Journal of Nutrition & Intermediary Metabolism 6 (2016) 22–25

Contents lists available at ScienceDirect

Journal of Nutrition & Intermediary Metabolism

journal homepage: http://www.jnimonline.com/

Toxicological and biochemical investigations in rats administered "kaun" (trona) a natural food additive used in Nigeria

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ARTICLE INFO

Article history:
Received 22 February 2016
Accepted 23 May 2016
Available online 1 June 2016

Keywords:
Trona
Acute toxicity
Liver
Kidney
MDA

ABSTRACT

Trona, a geological mineral, is often used as a natural food additive in many parts of Nigeria. This work was done to evaluate trona for metal content, acute toxicity and biochemical effects on vital organs such as the liver and the kidney. Consequently, graded doses of 10, 100, 1000, 1500 and 5000 mg trona per kg body weight were administered to determine their effects on body weight changes, relative organ weight, acute toxicity, liver and renal function indices and oxidative status of rats. Elemental analyses revealed the presence of high levels of sodium and iron, the presence of heavy metals such as cadmium, zinc and lead were also detected. There were losses in weights only at the 5000 mg/kg dose levels; relative liver and kidney weights were not affected. Acute toxicity tests recorded no mortality and no visible sign of toxicity. There were significant increases in ALT, AST and ALP activities at all dose levels except at the 10 mg/kg dose level. Liver MDA levels were significantly increased while catalase and SOD activities were significantly reduced in all the test rats compared with control. Kidney MDA levels were only affected at dose levels 5000 mg/kg; kidney SOD and catalase activities were not significantly affected. Creatinine, sodium and potassium levels were also not affected. These results show that trona may elicit toxic effects on the liver on prolonged administration, however no toxic effect was observed on the kidney within the duration of this study.

1. Introduction

Nigeria's government revitalized concern in the exploration of solid minerals in the past, could perhaps explain the reason for the use of naturally occurring inorganic substances (salts) by the people for various purposes (Aribido et al., 2001) [1]. One of such geological mineral is "kaun" erroneously called potash although it contains low amount of potassium compared to sodium. Kaun (trona) is the second most commonly used salt in Nigeria. In Hausa, it is called "kanwa."

Trona is a dry lake salt and it is largely a hydrated sodium carbonate (Oyeleke and Morton, 1981) [2]. It is a sesquicarbonate containing Na_2CO_3 and $NaHCO_3$ in equimolar proportion. Trona is a natural food additive, its main uses are in cooking tough food material such as skins, bones, beans, maize e.t.c., and it is also used in the preparation of a delicacy called owo in Edo and Delta States of Nigeria. Studies involving rats have indicated that high levels of trona in cooked foods and drinking water may be detrimental to health (Oyeleke, 1988) [3] and hemolytic to human (Sodipo et al., 1993) [4].

The toxic nature of trona was observed in nursing mothers around Zaria and Malumfashi areas of Northern Nigeria as far back as 1974; Davidson et al. (1974) [5] reported peripartum cardiac failure in foetus, forty days after birth. Bamaiyi and Momoh, (2010) [6] reported stomach upset and diarrhea in individuals after administration of high concentration of trona. A food additive is only approved for human consumption after studying its acute, subacute and chronic toxicity. To the best of our knowledge, no work has been done on the acute and sub-acute toxicity of this additive. This work was done to bridge this gap in knowledge.

2. Materials and methods

2.1. Source of materials

Trona was purchased from Uselu market, Benin City, Edo State, Nigeria and identified by Dr O.I. Imasuen, Department of Geology, University of Benin, Nigeria.

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http://dx.doi.org/10.1016/j.jnim.2016.05.003

2.2. Metal content analyses

Calcium and magnesium were determined using complexometric method as outlined in AOAC (1984) [7]. The Corning 421 flame emission photometer was used for the estimation of sodium and potassium content (Tietz, 1995) [8]. Zinc, iron, cadmium, manganese, lead and copper were determined using the Perkin-Elmer model 403 Atomic Absorption Spectrophotometer (Skoog, 2007) [9].

2.3. Animals

Male albino rats of wistar strain were obtained from the Animal House of the Department of Anatomy, University of Benin, Nigeria. The animals were acclimatized for two weeks; they were fed commercially formulated rat feed and water ad libitum. The principles of laboratory animal care (NIH publication, 1985) [10] were followed.

2.4. Chemicals

All chemicals used were of the analytical grade. Kits used for ALT, AST, ALP, total protein, albumin, total bilirubin, urea and creatinine were obtained from Randox Laboratories (Crumlin, Co Anthrim, Spain). Others were products of BDH Laboratories (BDH Chemicals Limited, Poole, England).

2.5. Acute toxicity determination

The Lorke,s method (1983) [11] was used. The procedure was conducted in two phases. In the first phase, nine rats were divided into three groups of three rats each. The groups were administered 10, 100 and 1000 mg trona (i.p) per kg body weight of rats. All the rats were kept under the same conditions and monitored for toxicity signs or mortality for 24 h. In the second phase, a total of three rats were used. The rats were divided into three groups of one rat each and were administered 1500, 3000 and 5000 mg/kg (i.p). They were also observed for toxicity signs and mortality after 24 h.

2.6. Sub-acute toxicity studies

A total of thirty-six albino rats were divided into six groups of six rats each. The first group serves as the control while the remaining five groups were administered 10, 100, 1000, 1500 and 5000 mg trona per kg body weight of rats (i.p). All animals were allowed free accesses to food and water. Weekly measurements of weights were recorded.

2.6.1. Blood sample collection

At the end of the treatment, blood samples were collected by direct cardiac puncture into sterile containers with or without anticoagulant.

2.6.2. Biochemical analysis

Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total proteins, albumin, total bilirubin, urea and creatinine were determined colorimetrically by using the standard ready-to-use kits and methods of Randox laboratories. The manufacturer's instructions for each biochemical parameter were strictly followed in the course of the investigation. Serum sodium and potassium was estimated using ion selective electrode (an automated method). Catalase activity was determined by the method of Sinha, (1972) [12], superoxide dismutase (SOD) by the method of Misra and Fridovich, (1972) [13] while malondialdehyde levels were measured by the method of Gutheridge and Wilkin, (1982) [14].

2.7. Statistical analysis

All data were expressed as mean ± SEM (n = 6). One way analysis of variance was used to test for difference among all the groups. Dunnett; s multiple range test was used to test for significant differences among the means, A p-value of <0.05 was considered statistically significant.

3. Results

The results of the metal content analyses of trona showed that it contain a high concentration of sodium and iron. Heavy metals such as cadmium, zinc and lead were also present.

Table 2 shows the effects of trona on body weight changes and relative organ weight. Groups of animals administered 10, 100 and 1000 mg/kg did not differ significantly in body weight gain when compared with the control, Animals administered 1500 mg/kg had significant reduction in body weight gain. Losses in weights were observed in rats administered 5000 mg/kg. Organ body weight ratios were not significantly affected in the test rats compared with the control.

Table 3 show the effect of graded doses of trona on acute toxicity of rats. No significant toxic effects were observed. Administration of trona to rats up to 5000 mg/kg resulted in no mortality of the test rats after 24 h. Hence the LD_{50} of trona is estimated to be greater than 5000 mg/kg.

ALT, AST and ALP activities were significantly increased at the 100, 1000, 1500 and 5000 mg/kg dose levels (Table 4). There were non-dose dependent effects on albumin and total bilirubin levels although their values were consistently high at the 5000 mg/kg dose levels.

Table 5 shows the effect of graded doses of trona on renal function indices; there were non-dose dependent increase in total protein levels at the 1000, 1500 and 5000 mg/kg dose levels. Creatinine, urea, sodium and potassium levels were not significantly affected.

There were significant increases in liver MDA levels and significant reduction in catalase and SOD activities of test rats compared with the control (Table 6).

Kidney MDA levels were significantly increased only at dose levels 5000 mg/kg. Catalase and SOD activities were not significantly affected (Table 7).

4. Discussion

Work done on the metal content analysis of trona (Table 1) revealed high sodium content followed by iron and relatively low levels of magnesium and calcium. Cadmium, zinc and lead were

Table 1
Metal content analysis of trona.

Element	Concentration (%)
Potassium	0.22 ± 0.01
Magnesium	0.02 ± 0.00
Calcium	0.05 ± 0.00
Sodium	23.47 ± 3.07
Zinc ($\times 10^{-3}$)	0.05 ± 0.06
Iron ($\times 10^{-3}$)	1.29 ± 0.27
Cadmium ($\times 10^{-3}$)	0.10 ± 0.01
Manganese ($\times 10^{-3}$)	0.30 ± 0.00
Lead ($\times 10^{-3}$)	0.10 ± 0.00
Copper ($\times 10^{-3}$)	0.57 ± 0.01

Results are expressed as mean ± sem.

Table 2
Effects of trona on body weight change and relative organ weights of rats.

Treatment	Wt change (g)	Liver/b wt ($\times 10^{-3}$)	Kidneys/b wt ($\times 10^{-3}$)
Control	30.20 ± 4.78[a]	30.40 ± 2.00[a]	8.70 ± 0.50[a]
10	27.34 ± 1.52[a]	32.40 ± 1.80[a]	6.80 ± 0.60[a]
100	31.90 ± 7.37[a]	33.00 ± 1.90[a]	7.60 ± 0.20[a]
1000	28.33 ± 5.94[a]	35.50 ± 2.90[a]	6.00 ± 0.40[a]
1500	8.10 ± 8.75[b]	29.10 ± 0.80[a]	6.00 ± 0.10[a]
5000	−9.63 ± 4.86[c]	27.70 ± 0.70[a]	7.60 ± 0.10[a]

Results are expressed in mean ± sem (n = 6). Values with different superscripts are significant ($p < 0.05$).

Table 7
Effects of trona administration on the kidney oxidative status.

Treatment	MDA ($\times 10^{-4}$)	Catalase (unit/mg)	SOD (unit/mg) $\times 10^{-3}$
Control	2.06 ± 0.60[a]	19.48 ± 0.87[a]	0.18 ± 0.05[a]
10 mg/kg	2.77 ± 0.16[a]	17.00 ± 0.76[a]	0.14 ± 0.03[a]
100 mg/kg	2.84 ± 0.33[a]	14.66 ± 0.24[a]	0.13 ± 0.02[a]
1000 mg/kg	2.82 ± 0.12[a]	17.75 ± 0.46[a]	0.16 ± 0.04[a]
1500 mg/kg	3.53 ± 0.26[ab]	19.93 ± 2.16[a]	0.19 ± 0.02[a]
5000 mg/kg	4.67 ± 0.72[b]	14.60 ± 0.31[a]	0.13 ± 0.03[a]

Results are expressed as mean ± SEM. (n = 6). Values with different superscripts are significant at $p < 0.05$.

Table 3
Results of acute toxicity test on rats administered trona.

Treatment	No of rats	Mortality recorded	Mortality rate	Observation
First phase				
10 mg/kg	3	Nil	–	No visible sign of toxicity
100 mg/kg	3	Nil	–	“
1000 mg/kg	3	Nil	–	“
Second phase				
1500 mg/kg	1	Nil	–	No visible sign of toxicity
3000 mg/kg	1	Nil	–	“
5000 mg/kg	1	Nil	–	“

Table 4
Effects of administration of trona on some liver function indices.

Treatment	ALT (u/l)	AST (u/l)	ALP (u/l)	Albumin (g/l)	T.bil (μmol/l)
Control	3.33 ± 0.74[a]	7.58 ± 1.26[a]	28.86 ± 2.05[a]	33.20 ± 0.36[a]	2.57 ± 0.03[a]
10 mg/kg	5.57 ± 1.07[a]	8.27 ± 0.39[a]	23.12 ± 0.94[a]	35.70 ± 0.32[a]	2.40 ± 0.04[a]
100 mg/kg	8.52 ± 1.52[b]	15.96 ± 1.13[b]	34.75 ± 0.07[c]	51.20 ± 1.09[b]	5.82 ± 0.13[b]
1000 mg/kg	9.90 ± 1.74[b]	17.11 ± 1.90[b]	34.96 ± 4.31[c]	61.10 ± 0.89[b]	3.60 ± 0.07[a]
1500 mg/kg	12.94 ± 2.46[c]	19.79 ± 1.59[bc]	36.54 ± 2.56[c]	47.40 ± 0.45[a]	3.94 ± 0.08[a]
5000 mg/kg	20.24 ± 2.51[d]	23.36 ± 2.09	39.75 ± 4.93[c]	68.10 ± 0.89[b]	10.62 ± 0.09[c]

Results are expressed as mean ± SEM (n = 6). Values with different superscripts are significant at $p < 0.05$.

Table 5
Effects of trona administration on renal function indices.

Treatment	T.prot. (g/l)	Urea (g/l)	Creat. (g/l)	Na (mmol/l)	K (mmol/l)
Control	64.80 ± 5.25	4.40 ± 0.10[a]	0.63 ± 0.06[a]	137.67 ± 5.09[a]	4.93 ± 0.35[a]
10 mg/kg	59.10 ± 3.11[a]	4.00 ± 0.35[a]	0.60 ± 0.10[a]	134.67 ± 5.03[a]	4.27 ± 0.25[a]
100 mg/kg	68.80 ± 4.47[a]	4.36 ± 0.68[a]	0.60 ± 0.04[a]	137.33 ± 1.15[a]	3.53 ± 0.12[a]
1000 mg/kg	95.10 ± 7.34[b]	5.00 ± 0.35[a]	0.61 ± 0.09[a]	132.67 ± 4.62[a]	5.00 ± 0.26[a]
1500 mg/kg	81.00 ± 5.60[ab]	4.63 ± 0.89[a]	0.67 ± 0.12[a]	135.33 ± 1.53[a]	4.60 ± 0.60[a]
5000 mg/kg	108.10 ± 8.25[b]	5.33 ± 0.44[a]	0.70 ± 0.03[a]	135.00 ± 4.00[a]	4.03 ± 0.60[a]

Results are expressed as mean ± SEM (n = 6). Values with different superscripts are significant at $p < 0.05$.

Table 6
Effects of trona administration on the liver oxidative status.

Treatment	MDA ($\times 10^{-4}$)	Catalase (unit/mg)	SOD (unit/mg) $\times 10^{-3}$
Control	1.84 ± 0.21[a]	52.28 ± 2.05[a]	36.90 ± 5.00[a]
10 mg/kg	8.28 ± 0.28[b]	40.44 ± 2.71[b]	25.40 ± 2.00[b]
100 mg/kg	6.54 ± 0.67[b]	28.75 ± 3.64[b]	20.80 ± 4.00[b]
1000 mg/kg	7.81 ± 0.10[b]	27.32 ± 3.48[b]	19.20 ± 2.00[b]
1500 mg/kg	7.85 ± 0.82[b]	30.04 ± 4.02[b]	21.80 ± 3.00[b]
5000 mg/kg	9.26 ± 0.81[b]	24.50 ± 3.01[b]	19.50 ± 1.00[b]

Results are expressed as mean ± SEM. (n = 6). Values with different superscripts are significant at $p < 0.05$ (MDA in moles/mg wet tissue).

also present. The presence in food and drugs of metals such as cadmium, zinc and lead which are injurious to health is regulated by law. These metals are known as heavy metals and are only allowed in trace amounts in food and drugs (Okunrobo et al., 2012) [15]. Toxicological assessment of rats administered graded doses of trona revealed significant reduction in weight gain of rats administered 1500 mg/kg and losses in weights of rats administered 5000 mg/kg (Table 2). This indicates that at these doses, this additive may be toxic especially when administered on long term basis.

Acute toxicity studies (Table 3) revealed no mortality and no visible sign of toxicity. Acute toxicity is toxicity elicited as a result of short term exposure to a toxicant. The LD_{50} was not calculated because there was no mortality at 5000 mg/kg dose level indicating that it is relatively safe under short term exposure. However, acute toxicity data are of limited clinical application since cumulative toxic effects do occur even at low doses. Sub-acute and chronic toxicity are useful in evaluating the safety profile of additives.

The enzymatic activity of ALT, AST and ALP were studied to evaluate liver malfunctions (Table 4). Significant increases in ALT, AST and ALP activities were observed in the test rats at all dose levels, except at the 10 mg/kg dose level compared with control.

This indicates that at these dose levels, there is a possibility of liver damage on long term exposure. Increases in ALP activities have been reported on administration of trona (Ajiboye et al., 2013) [16].

Ajiboye et al. (2015) [16] also reported a dose dependent reduction in the activities of SOD and Catalase in the liver of male wistar rats. This is consistent with the report of this study. The effect of trona on the kidney is not so pronounced; as most markers of renal function were not significantly affected.

5. Conclusion

No short term toxic effect was observed on administration of trona, there was also no toxic effect on renal function within the duration of this study. Results on liver oxidative status and function indicate the potential hepatotoxic effect of trona.

Conflict of interest statement

We declare that we have no conflict of interest.

Authors contribution

The conception and design of the study, interpretation of data, drafting the article and final approval of the version to be submitted was done by K. E. Imafidon; Iriagbonse D. E. and Omoregie I.P. were responsible for the acquisition of data and analysis and also contributed to the interpretation of data.

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