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Evaluation of hematological, electrolyte and lipid profiles of potash samples obtained from Anambra State, Nigeria: A comparative sub-acute study on the effects of low-dose trona and natron on Wistar rats.

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Abstract

Trona and natron are natural-occurring salts geologically referred to as evaporites and commonly known as potash. Both types of potash are most common among Nigerian users, especially as food softeners/additives and medicinal remedies. The sub-acute toxicological effects of the two primary varieties consumed in Anambra State, Nigeria, on hematological, electrolyte, kidney function, and lipid profiles at low doses remain unexplored. A 28-day sub-acute study used 120-140 g Wistar rats. Three groups of six (n=6) rats were assembled. Group A (control) received feed and water, group B received 500 mg/kg of reddish potash (natron) while group C received 500 mg/kg of greyish potash (trona). Standard methods were used in analyzing parameters. Data was analyzed with SPSS, version 27. Results were shown as mean ± SD. One-way ANOVA was performed to compare mean values. Hematological results in groups A and B reveal a significant ($p<0.05$) decrease in red blood cells and platelets and an increase in white blood cells ($p<0.05$) compared to the control. The electrolytes results recorded significant increase($p<0.05$) in Na^+ in group B while group C recorded a significant increase in K^+ and CL^- when compared to the control. Group B had a significantly higher creatinine and urea than group C and the control. The study revealed a significant increase ($p<0.05$) in total cholesterol and high density lipoprotein in group B, but not in triglycerides or low density lipoprotein. The study concludes that on chronic consumption, low dosages (10 % of LD50) of natron or trona can alter hematological parameters, electrolyte balance, renal function (particularly in natron), and lipid profile.

Keywords: Trona; Natron; Electrolytes; Lipid profile; Kidney; Blood

1. Introduction

In recent years, there has been a renewed interest in the effects of geologic materials and processes on the health of animals and people[1]. Examples of such geological mineral salts are trona and natron. Trona and natron are both evaporite minerals containing sodium carbonate, although they have distinct chemical compositions and characteristics. Trona is a carbonate mineral, whereas natron is a compound composed of sodium carbonate decahydrate and sodium bicarbonate. Both minerals occur in sedimentary evaporator deposits and are frequently linked with thermonatrite [2]. In Nigeria, either of each major types are generally referred as 'potash' by the local users. These salts are geographically dispersed across Nigeria's six geopolitical areas based on three major languages: Yoruba (*Kaun*), Igbo (*Akanwa*), and Hausa (*Kanwa)* [3].

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These salts are used in soups to soften tough meat and beans, saponification, and to improve viscosity in some soups [4][5] .According to Turner et al.[6], potash is regarded as second in importance to conventional table salt in Nigeria due to its popularity and widespread use in the home. The frequent use, especially among the locals, might be a result of its abundance, availability, affordability, and mainly economic purposes. This is because users of potash as a food softener reduce cooking time and gas, while others who use both types as additives in the preparation of medicinal remedies for coughs and contraceptives take advantage of its low cost and abundance.

Unfortunately, the growing local demand and unregulated use by food vendors raise health concerns for natural salt users. Some studies such as Tchounwou[7],Munir et al., [8] and Velarde [9] have expressed the contamination of natural salts such as trona and natron with heavy metals. If lead is present in such salts, it might accumulate and harm key organs or hematological indices. Moreover, comparison of these major types of 'potash' on electrolytes content has not been investigated at low doses. Although, studies and Katuzu et al.,[10] high sodium concentration of trona in the samples obtained in Nigeria where the studies took place, however it is unclear if this is similar in Anambra State, Nigeria. Moreover, the comparative effects of trona and natron forms of potash on kidney function and lipid profile markers at low doses have not been investigated, hence the study.

2. Materials and methods

- **Collection of Potash**: Two major potash types, trona and natron, were randomly selected from the three major markets in Anambra State: *Eke-Awka, Ekwulobia, and Nkwo Nnewi.* Anambra is one of Nigeria's 36 states. Situated in the South-Eastern region of the country, it is between 5 ° 32' and 6 °45'N and 6 °43' and 7 ° 22'E[11].
- **Animals**: The study utilized healthy young adult male and female Albino Wistar rats. Animals were nulliparous and not pregnant between 8 and 12 weeks. The optimal weight range for animals is 120-140 grams. Optimal environmental conditions were upheld, and unrestricted availability of commercially prepared rodent food and pure water was ensured.
- **Acute toxicity test(LD50):**This was carried out using modified Lorke's [12] method for each of the potash types (i.e trona and natron)**.**
- **Experimental Design:** This study used a randomized subject-control experimental design. The animals (Wistar rats) of both sexes were randomly selected and divided into the following groups:
    - Control group (A) received distilled water.
    - Group (B) received 500 mg/kg of natron (reddish potash).
    - Group (C) received 500 mg/kg of trona (greyish potash).

To make identification easier, all animals were marked with indelible ink. The animals were given an oral gavage every day for 28 days.

- **Animal Sample Size:** Animals were randomly selected and sample size was based on Charan & Kantharia [13] equation:

Corrected sample size = Sample size/ (1− [% attrition/100]). Taken 14 % attrition and 21 Wistar rats as the Corrected samples size; Sample size = 18 (i.e. n=6 per group). For possible attrition (death) within the 28 days study, one animals was added to each of the groups: A, B and C respectively. Therefore, the total animals required in the sub-acute study will be 21.

- **Preparation and administration of doses:** Potash was mixed based on types (similar types were collected from the three markets) after pulverization using the laboratory mortar and pestle. Test substances (trona and natron) were dissolved in deionized water to ensure no metal or ion contamination. Both potash types (natron and trona) were warmed at 60 ^{0}C to ward off microorganisms. Doses were freshly prepared prior to administration.

Administration of doses of the test substance was done on a daily basis and stopped after 28 days (4 weeks). Trona and natron administered to Wistar rats were based on milligrams per kilogram (mg/kg). The volume of deionized water administered at one time was dependent on the size of the test animal (i.e., 1 mL/100 g of body weight). The test potash (low dose) of 500 mg/kg was administered in a single dose daily with a suitable oral gavage such that the animals were not injured.

- **Hematological analysis:** This was carried out using Mindray BC-5300 Auto Haematology Analyser (Schenzhen, China) similar to Enemali[14] .

- **Electrolytes tests:** The serum electrolytes tested were Na+,K+,Ca^{2+}.Mg^{2+},$HCO3^-$ and Cl^- respectively. Serum electrolytes were processed using their respective kits. For sodium: Arena Bio Scien kit (BA-88A;. Arena Bio Scien, Egypt); potassium : Arena Bio Scien kit (Arena Bio Scien, kit, Egypt.) ;calcium: Randox reagent kit (BA-88A Randox reagent kit, United Kingdom);magnesium: Teco reagent kit (BA-88A.; Teco reagent kit, United State of America); Bicarbonate: Agappe reagent kit (BA-88A.; Agappe reagent kit, India) while Serum Chloride content was calorimetrically determined with Mindray chroride reagent(BA-88A**:** Mindray, China). All were read with Mindray (BA-88A) Semi-Auto chemistry analyzer similar to Muhammad et al., [15].
- **Renal function tests:** Serum urea and creatinine content was calorimetrically, determined using Randox reagent kits (BA-88A,; Randox reagent kit, United Kingdom) similar to El-Ishaq et al.[16].
- **Lipid profile test.** The serum was isolated from the whole blood by centrifugation. The whole blood was allowed to clot at room temperature for 15–30 minutes, followed by centrifugation at 1,000–2,000 x g for 10 minutes in a refrigerated centrifuge to separate the components. For *cholesterol:* A 5 µL serum was mixed with 500 µL cholesterol reagent (80 mmol/L Pipes buffer, pH 6.8, 0.25 mmol/L 4-aminoantipyrine, 6 mmol/L phenol, 0.5 U/mL peroxidase, 0.15 U/mL cholesterol oxidase, and 0.1 U/mL cholesterol esterase). After 10 minutes at room temperature, the Mindray Semi-Autochemistry Analyzer (BA-88, Shenzhen, China) was used to detect total cholesterol at 546 nm. *Triglyceride:* A mixture of 5 µL serum and 500 µL triglyceride reagent (Pipes buffer 40 mmol/l (pH 7.6), 5.5 mmol/L 4-chlorophenol, 17.5 mmol/L Magnesium ions, 0.5 mmol 4-aminophenazone, 1.0mmol/L ATP, Lipases, glycerol-kinase, glycerol-3-phosphate oxidase, peroxidase). After standing at room temperature for 10 minutes, the triglyceride content was measured at 546 nm using an auto chemistry analyzer calibrated at 210 mg/dl. *Low Density Lipoproteins (LDL*): A 200 µL serum was added to 500 µL of polyvinyl sulphate reagent and left to stand for 10 minutes at room temperature. It was centrifuged at 4000 rpm for 10 minutes using an Axiom C-90 bench centrifuge (USA). The supernatant (100 µL) was combined with 500 µL of cholesterol reagent and allowed to stand for 10 minutes before the LDL concentration was determined using an auto chemistry analyzer. *High density Lipoprotein (HDL)*: The serum (200 µL) was combined with 500 µL of Mindray HDL reagent and left for 10 minutes at room temperature. It was centrifuged at 4000 rpm for 10 minutes on an Axiom C-90 bench centrifuge (USA). The supernatant (100 µL) was combined with 500 µL of cholesterol reagent and allowed to stand for 10 minutes. The HDL concentration was determined using an auto chemical analyzer.
- **Determination of pH of Trona and Natron at Varied Temperatures:** The pH meter was calibrated with three buffer solutions (pH 4.0, 7.0, and 10). The process was similar to A 1000 mg of trona and natron potash types were dissolved in 50 ml of deionized water each. The initial pH was taken at 0 ^oC, followed by pH measurements at 20 ^oC, 40 ^oC, 60 ^oC, 80 ^oC, and 100 ^oC.

2.1. Statistical Analysis

Results were presented as mean ± standard deviation. Statistical package for social sciences (SPSS) version- 27) was used for data analysis. Statistical comparison between potash treated groups and control was made using one-way analysis of variance (ANOVA) followed by Dunnet test, which compared sample B and C against control group.

3. Results

Table 1 LD_{50}s of Natron (B) and Trona (C) Using Modified Lorke's (1983) Method

Phases	Dose(mg/kg)	Onset of Toxicity (hour)	Mortality	Remarks
Phase I	10	-	0/3	No visible sign of toxicity
	100	_	0/3	No visible sign of toxicity
	1000	-	0/3	No visible sign of toxicity
Phase II	2000	-	0/1	No visible sign of toxicity
	3000	-	0/1	No visible sign of toxicity
	4000	-	0/1	No visible sign of toxicity
	5000	Sample B: 17. 30-to 24. 48 hours. SampleC:19.12-to 26.15 hours	0/1	Soft stool from rats in samples B and C was observed

LD50> 5000 mg/kg.

Table 2 Effects of low dose potash types (natron and trona) on red blood cells and its parameters

Group	RBC (10^12/L)	HGB (g/dl)	HCT (%)	MCV (fL)
Sample A	7.02 ± 1.54	15.67 ± 9.03	37.95 ± 8.44	50.73 ± 16.69
Sample B	5.00 ± 1.14*	10.26 ± 1.92^{ns}	28.87 ± 5.84^{ns}	53.62 ± 3.04^{ns}
Sample C	6.15 ±0.24^{ns}	11.37 ± 0.95^{ns}	36.50 ± 6.79^{ns}	54.95 ± 0.75^{ns}

Values are presented as mean ±Standard Deviation, n = 6. nsP>0.05: Not statistically significantly different from sample A. *P<0.05: Statistically significantly different from sample A; Key: Sample A(Control), Sample B(natron), Sample C(trona)

Table 3 Effects of low dose potash types (natron and trona) red blood cell parameters (Contd.)

Group	MCH (pg)	MCHC (g/dl)	RDW CV (%)	RDW SD (Fl)
Sample A	18.42 ±1.84	32.37 ± 2.37	18.67 ± 1.49	34.20 ± 1.89
Sample B	18.70 ± 0.53^{ns}	34.15 ± 1.09^{ns}	18.67 ± 0.82^{ns}	30.72 ± 1.41**
Sample C	18.68 ± 0.33^{ns}	33.98 ± 0.73^{ns}	17.52 ± 0.72^{ns}	29.95 ± 1.56**

Values are presented as mean ±Standard Deviation, n = 6. ^{ns}P>0.05: Not statistically significantly different from sample A. *P<0.05, **P<0.01: Statistically significantly different from sample A. ^{a}P<0.05, ^{aa}P<0.01: Statistically significantly different from sample B.

Table 4 Effects of low dose potash types (natron and trona) on white blood cell and its parameters

Group	WBC (10^9/L)	LYM (%)	MID (%)	GRAN (%)
Sample A	9.05 ± 2.73	79.28 ± 4.39	11.73 ± 3.65	7.62 ± 1.98
Sample B	6.92 ± 2.00	79.27 ± 2.12^{ns}	10.90 ± 1.80^{ns}	8.23 ± 1.12^{ns}
Sample C	13.20 ± 1.22**^{aa}	81.15 ± 2.56^{ns}	9.27 ± 0.69^{ns}	7.12 ± 1.24^{ns}

Values are presented as mean ±Standard Deviation, n = 6. ^{ns}P>0.05: Not statistically significantly different from sample A. *P<0.05, **P<0.01: Statistically significantly different from sample A. ^{a}P<0.05, ^{aa}P<0.01: Statistically significantly different from sample B.

Table 5 Effects of low dose potash types (natron and trona) on Platelets

Group	PLT(10^9/L)	MPV(fL)	PDW(fL)	PCT (%)	P LCR (%)	P LCC (10^9/L)
Sample A	566.33 ± 85.00	5.72 ± 0.73	6.72 ± 0.68	0.29 ± 0.08	3.95 ± 1.20	22.83 ± 5.42
Sample B	460.67 ± 25.06*	5.35 ± 0.30^{ns}	6.17 ± 0.70^{ns}	0.21 ± 0.06^{ns}	3.82 ± 0.79	17.25 ± 2.23
Sample C	612.83 ± 51.40^{a}	5.63 ± 0.37^{ns}	6.68 ± 0.41^{ns}	0.35 ± 0.05^{ns}	6.00 ± 0.18^{*aa}	37.17 ± 3.66^{**aa}

Values are presented as mean ±Standard Deviation, n = 6. ^{ns}P>0.05: Not statistically significantly different from sample A. *P<0.05, **P<0.01: Statistically significantly different from sample A. ^{a}P<0.05, ^{aa}P<0.01: Statistically significantly different from sample B.

Table 6 Effects of low dose potash types (natron and trona) on body weight

	Body weight (g)					
Group	**Week 0**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Weight gain (%)**
Sample A	122.93 ± 3.51	129.40 ± 5.09	136.45 ± 3.28	139.55 ± 4.74	142.97 ± 4.51	13.96 ± 3.05^{ns}
Sample B	121.48 ± 1.95	124.18 ± 2.58^{ns}	124.17 ± 7.53*	137.80 ± 8.53*	136.12 ± 3.00*	10.73 ± 1.85^{ns}
Sample C	122.70 ± 2.88	126.95 ± 3.67 ^{ns}	131.23 ± 10.38^{ns}	133.33 ± 5.98^{ns}	137.78 ± 4.00^{ns}	10.88 ± 3.38^{ns}

Values are presented as mean ± Standard Deviation, n = 6. ^{ns}P>0.05: Not statistically significantly different from sample A. *P<0.05: Statistically significantly different from sample A.

Table 6 Effects of pH of low dose potash types (natron and trona) on Temperature Changes

pH at varied temperatures	**Samples**	**Average pH**	**p-value**	**Decision**
pH at 0℃	Sample A	8.52 ± 0.38	0.953	Not significant
	Sample B	8.51 ± 0.36		
pH at 20℃	Sample A	8.69 ± 0.29	0.834	Not significant
	Sample B	8.67 ± 0.28		
pH at 40℃	Sample A	8.75 ± 0.27	0.771	Not significant
	Sample B	8.78 ± 0.18		
pH at 60℃	Sample A	8.80 ± 0.26	0.805	Not significant
	Sample B	8.82 ± 0.19		
pH at 80℃	Sample A	8.82 ± 0.27	0.637	Not significant
	Sample B	8.87 ± 0.20		
pH at100℃	Sample A	8.87 ± 0.28	0.734	Not significant
	Sample B	8.91 ± 0.21		

Statistically analyses by independent student's t-test revealed no significant difference (p>0.05) in pH of sample A and B at various temperatures ranging from 20 ℃ to 100 ℃.

Table 8 Effects of low dose potash types (natron and trona) on electrolytes

Samples	**K+ (mEq/L)**	**Na+ (mEq/L)**	**Cl- (mEq/L)**	**HCO3 (mEq/L)**
Sample A	5.08 ± 0.25	65.84 ± 15.27	68.39 ± 5.19	17.88 ± 5.08
Sample B	31.77 ± 11.62***	130.50 ± 16.24***	73.24 ± 5.39ns	17.22 ± 3.05ns
Sample C	33.11 ± 12.21***	74.45 ± 29.16aa	80.63 ± 5.79**	19.34 ± 4.70ns

Values are presented as mean ± Standard Deviation, n = 6. ^{ns}P>0.05: Not statistically significantly different from sample A. *P<0.05,**P<0.01,***P<0.005: Statistically significantly different from sample A. ^{aa}P<0.01: Statistically significantly different from sample B.

3.1. Effects of low dose potash types (natron and trona) on urea and creatinine

Group	Urea (mg/dl)	Creatinine (mg/dl)
Sample A	10.81 ± 0.69	0.88 ± 0.51
Sample B	14.62 ± 1.24*	1.35 ± 0.13*
Sample C	12.97 ± 3.42^{ns}	1.16 ± 0.13^{ns}

Values are presented as mean ± Standard Deviation, n = 6. ^{ns}P>0.05: Not statistically significantly different from sample A. *P<0.05: Statistically significantly different from sample A.

Table 10 Effects of low dose potash types (natron and trona) on lipid profile

Group	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Sample A	107.10 ± 13.31	76.80 ± 26.68	26.90 ±8.15	66.60 ± 15.01
Sample B	137.43 ± 10.59**	95.23 ± 24.25^{ns}	45.40 ± 5.44**	72.67 ± 10.82^{ns}
Sample C	111.90 ±6.40^{aa}	113.43 ± 24.39^{ns}	29.92 ± 3.14^{aa}	60.92 ± 10.64^{ns}

Values are presented as mean ±Standard Deviation, n = 6. ^{ns}P>0.05: Not statistically different from sample A. **P<0.01: Statistically different from sample A.

4. Discussion

The acute toxicity study (LD50) in Table 1 of the preliminary studies shows that the two main potash types (natron, Sample B, and trona, Sample C) were greater than 5000 mg/kg, indicating that acute exposure is unlikely to cause harm even though rats in both groups had loose stools from 17 hours after administration. The LD50 results align with Imafidon et al. [17].Results of the effects of low dose potash types (natron and trona) on red blood cells and its parameters in Tables 2 and 3 suggests that samples(B) of natron collected from the three major markets in Anambra State could cause reduction of the red blood cells even at low dose of 500 mg/kg. The RBCs and RDW SD recorded in Wistar rats administered natron were found to be statistically significantly ($p<0.05$) lower than samples A (control) and C (trona).This indicate possible anemia, since anemia is characterized by a decrease in the production of red blood cells,[18], [19].

In Table 4, there was statistically significant increase ($p<0.05$) in WBCs in sample C (trona) when compared to samples A and B. This implies that trona, even at 500 mg/kg would cause immune stimulatory effects leading to elevated WBCs. According to Chmielewski et al., [20], total leukocyte count rises considerably in response to infection, trauma, inflammation, and some disorders. Moreover, in Table 5, there was a statistically significant decrease ($p<0.05$) in platelet count caused by natron (sample B) but increased platelet counts in Wistar rats administered trona (sample C) when compared to the control (sample A). This might be as a result of damaged hematopoietic stem cells which age into self-reactive T-helper cells (T1) that release IFN and TNF to start a cytotoxic cascade that kills and suppresses other stem cells, which might result in thrombocytopenia[21] in sample B (natron) recipients. Although the mechanism is unclear, natron might have a similar drug-induced thrombocytopenia effect as amiodarone, captopril, and sulfonamides. As recorded in sample C of Table 5, thrombocytosis-inducing natural salts such as trona might have both favorable and unfavorable consequences. Chemicals that cause an increased platelet count can aid in blood clotting and wound healing but can be harmful if co-administered with drugs or chemicals producing similar effects. Chemicals that cause thrombocytosis may increase the platelet count and risk blood clots[22],especially on chronic consumption.

Table 7 demonstrates that there were no statistically significant changes ($p > 0.05$) in the pH of low-dose potash types (natron and trona) when temperature changed. However, our findings indicated that both natron and trona are alkaline, as evidenced by pH ranges. The comparison of the effects of two types of low-dose potash (natron and trona) on electrolytes in Table 8 shows that Trona is much safer. The serum Na+ content of Wistar rats treated with trona was significantly lower ($p<0.0$) than trona (Sample B). It is also richer in potassium and chloride when compared to natron (sample B). In Table 9, sample B showed a significant increase ($p<0.0$) in creatinine and urea levels compared to samples A and C, indicating potential kidney impairment with prolonged exposure. Renal failure is characterized by an increase in serum urea and creatinine levels[23]. Comparative effects of low-dose potash types (natron and trona) on lipid profiles, as shown in Table 10, indicate that sample B is statistically significantly (**P<0.01) higher than samples A and C in total cholesterol and HDL contents. High HDL cholesterol levels are often related with a lower risk of coronary heart disease. However, recent research suggests that very high HDL levels may not provide additional protection and may

even accelerate atherosclerosis[24]. This therefore indicates that trona (sample C) is more heart-friendly than sample B, having lower sodium, higher potassium, and low LDL-C.

5. Conclusion

Using Wistar rats, the study successfully compared the effects of two major types of potash commonly used as food softeners, additives, and herbal preparations in Anambra State, Nigeria. The findings of the study show that even at low doses of potash (500 mg/kg), representing 10 % of the LD50, visible effects on acute consumption abound. Natron (Sample B) caused a reduction in RBCs, implying possible anemia from chronic consumption. Although both potash types have strong effects on WBCs and platelets, sample C (trona) has more stimulatory effects on WBCs and platelets. Comparative effects on electrolytes show that both, on chronic consumption, can cause electrolyte imbalance. However, trona contains high potassium but low sodium. Chronic consumption of natron at a low dose (500 mg/kg) will eventually impair the kidney, while low-dose consumption of trona might not. Furthermore, both have good alkaline properties, as an increase in temperature does not have any significant effect on their pH values. Trona has a better lipid profile than natron. Therefore, low-dose consumption of trona, even below 500 mg/kg on irregular bases, might be heart-friendly.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The Animal Research Ethics Committee (AREC) on Animal Use Policy at Nnamdi Azikiwe University, Awka, approved the study with approval number NAU/AREC/2023/00071. Furthermore, all other protocols in the study strictly followed the OECD (2001) guidelines for research involving animal use.

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