



Subacute Toxicity of Wood Ash (Toka) on Behaviour, Haematological and Biochemical Parameters of Wistar rat

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ABSTRACT

Toka is mostly produced from maize cob by the process of leaching, diluting decanting and removal of water residue. It is a commonly used food additive in northern Nigeria. Thirty-three Wistar rats were used to determine the acute and sub-acute toxicity of toka. The median lethal dose or LD₅₀ of toka using the up and down procedure was discovered to be above 2000 mg/kg since administering 175 mg/kg, 500 mg/kg and 1000 mg/kg did not trigger death. However, during the 28-d study for sub-chronic toxicity study of Toka on the four test groups with graded doses of 0 mg/kg, 250 mg/kg, 500 mg/kg and 1000 g/kg. Physical behaviour such as redness of the eye started on the seventh day of the experiment while blisters on the mouth were observed from the fourteenth day of the experiment. There was no significant difference between the parameters and test groups. The findings of the current investigation revealed that there is not any significant difference in all the haematological parameters except haemoglobin. It also reveals various changes in the haematological parameters; White blood cell (RBC), packed cell volume (PCV), haemoglobin (HB), MCV, MCH, neutrophil (N) and Lymphocyte (L), did not show any significant difference with the control. Red blood cells (RBC) decreased significantly; this could be due to the increase in the breakdown of haemoglobin. There was an increase in the activity of ALT during the oral administration of Toka at different doses, this is an indication of the Liver malfunctioning. The outcome of this research indicated the toxicity of Toka on Wistar rats and therefore prolonged use of Toka as a food additive may have a toxic effect on some organs of the user.

INTRODUCTION

In some parts of Nigeria maize cub is processed into potash commonly known as "Toka". This is locally done by burning the maize cub into wood ash and allowing it to go through the process of traditional distillation. Maize is not the only source of "Toka" as it is also obtained from other plants [1]. "Toka" is a Hausa name for crude Potassium carbonate obtained by leaching the ashes of burnt plants and animal bones with water and evaporation resulting solution to dryness [2]. With impurities, it is brownish or blackish while in a pure state, it is whitish [3]. It is a translucent (granular) or white odourless deliquescent solid known in the anhydrous form [4]. The old method of making potassium carbonate (K₂CO₃) was by collecting or producing wood ash (an occupation carried out by ash burners), leaching the

ashes and then evaporating the resulting solution in large iron pots, leaving a white residue called *potash*. Approximately 10% by weight of common wood ash can be recovered as potash [5]. "Toka" is also a hausa/local name for aqueous burnt wood ash extract among the inhabitants of Gombe. Pappa *et al.*, [6] investigated the effects of potash on cooking time since it is commonly used by most Nigerians in rural areas for cooking vegetables and legumes. It was observed that it increases the green colour and texture of vegetables as well as reduces the cooking time of legumes. Cowpea which is noted for its prolonged cooking time of 40-65 minutes was reduced to 10-15 minutes when cooked with a high concentration of potash [6]. Another distinct property is that Toka is highly alkaline. In some cases it is applied on open wounds which helps in the healing process, It particularly helps in blood clotting. Locals commonly

apply a small quantity when they have a cut thereby stopping the bleeding and healing the wound [7]. Toka was also reported to have several other industrial uses, including glass manufacturing, soaps, plastics and pharmaceuticals [8].

Another study by [9] found that Toka has a high pH and contains varying amounts of other nutrients such as calcium (Ca). Phosphorus (P) and magnesium (Mg). Studies have also shown that Toka is an effective fertilizer and liming material for increasing soil fertility, pH and nutrient uptake by crops such as maize and cassava [10]. However, many people consume this product without knowing if it has side effects, particularly after a long period of consumption. Due to growing concern over the consumption of mineral salts. It is therefore necessary to understand the dose-effect of “Toka” on the human system since it is a very important seasoning among the inhabitants of Northern Nigeria.

There is an increased use of geological minerals in human and animal feed. Potash is being administered as medicine for all sorts of ailments. As medicine, in its grounded form, it is mixed with tobacco and used as snuff. In the Northern part of Nigeria, it is also administered in large doses by some people in preparing traditional soups in what is popularly called “miyan Toka” in Hausa. This is a special traditional delicacy served with Tuwo. It is also grounded with *seamase* seed. This is part of the items shared with people as a wedding notification in some tribes.

The addition of “Toka” in cooking reduces cooking time, thereby serving as a tenderizer. Most often this salt is very common in households and serves as the second most commonly used salt in households. It is also used for other purposes apart from consumption in this part of the country without knowing the effect of prolonged consumption of the product. From documented literature however, it has been observed that increased potash intake leads to decreased food and water intake in experimental animals which was followed by the presence of wrinkled skin with some hair losses [11]. More so, the toxic nature of potash was noticed in the nursing mothers around Zaria and Malunfashi areas of Northern Nigeria due to the intake of 30 mg per day [11]. Looking at the uncertainties in the uptake of Toka to human health, this study is of immense importance.

The study will help to determine the toxicity of wood ash otherwise processed and known as “Toka” on human health. Being a commonly used food additive in Northern Nigeria, most of the inhabitants do not know the effect it has on consumption. Wood ash has a high pH and contains varying amounts of other nutrients such as calcium (Ca), phosphorus (P), and magnesium (Mg). Few studies have demonstrated its effect on the body following its use as a food tenderizer. Thus, the continuous and indiscriminate use of “Toka” as a food additive in many African communities following the reported deleterious effect of trona/kaun precipitated this study.

MATERIALS AND METHOD

Study Population

The study was carried out on 33 Wistar rats obtained from the National Root Crops Research Institute, Kuru Wistar rats of both sexes weighing 170 – 200 gm were selected for sub-acute toxicity studies. The animals had free access to food and water and were maintained under standard laboratory conditions which included a 12-hour light-dark cycle and temperature of 28-30 °C. Animals were allowed a one-week acclimatization period before the study.

Animal procurement and acclimatization

The rats for this research were purchased from National Root Crops Research Institute in Kuru, Plateau State. Before the experiment commenced, the rats were allowed to acclimatize for one week. The rats are kept under standard environmental conditions such as temperature and relative humidity in a room. All the animals were fed with the starter that is produced by Chukun Feed Nigeria plc; water was given liberally. Rats were fed two times daily, Toka was mixed with the feed and ensured that it was a homogeneous mixture, and the feeding continued for 28d. Water was given to the animals under strict hygienic conditions throughout the week. The rats were handled according to the guidelines contained in the Guide for the Care and Use of Laboratory Animals [12]

Preparation of Toka (Potash) before administration

The maize cobs were processed locally by burning in a drum. The ash was collected after leaching and diluted with water. The product is allowed to go through a locally made distillation where the extracted water. The liquid produced was further subjected to heating to remove excess water. After boiling the residual substance is poured into containers of desirable shapes and allowed to cool, this further becomes solidified. The end product is known as Toka.

Toka is ground to powder and weighed from the range of 50-1000 grams using a weighing balance. This is mixed with the animal feed and administered to the rats [9] The animals in the control group were fed with only the starter feed and water liberally.

Experimental Design

The 24 Wistar rats of relatively similar sizes and weights of approximately 170-200g were selected for the experiment. These were randomly assigned into four groups: A, B, C and D of six animals in each group. Animals in groups B, C, and D served as treatment groups while those in group A served as the control. The group A animals (controls) were fed normally with starter while those in groups B, C, and D were administered with Toka in graded dosages (250 mg/kg, 500 mg/kg and 1000 mg/kg) mixed with 1kg of starter respectively. The feeds were available for the animals and changed daily with fresh ones after cleaning the containers and the animal cages.

Physical observation: The rats were observed for physical changes such as aggression, rashes and blisters, during the experiment there was no significant change in initial and final weight. However, a significant increase in water intake was observed in various test groups as the concentration increased.

Sub-acute toxicity studies

Sub-acute toxicity study (28-day repeated oral toxicity study) was carried out according to OECD 407. Both sexes of rats (170-200 g) were divided into four groups with 6 animals. Group A received no treatment whereas the rats in groups B, C and D were treated with Toka at the doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg body weight continuously up to 28 d.

All the groups of rats were observed twice daily for clinical signs and the time of onset, duration of these symptoms, mortality and morbidity till the completion of the experiment. Body weights of the rats in all groups were recorded once before the start of dosing, once weekly during the treatment period and finally on the day of sacrifice. The quantities of food and water intake were recorded daily. At the end of the experiment (on the 29th day), blood samples were collected from the rats after an overnight fast (but with drinking water allowed) by retro-orbital bleeding into heparinized and non-heparinized tubes for haematological analysis and biochemical analysis, respectively.

Hematological analysis

Hematological analysis was done to determine PCV, WBC, RBC, HB, Differentials, Platelets, MCH and MCHCB as described by

The heparinized blood was used for the determination of haematological parameters such as haemoglobin, red blood cell count, white blood cell count, and platelet count using a fully automated haematology analyzer (PE 6000).

Biochemical analysis

The serum was separated from non-heparinized blood and the serum biochemical parameters including total cholesterol, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), triglycerides, total cholesterol, albumin, bilirubin and total protein were analyzed using semi-automatic biochemical analyzer (Star 21plus, china).

Data analysis

The results obtained were subjected to analysis of variance (ANOVA), followed by Dunnett's post hoc test. The ANOVA was done using SPSS version 22.

RESULTS AND DISCUSSION

Behavioural and Physical changes of Wister rats due to the administration of Toka

The response of the effect of the administration of Toka on the biological and physical changes in Wister rats varies with the number of d and concentration administered to the rats as presented in **Table 1**. There was no death recorded at all concentration. Aggressive behaviour was also not observed in all the graded doses. From the second day to the twenty-eight day of the experiment, there was an increase in the redness of the eye. Irritation on the mouth/blisters was observed from the fourteenth day of the experiment and gradually increased at the various graded doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg respectively.

During the study on the effect of Toka on various graded doses on Wister rats as compared to the control group. The acute dose (LD_{50}) is discovered to be above 2000mg using the up and down method, at the dose limit of 175, 500, and 1000 mg/kg of Toka did not trigger death in Wister rats. The whole course of the short and long-term observation period was not observed with any lethal effects. In the entire 14-day study period, no signs of poisonousness were detected in the animals. However, during the 28 d study for sub-chronic toxicity study of Toka on the four test groups with graded doses of 0 mg/kg, 250 mg/kg, 500 mg/kg and 1000mg/kg. There was a significant ($p < 0.05$) difference between the parameters of the test groups. Potash was reported to cause changes in physical activity by causing a decrease in the weight of wister rats that in turn induce retardation in their growth [13,14].

Table 1. Behavioural and Physical change of wister rats due to Toka administration.

Physical change /Observation	Days						
	0	8	24	28	7	14	28
Death	N	N	N	N	N	N	N
Aggressive behaviour	N	N	N	N	N	N	N
Redness of the eye	N	N	N	Y	Y	Y	Y
Irritation of the mouth/blisters	N	N	N	N	N	Y	Y

Key: N= absent Y=present

Sub-acute toxicity of Toka on Wister rats.

During 28 d of treatment, all of the treatment rats of both sexes at the 250, 500, and 1000 mg/kg doses survived. Compared with the control, slight observable toxicity signs were noticed in the rats administered with Toka. The effect of Toka on different hematological parameters of wister rats after 28d treatment was investigated (**Table 2**). There was no significant difference ($p > 0.05$) in the values, PCV, HB, MCV, MCH, N, L, test group treated with 250mg/kg, 500mg/kg and 1000mg/kg. In WBC, a significant difference ($p < 0.05$) existed between the control and the group treated at 1000mg/kg. This could be due to inflammation, or excessive physical and emotional stress.

Hematological parameters in the rats are considered important biomarkers in assessing the effect of a toxicant on rats, this is because these parameters offer information on the physiology and health status of the animal under investigation. Some pathological alterations may appear in the blood before the appearance of external signs of toxicity. The findings in the current investigation revealed various degrees of changes in the haematological parameters.

Packed cell volume (PCV), hemoglobin (HB), MCV, MCH, Neurophil (N) and Lymphocyte (L), did not show any significant difference with the control. Red blood cells (RBC) decreased significantly, this could be due to the increase in the breakdown of haemoglobin. A similar trend was reported after a sub-chronic exposure of palm bunch ash in the liver of mice [15]. There was a significant increase in the white blood cells (WBC) this could be as a result of injury and emotional stress on the test organism.

It is normal for such occurrence in the blood of an organism exposed to injury or foreign organism. In a study carried out by [16], the effect of potash was carried out on the heart function of rabbits. As a result of the toxicity cases that had been observed in potash intake. The study revealed that continuous consumption of potash resulted in varying degrees of distortion and disruption of the cytostructure of the heart. This indicates that prolonged consumption of aqueous wood ash could have a detrimental effect on the heart of humans.

Exposure of rats to trona was also reported to cause a reduction in their body weight and is said to be dose-dependent and is in agreement with the study carried out by [17] who reported a similar effect. This is consistent with the result of the study carried out by [18] 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.

Table 2. Effect of sub-acute and oral administration of Toka (mg/kg) on haematological parameters of rats.

Parameter	Normal range	0	250	500	1000
RBC	6.3-10.0	4.27±0.21 ^a	4.07±0.06 ^a	3.17±0.10 ^a	4.13±0.58 ^a
WBC	5.0-23.0	5.23±0.97 ^a	4.47±0.63 ^a	4.33±0.63 ^a	5.10±0.17 ^b
PCV	35-45	33.00±2.65 ^a	34.33±4.16 ^a	27.67±5.87 ^a	30.67±3.79 ^a
HB	12.0-18.0	11.03±0.83 ^a	11.50±1.44 ^a	9.20±1.93 ^a	10.20±1.23 ^a
MCV	58-67	77.33±7.09 ^a	82.00±5.57 ^a	65.67±13.58 ^a	77.67±8.96 ^a
MCH	17-24	26.00±2.65 ^a	27.33±2.08 ^a	22.00±4.36 ^a	25.67±2.89 ^a
N	8-40	44.67±6.51 ^a	41.67±1.53 ^a	56.00±5.29 ^a	53.00±12.53 ^a
L	50-80	55.00±7.00 ^a	54.67±5.03 ^a	42.33±6.81 ^a	46.00±11.00 ^a

Note: All values are expressed in Mean ± SD, n=6 animals/group, $p < 0.05$ (ANOVA/ post hoc test). Different letters on the same row indicate a significant difference.

The effect of Toka on the biochemical parameters of Wister rats after 28d treatment was investigated (Table 3). Indicated that there was no significant difference ($p > 0.05$) in the values of ALP, ALB and TP in the test group treated with 250mg/kg, 500mg/kg and 1000mg/kg. More so a significant difference ($p < 0.05$) existed in the ALT between the control and the group treated at 1000mg/kg.

Alkaline aminotransferase (ALT) is quite important in the production and deamination of amino acids, mostly during stress conditions to adjust to high energy demand. There was an increase in the activity of ALT during the oral administration of Toka at different doses this is also similar to the experiment carried out by [15]. Aspartate Aminotransferase (AST) is also considered an enzyme of the liver, a high level of AST indicates liver disorder or inflammation. The findings of the current investigation revealed a significant change between the control and the group tested at 1000mg/kg. This is similar to the experiment carried out by [19]. Alkaline phosphates are regarded as an important enzyme in the liver, in the study there was no increase in ALP. Total protein (TP), and Albumin (ALB) also showed no significant increase with the control group. A similar trend was reported after exposure of mice to the herbicide glyphosate-Roundup [20].

Biochemical analyses of the diseased animal showed a decrease in the levels of different biochemical parameters including the total serum protein [21]. The quantity of protein in the blood is highly important in the diagnosis of a wide range of disorders. Decreased total protein concentrations can be found in defective protein synthesis in various organs such as the liver, kidney and intestine.

Table 3. Effect of sub-acute and oral administration of Toka (mg/Kg) on biochemical parameters of rats.

Parameter	Normal range	0 mg/L	250 mg/L	500 mg/L	1000 mg/L
ALT	48-80	58.33±12.42 ^a	55.67±4.04 ^a	63.00±12.49 ^a	71.50±4.95 ^b
AST	14-113	64.33±34.08 ^a	79.17±33.92 ^a	48.33±28.87 ^a	45.00±10.00 ^a
ALP	4-16	11.33±2.08 ^a	9.67±1.53 ^a	11.33±3.06 ^a	10.67±1.53 ^a
ALB	24-46	48.17±2.06 ^a	47.40±1.97 ^a	41.87±6.67 ^a	46.53±3.03 ^a
TP	56-83	70.67±10.02 ^a	68.00±11.79 ^a	60.67±8.51 ^a	62.67±10.02 ^a

Note: All values are expressed in Mean \pm SD, n=6 animals/group, $p < 0.05$ (ANOVA/ post hoc test). Different letters on the same row indicate a significant difference.

CONCLUSION

Based on the findings of this study, it can be concluded that prolonged and uncoordinated consumption of Toka can result in to lethal effects on the human system. Toka causes physical and behavioral changes, hematological, and biochemical changes in Wister rats. It can therefore be recommended that further investigation into acute toxicity of Toka in vitro should be carried out. Sub-acute toxicity tests should also be carried out for a longer duration to give more expansive information on the effect of the long-period consumption of Toka.

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