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(RESEARCH ARTICLE)

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Sub-acute studies on the effects of trona consumption at varied low doses on male fertility and antioxidant properties using Wistar Rats

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Abstract

Geologically known as trona, evaporite, or locally known as greyish potash, *akanwu*, or *kaun*, it serves a multitude of purposes in Nigeria. These include, but are not limited to, softening legumes and flesh, augmenting the therapeutic attributes of herbal expectorant remedies, and reducing male libido or fertility. The unrestricted consumption and accessibility of this salt in the local markets of Anambra State, Nigeria, are due to its affordability and accessibility. Currently, there is no investigation into the comprehensive impact of potash's antioxidant properties at various low doses on male fertility. This led to a study that lasted for 28 days and involved rats weighing between 120 g and 140 g. The rats were separated into four groups, with each group consisting of seven rats. Group A was the control group, while groups B, C, and D were the test groups administered doses of 150 mg/kg, 300 mg/kg, and 450 mg/kg of potash, respectively. At the end of the study, the antioxidant properties of potash were tested. Prostate, gland, testis, and blood were collected and analyzed. Data was analyzed with the statistical social science (SPSS) version 27. Results were presented as mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) was used to compare the mean values. Our study found a significant ($p<0.05$) dose dependent decrease in fertility hormones and semen parameters. Prostate specific antigen (PSA) increased dose-dependently compared to the control. Our findings indicate that trona is rich in antioxidants.

Keywords: Anambra; Prostate; Fertility; Potash; Antioxidant

1. Introduction

Geologically known as evaporite, or locally known as grayish potash, *akanwu,* or *kaun,* trona is a popular natural salt that serves a variety of purposes in Nigeria. These include, but are not limited to, softening legumes and meat, enhancing the medicinal properties of herbal expectorants, and lowering male libido or fertility in the northern states of Nigeria. It can be easily purchased in the market by using the name 'greyish potash'. Potash is the second most popular used salt in Nigeria based on studies by Kutshik et al.[1]. It is a hydrated sodium carbonate that is found in dry lakes. It is available in both local and semi-urban markets. It's a sesquicarbonate with an equimolar ratio of Na2CO3 and $NaHCO_3$ [2].

Although many types of potash abound, trona is used as a food additive because of its mechanism of softening food, thereby saving cooking gas and time. This economic purpose makes trona popular in the homes of food vendors and many households in Anambra State, Nigeria. In Anambra State, potash is also used for local saponification purposes, especially for the preparation of local delicacies such as *Abacha, Nkwobi, Akidi*, and *Ukwa* [3].

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Infertility is estimated to impact 8-12 % of couples worldwide, with a male factor being the primary or contributing reason in roughly 50 % of cases[4]. Male subfertility is caused by a variety of causes that affect spermatogenesis, which can be hereditary, acquired, or idiopathic [5]. Studies in Anambra and the rest of south-east Nigeria have shown that male variables are more strongly associated with infertility[6]. Furthermore, studies have linked biologically and physiologically reduced sperm concentrations to male infertility [7].

Although studies have also linked male infertility to hormonal imbalances or abnormalities, it remains unclear whether trona contains chemicals or metals that affect male fertility. Because potash is a natural salt, particularly in raw form, it may contain some heavy metals, the most prominent of which is lead (Pb). Lead, through mechanisms such as sperm motility and count reduction, aberrant morphological induction, and functional parameter disruption, has the potential to diminish male fertility [8]. However, the potential for lead to be present in a minute or large amount in potash ingested in Anambra State, Nigeria, remains unclear. But there is a linkage between the existence of trace levels of Pb in potash feldspars and the emergence of Pb ore deposits that are associated with igneous rocks [9].

Moreover, a number of prevalent disorders target the prostate, affecting male fertility at various ages. Prostatic disorders or an unhealthy prostate in both young and elderly men can impair spermatozoa function and, consequently, male fertility [10]. In addition, it is not known if there is any effect of potash on prostate grand. The study seeks to evaluate clearly, even at varied low doses, the effects of trona on prostate-specific antigen (PSA) in Wistar rats fed with potash (trona) to extrapolate its possible effects in man. Although limited evidence suggests that antioxidants improve fertility[11], antioxidants can assist in neutralizing free radicals within the body. The study also instigated the antioxidant effects of potash at varied doses using Wistar rats.

2. Materials and methods

2.1. Instruments & Equipment

The following instruments and equipment were used in the course of this study: Microplate shaker, Plate reader scalpel, blades, Forceps, Bunsen burner, Microscope, Hypodermic needles, hand gloves, test tubes, Centrifuge, Leica binocular microscope (DM750P, Leica, Germany), wintrobe hematocrit tube ,Analytical weighing balance (PA214,Ohaus,USA), Electronic weighing balance (SPU 401,Ohaus,USA), and haemocytometer (CTL-HEMM-GLDR, LW Scientific, USA), Compound digital light microscope(Motic™-BA210 ,China) centrifuge (Vanguard V 6000,Germany), Acurex Chemistry Analyzer (SR NO: 7047,England), Semi-auto analyzer ,EMP 165 ,China)

- **Elisa Kits:** AccuBind enzyme-linked immunosorbent assay microwell (4925-300, Monobind, USA microwell), AccuBind ELISA (4825-300, Monobind, USA), AccuBind ELISA microwell(625-300, Monobind, USA), AccuBind ELISA microwell (5325-300, Monobind, USA).
- **Sample Collection (Wistar rats, potash and feed):** Grey-colored potash (trona) samples were collected at random from three main markets (*Eke-Awka*, *Ekwulobia,* and *Nkwo Nnewi*) in Anambra State, Nigeria. The GPS coordinates of the various markets include: *Nkwo Nnewi* (6.0196195, 6.9067093), *Eke Awka* Market (6.20781, 7.069655), and *Ekwulobia* Market (6.0197876, 7.0826180). Only healthy male adult Wistar rats aged 10 to 12 weeks and weighing 120–140 g were utilized. The rats were procured from the Animal House of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu campus. Standard, commercially available rodent feed and distilled water were utilized.

2.2. Experimental Design

The study used a randomized subject-control experimental design, with Wistar rats randomly assigned to four groups: Group A received only distilled water and feed for 28 days, Group B received 150 mg/kg greyish-colored potash (trona) with feed and water daily for 28 days, Group C received 300 mg/kg greyish-colored potash (trona) with feed and water daily for 28 days, and Group D received 450 mg/kg greyish-colored potash (trona) with feed and water daily for 28 days.

2.2.1. Animal Sample Size

Animals were randomly selected and sample size were based on Charan & Kantharia,[12] equation: *Corrected sample size=Sample size/ (1− [% attrition/100]).* Taken 12 % attrition and 28 Wistar rats as the Corrected samples size, Sample size = 28 (i.e. n=7per group). For possible attrition (death) within the 28days study, 1 animals will be added to each of the groups: A, B and C and D respectively. Therefore, the total animals used in the sub-acute study was 32.

2.2.2. Animal Selection

The study included thirty-two (32) sexually mature male adult Wistar rats. The criteria provided in "Reporting Animal Research: Explanation and Elaboration for the Animal Research, Reporting of In Vivo Experiments (ARRIVE) Guidelines 2.0" were strictly followed while selecting the animal. The study also followed the method of du Sert et al.[13].The animals were 10–12 weeks old and had weights within a ±20% range of the mean beginning weight of previously dosed animals[14].The animals were picked at random and marked to facilitate individual identification. They were kept in their cages for around two weeks before they were administered the dose to allow them to adjust to the laboratory environment. Throughout the study, care was taken to ensure that animals of the appropriate size and age range were accessible.

2.2.3. Housing and feeding conditions

The temperature in the experimental animal room was 22 °C (±3 °C). Although the relative humidity was at least 30% and ideally did not exceed 70 % during room cleaning, the goal is to achieve 50-60 %. Lighting was artificial, with 12 hours of light followed by 12 hours of darkness. Conventional rodent feed was used, along with an unrestricted supply of deionized drinking water.

2.2.4. Preparation and Administration of Trona

The doses of potash administered to each group were: 150 mg/kg, 300 mg/kg, and 450 mg/kg. The size of the test animal also determined the amount of deionized water to dissolve the potash at once, with a dosage of 1 mL per 100 grams of body weight. Fresh daily dosages were prepared, warmed to 70 degrees Celsius, and allowed to cool before administration. Daily dosing was administered with a suitable intubation cannula so that the animals were not injured.

2.3. Laboratory procedures

2.3.1. Semen Sample Collection

Each rat was sacrificed by cervical dislocation, and its epididymis was harvested. Sperm samples were collected from the cauda epididymis with the use of a capillary tube or test tube. The samples collected were labeled according to the dose administered and sample group.

2.3.2. Semen color and consistency

The color and consistency of the sperm samples were evaluated macroscopically by mere observation and recorded. The consistency scale (1-4) adopted by Orieke et al. [15] was used. The adopted color scale (1-3) represents 1 (white), 2 (milky white), and 3 (creamy white). For consistency, the scale was: 1 (watery), 2 (slightly thick), 3 (thick), and 4 (very thick).

2.3.3. Determination of Semen pH

The semen pH was determined according to Angélica Lucio et al., [16]. To evaluate pH (semen acidity and alkalinity), 10 microliters of fresh semen were deposited on pH indicator paper. The acquired color was directly compared to the printed standard color. The semen pH of the male Wistar rats were determined by comparing the paper's color shift to the standard color chart.

2.3.4. Determination of Semen Morphology and Abnormalities

The preparation of the eosin-nigrosin stain was determined according to World Health Organization guideline(WHO) as demonstrated by Agarwal et al., [17].

2.3.5. Determination of Sperm Concentration and Sperm Count

The procedure of sperm concentration and sperm count was determined according to World Health Organization guideline similar to Björndahl [18]

2.3.6. Analysis of Prostate Specific Antigen

The assay of Prostate Specific Antigen (PSA) was determined with process specified by Sandwich Enzyme Immunoassay (EIA) test kit method similar to Liu et al., [19].

2.3.7. Analysis of Follicle Stimulating Hormone, Luteinizing Hormone, Estrogen and Testosterone

For analysis of follicle stimulating hormone , blood was collected from the test animal via direct cardiac puncture and placed in tubes containing the anticoagulant EDTA. To obtain serum, the blood sample was centrifuged at 3000 rpm for 10 minutes. The AccuBind ELISA microwell (4925-300; Monobind, USA) was employed. A 20 µL of the reference concentration, control, and test sample was added to the assigned well of the microtiter plate. This was added 100µL of follicle stimulating hormone-specific antibodies, gently swirl, and incubate for five minutes at room temperature. This allows the antigen to unspecifically adhere to the plastic through hydrophobic bonds. Following that, all liquids were taken from the wells with a transfer pipette, and each well thoroughly rinsed with phosphate buffer saline. A blocking agent, casein protein, was introduced to prevent non-specific protein binding to the plate. Then, 100 µL of secondary antibody conjugated with horseradish peroxide was added to each well and incubated for 15 minutes at 37℃. This allows the secondary enzyme to bind to the testosterone in the walls while the enzyme catalyzes a reaction with the substrate, resulting in a colored product. After 15 minutes, the secondary enzyme was removed with a transfer pipette and the wells were rinsed with phosphate buffer saline. Ultimately, the substrate tetra methyl benzidine (TMB) was added; the substrate reacted with the enzyme to generate a blue color shift, providing a quantitative determination of the follicle-stimulating hormone concentration in the sample using a spectrophotometer. The serum concentrations of luteinizing hormone, estrogen, and testosterone were analyzed using the same approach.

2.3.8. Determination of biochemical markers of oxidative stress

The activity of serum catalase was assayed using a UV recording spectrophotometer to measure the decrease in absorbance at 240 nm caused by H_2O_2 breakdown, in accordance with the Beers and Sizer method described by Usoh et al. [20].The reduced glutathione (GSH) was determined similar to Hussein et al [21] while malondialdehyde (MDA) was determined using the method of Buege & Aust [22] described in Vo et al. [23].

2.3.9. Histological examination of testis and prostate

Tissue preparation method written by Alturkistani et al., [24]was used in the study. Histopathological examinations were performed on sections of the testis and prostate from the experimental Wistar rats used. For at least 48 hours, the samples were fixed in 10% phosphate buffered formalin. The tissues were then sliced, dehydrated in four grades of alcohol (70 %, 80 %, 90 %, and pure alcohol), cleansed with three grades of xylene, and embedded in molten wax. Following solidification, the blocks were sectioned into 5m thick slices with a rotary microtome, floated in a water bath, and incubated at 60 °C for 30 minutes. The 5m thick sections of tissues were then cleaned in three grades of xylene and rehydrated in three grades of alcohol (90 %, 80 %, and 70 %). Following that, the sections were stained for 15 minutes with hematoxylin. The blueing process employed ammonium chloride. Before being counterstained with Eosin, the cells were differentiated with 1% acid alcohol. Permanent mounts on degreased glass slides were made using a mountant known as DPX(a mixture of Distyrene, a plasticizer, and xylene).

2.4. Statistical Analysis

All information and data generated were evaluated with the Statistical Package for Social Sciences (SPSS) software (Version 27).All data was reported as mean ± SD. ANOVA was used to compare the mean values of the measured parameters in the control and experimental groups. Values with $p < 0.05$ are deemed statistically significant.

3. Results

3.1. Effects on Relative Organ Weight and Prostate Specific Antigen of Wistar Rats

Table 1 Effects of varied doses of trona on organ weights and PSA

	Testis weight(g)	Relative testis	Prostate weight(g)	Relative prostate	Total PSA (mg/dl)
Control	1.08 ± 0.24	0.83 ± 0.17	0.44 ± 0.203	0.34 ± 0.15	1.19 ± 0.09
150 mg/kg	0.50 ± 0.17**	0.44 ± 0.11*	0.40 ± 0.26^{ns}	0.35 ± 0.20^{ns}	1.43 ± 0.10*
300 mg/kg	0.73 ± 0.25*	0.71 ± 0.24*	0.37 ± 0.17^{ns}	0.36 ± 0.16^{ns}	1.57 ± 0.23**
450 mg/kg	0.90 ± 0.25^{ns}	0.78 ± 0.31^{ns}	0.42 ± 0.23^{ns}	0.36 ± 0.21^{ns}	1.76 ± 0.14**

Values are presented as mean ± Standard Deviation, n = 7. **P<0.01, *P<0.05: Statistically significantly different from control group. ^{ns}P>0.05: Not statistically significantly different from control group.

Table 1 shows no statistically significant ($p>0.05$) difference in prostate weight following varied dosages of trona compared to the control group. Significant ($p<0.01$) increase in testes weight was observed at 150 and 300 mg/kg doses, but not at 400 mg/kg, compared to the control group. PSA values increased dose-dependently at 150, 300, and 400 mg/kg compared to the control group.

3.2. Effects on semen count, motility and morphology

Table 2 shows a statistically significant ($p<0.0$) dose-dependent reduction in AM (Actively motile) semen at 150, 300, and 450 mg/kg compared to control group. There was also a significant dose-dependent increase in NM (None motile) semen at 150, 300, and 450 mg/kg compared to control group. Moreover, 300 mg/kg recorded a significant ($p<0.0$) reduction in normal semen morphology.

Table 2 Effects of varied doses of trona on semen count, motility and morphology

	AM (%)	**NM (%)**	**Count (x10^6/ml)**	**Normal (%)**	**Abnormal (%)**
Control	82.43 ± 2.07	17.57 ± 2.07	484.29 ±22.40	82.29 ± 2.56	17.71 ± 2.56
150 mg/kg	67.29 ± 7.30**	31.29 ± 8.36**	345.14 ± 28.40**	80.71 ± 6.02^{ns}	19.29 ± 6.02^{ns}
300 mg/kg	60.71 ± 5.56**	39.29 ± 5.56**	327.29 ± 36.11**	75.57 ± 7.76*	25.86 ± 8.36*
450 mg/kg	46.71 ± 6.07**	51.86 ± 5.96**	197.29 ± 33.47**	78.29 ± 3.82^{ns}	21.71 ± 3.82^{ns}

Values are presented as mean ± Standard Deviation, n = 7. *P<0.05, **p<0.001: Statistically significantly different from control group. ^{ns}p>0.05: Not Statistically significantly different from control group. AM (Actively motile), NM (None motile).

3.3. Effects on Semen Color, Semen Consistency and Semen pH

Compared to the control group, there was no statistically significant difference ($p>0.05$) in the color of sperm at concentrations of 150, 300, and 450 mg/kg, as shown in Table 3. In addition, neither the pH nor the consistency of sperm at concentrations of 150, 300, and 450 mg/kg varied significantly ($p>0.05$) from the control group.

Table 3 Effects of varied doses of trona on Semen Color. Semen Consistency and Semen pH

	Semen Colour	**Decision**	**Semen Consistency**	**Decision**	**Semen pH**	**Decision**
Control	1	Not Significant	3	Not Significant	Neutral	Not Significant
150 mg/kg	1	Not Significant	3	Not Significant	Neutral	Not Significant
300 mg/kg	1	Not Significant	3	Not Significant	Neutral	Not Significant
450 mg/kg	1	Not Significant	3	Not Significant	Neutral	Not Significant

Adopted Semen color scale (1-3) represent 1 (white); 2 (Milky White); and 3 (Creamy white). For Semen consistency, the scale was 1 (watery), 2 (slightly thick), 3 (thick) and 4 (very thick).

Semen pH, the scale range includes; 0-5 (Acidic), 6-8 (Neutral), 9-14 (Alkaline).

3.4. Effects of trona on male reproductive hormonal parameters

Table 4 shows the effects of graded doses of trona on male reproductive hormones. Administration of trona caused dose dependent statistically significant ($p<0.05$) increase in estrogen, and statistically significant reduction ($p<0.05$) in testosterone and luteinizing hormone (LH). Highest dose of trona caused a statistically significant reduction in follicle stimulating hormone (FSH) when compared to control group.

Table 4 Effects of varied doses of trona on male reproductive hormones

	Estrogen (pg/ml)	Testosterone (ng/dl)	LH (IU/L)	FSH (mIU/L)
Control	28.23 ± 0.81	2.51 ± 0.17	2.50 ± 0.14	1.71 ± 0.16
150 mg/kg	31.56 ± 2.34*	2.33 ± 0.11	2.30 ± 0.14^{ns}	1.54 ± 0.29^{ns}
300 mg/kg	46.93 ± 2.03**	2.17 ± 0.11*	2.10 ± 0.08**	1.41 ± 0.34 ^{ns}
400 mg/kg	52.11 ± 3.43**	1.70 ± 0.37**	1.87 ± 0.40**	0.97 ± 0.26**

Values are presented as mean ± Standard Deviation, n = 7. **P<0.01, *P<0.05: Statistically significantly different from control group. ^{ns}P>0.05: Not statistically significantly different

3.5. Effects on Antioxidant Parameters

Table 5 shows a statistically significant dose-dependent reduction (p<0.05) in malondialdehyde (MDA) levels in the 150, 300, and 400 mg/kg treated groups compared to the control group. When compared to the control group, the 150, 300, and 400 mg/kg treatment groups showed a statistically significant dose-dependent increase (p<0.05) in glutathione (GSH). In comparison to the control group, the concentrations of catalase in the groups treated with 150, 300, and 400 mg/kg were statistically significantly (p<0.05) elevated.

Table 5 Effects of varied doses of trona on antioxidant parameters

	MDA(x10^-4 um/L)	GSH (x10^-4 umol/L)	Catalase(x10^-4 u/L)
Control	7.83 ± 0.54	3.78 ± 0.40	3.14 ± 0.26
150 mg/kg	7.13 ± 0.60*	4.85 ± 0.53**	3.71 ± 0.36**
300 mg/kg	6.51 ± 0.32**	5.35 ± 0.43**	3.92 ± 0.25**
450 mg/kg	5.55 ± 0.44**	6.87 ± 0.39**	4.30 ± 0.37**

Values are presented as mean ± Standard Deviation, n = 7. **P<0.001 *P<0.05: Statistically significantly different from control group.

3.6. Photomicrographs of Testes of Wistar rats

Figures 1a and 1b shows photomicrograph of testes of rats in control group. Sections show morphology consistent with testicular histology. The ductus epididymis (curved arrow) and the connective tissue are shown with normal architecture. The seminiferous tubules (arrowhead) show active and normal spermatogonia and spermatids (H&Ex100). Figures 2a and 2b shows photomicrograph of testes sections show morphology consistent with testicular histology. The ductus epididymis (curved arrow) and the connective tissue are shown with normal architecture. The seminiferous tubules (arrowhead) show active and normal spermatogonia and spermatids (H&Ex100). Figure 3a and 3b show photomicrograph of testes sections show morphology consistent with testicular histology.

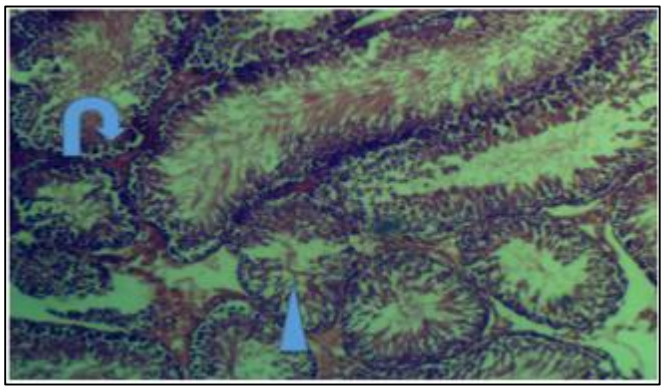

Figure 1a Photomicrograph of testes (Control). (H&Ex100)

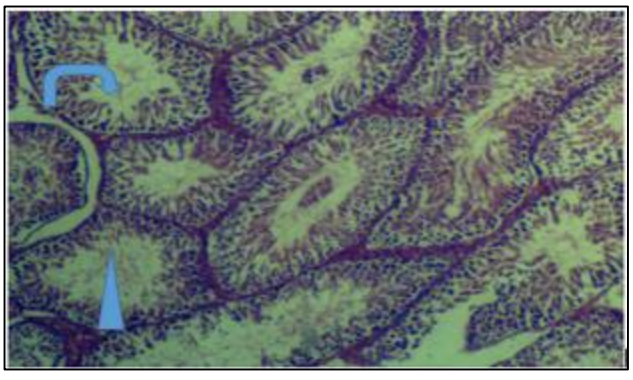

Figure 1b Photomicrograph of testes (Control) (H&Ex100)

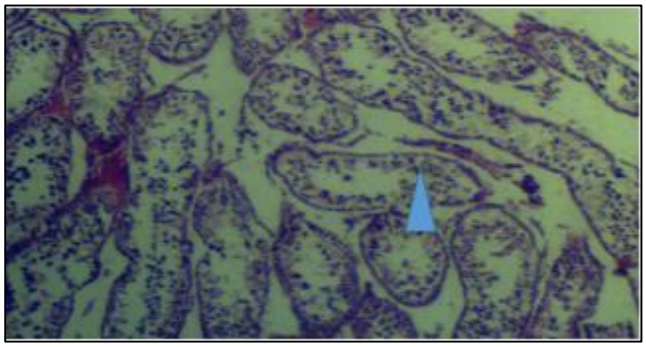

Figure 2a Photomicrograph of testes (150 mg/kg) (H&Ex100)

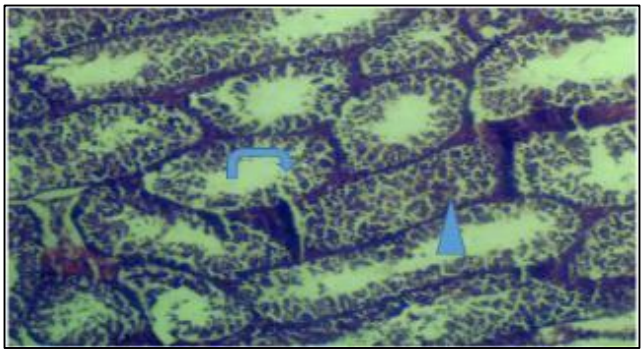

Figure 2b Photomicrograph of testes (150 mg/kg). (H&Ex100)

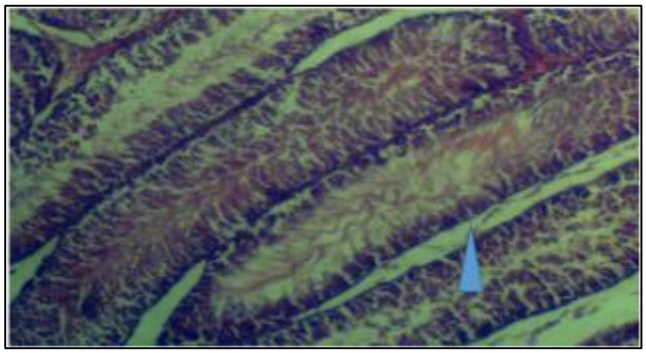

Figure 3a Photomicrograph of testes (300 mg/kg) (H&Ex100)

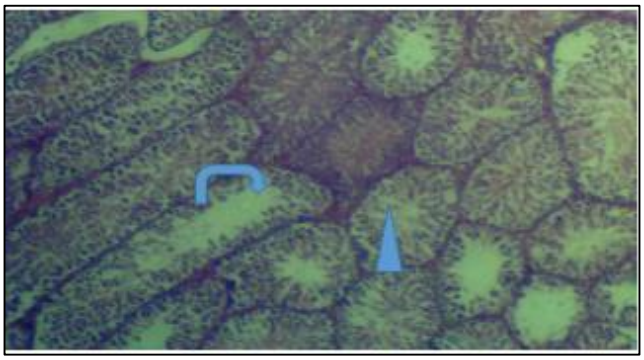

Figure 3b Photomicrograph of testes (300 mg/kg) (H&Ex100)

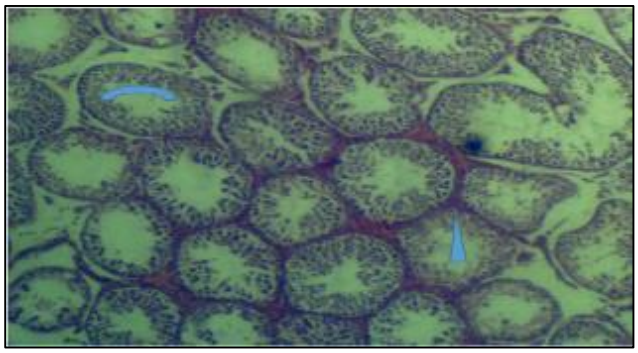

Figure 4a Photomicrograph of testes (450 mg/kg) (H&Ex100)

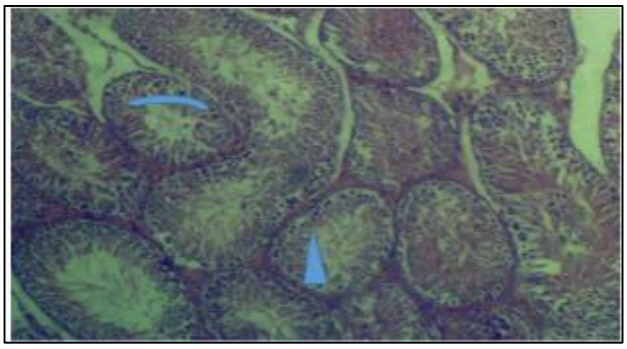

Figure 4b Photomicrograph of testes (450 mg/kg). (H&Ex100)

Figures 1-4 Photomicrographs of testes of Wistar rats administered varied doses of trona

The ductus epididymis (curved arrow) and the connective tissue are shown with normal architecture. The seminiferous tubules (arrowhead) show active and normal spermatogonia and spermatids (H&Ex100). Figure 4 (a) and (b) below show photomicrographs of testes sections show morphology consistent with testicular histology. The ductus epididymis (curved arrow) and the connective tissue are shown with normal architecture. The seminiferous tubules (arrowhead) show less active and normal spermatogonia and spermatids (H&Ex100).

3.7. Photomicrographs of Prostate of Wistar rats

Figures 5a and 5b depict a photomicrograph of a prostate tissue segment from the control group, revealing morphology consistent with prostate histology. The coagulating glands (arrows) are located within the fibromuscular stroma. The vas deference has intact histology (arrowhead) (H&Ex100).Figures 6a and 6b depict photomicrographs of prostate tissue sections with morphology consistent with prostate histology. The prostatic glands are located within the fibromuscular stroma. The seminal vesicles are functional, with intact histology (arrowhead) (H&Ex100).Figures 7a and 7b show a photomicrograph of a prostate tissue segment with morphology similar to prostate histology. The prostatic

glands (arrow) are located within the fibromuscular stroma. The urethra has intact histology (arrowhead) (H&Ex100).Figures 8a and 8b show a photomicrograph of a prostate tissue segment that displays morphology compatible with prostate histology. The prostatic glands (arrow) are located within the fibromuscular stroma. Some glands (arrowhead) contain amylase (H&Ex100).

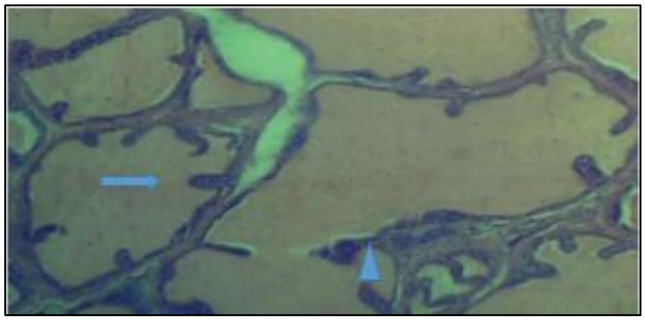

Figure 5a Photomicrograph of prostate (Control). (H&Ex100)

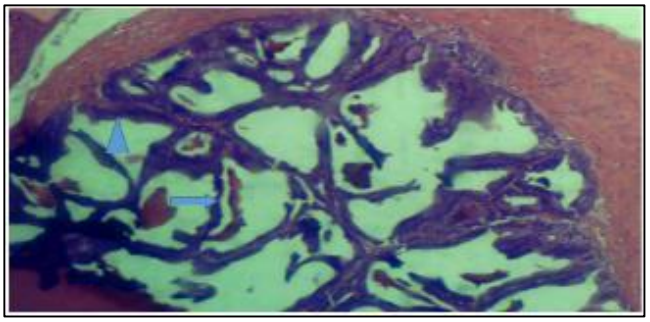

Figure 5b Photomicrograph of prostate (Control)(H&Ex100)

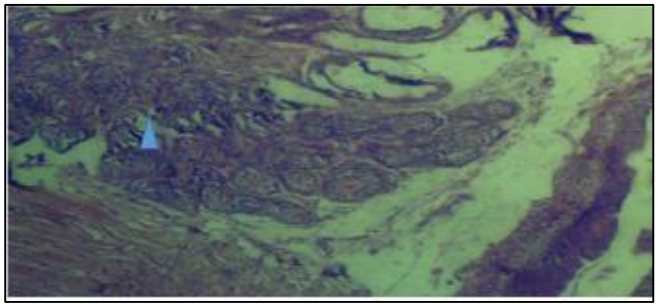

Figure 6a Photomicrograph of prostate (150 mg/kg (H&Ex100)

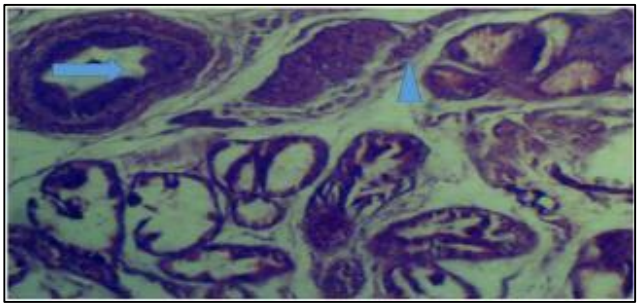

Figure 6b Photomicrograph of prostate (150 mg/kg). (H&Ex100)

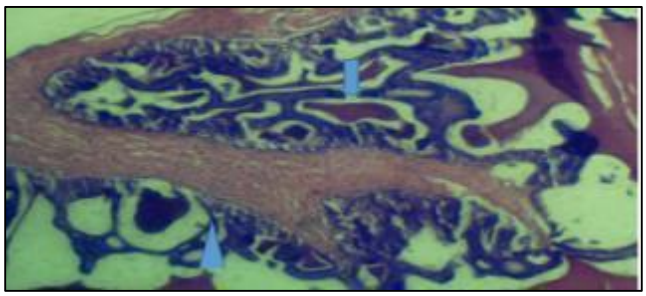

Figure 7a Photomicrograph of prostate (300 mg/kg) (H&Ex100)

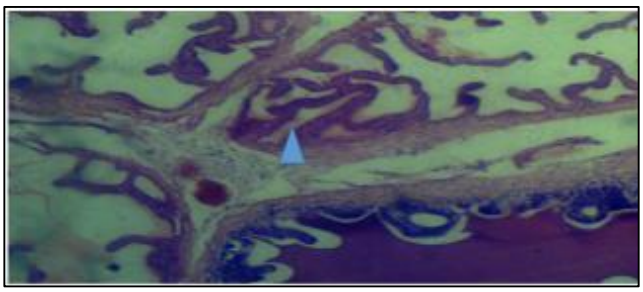

Figure 7b Photomicrograph of prostate (300 mg/kg) (H&Ex100)

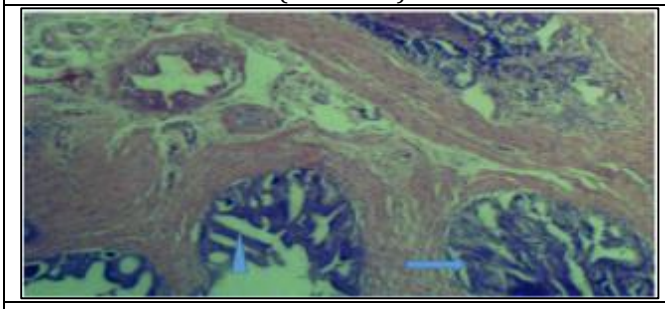

Figure 8a Photomicrograph of prostate (450 mg/kg) (H&Ex100)

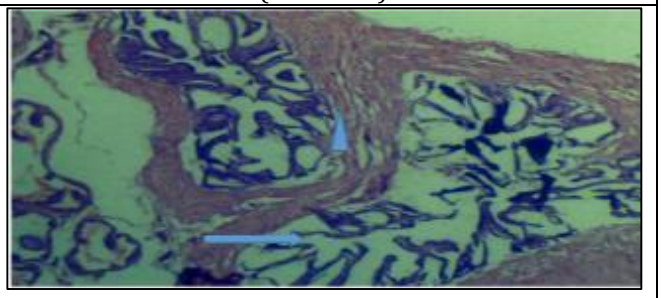

Figure 8b Photomicrograph of prostate (450 mg/kg). (H&Ex100)

Figures 5-8 Photomicrographs of prostates of Wistar rats administered varied doses of trona

3.8. Effects of trona on body weights of Wistar rats

Table 3 displays the impact of trona on the weekly body weights of Wistar rats. The body weight of the control group progressively increased from week 0 to week 4, with a weight gain of 4.96 ± 3.83 %. Conversely, there was a gradual decrease in the body weights of animals given doses of 150, 300, and 400 mg/kg starting from week 4. This resulted in a decrease in the rate of change in body weight compared to the control group.

Table 6 Effects of varied doses of trona on weekly body weight changes of Wistar rats

	Body weight (g)					
	Week 0	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Weight change (%)**
Control	134.83 ± 4.78	136.40 ± 4.37	137.46 ± 3.84	139.19 ± 5.59	141.97 ± 5.14	4.96 ± 3.83
150 mg/kg	137.43 ± 2.82	134.06 ± 2.75	131.83 ± 3.68	126.97 ± 5.67	123.56 ± 6.09	-11.39 ± 4.00**
300 mg/kg	133.36 ± 7.47	126.70 ± 6.06	118.96 ± 12.25	112.53 ± 12.69	115.74 ± 14.21	-16.26 ± 11.39**
450 mg/kg	134.96 ± 3.00	123.83 ± 5.45	118.30 ± 12.89	115.13 ± 11.19	113.33 ± 10.83	-20.06 ± 4.04**

4. Discussion

In table 1, the result shows dose-dependent increases in PSA of Wistar rats administered trona when compared to control. Although there are factors that can cause increased serum prostate in humans, both normal and malignant prostatic epithelium produce elevated PSA levels [25].According to Nepal et al.,[26], elevated PSA values usually indicate prostate cancer, although they can also indicate non-cancerous prostatic disorders. Despite the elevated PSA levels in the trona-administered groups, rats' testes and prostates showed no signs of inflammation or alterations. The short study period (28 days) may have resulted in the absence of these conditions, leading to the conclusion that a prostate biopsy was not necessary, as it would be more beneficial to subject the rats to sub-chronic or chronic studies. Furthermore, Fang et al.[27] conducted human studies involving 5089 American males and found that overdosed dietary zinc can substantially increase serum prostate-specific antigen levels in response to heavy metal exposure. Because trona is a natural salt, it may not be completely devoid of heavy metals, and our ongoing comprehensive study on the metal content of trona may provide further clarification.

Table 2 displays how different doses of trona altered spermatozoa count, motility, and morphology. At 150, 300, and 450 mg/kg, there was a substantial reduction in actively motile spermatozoa compared to the control group. This led to a significant dose-dependent increase in non-motile spermatozoa at similar doses across groups. More importantly, the dose-dependent decreases in sperm count recorded in the same table suggest that trona can affect sperm count. As a result, this study agrees with those of Ajayi and Akhigbe[28]. However, our study recorded related results at lower doses, suggesting trona's antispermatogenic effects even at very low doses. As shown in Table 3, there were no significant changes in semen color, semen consistency, or semen pH across the groups, indicating that trona has no effect on these parameters.

In Table 4, the effects of trona on male reproductive hormonal parameters showed a dose-dependent increase in estrogen but decreases in testosterone and luteinizing hormones at 300 and 450 mg/kg. The follicle-stimulating hormone showed slight decreases at 150 and 300 mg/kg, but was not statistically significant except at 450 mg/kg when compared to the control. Estrogen and testosterone are endogenous hormones present in both male and females[29]. Reproductive system regulation depends on the complex interplay between estrogen, testosterone, LH, and FSH. While the precise reason for the surge in estrogen levels may be difficult to determine, one hypothesis put forth by Spratt et al.[30] is that the increase in serum estrogen is due to accelerated aromatization rates of androgens to estrogens.

Furthermore, the binding of sex hormone-binding globulin (SHBG) to testosterone can change the availability of testosterone for aromatization, affecting estrogen levels independently of free testosterone levels[31].On the other hand, testosterone is crucial for spermatogenesis, the process of sperm production. Low testosterone levels can lead to decreased sperm production, affecting male fertility[32]. It can reduce sperm production directly and cause erectile dysfunction indirectly, therefore affecting fertility[33]. In males, testosterone is produced by Leydig cells while FSH

promotes the growth and spermatogenesis of Sertoli cells [34]. Our findings suggest that chronic consumption of trona can lead to secondary hypogonadism. Secondary hypogonadism is frequently accompanied by comparable reductions in the production of sperm and testosterone [35]. This occurs because intratesticular testosterone, the principal hormonal stimulus to sperm production, decreases in response to a reduction in LH secretion and, consequently, testicular testosterone production [36].

In Table 5, the effects of varied low doses of trona on antioxidant parameters were shown. Antioxidants are essential for preventing the creation and counteracting the effects of reactive oxygen and nitrogen species. These species are produced within the body and can cause harm to DNA, lipids, proteins, and other biomolecules[37],[38]. A dose-dependent reduction in serum MDA in Wistar rats treated with trona suggests that it may have antioxidant properties. This is because studies have shown that decreasing MDA levels are associated with increasing amounts of antioxidants such as vitamin A, C, and E[39]. Reduced MDA levels can lead to enhanced non-enzymatic antioxidants, reducing oxidative stress. This balance of MDA and antioxidants is critical for preventing oxidative damage in the body. Moreover, a dose-dependent rise in serum GSH and Catalase levels can boost antioxidant capacity, reduce oxidative stress, lower MDA generation, and help to maintain a healthy redox balance in the body. According to Tang et al., [40], increased levels of GSH and Catalase have been linked to lower MDA generation. MDA is an indicator of oxidative stress, and lower levels imply a more balanced redox state with less oxidative damage.

5. Conclusion

The study successfully investigated the effects of varied low doses of trona (grey-colored potash) administered to male Wistar rats for 28 days. When extrapolated to humans, the study's findings may have similar effects. The benefits of consuming trona as a food additive and in the preparation of local herbal remedies are that it is loaded with good antioxidant properties even at the lowest dose of 150 mg/kg. When used in very low doses, it is a cheaper source of natural antioxidants. This study also confirmed the use of trona as a local male contraceptive and to douse libido. Unfortunately, the cons of chronic consumption of trona, especially at doses above 300 mg/kg and 400 mg/kg, are alterations in male fertility hormones, posing a danger of infertility that could arise from secondary hypogonadism. Moreover, the elevated PSA recorded in this study also indicates that regular trona intake may worsen the health conditions in already existing prostate-related problems.

Compliance with ethical standards

Acknowledgement

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Disclosure of conflict of interest

The authors have no conflict of interest for publishing this article.

Statement of ethical approval

The study sought and obtained clearance from the Animal Research Ethics Committee (AREC) on the use of animal policy at Nnamdi Azikiwe University, Awka. The approval number is : ***NAU/AREC/2024/0016***. Furthermore, all other procedures in this study fully adhered to the rules for conducting research involving the use of animals, as detailed in Organization for Economic Co-operation and Development OECD [41] guidelines.

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