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Effects of trona (sodium sesquicarbonate) on physio-biochemical profiles and cardiovascular risk indices in Wistar rats

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ABSTRACT

Purpose of the study: This investigation aimed to assess the impact of trona on the cardiovascular risk markers and physio-biochemical profiles of rats administered graded dosages over 28 days. Five groups (n = 5) of rats—A, B, C, D, and E—were randomly assigned, with E acting as the control. For 28 days, rats in groups A, B, C, and D were administered trona at doses of 50, 100, 200, and 400 mg/kg, correspondingly. All over the investigation, distilled water was provided to the animals in the control group. We measured body weights, oxidative stress, hematology, hepatorenal profiles, somatic organs, fasting blood glucose (FBG) levels, and cardiovascular risk indices (CVRI). Findings: Rats administered 400 mg/kg had higher FBG levels ($p < 0.05$) than the control group. Group D showed decreases ($p < 0.05$) in erythrocytic indices, total protein level, and heart-body index with concurrent increases ($p < 0.05$) in total leucocyte counts (TLC) and creatinine levels in comparison to the control group. Rats in groups B, C, and D showed higher levels of catalase activity ($p < 0.05$) compared to the control group. Rats given 100 mg/kg or above of trona showed increases ($p < 0.05$) in CVRI compared to controls. Conclusions: There were dose-dependent harmful effects on the erythrocytic indices, high exposure to T2DM (type-2 diabetes mellitus), and increased CVRI levels in rats dosed orally with ≥100 mg/kg trona for 28 days.

Keywords

Cardiovascular indices, hepatorenal profiles, oxidative stress, rats, trona

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Abbreviations

AI: Atherogenic index
ALP: Alkaline phosphatase
ALT: Alanine aminotransferase
ANOVA: One-way analysis of variance
BUN: Blood Urea Nitrogen
CAT: Catalase
CHD: Coronary heart disease
CRI: Coronary risk index

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Introduction

In several parts of Nigeria, trona, an earthly mineral, is utilized as an intrinsic food enhancer. [1]. It is traditionally known by different names among various tribes in Nigeria. Kaun (Yoruba in Southwest Nigeria; Igbos in Eastern Nigeria); 'Kanwa' or 'Karu' (Hausa in Northern Nigeria); and 'Okanwa' and 'Ikoro' (Igalas and Egbira, respectively, in the Middle Belt of Nigeria), [2]. There is evidence of trona in the northern regions of Nigeria, especially in Kano and Maiduguri, as well as in neighboring countries like Niger and Chad [2]. Trona is erroneously called 'potash', although, in comparison to sodium, it has very small potassium [2,1]. Calcite makes up the majority of trona's chemical composition; the remaining constituents are hanksite, halite, pirssonite, and borax [3]. According to [4], sodium sesquicarbonate is the primary ingredient in trona. Conventionally, the reddish white (20% sodium carbonate) and whitish (80% sodium carbonate) kinds of trona have different make-ups when it comes to sodium salt. Trona (sesquicarbonate) is said to have sodium carbonate and sodium bicarbonate in an equal molar concentration [2]. As an intrinsically occurring food additive, trona is mostly utilized to soften tough foods including skins, bones, beans, and maize. In the states of Edo and Delta, it is also explored in making a delicacy known as "owo." [2, 1]. It is used broadly in ethno-veterinary practices for the treatment of skin diseases and digestive problems. Trona also serves as a salt lick and decoction for the treatment of reproductive ailments such as retained placenta [5]. The widespread use of trona (sodium sesquicarbonate dihydrate) in Africa, especially Nigeria, as an essential culinary additive and its gross applications in ethno-veterinary practices without minding its impacts on the biological systems of the end users could expose them to high toxicity during prolonged usage. The purpose of the study was to determine a safe dosage for trona to be administered sub-acutely orally for 28 days while also assessing the effects of the drug on the physio-biochemical profiles of rats.

Abbreviations-Cont'd

CVD: Cardiovascular disease
CVRI: Cardiovascular risk index
DLC: Differential leucocyte count
EDTA: Ethylenediaminetetraacetic acid
FBG: Fasting blood glucose
HbC: Haemoglobin Concentration
HDL-C: High-density lipoprotein cholesterol
LD50: Median lethal dose
LDL-C: Low-density cholesterol
LPH: Lipid hydroperoxide
LSD: Least significant different
MDA: Malondialdehyde
NOAEL: No observed adverse effect level
OECD: Organization of Economic Cooperation and Development
PCV: Packed cell volume
RBC: Red blood cell
SEM: Standard error of the mean
SPSS: Statistical Package for Social Science
T2DM: Type-2 diabetes mellitus
TAG: Triacylglycerols
TB: Total bilirubin
TC: Total cholesterol
TLC: Total leukocyte count

Result

The findings of the metal content analysis of trona are displayed in Table 1. Heavy metals like iron, zinc, arsenic, cadmium, lead, and copper were also present, although sodium had the highest concentration.

Rats' acute toxicity to different trona dosages is displayed in Table 2. The behavioral reactions of the experimental rats did not exhibit any notable negative clinical effects. Rats administered trona orally at a dose of 5000 mg/kg for 24 hours did not exhibit any mortality. As a result, it is anticipated that trona's LD50 is greater than 5000 mg/kg.

The effect of different trona dosages on the fasting blood glucose levels of rats on days 14 and 28 is depicted in Figure 1. When comparing the blood glucose levels of rats exposed to different dosages of trona on day 14 to day 28, there were no observable changes ($p > 0.05$). On both days 14 and 28, the rats in group D had significantly higher fasting blood glucose levels ($p < 0.01$) (67.00 ± 5.24 and 73.25 ± 4.87) than the control group (49.00 ± 4.55 and 55.75 ± 3.90). The effect of weekly graded doses of trona on rats' body weight and the relative organ-body weights of the liver, spleen, heart, and kidneys is depicted in Figures 2 and 3. Rats' mean body weights before trona administration (day zero) were markedly lower ($p < 0.05$) than their mean body weights on day 28 post-trona administration in all groups, and their body weights showed increasing trends overall. Relative weights of the kidney and liver of rats treated with trona did not markedly differ ($p > 0.05$) from those of the rats' control group. Rats given 400 mg/kg (group D) of trona had a discernably lower relative heart weight ($p < 0.05$) than the control group.

Following oral treatment of trona for 28 days, the effects of different dosages on the hematological profiles of rats are displayed in Table 3 and Figure 4. Rat mean PCV values in groups A, B, and C were noticeably higher ($p < 0.05$) than those of the control. When comparing the PCV levels of the rats in group D to the control, there was no discernible difference ($p > 0.05$). Rats in the trona-treated groups showed no appreciable differences ($p > 0.05$) in their HbC when compared to the controls. When compared to the control, the RBC count of the rats in groups A, B, and C increased

Table 1.
Metal contents analysis of tronaa

Metal	Concentration[b] (%)
Potassium	0.008
Sodium	0.150
Calcium	0.012
Lead (x 10^{-3})	0.233
Iron (x 10^{-3})	1.104
Copper (x 10^{-3})	0.189
Zinc (x 10^{-3})	1.430
Arsenic (x 10^{-3})	0.015
Cadmium (x 10^{-3})	0.003

a. Sodium sesquicarbonate ore
[b] Dry matter basis

Table 2.
The outcomes of a test on the acute toxicity of trona in rats

Treatment	No of rats	Mortality recorded	Observation
First phase			
50 mg/kg	3	Nil	No visible signs of toxicity
300 mg/kg	3	Nil	No visible signs of toxicity
2000 mg/kg	3	Nil	No visible signs of toxicity
Second phase			
5000 mg/kg	3	Nil	No visible signs of toxicity

Visible signs of toxicity such as changes in gait, drowsiness, hyperexcitability, diarrhea, vomiting, and nose bleeding were watched out for during the acute toxicity test.

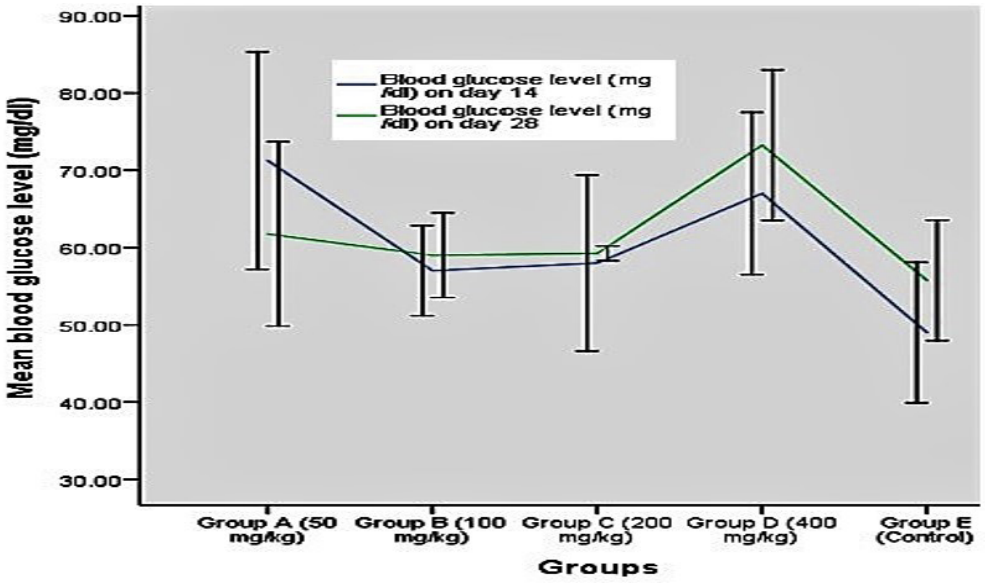

Figure 1.
Fasting blood glucose levels of rats exposed to varying doses of trona on days 14 and 28. Results are shown as mean ± SEM (n = 5)

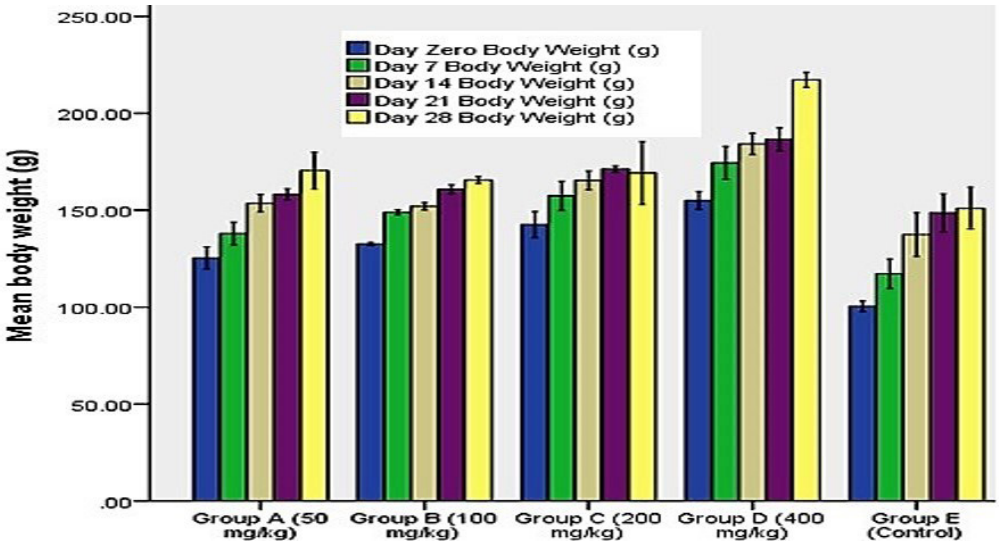

Figure 2.
Mean weekly body weight of rats exposed to varying doses of trona after 28 days oral administration. Results are shown as mean ± SEM (n = 5).

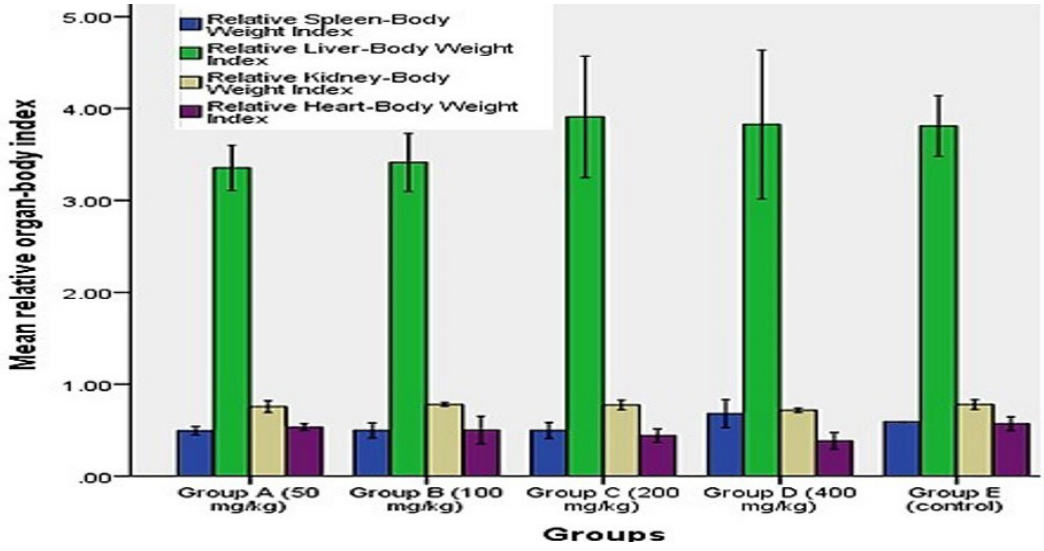

Figure 3.
Mean relative organ-body weight index of rat exposed to varying doses of trona after 28 days oral administration. Results are shown as mean ± SEM (n = 3).

Table 3.
Rats' hematological parameters following 28 days of oral trona treatment at different doses

Group	PCV (%)	HbC (g /dl)	RBC (x 106 / µl)	TLC (x 10^3 / µl)
A (50 mg/kg)	45.50 ± 2.96[a]	18.62 ± 1.34[a]	3.33 ± 0.18[a]	9.97 ±0.32[a]
B (100 mg/kg)	47.50 ± 3.97[a]	16.72 ± 3.40[a]	3.25 ± 0.33[a]	10.27 ± 0.84[a]
C (200 mg/kg)	46.25 ± 3.47[a]	14.69 ± 1.15[a]	2.82 ± 0.20[a]	8.07 ± 0.52[a]
D (400 mg/kg)	36.00 ± 3.11[b]	15.17 ± 1.55[a]	1.98 ± 0.50[b]	13.07 ± 1.10[c]
E (Control)	38.25 ± 1.15[b]	15.00 ± 1.25[a]	0.83 ± 0.2 8[c]	6.80 ± 0.81[b]

The means ± SEM of the four groups is displayed. A one-way ANOVA was used, and post hoc LSD was applied. LSD stands for least significant difference. A different superscript letter (s) in the same column indicates a significant difference of $p < 0.05$ when comparing all the groups. PCV = Packed cell volume; HbC = Hemoglobin concentration; RBC = Red blood cell; TLC = Total leucocyte count.

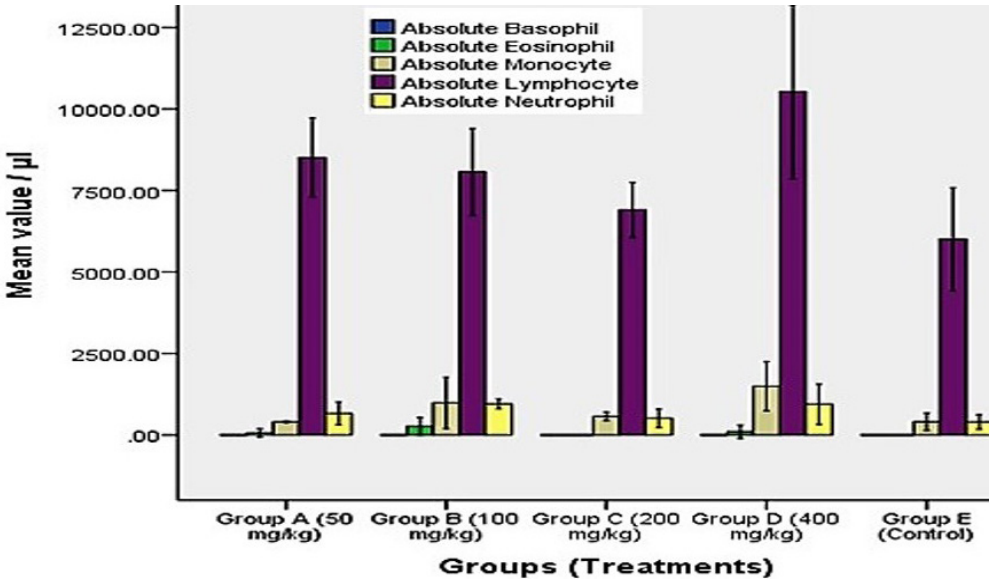

Figure 4.
Mean absolute differential leukocyte counts per of rat exposed to varying doses of trona after 28 days oral administration. Results are shown as mean ± SEM (n = 5).

significantly ($p = 0.000$). Rats in group D had a substantially lower ($p < 0.05$) RBC count compared to rats in groups A, B, and C, but a markedly higher ($p = 0.001$) RBC count when compared to the control. Rats in group D had a considerably higher TLC ($p = 0.000$) than rats in the control group.

Table 4.
Rats given different dosages of trona orally for 28 days and their hepatorenal profiles

Groups	ALT (IU/L)	ALP (IU/L)	TP (g /dl)	TBIL(mg/dl)	BUN (mg/dl)	Creatinine (mg/dl)
A (50 mg/kg)	8.00 ± 0.41[a]	29.25 ± 0.25[a]	4.65 ± 0.16a	0.56 ± 0.16[a]	60.50 ± 5.56[a]	2.18 ± 0.19[ab]
B (100 mg/kg)	8.75 ± 0.48[a]	28.50 ± 0.87[a]	5.13 ± 0.10[b]	0.73 ± 0.10[a]	52.52 ± 4.56[a]	1.88 ± 0.16[a]
C (200 mg/kg)	8.50 ± 0.29[a]	28.50 ± 0.50[a]	5.25 ± 0.20[bc]	0.71 ± 0.12[a]	59.75 ± 4.64[a]	2.13 ± 0.17[ab]
D (400 mg/kg)	8.50 ± 0.29[a]	28.75 ± 0.48[a]	4.58 ± 0.27[a]	1.14 ± 0.23[b]	68.50 ± 5.70[a]	2.45 ± 0.19[bc]
E (Control)	8.50 ± 0.29[a]	29.85 ± 0.48[a]	5.75 ± 0.21[c]	1.06 ± 0.17[b]	59.75 ± 6.14[a]	2.13 ± 0.22[ab]

The means ± SEM of the four groups is displayed. A one-way ANOVA was used, and post hoc LSD was applied. LSD stands for least significant difference. A different superscript letter (s) in the same column indicates a significant difference of $p < 0.05$ when comparing all the groups. ALT= Alanine aminotransferase; ALP= Alkaline phosphatase; TP= Total protein; TBIL= Total bilirubin; BUN = Blood urea nitrogen.

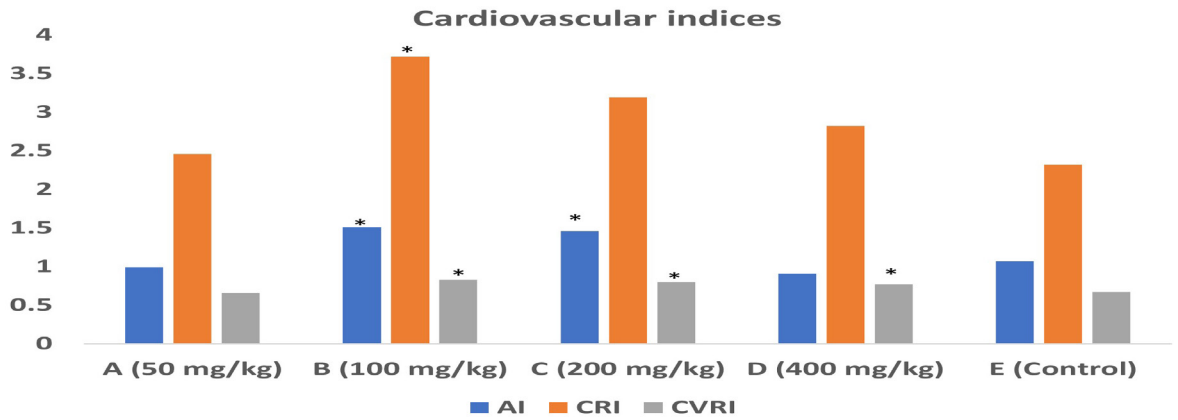

Figure 5.
Effects of varying doses of trona on cardiovascular risk indices of rats after 28 days sub-acute administration. Results are shown as mean ± SEM (n = 4). *AI significant when compared with A, D, and control ($p < 0.05$). *CRI significant when compared with A and control ($p < 0.05$). *CVRI significant when compared with A and control ($p < 0.05$). AI = Atherogenic index; CRI = Coronary risk index; CVRI = Cardiovascular risk index.

When compared to rats in groups C and control, the mean absolute lymphocyte count of rats in group D increased significantly ($p < 0.05$). Rats in group D had significantly higher mean absolute monocyte counts (p =0.000) than rats in groups A, C, and control. When comparing the mean eosinophil and neutrophil counts of rats in the trona-treated groups to the controls, there were no observable differences (p> 0.05).

Following 28 days of oral dosing, Table 4 displays the effect of different dosages of trona on biomarkers of hepatorenal function in rats. Comparing the ALT and ALP activities of the trona-treated groups to those of the control group revealed no marked changes ($p > 0.05$). When compared to the control, the rats in group A (50 mg/kg trona) had a significant ($p < 0.05$) drop in their total bilirubin level. Rats treated with trona showed no appreciable differences ($p > 0.05$) in their blood urea nitrogen (BUN) and creatinine levels when compared to controls. When compared to the control, the total protein of the rats in group D was substantially ($p < 0.05$) lower.

Table 5 displays the results of antioxidant enzyme activity and serum lipid peroxidation in rats given different dosages of trona orally for 28 days.

While the CAT activity of rats in groups B, C, and D increased significantly ($p < 0.05$) when compared with control and group A, there were no discernible changes ($p > 0.05$) in the activity of SOD in trona-treated groups compared with control. When compared to control and group D, the serum lipid peroxidation levels of rats in groups A, B, and C showed a marked decrease ($p < 0.05$).

Following 28 days of oral dosing, Table 6 displays the effects of different dosages of trona on the serum lipid profiles of rats. No significant changes ($p > 0.05$) were observed in triacylglycerols (TAG) or high-density lipoprotein cholesterol (HDL-C) when the trona-treated rat group was compared to the control group. Rats in groups B, C, and D had substantially higher total cholesterol (TC) levels ($p < 0.05$) than the controls. Rats in groups B and C showed a marked (p

Table 5.
Serum lipid peroxidation and antioxidant enzyme activity of rats subjected to different doses of trona orally for 28 days

Group	MDA (mg/ml)	SOD (U/ml)	CAT (U/ml)
A (50 mg/kg)	2.73 ± 0.41[a]	10.60 ± 0.28[a]	3.92 ± 0.35[a]
B (100 mg/kg)	2.84 ± 0.34[a]	10.09 ± 0.51[a]	5.94 ± 0.50[b]
C (200 mg/kg)	3.64 ± 0.49[a]	10.83 ± 0.11[a]	5.71 ± 0.78[b]
D (400 mg/kg)	4.09 ± 0.49[b]	10.70 ± 0.22[a]	5.17 ± 0.19[b]
E (Control)	4.96 ± 0.23[b]	10.64 ± 0.28[a]	4.48 ± 0.33[a]

The means ± SEM of the four groups is displayed. A one-way ANOVA was used, and post hoc LSD was applied. LSD stands for least significant difference. A different superscript letter (s) in the same column indicates a significant difference of $p < 0.05$ when comparing all the groups. MDA= Malodialdehyde; SOD= Superoxide dismutase; CAT= Catalase.

< 0.05) rise in their levels of low-density lipoprotein cholesterol (LDL-C) when compared to the control group. Following oral administration of trona for 28 days, Figure 5 illustrates the effect of different dosages on the atherogenic, coronary risk, and cardiovascular risk indices in rats. In comparison to the controls, the atherogenic index level was substantially ($p < 0.05$) elevated in rats in groups B (100 mg/kg) and C (200 mg/kg). Rats in group B had a higher coronary risk index level ($p < 0.05$) than the control group. On the other hand, when compared to controls, rats administered trona at doses more than 100 mg/kg showed a substantial ($p < 0.05$) elevation in their cardiovascular risk index (CVRI).

Discussion

Only following acute, subacute, and chronic toxicity testing is a food additive safe for human consumption [1]. Therefore, the purpose of this study was to assess how trona affected the physio-biochemical profiles of rats when it was administered orally over a short period.

High amounts of sodium and potassium are found in the metal analysis of trona (Table 1), but calcium and iron values are comparatively low. There was also zinc, cadmium, lead, copper, and arsenic. It has been determined that toxic metals pose a serious risk to human health, mostly due to their capacity to harm DNA and membranes as well as to interfere with the activity of enzymes and proteins [6]. The current study's findings demonstrated that, although there is a possibility of bioaccumulation, the levels of metal pollution, as measured against the FAO/WHO [7] standard, were below the upper limit that was permitted.

The acute toxicity investigation (Table 2) revealed no deaths and observable harm. Since there was no mortality at 5000 mg/kg, suggesting that it is reasonably safe for short-term exposure, the LD50 was not determined.

When comparing the FBG levels of rats in group D (400 mg/kg) on days 14 and 28 to those of the control group, the results (Figure 1) show a substantial rise ($p < 0.01$). T2DM is a possibility for the rats given a 400 mg/kg dosage, based on their hyperglycemia. Following a carbohydrate-rich meal, T2DM is known for a persistent rise in blood glucose [8]. Figure 2 illustrates that the body weights of the rats in each group significantly increased when compared to their baseline weights. This suggests that trona has no negative effect on the rats' body weight. This is consistent with findings from [9], who reported that trona had no negative effects on body weight or testicular shape. As seen in Figure 3, the heart-body weight index of rats in group D (400 mg/kg) decreased significantly ($p < 0.05$) in comparison to the control group. This may be a sign of inflammation in the heart caused by elevated salt levels from a high trona dosage. High sodium concentrations are associated with increased production of reactive oxygen species in cardiac muscles, which is a significant factor in the development of cardiovascular disease (CVD) [10].

The results of this investigation clearly showed that trona damages erythrocytic parameters in a dose-dependent manner (Table 3). This could be because of a high rate of erythrocyte destruction that lowers PCV and RBC levels because of a rise in lead bioaccumulation in bone marrow with increasing doses. Lead disrupts the proper maturation of erythroid components in the bone marrow and is primarily found in bones in both humans and animals [11]. In the present investigation, we found higher TLC in rats dosed with 400mg/kg of trona which was linked to both lymphocytosis and monocytosis in comparison to the control and other groups as shown in Table 3. The monocytosis observed in our study agrees with the previous reports that monocyte recruitment is crucial in the host response to metabolic, atherogenic, and neoplastic stimuli, attributing to wound repair and fibrosis [12]. The concurrent lymphocytosis and monocytosis observed could be attributed to potentiation of B and T lymphocytes by interleukin secreted by monocytes as was demonstrated in previous reports [13].

Analysis of serum liver function enzymes in our present study (Table 4) showed that the dosage of trona could not have any significant effect on the activities of ALT and ALP suggestive of no hepatic tissue injury. However, the marked reduction in the total bil-

irubin level of rats dosed with trona below 400 mg/kg suggests an enhanced glucuronidation process. This is in line with previous studies that increased total bilirubin level above normal (0.3 to 0.8 mg/dl) could be a pointer to liver damage mostly in hepatocytes that affect the glucuronidation process and the presence of erythrocyte hemolysis [14]. Our study's findings (Table 4) demonstrated that, in comparison to controls, rats given 400 mg/kg trona had a significantly lower level of total protein and an increased amount of creatinine. This implies that rats given 400 mg/kg of trona may be susceptible to decreased renal function after extended treatment. This supports a previous study that found a higher blood creatinine level to be a diagnostic marker for a decline in glomerular filtration rate [15].

One important enzyme called catalase uses hydrogen peroxide, a nonradical ROS, as a substrate. It oversees neutralization through the breakdown of hydrogen peroxide, preserving the molecule at the optimal amount in the cell, which is essential for signaling events within the cell [16]. The current study (Table 5) showed elevated catalase activity in dose dose-dependent manner with concurrent elevation of MDA level. This suggests that an increase in the dosage of trona could lead to the suppression of the antioxidant systems with the consequential effect of an increase in lipid peroxidation. This finding is consistent with previous reports that antioxidant enzyme activities are overwhelmed when the levels of MDA and LPH become enhanced [2].

Due to its correlation with the quantity of cholesterol contained in lipoprotein, total serum cholesterol plays a crucial role in the development of cardiovascular disease (CVD) [17]. There is a dearth of knowledge regarding trona's effects on rats' lipid profiles, hence this study is necessitated. This current study (Table 6) showed elevated total cholesterol in trona-treated rats with associated increased low-density lipoprotein cholesterol compared with control. There were no significant effects on triglycerides and high-density lipoprotein cholesterol in trona-treated rats compared with control. This implies that trona administration promotes LDL cholesterol oxidation by increasing total serum cholesterol, a hallmark in the development of atherosclerotic plaque.

The current investigation assessed the impact of trona administration on the experimental rats' lipid ratios (TC/HDL cholesterol, TG/HDL cholesterol, and LDL/HDL cholesterol). An effort is currently being made to maximize the predictive power of lipid profiles against the risk of atherogenicity, coronary heart disease (CHD), and cardiovascular disease (CVD) by evaluating lipid ratios, or cardiovascular risk indices [18]. Rats given 100 mg/kg trona showed significantly higher levels of both the coronary risk index (CRI) and the atherogenic index (AI) when compared to the control group. This suggests that trona (100 mg/kg) treatment in rats may increase the risk of coronary heart disease and atherosclerosis. The LDL/HDL cholesterol ratio takes AI into account [18]. Compared to separately used total cholesterol, LDL cholesterol, and HDL cholesterol of coronary heart disease, CRI is a more powerful coronary risk predictor [19]. In a similar vein, rats administered trona at doses greater than 100 mg/kg showed a significant rise in their cardiovascular risk index (CVRI) values relative to the control group, hence raising the risk of cardiovascular illness. This implies that trona administration at doses greater than 100 mg/kg is associated with increasing levels of sd LDL cholesterol, a tiny and dense subclass of LDL cholesterol linked to cardiovascular risk. A significant risk of cardiovascular disease is associated with elevated levels of sd LDL, a small and dense subclass of LDL, which is indicated by an elevated TG/HDL cholesterol ratio [20].

It is also hypothesized that an increase in CVRI level is associated with hyperglycemia [21] which is in tandem with our finding as shown in Figure 1. The present study reveals that T2DM observed in rats dosed 400 mg/kg trona serves as a predisposing factor to cardiovascular disease which agrees with previous reports that T2DM correlates positively with cardiovascular disease risk factors [22]. In summary, the present study has revealed that animals given high doses of trona 400 mg/kg might be at risk of T2DM, and a dose-dependent deleterious effect on the erythrocytic indices. Our study also reveals that trona at 100mg/kg and above when given to animals could raise their chances of developing myocardial infarction. Thus, for this present study, the no observed adverse effect level (NOAEL) dose for oral administration of trona in rats is estimated to be 50 mg/kg per day in 28-day sub-acute toxicity studies. Hence, caution has to be taken when consuming trona continuously and indiscriminately due to its potential toxicity risk that is associated with cardiovascular disease.

Accordingly, further investigations are recommended for a better understanding of the dose-response correlation of trona, the bioavailability of heavy metals content of trona in the body, and the toxicological effect of trona on other organs such as the brain and lungs over a long period based on the NOAEL dose (50 mg/kg).

Materials and Methods

Ethical approval

The EU Directive 2010/63/EU and the rules of the University of Nigeria, Nsukka's Animal Ethics Committee (UNEC/21/190304) were followed in the conduct of this study. The Faculty of Veterinary Medicine, University of Nigeria, Nsukka's Institutional Animal Care and Use Committee finally approved this study (FVM-UNN-IACUC-202306108).

Materials

The trona was bought at Ogige market in Nsukka LGA, Enugu State, Nigeria. The trona was properly recognized by a geologist at the Department of Geology, University of Nigeria, Nsukka. It was then pulverized into powder form and properly stored in an airtight container for the study.

Metal content analyses

Calcium, magnesium, sodium, potassium, zinc, iron, lead, cadmium, arsenic, and copper were analyzed using standard procedures (23, 24, 25).

Chemicals

The assay kits for ALP, ALT, AST, ALP, total bilirubin, total protein, urea, and creatinine were provided by Randox Laboratories Ltd. (Antrim, United Kingdom). Additional kits for total cholesterol, triacylglycerols, high-density lipoprotein choles-terol (HDL-Chol), and low-density lipoprotein cholesterol (LDL-Chol) were also acquired from Randox Laboratories (Antrim, United Kingdom) as well as catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA) were also determined All chemicals utilized were of analytical grade.

Animals

The Department of Zoology's Animal House provided male Wistar strain albino rats that were 5–6 weeks old. They were kept at the University of Nigeria, Nsukka's Laboratory Animal Unit of Veterinary Physiology and Pharmacology. During the two weeks of acclimatization, the animals were given commercially prepared rat food and unlimited water. Because female rats' cycles are characterized by hormonal fluctuations, this study used male rats instead of females to avoid inconsistent responses to the same stimuli. Adopted were the guidelines for the management of laboratory animals [26]. The Institutional Animal Care and Use Committee (IACUC) of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, examined and ultimately accepted this study protocol with scientific reason (FVM-UNN-IACUC-202306108).

Acute toxicity study

Organization for Economic Cooperation and Development (OECD) guideline 423 [27] was used to determine the oral acute toxicity and median lethal dosage (LD50) of trona. The investigation involved the use of twelve male Wistar rats, three of which were employed for testing at 50, 300, 2000, and the 5000 mg/kg limit, respectively. Clinical and behavioral signs of toxicity such as changes in gait, drowsiness, hyperexcitability, diarrhea, vomiting, and nose bleeding were checked out for during the acute toxicity test. With the LD50 shown to be greater than 5000 mg/kg, the oral dosages of 50 mg/kg, 100 mg/kg, 200 mg/kg, and 400 mg/kg were selected for the trona-treated groups.

Sub-acute toxicity studies

For the investigation, rats weighing between 110 and 135 g were employed. Five groups (n = 5) consisting of A, B, C, D, and E, were randomly assigned to them, with E acting as the control.

For 28 days, the rats in groups A, B, C, and D received daily doses of trona of 50 mg/kg, 100 mg/kg, 200 mg/kg, and 400 mg/kg, respectively. Rats in group E were given distilled water throughout the study. Body weights of the rats in the treatment groups and control group are taken at day one (zero) and 7-day intervals for 28 days using a Metler weighing balance.

Blood sample collection and animal sacrifice

The animals were allowed to fast the night before the experiment concluded, and in the morning, blood samples were taken from the retrobulbar plexus of the median canthus of the eye and placed into sterile containers either with or without ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. The serum utilized for the examination of lipid and hepatorenal function indicators was separated from the blood in the sterile bottles by centrifuging the mixture at 3000 g for 15 minutes. Blood was utilized for hematological analyses in EDTA sample vials. Following their euthanasia, three (3) rats from each group were slain by intraperitoneal injection of five milligrams of xylazine (Kepro Holland) and ninety mg of ketamine hydrochloride (Laborate Pharmaceutical, India) [28]. Following the animals' dissection, relevant organs including the liver, spleen, heart, and kidney were removed and weighed for organosomatic research using a Metler weighing scale.

Fasting blood glucose level

On days 14 and 28 of the trial, after an overnight fast, blood samples were taken through the tail. The digital blood glucose monitor with microprocessor was filled with blood after drops of blood were placed onto the dextrostix reagent pad. Data were then collected. On days 14 and 28 of the trial, all groups' FBG levels were measured.

Hematological analyses

The hemocytometer method was used to determine the TLC and RBC whereas the microhematocrit method was used to calculate PCV [29]. The stained blood film was used to perform differential leucocyte counts (DLC) [29], and Drabkin's reagent assay method was used to measure hemoglobin (Hb) content [30].

Serum biochemical analyses

Following the manufacturer's instructions, commercial kits from Randox® were used to analyze serum stored at 4 °C for liver and kidney function biomarkers such as alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TB), creatinine, blood urea nitrogen (BUN), and total proteins (TP). Utilizing Randox® kits, lipid profiles including total cholesterol (TC), triacylglycerols (TAG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) levels were also assessed. Superoxide dismutase (SOD) and catalase (CAT) activities were measured using conventional techniques [31]. Using the spectrophotometric approach, the lipid peroxidation biomarker malondialdehyde (MDA) was identified following Ohkwa [32].

Organosomatic indices

Vital organs of interest, such as the spleen, heart, liver, and kidneys, were harvested, dissected, and weighed.

Organosomatic index = Weight of organ ÷ weight of animal X 100%

Cardiovascular risk indices

Using the following formulas, cardiovascular risk indices, including atherogenic index (AI), coronary risk index (CRI), and cardiovascular risk index (CVRI), were calculated: [33, 34].

AI (atherosclerotic index) = LDL/HDL cholesterol

TC divided by HDL cholesterol is the coronary risk index (CRI).

TAG divided by HDL cholesterol is the cardiovascular risk indicator (CVRI).

Data analysis

With the use of SPSS version 23, the acquired data were examined using One-Way Analysis of Variance (ANOVA). To distinguish between the variant means, a least significant difference (LSD) post hoc test was employed. It was determined that the probability level ($p < 0.05$) was significant. The means of the SEM are displayed in tabular and graphical style for the results.

Data availability statement

The raw data were produced by the Department of Veterinary Physiology and Pharmacology at the University of Nigeria, Nsukka's Faculty of Veterinary Medicine. On request, the corresponding author (H. E. U.) will provide derived data supporting the study's findings.

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Authors' Contributions

H.E., and O.B. were involved in research conceptualization; supervision; project administration; validation; visualization; drafting, reviewing, and editing the original manuscript while H.E., and M.Z. were involved in data curation; formal analysis; investigation; methodology; resources, and software. All authors read and approved the final manuscript.

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Competing Interests

The authors declare no conflict of interest.

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