental animals by oral gavage. Based on the calculated mean weight (162g) of the animals used in this study the following doses were administered: Group 1; 2ml of normal saline, Group 2; 486 mg/kg of trona, Group 3; 972 mg/kg of trona, Group 4; 486 mg/kg palm bunch and Group 5; 2ml high dose of (972 mg/kg) palm bunch.

Sample Collection and Analyses

The animals were subjected to an overnight fast after 21 days of treatment, weighed using a weighing balance (doran-PC 500) and anesthetized using the "Drop method" (JHU, 2009). Blood samples were taken for PCV and haemoglobin estimation by the Cyanmethhaemoglobin. The sera were analysed for AST and ALT (using the Reitman and Frankel method), ALP, LDH and Amylase (using the kinetic method as described by Sood (2009). Their absorbencies were read using a spectrophotometer (APEI PD-303S). The liver was excised, blotted dry, weighed on a microbalance sensitive at 0.001mg (Precisa 125A, Switzerland) and fixed in 10% formal saline for histopathological analysis.

Tissue Processing

Tissues were processed by the paraffin wax method, sections cut at 4µ, stained using haematoxylin and eosin, and photomicrographs taken for documentation (Avwioro, 2002).

Statistical Analysis

The biochemical data were subjected to some statistical analysis: analysis of variance (ANOVA) and Post Hoc test was carried out on

the data using the Statistical Package for Social Sciences (SPSS; version 18). Values were reported as Mean ± SD. A value of P<0.05 was accepted as significant.

RESULTS

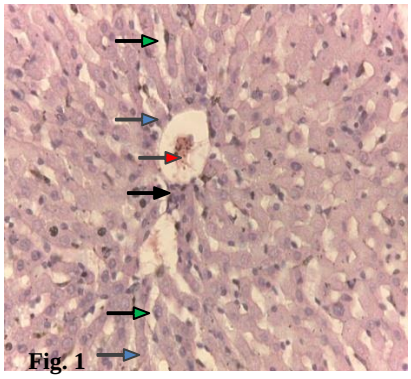
Fig. 1

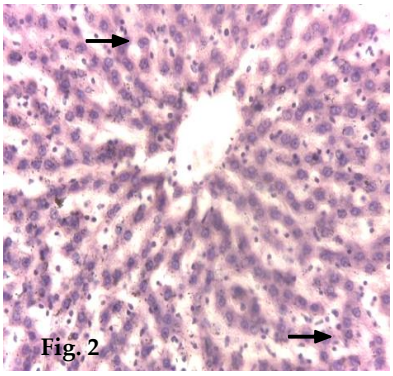
Fig. 2

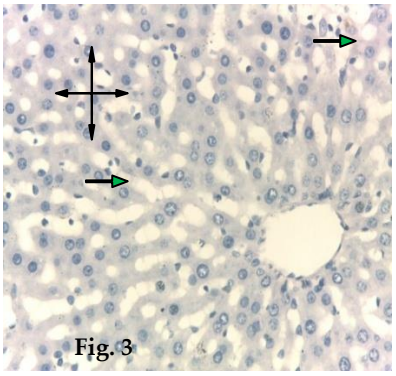
Fig. 3

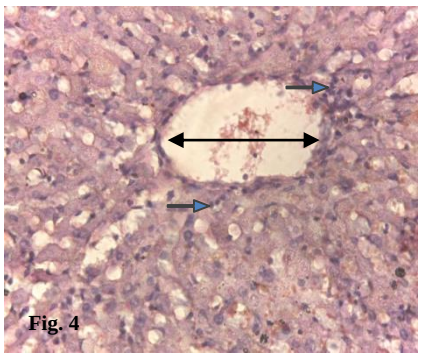
Fig. 4

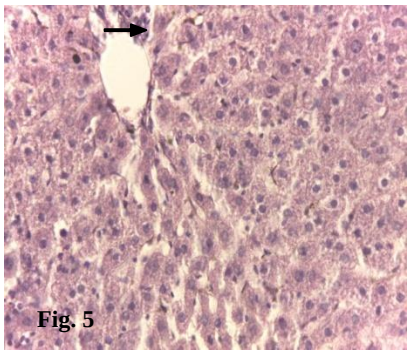
Fig. 5

Figure 1: 2ml of distilled water
Figure 2: 486 mg/kg of Trona
Figure 3: 972 mg/kg of Trona
Figure 4: 486 mg/kg of PBA
Figure 5: 972 mg/kg of PBA

Figure legends

Figure 1 (Group 1; control): Liver section with normal microarchitecture. Its shows distinct liver sinusoids (marked by green arrows), hepatocytes (marked by blue arrows), blood cells (marked by red arrow) and central vein (marked by black arrow). H&E X400

Figure 2 (Group 2): Liver section showing mild change in tissue architecture. The stained section shows moderate influx of numerous inflammatory cells in the stroma (an evidence of hepatitis; marked by black arrows) and mild cytoplasmic necrosis. The integrity of the central vein appears to be mildly compromised in some areas. H&E X400

Figure 3 (Group 3): Liver section showing moderate signs of necrosis. Some areas appear vacuolated (marked by four headed arrows) with evidence of karyolysis and inflammatory cells (marked by green arrows). H&E X400

Figure 4 (Group 4): Liver section showing dilated central vein (marked by double headed black arrow), less identifiable sinusoids (an evidence of stromal erosion or hepatitis), a few inflammatory cells (marked by blue arrows) and iron deposits (appear as dark-brown deposits), all pointing towards a medical condition known as cholestasis. H&E X400

Figure 5 (Group 5): Liver section showing mild cytoplasmic necrosis, very few inflammatory cells (marked by black arrow) and mild stromal erosion (with few distinct sinusoids). H&E X400

Table 1: Comparison of Trona and palm burnt ash effects on animal body weight based on group treatments

Groups	Day 1	Day 5	Day 9	Day 13	Day 17	Day 21
Group 1	169.67 ± 26.52	194.67 ± 17.65	183.33 ± 21.12	187.67 ± 20.18	195.00 ± 20.54	195.00 ± 20.50
Group 2	159.00 ± 23.18	169.00 ± 13.90	157.67 ± 16.61	162.00 ± 14.59	169.67 ± 15.82	168.67 ± 19.91
	0.437	0.029	0.026	0.023	0.035	0.051
Group 3	163.20 ± 20.18	165.20 ± 22.65	154.00 ± 16.31	158.00 ± 20.88	161.60 ± 26.17	152.80 ± 27.81
	0.652	0.018	0.016	0.013	0.010	0.249
Group 4	165.00 ± 28.42	161.67 ± 21.22	151.67 ± 21.96	160.00 ± 20.78	164.67 ± 20.23	163.33 ± 16.13
	0.733	0.006	0.007	0.015	0.013	0.680
Group 5	162. 33± 15.82	172.67 ± 19.58	173.33 ± 16.23	182.67 ± 14.68	182.67 ± 14.68	180.00 ± 25.71
	0.592	0.057	0.364	0.641	0.287	0.384
Group 2 vs Group 3	0.769	0.745	0.749	0.722	0.503	0.249
Group 2 vs Group 4	0.661	0.512	0.584	0.852	0.663	0.680
Group 4 vs Group 5	0.845	0.328	0.056	0.043	0.125	0.205
Group 3 vs Group 5	0.952	0.524	0.101	0.036	0.089	0.054
F-value	1.168	2.736	3.153	3.365	2.902	3.008
P-value	0.952	0.052	0.032	0.025	0.048	0.038

The mean difference is significant at P<0.05, N=30, n=6, Analysis of variance and Post Hoc test

Key: vs= versus; Group 1=2 ml of normal saline; Group 2= 486 mg/kg body weight of Trona (cooking potash); Group 3= 972 mg/kg body weight of Trona (cooking potash); Group 4= 486 mg/kg body weight of Palm burnt ash; Group 5= 972 mg/kg body weight of Palm burnt ash.

Table 1 above shows insignificant changes in the weight of the animals from day 1 through day 3 ($p>0.05$) but shows significant changes from day 6 through day 12 ($p<0.05$) when compared with the control. There was no significant change ($p>0.05$) observed in the weight of group 2 when compared with group 4 despite fact that they had different type of food softener, though of the same quantity (486 mg/kg). The same effect was observed in group 3 when compared with group 5 (administered 972 mg/kg), except on day 9 which showed significant change in weight.

Table 2: Mean comparison of Trona (cooking potash) and palm burnt ash (Ngu) effects on organ weight and organ against body weight based on group treatments

Groups	Liver	O/B Wt %
Group 1	9.13 ± 1.28	4.68 ± 0.98
Group 2	7.91 ± 1.37	4.74 ± 0.98
	0.133	0.864
Group 3	7.38 ± 1.59	4.81 ± 0.33
	0.043	0.707
Group 4	7.75 ± 0.66	4.76 ± 0.34
	0.090	0.817
Group 5	8.76 ± 1.70	4.83 ± 0.34
	0.637	0.640
Group 2 vs Group 3	0.522	0.831
Group 2 vs Group 4	0.835	0.952
Group 4 vs Group 5	0.211	0.813
Group 3 vs Group 5	0.107	0.944
F- value	1.648	0.069
P-value	0.195	0.991

Mean difference is significant at $P<0.05$, N=30, n=6, Analysis of variance and Post Hoc test

Keys: vs= versus; O/B wt %= Organ: body weight ratio in percentage (relative weight), Group 1=2 ml of normal saline; Group 2= 486 mg/kg body weight of Trona (cooking potash); Group 3= 972 mg/kg body weight of Trona (cooking potash); Group 4= 486 mg/kg body weight of Palm burnt ash; Group 5= 972 mg/kg body weight of Palm burnt ash.

Table 2 above shows insignificant changes in the organ and body weights of the animals in the test group when compared with the control group ($p>0.05$).

Table 3: Mean comparison of Trona and palm burnt ash effects on some biochemical parameters based on group treatments

	Hb	**PCV**	**ALP**	**AST**	**ALT**	**LDH-P**	**Amylase**
Group 1	17.48 ± 3.02	52 ± 9.02	165.83 ± 16.73	71.67 ± 19.87	21.33 ± 1.75	628.00 ± 295.03	171.83 ± 41.12
Group 2	18.30 ± 2.28	55 ± 6.84	231.83 ± 63.52	73.00 ± 17.61	16.33 ± 4.68	852.33 ± 343.07	174.00 ± 66.03
	0.653	0.634	0.332	0.890	0.274	0.173	0.942
Group 3	19.52 ± 4.74	59 ± 14.24	233.40 ± 48.07	69.80 ± 19.12	17.00 ± 4.30	710.80 ± 314.09	165.20 ± 36.20
	0.289	0.282	0.343	0.854	0.364	0.625	0.833
Group 4	15.67 ± 2.28	47 ± 6.78	245.83 ± 130.08	73.67 ± 15.20	21.33 ± 11.99	568.17 ± 220.30	168.33 ± 47.87
	0.324	0.327	0.241	0.836	1.000	0.711	0.907
Group 5	17.67 ± 2.22	53 ± 6.63	349.67 ± 201.99	49.50 ± 9.65	17.67 ± 10.15	239.17 ± 186.66	168.17 ± 57.19
	0.918	0.912	0.011	0.030	0.419	0.023	0.903
Group 2 vs Group 3	0.504	0.511	0.982	0.753	0.888	0.406	0.780
Group 2 vs Group 4	0.138	0.133	0.835	0.945	0.274	0.088	0.850
Group 4 vs Group 5	0.256	0.255	0.132	0.019*	0.419	0.050	0.996
Group 3 vs Group 5	0.314	0.309	0.109	0.055	0.888	0.010*	0.925
F-value	1.239	1.259	1.973	2.256	0.575	4.038	0.025
P-value	0.322	0.315	0.131	0.093	0.683	0.012*	0.999

*Mean difference is significant at $P<0.05$, N=30, n=6, Analysis of variance and Post Hoc test

Key: vs= versus, Hb= Haemoglobin, PCV= Packed Cell Volume, ALP= Alkaline Phosphatase, AST= Aspartate transaminase, ALT= Alanine transaminase, Lactate Dehydrogenase , Group 1=2 ml of normal saline; Group 2= 486 mg/kg body weight of Trona (cooking potash); Group 3= 972 mg/kg body weight of Trona (cooking potash); Group 4= 486 mg/kg body weight of Palm burnt ash; Group 5= 972 mg/kg body weight of Palm burnt ash.

Overall, table 3 above show insignificant differences in haematological and biochemical parameters within and among the groups ($p>0.05$), except in lactate dehydrogenase which had significantly increased activity ($p<0.05$). Significant differences were observed when group 5 was compared with group 1 and group 3 ($p<0.05$). Insignificant higher levels of Hb, PCV and ALP with corresponding insignificant lower levels of ALT, LDH-P and Amylase activity were observed in group 5 when compared with group 4 ($p>0.05$), while significant lower levels of AST was also observed in the group 5 ($p<0.05$). However, insignificant higher levels of Hb, PCV and AST ($p>0.05$) with significant higher level of LDH activity ($p<0.05$) and lower insignificant level of ALP, ALT and Amylase activity were observed in group 5 when compared with group 3 ($p>0.05$). Insignificant increase in Hb, PCV, ALP, AST, LDH-P and serum amylase with insignificant decrease in ALT were observed when group 2 was compared with the control group 3 ($p>0.05$). Higher insignificant levels of Hb, PCV, LDH-P and serum amylase with corresponding lower insignificant levels of ALP, AST and ALT were observed in group 2 when compared with group 4 ($p>0.05$). However, lower levels of Hb, PCV, ALP, ALT and higher levels of AST, LDH-P and Amylase were observed in group 2 when compared with group 3 ($p>0.05$).

Table 4: Correlation analysis between some affected haematological and biochemical parameters

Groups Control variable (Parameters)	r-value	Correlation P-value	Remark
Hb vs PCV	1.000	0.000**	Strong positive correlation
LDH vs AST	0.528	0.005**	Strong positive correlation

**Correlation is significant at $P<0.01$ and $P<0.05$ (two tailed test), N=30, n=6
Table 4 above shows significant strong positive correlation between Hb and PCV, LDH and AST ($p<0.01$).

DISCUSSION

The traditional use of food tenderizers (Trona and Palm bunch ash) cannot be abated in local communities, especially in developing countries with low income earners. These food tenderizers are believed to reduce cooking time and thus, reduce expenditure (Nasiru et al., 2011). This study investigated the effect of Trona (Akaun) and Palm bunch ash (Ngu) in relation to body weight, haematological, biochemical and liver microarchitecture of male albino rats. The reduction in body weight in the test groups when compared with the control group suggests that food tenderizers may cause some health problems. However, the body weights of the test groups administered with Trona were lower than those administered with Palm bunch ash (table 1). The latter suggest that the degree to which the compared food softeners inflict injury to the entire body system varies. This reduction in body weight of the test groups fed with Trona is dose dependent (Table 1) and is in agreement with the study carried out by Ebadan et al., (2014) who reported similar effect. The reduction in body weight could be as a result of chemical nature of the substance (trona). More so, animals fed with Trona had lower liver weight and relative weight (table 2) when compared with the control group which had no pathological change in their liver sections (Figure 1). The difference could be adduced to the mild toxic effect of trona on the liver, evident in Figure 2. Polycythemia (erythrocytosis) is refers to increased Hb and red cell count and is associated with chronic chemical exposure, renal disease and dehydration among others (Sood, 2009). This is consistent with the result of this study which revealed dose dependent higher haemoglobin (Hb) and packed cell volume (PCV) estimates in rats fed with Trona when compared with those fed with palm bunch ash and the control group. This suggests that trona may have the potency of dehydrating the body, hence the higher Hb and PCV and the strong positive correlation between them (tables 3 and 4). This is in consonance with the findings of Oyeleke et al., (1981) who observed that high potash intake result in reduction of food and water consumption. This dehydrating effect of trona may be linked to its chemical components which include: calcite, hanksite, halite, pirssonite, borax, sodium sesquicarbonate, sodium carbonate, sodium sulphate (Copenhafer, 2000; Lu et al., 2005; Ajiboye et al., 2013). The enzymatic activity of ALT, AST, ALP and LDH are usually raised in acute hepatotoxicity but tend to decrease with prolonged intoxication due to damage to the liver (Obi et al., 2004; Mcmillin and Johnson-Davis, 2010). Higher ALP activity was observed in the test groups fed with Palm bunch ash when compared with those

fed with Trona and the control group. This correlates well with the studies carried out by Omajili et al., (2010) and Ajiboye et al., (2013) who reported increase in serum levels of ALP following the administration of Trona. This may be suggestive of liver damage as figure 4 and 5 show evidence of hepatitis. This is further supported by the higher liver weight and organ versus body weight ratio observed in animals fed with PBA. Since AST and LDH have also been reported to be elevated in disease condition such as pulmonary infarction, acute renal infarction and progressive muscular dystrophy (Sood, 2009), the higher serum AST and LDH observed in the groups fed with Trona when compared with the other groups suggests that Trona may also have extrahepatic effect. The strong positive correlation between AST and LDH (Table 4) further lends support to the latter suggestion. The reports of Bankole et al., (2015) and Ebadan et al., (2014) who observed renal toxicity following the administration of trona (potash) further buttresses the argument. More so, the elevated level of serum AST and increased activity of LDH may be adduced to the cytotoxic effect of trona on the heart muscle as earlier reported by Ochei et al., (2014). Elevated levels of amylase are found in acute pancreatitis in first 24 hours (Kumar et al., 2010) while decreased levels are found hepatitis (Sood, 2009). With acute pancreatitis and hepatitis occurring simultaneously in a system it could be difficult to come up with a diagnostic value for amylase, as one disease condition counters the effect of the other. This might be the explanation for the insignificant differences observed in the test groups fed with trona when compared with the control group (Table 3). Since the serum levels of amylase in the test groups fed with PBA are relatively the same (Table 3), it could be argued that PBA had little or no effect on the pancreatic function at the administered dosages. The iron deposits observed in figure 4 may be linked to the decreased Hb and PCV observed in Group 4. The decrease in these haematological parameters may be due to cholestasis caused by intrahepatic haemolysis. Cholestasis has been reported to result in nutritional deficiencies of the fat-soluble vitamins A, D, or K and characterized by elevated serum alkaline phosphatase (Kumar et al., 2010). The latter report is consistent with the results of this study. Surprisingly, lower haematological values were not observed in group 5. This, yet unclear, suggests that the effect of PBA may be dose and duration dependent. In general, considering the microarchitectural changes observed in the liver sections of the test groups when compared with the control group (Figures 1), it is agreeable that PBA (Figures 4 and 5) had as much hepatotoxic effect as trona (Figures 2 and 3) but the observe differences in the biochemical and haematological effect suggests that trona was more toxic.

Conclusion

Based on the body weight, biochemical, haematological and histological evaluations, this study suggests that both Trona and Palm bunch ash are more toxic at 972 mg/kg body weight. Minor architectural changes were observed in the experimental animals given both tenderizing agents at 486 mg/kg body weight, thus lower concentrations should be used when preparing meals.

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