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Toxicological effects of two major types of potash used as food additives in Nigeria: Biochemical, hematological, and histopathological analysis of major organs in Wistar rats

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Abstract

Trona or natron, often called potash, are evaporites, which are natural salts used unregulated in Nigeria as food additives, particularly to soften hard beans or tenderize tough cow meat, and for medical purposes. A 90-day sub-chronic toxicological study was conducted on Wistar rats weighing 120-140g to investigate the effects of consumption of these natural salts on hematological, histological and biochemical parameters. The experimental design involved 30 rats (n=10), considering attrition of two rats per group. The animals were divided into 3 groups. Group 1 was administered potash type A, group 2 administered potash type B while group 3(control) received only food and water. Standard methods were used to analyze all parameters. All results were presented as mean ± SD, with p-values < 0.05 considered significant. Each potash type has LD50s exceeding 5000mg/kg. Potash types significantly increased AST and ALT levels ($p<0.05$) in liver function measures compared to the control group. In both potash types, creatinine levels were considerably higher ($p<0.05$) than the control, but Urea levels were not statistically different ($p>0.05$). Sample A (Trona) reduced RBCs, Hb, and PCV more than sample B (natron) in rats. Both types had WBCs and Platelets that were similar to controls ($p>0.05$). Effects of potash on four electrolytes :Na+, K+, Cl- and HCO3- shows that potash type B contained elevated ($p<0.05$) levels of sodium than type A. Our results indicate that chronic consumption of each potash type or even both types, might expose users to hypernatremia, electrolyte imbalance, hematological problems, liver and kidney failures.

Keywords: Potash; Additives; Kidney; Liver; Electrolytes

1. Introduction

Trona or natron commonly known as potash are evaporites which are naturally occurring mineral deposit found in potassium salt rocks in the forms of Sylvite (KCl), Carnallite (KMgCl3.6H2O), Kainite (4KCl.4MgSO4.11H2O), and Langbeinite (K2Mg2(SO4)3)[1] . Trona and natron are natural salts that share the common characteristic of containing sodium carbonate. However, they can be distinguished by their distinct chemical compositions, water of crystallization, and applications. Trona is a compound consisting of sodium sesquicarbonate, whereas natron is a compound composed of sodium carbonate decahydrate. Potash is a broad term that covers all the bases when looking at potassium-bearing minerals [2] . It appears in a variety of colors: white (complex potash), grey/reddish brown (lite potash), and a wide

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range of particle sizes[3]. The presence of trace amounts of other elements, such as iron, determines its grey/reddish brown color. It appears in the form of salt deposits and emerges from the soil during the wet season when sea water/ocean evaporites, but it tends to fall off, solidify, and dry out during the dry season, forming a bed of potash ore [4]. Potash is widely used in Nigeria's six geopolitical zones, especially in the South-West, South-South, South East, and North-West, as well as the North-Central and North-East. It is referred to as *Kaun* (Yoruba), *Akanwa* (Igbo), and *Kanwa* (Hausa) in the three major languages of the country respectively. According to Kutshik et al[5], potash is the second most often used salt in Nigeria, mostly as an additive and food softener. The rising demand and use of either form of potash is due to the high cost of cooking gas, as potash reduces cooking time and makes food softer. Although the mechanism of action is unknown, it does speed up cooking time, particularly for hard beans and difficult meat.

While the two primary potash forms are employed for various purposes, including antifungal preservatives, medicinal purposes (herbal concoction), relief from constipation and congestion, and lactation support[6] ,their usage is not regulated and varies from meal to meal. Concerns have been raised about the toxic health effects of potash, which is widely used as a food additive and in herbal products. Potash is an earth-derived, naturally occurring evaporate that may contain or be contaminated with heavy metals [7]. Some of these heavy metals, such as cadmium, mercury, lead, chromium, silver, and arsenic, have delirious effects in the body, causing acute and chronic toxicities in humans [8].

Various harmful and terminal diseases with unknown causes and damaging effects have recently infringed on human life. According to Asrani et al., [9], liver disease causes nearly two million deaths per year worldwide, whereas chronic kidney disease (CKD) is the 16th leading cause of lost years of life[10]. Chronic ingestion of chemicals and other edible earth materials over time may cause renal failure in humans[11].Because the liver is involved in the metabolism of orally taken xenobiotics, putting stress on the liver by indiscriminately consuming a natural substance whose components are unknown could result in hepatic insufficiency and damage [12]. The study aimed to determine the long-term effects of either or both types of potash on hematological and biochemical markers using Wistar rats.

2. Material and methods

2.1. Materials

2.1.1. Equipment

The following equipment were used in the study: Analytical weighing balance (PA214,Ohaus,USA), electronic weighing balance (SPU 401,Ohaus,USA), Compound digital light microscope(Motic™-BA210 ,China) centrifuge (Vanguard V 6000,Germany), Acurex Chemistry Analyzer (SR NO: 7047,England), Semi-auto analyzer ,EMP 165 ,China), Humalyzer 2000 (China), Ion selective electrode (ISE) analyzer(ISE 4000,France), Selectra Junior (Semi- robotics, England), Sysmex Automated Hematology Analyzer (Sysmex Kx-21N, United States).

2.1.2. Chemicals/Reagents/Kit

The following reagents and chemicals were used in the biochemical analysis: 5,5- dithio-bisnitro benzoic acid (DNTB), Glacial acetic acid, Sodium azide and Sodium nitrate; Biuret (Randox), Bromocresol green (Randox), Detergent: Cell Clean, Diacetyl monoxime reagent (EMD Biosciences), Diluent (Cell Pack) ,Glucose oxidase reagent (randox), ISE Pack (Teco), Jaffes Reagent (EMD Biosciences), Standard biochemical kits (Randox, United Kingdom) ,Lipid Profile reagents: (Randox and Agappe), SOD Kit (Randox), Uric Acid reagent (Teco),WBC/HGB lyse reagent: Stromatolyser-WH approximately 1.0ml, HCl , H 2 O 2, Phosphate buffered formalin , TCA, Potassium dichromate, Phosphate buffer pH 7.0 and Thiobarbituric acid.

2.1.3. Rodent Feed

Ccommercial standard rodent feed (Vital Feeds®, Nigeria Limited) was used.

2.1.4. Potash (Trona and Natron)

Samples A (500g) and B (500g) were collected from each of the six zones of the country.

2.1.5. Animals

Healthy young adult male and female Albino Wistar rats weighing between 120-140g. The rats were in-bred at the Animal House facility of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria.

2.2. Methods

2.2.1. Research Design

A randomized subject-control experimental design was used in this study. Animals of both sexes (Wistar rats) were chosen at random and categorized accordingly. The study involved two test groups, with the third serving as a control group. Potash sample (A) was assigned to group (1), potash sample (B) to group (2), and control to group (3). To facilitate identification, all animals utilized in the study were marked with indelible ink.

2.2.2. Sample size for Animal Toxicological Studies

Animals were chosen at random, and the sample size was calculated using Charan and Kantharia, [13]formula: Corrected sample size = Sample size/ (1-[% attrition/100]). Sample size = 24 (i.e. n=8 per group) with 20% attrition and 30 animals as the Corrected sample size. Two animals were added to each of the groups: 1, 2, and 3 to account for possible attrition (death) over the 90-day study. As a result, a total of 30 rats were required for the sub-chronic study.

2.2.3. Sample Collection of Potash Samples

Each potash sample was randomly selected from major markets in states representing each of the country's six geopolitical zones. Seven samples (n=7) of each type of potash (14 samples for both types) were collected in a popular market where potash is abundant in each state and region. This study employed 500g samples of the two most common potash samples, A and B.

2.2.4. Animal Housing and Feeding Conditions

Healthy adult male & female Wistar rats used in the study were obtained and housed similar to Orji *et al* .[14]. At the commencement of study, animals were between 8 and 12 weeks old and their weights were within ± 20 % of the mean initial weight of any previously dosed animals according to [15]. They were kept in their cages for roughly two weeks before dosing to allow for acclimatization and to adjust to laboratory conditions. The animals were selected with care to ensure that they were available in the right sizes and age range for the duration of the study. The animal house were be kept at a temperature of 22°C (± 3°C) as recommended by National Research Council(NRC),[16]. Although the relative humidity should be at least 30% and preferably not exceed 70% during room cleaning, the goal were between 50-60%[16].Lighting were artificial, the sequence being 12 hours light and 12 hours dark. They were fed with commercial rat feed ad libitum, and liberally supplied with de-ionized water. The animals were handled in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals. Standard rodent laboratory diets were employed for feeding, along with an unlimited supply of de-ionized drinking water.

2.2.5. Preliminary test (LD_{50})

Modified Lorke [17] method was used in the determination of oral acute toxicity of each type of potash .Two LD_{50}s of the potash samples(A and B) were determined using deionized as solvent media respectively. A total of 13 male Albino Wistar rats were used in determination of each of the LD_{50}s.In phase I, Wistar rats were divided into three groups (n = 3). Solution of potash (i.e dissolved in deionized water) was orally administered to each of the animals using cannula in the dosages of 10, 100 and 1000 mg/kg per group respectively. The rats were kept under the same conditions and examined for toxicity signs for six hours following administration. They were monitored for mortality and general behavior for 24 hours and kept for 7 days for possible delayed death.

In phase II, four groups (n = 1) of rats were administered with 2000, 3000, 4000 and 5000 mg/kg of each potash sample solution in their different groups (A and B) respectively. The LD_{50} of each of the potash samples were determined in each group by taking the geometric mean of highest non-toxic dose (a) and the least lethal dose(b),[$LD_{50} = \sqrt{(a \times b)}$] Ngulde et al, [18].

2.2.6. Preparation and Administration of doses

All potash samples collected (500 g) from each of the popular markets representing the states in each of the six geopolitical regions were separated based on the two major types, pulverized, and properly mixed to ensure uniformity. Each potash type was heated to 70^{o}C to kill any microbial contaminants. The rats were administered potash samples on a daily basis until the end of the 90-day (12-week) period. Similar to Kumar and Sinha [19], 1000 mg/kg potash representing one-fifth of the LD_{50} (5000 mg/kg) was administered daily for 90 days using a suitable intubation cannula so that the animals were not injured. The volume of potash solution administered at one time was based on size of the test animal(i.e10ml/kg of body weight) as recommended by Turner et al., [20]. Prior to dosing, the animals were

weighed, and weekly weights were recorded on the following days: 0, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70..., and 90 days prior to the sacrifice of the rats, respectively.

2.2.7. Haematological test

All haematological parameters were analyzed using automated hematology analytical method with Sysmex Kx-21N auto-analyzer.

2.2.8. Electrolytes test

An automated method was used to carry out this procedure. Analyzer ISE 4000, an ion selective electrode (ISE), was the preferred equipment for determining serum electrolytes. This was carried out similar to Bolarin and Azinge [21].

2.2.9. Renal function test

Serum creatinine was determined using Jaffes' method while serum urea was diacetyl monoxime technique similar to Michael et al .,[22].

2.2.10. Liver function test

Aspartate aminotransferase (AST) and alanine transaminase (ALT) were carried out using test kit method (Enzymatic and Colorimetric methods) as adopted by International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

2.2.11. Histological examination

Tissue preparation and examination were prepared similar to Alturkistani et al., [23]. Histopathological examinations were performed on the stomachs, livers, and kidneys of the experimental animals. For at least 48 hours, the samples were fixed in 10% phosphate buffered formalin. The tissues were then sliced, dehydrated in four grades of alcohol (70%, 80%, 90%, and pure alcohol), cleansed with three grades of xylene, and embedded in molten wax. Following solidification, the blocks were sectioned into 5m thick slices with a rotary microtome, floated in a water bath, and incubated at 60°C for 30 minutes. The 5m thick sections of tissues were then cleaned in three grades of xylene and rehydrated in three grades of alcohol (90%, 80%, and 70%). Following that, the sections were stained for 15 minutes with hematoxylin. The blueing process employed ammonium chloride. Before being counterstained with Eosin, the cells were differentiated with 1% acid alcohol. Permanent mounts on degreased glass slides were made using a mountant known as dibutylphthalate polystyrene xylene (DPX).

2.2.12. Statistical Analysis

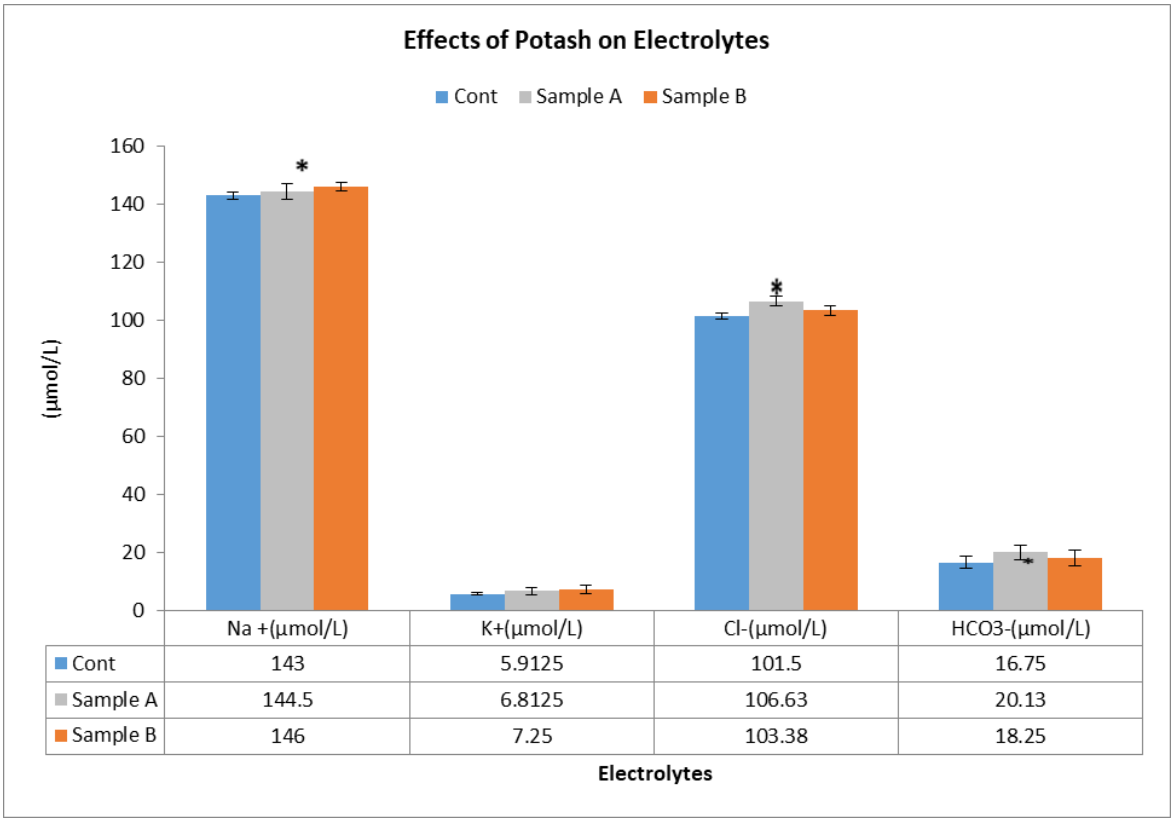
Statistical package for social sciences (SPSS) version- 27) was used for data analysis. Results were presented as mean ± standard deviation. Statistical comparison between potash treated groups and control was made using one way analysis of variance (ANOVA) followed by dunnet test, which compared sample A and B against control group. A p-value < 0.05 was deemed statistically significant, whilst a p-value > 0.05 was deemed non-significant.

3. Results and discussion

Results of the preliminary study on acute toxicity (LD_{50}) of either types of potash shows no death at 5000mg/kg. However, both types of potash induced watery stool beginning at 17 hours and returning to normal within 24 hours of treatment. This implies that it is unlikely to cause harm on acute exposure.

3.1. Effects of potash on electrolytes

Electrolytes are required for basic life functions like maintaining electrical neutrality in cells and generating and conducting action potentials in nerves and muscles [24]. Fig.1 shows electrolytes: sodium(Na+),potassium(K+) ,Chloride(Cl-)and bicarbonates(HCO_{3^-}) detected in both potash samples.



	Na +(µmol/L)	K+(µmol/L)	Cl-(µmol/L)	HCO3-(µmol/L)
Cont	143	5.9125	101.5	16.75
Sample A	144.5	6.8125	106.63	20.13
Sample B	146	7.25	103.38	18.25

Figure 1 Graphical Representation of Effects Two Samples of Potash on Electrolytes

The concentrations of Na^+(µmol/L) recorded in animals that received potash sample B(146.00 ± 1.31*) were found to be statistically significantly higher than the control(143.00 ± 1.31).On the other hand, concentration of Na+ recorded in sample B(144.50 ±2.73$^{ns)}$ is not statistically different from Na+ concentration recorded in the control(143.00 ± 1.31).This implies that between the two potash samples analyzed, Na+ content of sample A is higher even though sample B contains sodium. It is a known fact that sodium helps the body keep fluids in a normal balance[25] but high sodium consumption can raise blood pressure, and high blood pressure is a major risk factor for heart disease and stroke [26].The implication of this poses high risk of hypertension on the consumers of potash. The result obtained is agreement with findings by [27].It therefore confirms that potash (*Akanwa* or *kaun*) has high sodium content and very little potassium as reported by Imafidon et al.[28].

On the other hand, the K^+ serum concentration detected between the two groups of animals administered potash samples A(7.25 ± 1.41^{ns}) and B(6.81 ± 1.25^{ns}) shows that they are not statistically significantly different($^{ns}P>0.05$) from control(5.91 ± 0.37). Even though concentrations of serum k+ detected in both samples are not statistically different from each other, increased values of K+ recorded in sample A shows it contains more potassium than sample B .Low concentration of K+ recorded is expected since high sodium is already recorded in serum. This is due to the fact that potassium is primarily an intracellular ion, and there is a strong relationship between sodium and aldosterone, but aldosterone also increases potassium secretion.The activities of the sodium and potassium pump (Na+-K+-ATPase), which facilitates the active transport of sodium and potassium ions across the cell membrane against concentration gradients, have a significant impact on potassium homeostasis [29].The Na+-K+-ATPase is found in almost all animal cell membranes and pumps sodium ions (Na+) out of the cell while bringing potassium ions (K+) into the cell. This pump maintains the K+- balance between the ICF and ECF primarily through buffering, which involves the hydrolysis of ATP to generate energy, and for each ATP hydrolyzed, two K+ are transported inside the cell while three Na+ are pushed out[29]. This keeps the Na+ in the ECF high and the K+ in the ICF high.

Serum concentration of Chloride recorded in sample A(106.63 ± 1.51*) is not satistically significantly different from sample B (103.38 ± 1.77^{ns}) but the control (101.50 ± 1.20) . Furthermore, high serum concentration of serum bicarbonate was recorded in animals administered potash sample A (20.13 ± 2.42*) which is statistically significantly different from serum concentration of HCO_3^- recorded in animals administered sample B(18.25 ± 2.76^{ns}) and the Control(16.75 ± 1.91). The reason for more elevated bicarbonate in sample A could be associated with the fact that potash exists in sodium bicarbonate forms and on dissociation in the body fluid could release more serum bicarbonate.

Also, excess bicarbonate can lead to metabolic alkalosis resulting from loss of acidic radical (Cl-) and gain of alkali such as Na+ observed from the results of the study. According to Tinawi [30], metabolic alkalosis is caused by an increase in alkali or a decrease in acid, and it is prolonged by the kidneys' inability to excrete excess alkali. This therefore, suggests possible kidney impairment caused by chronic consumption of potash resulting in high serum bicarbonate concentration.

3.2. Effects of potash on kidney & liver function parameters

Figure 2 examined liver function tests such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), as well as two important kidney function parameters: urea and creatinine ..Although there are a few studies with prior information on the effects of potash on the above parameters, ours is a kind of confirmatory study to get an understanding of these parameters when the potash dose is 1000mg/kg for 90 days.

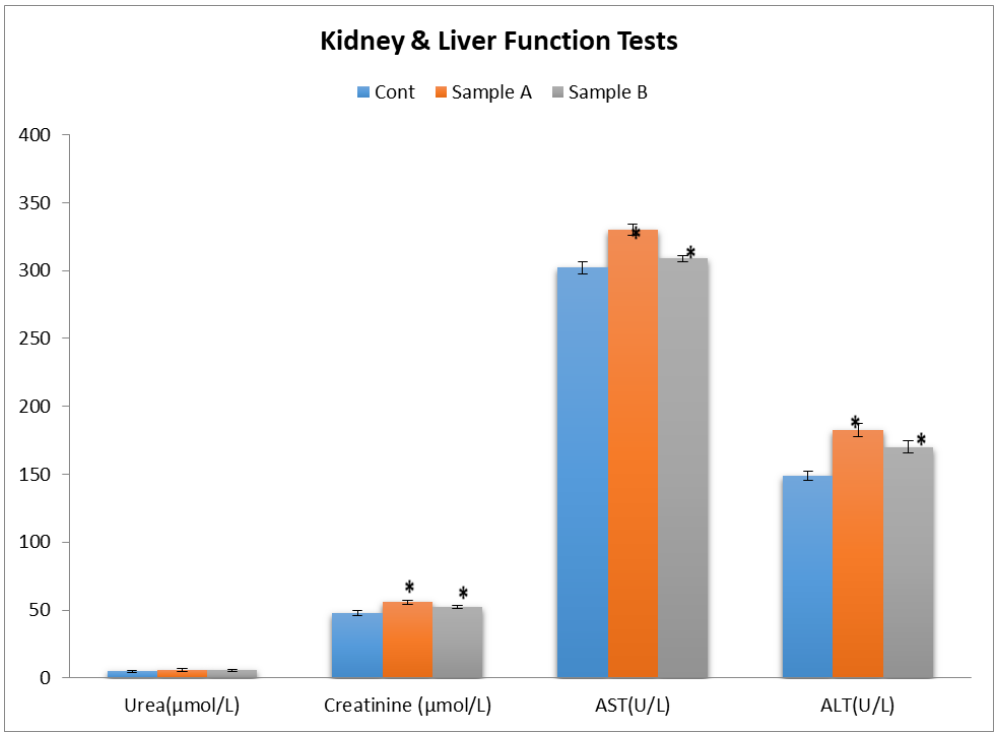


Figure 2 Graphical Representations of Effects of Potash on Kidney & Liver Function Parameters

The concentration of Urea recorded in animals administered sample A (5.66 ± 1.14^{ns}) and sample B (5.53 ± 0.82^{ns}) are not statistically different from the control(4.80 ± 0.68). The kidneys normally eliminate urea, which is created in the liver during the catabolism of amino acids and other nitrogenous metabolites, as quickly as it is produced. When renal function is weakened, blood urea values steadily rise [31].Even though that comparison of the urea recorded in among the three groups of rats are not statistically significantly different from on another, yet there is elevated urea in the samples A and and B than low urea concentration recorded in the control group. It could not be established that potash does not harm the kidneys.

According to Higgins [32], a number of non-renal factors materially affect urea production/concentration including: state of hydration ,amount of dietary, protein and liver diseases. The serum concentration of creatinine recorded in both samples A (55.75 ± 1.39*) and B (52.13 ± 1.23*) were statistically significantly higher than the control (48.13 ± 1.89). According to Wilson [33], Creatinine is a more specific indicator of renal glomerular filtration rate than BUN, which is more susceptible to non-renal influences.

When the kidneys become impaired for whatever reason, the creatinine level in the blood rises due to poor creatinine clearance by the kidneys [34]. Creatinine levels that are abnormally high indicate that the kidneys may be malfunctioning or failing. The liver parameters AST and ALT were equally elevated in the study carried out. The concentration of AST recorded in animals administered in sample A(330.25 ± 4.20*) and B

(309.00 ± 2.14*) were statistically significantly higher than concentration of AST recorded in control (302.13 ± 4.52).Similarly, Samples A (182.50 ± 5.04*) and B(170.13 ± 4.64*) recorded ALT values which are statistically significantly higher than the control(149.00 ± 3.12).This indicate therefore that both potash samples might have caused injury to liver cells leading to expression of high concentration of AST and ALT in the blood. Our findings on liver function parameters are consistent with results of a study by Okunrobo [35].

3.3. Effects of potash on haematological parameters

In figure 3, the RBCs count of animals administered samples A(4.65 ± 0.15*) and B(5.03 ± 0.42*) were statistically significantly different from RBCs count recorded in the control(6.15 ± 0.40).This may suggest that such drop in RBCs when compare to samples(A &B) might due to anemia. But one would think that more RBCs should be recorded since potash contains high iron content which plays useful role in RBCs formation. There have been reports of a significant correlation between lead poisoning and iron deficiency in children in humans [36].According to Hsieh et al., [37], chronic lead poisoning impairs hemoglobin production by interfering with enzymatic steps in the heme synthesis pathway and reduces red blood cells, increasing the risk of anemia . Lead absorption can result in iron deficiency and, in turn, anemia. Chronic lead exposure causes anemia by interfering with heme biosynthesis as well as decreasing red blood cell survival[38]. This mechanism might be responsible for decrease RBCs recorded in potash samples A and B respectively.

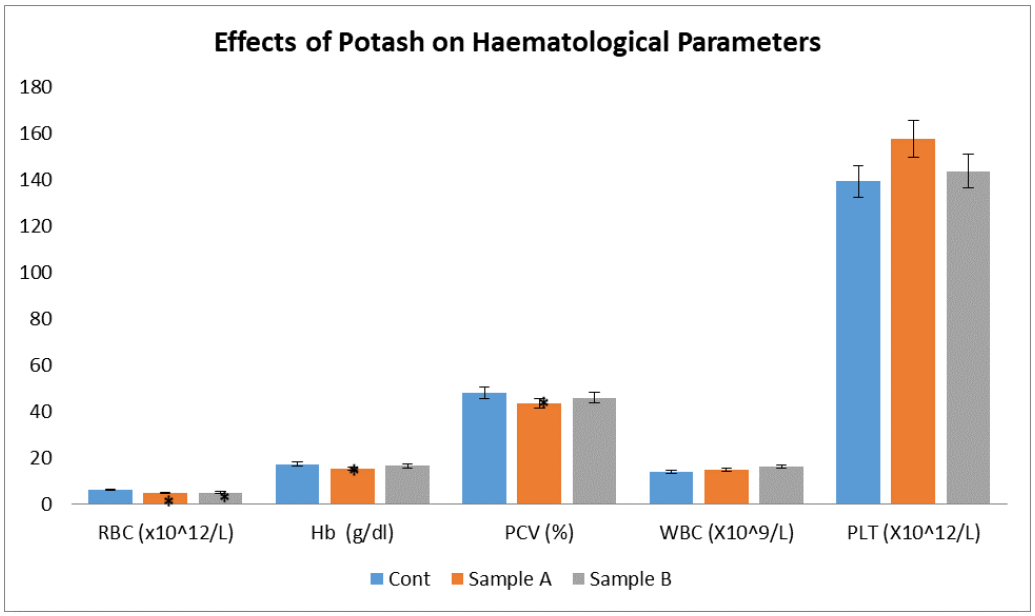


Figure 3 Graphical Representations of Effects of Potash Samples (A and B) on Haematological Parameters.

Moreover, the concentration of Hb recorded in samples A (15.33 ± 0.52*) was statistically significantly lower than samples B(16.44 ± 0.90^{ns}) and control(17.15 ± 2.06) respectively. Similar results were observed in the percentage of PCV recorded in sample A which was found to be statistically significantly ($p<0.05$)lower than the PCV recorded in sample B(45.76 ± 2.30^{ns}) when compared with the control(47.96 ± 5.69).This implies that ,even though the two potash sample decrease blood cells, sample A seems to lyse the blood cells more. The no of WBCs and Platelets recorded in rats administered each of the samples of potash were not statistically significantly different from their controls.

3.4. Histological examination of rats' tissues

Photomicrographs of the liver, heart, and kidney show no visible necrosis or structural alteration. However, photomicrograph of section of rats' stomach in fig. 4(a) shows multi-focally extensive areas of mucosal necrosis on rats administered sample A potash.

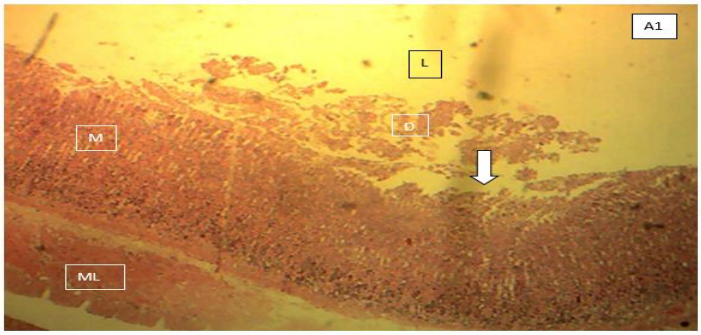


Figure 4 (a) Group (A).Photomicrograph of rat's stomach section (H&E x 40). Shows multi-focally extensive areas of mucosal necrosis (white arrow). Mucosa (M); Muscularis layer (ML); Lumen (L); Necrotic debri (D)

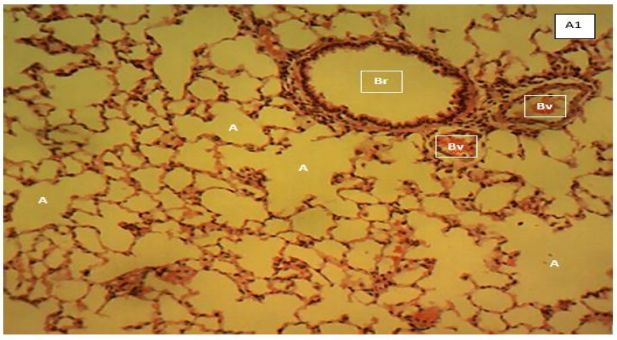


Figure 4(d) Group (A).Photomicrograph of rat's Lung section (H&E x 160). Shows normal bronchioles (Br) which opens into the alveoli (A) and expressing blood vessel (Bv).

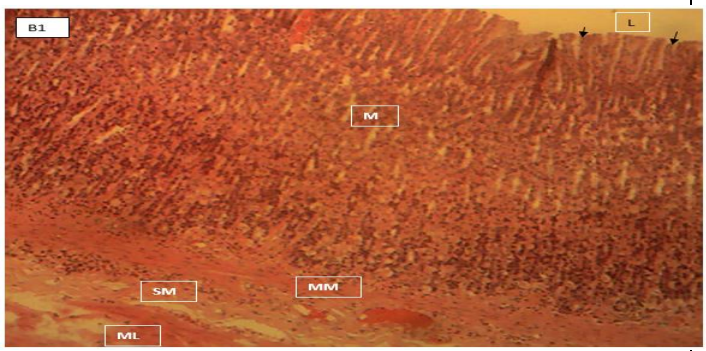


Figure 4(b) Group (B).Photomicrograph of rat's stomach section(H&E x100).Stomach section shows normal gastric histo-architecture. Gatric pits (black arrow); Mucosa (M); Muscularis mucosae (MM); Submucosa (SM); Muscularis layer (ML); Lumen (L).

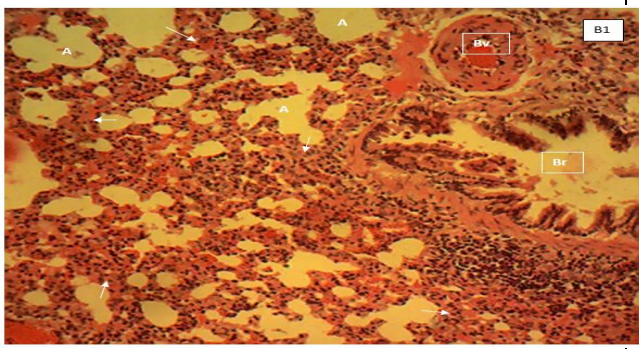
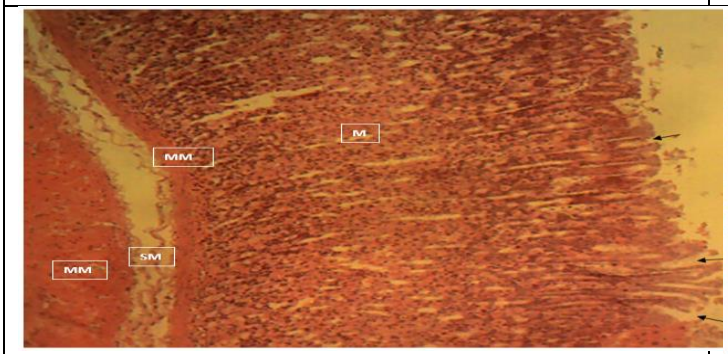


Figure 4(e) Group (B).Photomicrograph of rat's Lung section (H&E x 160).Shows a marked, widespread, interstitial pneumonia. The alveolar interstitium (arrow) were markedly thickened by inflammatory cells and varying amounts of protienaceous exudates. Bronchiole (Br); Blood vessel (Bv); Alveoli (A).



Figure(4c) Group(C, Control).Photomicrograph of rat's stomach section (H&E x100).Shows normal gastric histo-architecture. Gastric pits (black arrow); Mucosa (M); Muscularis mucosae (MM); Submucosa (SM)

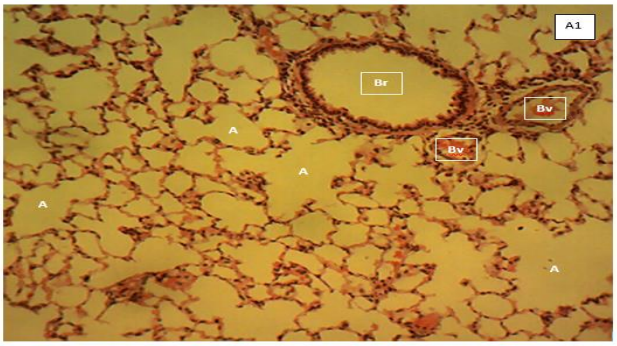


Figure 4(f)Group (C).Photomicrograph of rat's Lung section (H&E x 160).Shows normal pulmonary histomorphology. The bronchioles (Br) lined by a single layer of epithelium and open into the alveoli (A) and adjoining blood vessel (Bv).

Figure 4(a-f) Photomicrograph of rats' stomach and lung (Fig. 4a and 4e) necrotized by either type of potash.

In fig.4 (e) sample B potash caused a marked, widespread, interstitial pneumonia. The alveolar interstitium were markedly thickened by inflammatory cells and varying amounts of proteinaceous exudates. The necrosis on the stomach of rats administered potash type A could be due to highly alkaline nature of potash leading to liquefactive necrosis. As written by Judkins et al., [39]. Highly alkaline salts in its liquid form such as potash can cause serious damage to the stomach and esophagus, resulting in a condition known as liquefaction necrosis. This occurs because to the joining of

alkaline chemicals with tissue protein, generating fast damage that last until the alkaline substance is neutralized by the tissue fluids [40].Sample B caused a marked, widespread, interstitial pneumonia in the lungs, characterized by varying amounts of proteinaceous exudates. This might as a result of potash containing heaving metals such lead and chromium. Cobalt exposure can lead to a variety of lung diseases, including asthma and different interstitial patterns in the lungs [41]. The most well-known and common histological manifestation is giant cell interstitial pneumonia. Therefore, it can be assume that potash sample B(natron)contains more heavy metals than sample A(trona).

3.5. Effects of potash on weekly changes in weights of rats

Table 1 shows the effects of either type of potash on the weekly weight changes of Wistar rats. The average weekly weight changes of Wistar rats in the control group increased continuously from weeks 1–12. Although there were incremental average weekly weight changes in Wistar rats in group A, which received potash type 1 (Trona) from weeks 1–9, the weekly average weight recorded was statistically significantly lower ($p<0.05$) than the average weekly weights of rats recorded in the control group. However, average weekly weight changes recorded in potash sample A-treated rats show no statistically significant ($p > 0.05$) difference when compared to weights in the groups administered potash sample B. From weeks 10–12, the average weekly weights of the rats recorded in both potash samples (A and B) were statistically significantly ($p<0.05$) lower than the weights of rats in the control group. A careful comparison of the initial average weights of rats in all the groups and the average weights recorded at week 12 shows that samples A and B treated groups caused weight loss in the rats when compared to the control group.

Since potash is a natural salt that might be contaminated with heavy metals such as lead, it is possible that the weight loss recorded in either type of potash might be due to lead contaminants. Although lead-induced reductions in body and kidney weight in Wistar albino rats have been reported by Amjad [42]. The reduction of RBCs in rats administered both potash types can be a pointer to possible anemia. Chronic lead exposure causes anemia by disrupting the production of heme and reducing the lifespan of red blood cells. Loose stools observed in the animals during the 90-day study might have led to electrolyte loss. Depletion of electrolytes can cause rats' weight loss. Although the cause of weight loss in rats administered either of potash samples is not clearly defined, increased serum sodium levels as recorded in the study could promote water loss in rats [43].

Table 1 Effects of Potash Types (A and B) on Weekly Weight Changes in Rats

No. of Weeks		Control	Sample A	Sample B
Initial Ave. Wt.		128.55 ± 3.41	136.26 ± 2.67	134.89 ± 2.83
Week 1		3.95 ± 3.49	2.94 ± 2.79^{ns}	2.59 ± 3.95^{ns}
Week 2		5.95 ± 2.92	6.59 ± 4.35^{ns}	6.84 ± 4.36^{ns}
Week 3		8.05 ± 5.09	9.64 ± 4.24^{ns}	10.09 ± 3.00^{ns}
Week 4		10.75 ± 4.83	14.79 ± 3.58^{ns}	14.01 ± 4.52^{ns}
Week 5		15.55 ± 3.33	19.44 ± 1.33^{ns}	17.56 ± 2.61^{ns}
Week 6		18.85 ± 3.15	21.74 ± 3.51^{ns}	20.01 ± 1.85^{ns}
Week 7		23.25 ± 3.99	24.56 ± 1.46^{ns}	24.96 ± 2.98^{ns}
Week 8		26.85 ± 3.06	25.49 ± 3.42^{ns}	27.79 ± 4.33^{ns}
Week 9		33.65 ± 4.53	26.99 ± 4.60**	29.61 ± 3.05^{ns}
Week 10		37.95 ± 4.56	28.16 ± 4.08***	30.71 ± 3.27**
Week 11		43.35 ± 2.87	29.69 ± 3.77***	32.39 ± 3.50***
Week 12		46.20 ± 2.83	28.53± 3.13***	30.75 ± 2.27***

Values are presented as Mean ± SD, n=8. *P<0.05, **p<0.01, ***p<0.0005: Statistically significantly different from control. ^{ns}P>0.05: Not statistically significantly different from control.

4. Conclusion

The main types of potash samples were found to have LD50 values greater than 5000mg/kg in the preliminary acute toxicity test, suggesting that they are relatively safe for acute consumption. Based on our findings, it appears that both

forms of potash have the potential to impact the kidneys and liver, particularly when consumed on a regular basis. Also, our study shows that both potash types contained more sodium than potassium, which might result in an increase in blood pressure, especially in people who are predisposed to high blood pressure. Elevated bicarbonate could cause metabolic alkalosis or result in an electrolyte imbalance during chronic consumption. Moreover, our findings recorded a reduction in RBCs, PCV, and Hb, indicating that both types of potash samples collected from the six geopolitical regions of the country could cause anemia in humans if chronically consumed. Moreover, our study confirmed the traditional use of both potash types in weight-loss medicinal preparations. Chronic ingestion of potash sample A (trona) as a food additive in humans might result in greater weight loss compared to sample B (natron).

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

All authors disclose no competing interest.

Statement of ethical approval

Ethical approval of the study was obtained from the Animal Research Ethics Committee (AREC) of Nnamdi Azikiwe University, Awka with reference number: *NAU/AREC/2021/00009A,*

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