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# The mitigating effect of *Ananas comosus* on aluminum-induced oxidative stress on the testes of adult male Wistar rats

Bankole J. Leko<sup>1</sup>, Solomon T. Olawuyi<sup>2\*</sup>  and Lawrence U. Okon<sup>1</sup>

## Abstract

**Background:** The mitigating effect of *Ananas comosus* (pineapple juice) extract on an aluminum-induced testicular toxicity in male Wistar rats was examined in this study. Thirty healthy adult male Wistar rats with an average weight of 200 g were grouped into six groups; distilled water and 1 ml of pineapple juice extracts were administered to the control and treated animals respectively for 3 weeks. The control group was given rat pellets and distilled water. Negative control was given 100mg/kg/d Aluminum Chloride and pellets; Group 1 was given 100mg/kg/d and 1ml of pineapple juice in distilled water orally; Group 2 was given 100mg/kg/d Aluminum Chloride and 1.5 ml of in distilled water orally; group 3 was given 100 mg/kg/day aluminum chloride and 2 ml of pineapple juice in distilled water orally; group 4 was given 100 mg/kg/day aluminum chloride and 2.5 ml of pineapple juice in distilled water orally. Testicular histology, semen parameters, and testosterone were assessed.

**Results:** This study showed a significant ( $P < 0.05$ ) decrease in testicular volume, motile sperm count, concentration, total count, progressive evaluation, and morphology in the negative control group relative to the normal control and extract control groups. In the groups co-treated with aluminum chloride and *Ananas comosus* extract, there was improvement in sperm volume, motility, total count, progressive assessment, and morphology. There was also a statistical decrease ( $P < 0.05$ ) in testosterone hormone in the negative control group, but there was an increase in the aluminum chloride and *Ananas comosus* extract co-treated groups. Similarly, in co-treated aluminum chloride and *Ananas comosus* extract, the degenerative seminiferous tubule histoarchitecture due to aluminum chloride in the negative control group was enhanced.

**Conclusion:** Based on this current study, it was evident that aluminum chloride induced oxidative stress and retarded reproduction in males whereas *Ananas comosus* mitigated reproduction in males by improving sperm parameters and microarchitecture of the testes.

**Keywords:** *Ananas comosus*, Asthenospermia, Peroxidation, Teratospermia

## Background

Aluminum has been reported to have a toxic impact on the nervous system and has also caused damage to DNA (Mowry, Spyker, Brooks, Zimmermann, & Schaubenf, 2016; Olawuyi, Ukwanya, Jimoh, & Akinola, 2019). This was demonstrated through nanotechnology findings that

human exposure to engineered aluminum nanomaterials (NMs) could alter an individual's genetic makeup (Priyam, Singh, & Gehlout, 2018).

The bioavailability and oral bioavailability of aluminum ions in experimental animals and man's drinking water ranged from 0.1 to 0.3% (Mowry, Spyker, Cantilena Jr., McMillan, & Ford, 2013). Aluminum accumulates in a certain bone after being absorbed and distributed in animals and humans' tissues (Walker, Sharman, & Cody, 1990). Not less than 95% of

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aluminum ions bind to albumin transvascularly and are eliminated in the kidney (Macedo & De-Sousa, 2008).

Rapid industrialization and urbanization have brought about the increased use of metals and resulted in serious reproductive abnormalities which are now a major concern for global health (Mohammadirad & Mohammad, 2011; Turgut, Abban, Turgut, & Take, 2003). Oxidative stress is likely when imbalances exist between the produced reactive oxygen species (ROS) and their efficient elimination by available antioxidant systems (Papa & Skulachev, 1997). The most abundant ROS is the hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), hydroxyl ion (OH•), and the superoxide anion (O<sub>2</sub><sup>-</sup>). Researchers have documented the chemistry of oxygen-derived free radical generation and its scavenging effect. Chemical initiation of oxidative stress is a result of oxidation and peroxidation of cell macromolecules (Pryor, 1989; Szabo, Ischiropoulos, & Radi, 2007). The conditions that initiate oxidative stress in the testis as well as its effects on testicular function have been investigated, with much focus placed on semen quality; however, the pathophysiology of this phenomenon has been clearly understood (Agarwal, Prabakaran, & Said, 2005; Aitken & Clarkson, 1987; Bennetts & Aitken, 2005).

Oxidative stress is caused by exposure to heavy metals. For example, high concentrations of iron in testicular tissue cause depletion of antioxidants which will increase oxidative damage in the rat testes (Lucesoli & Fraga, 1995; Wellejus and Poulsen, 2000). Exposure to aluminum, cadmium, and lead may induce testicular oxidative stress and decrease rat testicular sperm output, increase ROS formation in epididymal sperm, and decrease sperm motility induced (Hsu, Liu, Hsu, Chen, & Leon-Guo, 1997; Oteiza, Adonaylo, & Keen, 1999). It is also found to decrease the antioxidant capacity of the testis and increase lipid peroxidation. Testicular oxidative stress is responsible for cellular changes (ranging from aging, varicocele to testicular torsion) that could be detrimental to male fertility.

It has been reported that medicinal plant has been used in folkloric medicine for the treatment of various diseases. Herbal medicine is an integral part of “traditional medicine” (TM). TM is the sum of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures used in the maintenance of health as well as in the prevention, diagnosis, improvement, or treatment of illness. TM involves the use of herbal, animal, and minerals with herbal medicines being the most widely used (World Health Organization, 2000).

Pineapple (*Ananas comosus*) is a tropical plant with edible multiple fruits also called pineapples and the most economically significant plant in the family Bromeliaceae (Coppens d’Eeckenbrugge and Leal, 2003). Raw

pineapple pulp is 86% water, 13% carbohydrates, 0.5% protein, and contains negligible fat (Felicity, 2010). In a 100-g reference amount, raw pineapple supplies 50 cal and is a rich source of manganese (44% daily value, DV) and vitamin C (58% DV), but otherwise contains no micronutrients in significant amounts (Hossain, Akhtar, & Anwar, 2015).

Phytochemical analysis of pineapple fruits and peels showed that it contained valuable compounds such as polyphenols, gallic acid, syringic acid, vanillin, ferulic acid, sinapic acid, coumaric acid, chlorogenic acid, epicatechin, and arbutin (Hossain et al., 2015). Pineapple is easy to cultivate and can be ripened for harvest after 6 months of cultivation (Morton, 1987). A report shows that in 2016, Costa Rica, Brazil, and the Philippines produce about one-third of the world’s pineapples.

Pineapple is short and can be about 1.0 to 1.5-m high. It is an herbaceous perennial plant that has a stocky stem with tough, waxy leaves. Pineapple plants produce many flowers up to 200 during fruit-bearing. Once it flowers, the individual fruits of the flowers join together to create what is commonly referred to as a pineapple (Joy & Anjana, 2015). Pineapple contains bromelain which produces sore mouth feeling often experienced when consumed. It also contains raphides needle-shaped crystals of calcium oxalate that occur in pineapple fruits and leaves, likely cause micro-abrasions, contributing to mouth discomfort (Bartholomew, Paull, & Rohrbach, 2003). Bromelain is an active component of the pineapple plant, found in a mixture of proteolytic enzymes. Bromelain is associated with some clinical disorders, but up to date its direct effect on humans has not been adequately defined (Oyesola, Oyesola, & Izagbo, 2013).

This study aims to investigate the mitigating effect of *Ananas comosus* extract on aluminum-induced oxidative stress on the testes of adult male Wistar rats.

## Methods

### Chemicals and reagents

All chemicals used in the course of the study were of pure analytical grade. Aluminum chloride was obtained from Pascal scientific limited, plot 21, Block Q, Ondo State industrial estate, Akure, Nigeria. The histological staining was done in Anatomical-Pathology Department, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

### Preparation and concentration of pineapple fruit extract

The pineapple used for the experiment was obtained from the central mart, Madonna University, Elele, Rivers State, Nigeria. The extraction of pineapple pulp was achieved by sieving to separate the pulp from the seeds according to the method described by Bates, Morris, and Crandall (2004).

### Experimental animals

A total of thirty healthy male Wistar rats with average weight of 200 g were used for this study. They were obtained from animal house of the Department of Anatomy, University of Port Harcourt Rivers State, Nigeria. The clearance was obtained from ethical committee on the care and use of animal experiments in the animal houses of the Department of Anatomy Madonna University, Elele, Rivers State, Nigeria, before the commencement of this study. The Wistar rats were weighed using an electronic weighing scale (WH-B Series, China) and housed in six-compartment cages (made of wire mesh) of five rats each, kept at room temperature with 12 h light and 12 h dark cycle. The rats were allowed to acclimatize for 2 weeks before the administration of aluminum and *Ananas comosus* extract. Animals in all groups were fed on rat pellet feed obtained from Madonna University feeds, Elele, and the rats were provided with water ad libitum throughout the experiment. The Principles of Humane Experimental Technique was followed: National Advisory Committee for Laboratory Animal Research (NACLAR, 2004), National Institute of Health, for the care and treatment of laboratory animals.

### Experimental design

The thirty male Wistar rats were randomly assigned into four (4) experimental groups, one (1) positive control, and one (1) negative control with five (5) rats per group. Using a feeding tube (size 6), distilled water and pineapple juice (PJ) extracts were administered to the control and treated animals respectively for 3 weeks (Olawuyi, 2020).

Control: given rat pellets and distilled water.

Negative control: given 100 mg/kg/d aluminum chloride and pellets.

Group 1: given 100 mg/kg/day of aluminum chloride and 1 ml of pineapple juice (PJ) in distilled water orally.

Group 2: given 100 mg/kg/day of aluminum chloride and 1.5 ml of pineapple juice (PJ) in distilled water orally.

Group 3: given 100 mg/kg/day of aluminum chloride and 2 ml of pineapple juice (PJ) in distilled water orally.

Group 4: given 100 mg/kg/day of aluminum chloride and 2.5 ml of pineapple juice (PJ) in distilled water orally.

### Epididymal sperm concentration

The caudal epididymis was removed and minced with anatomic scissors in 10 ml of normal saline, placed in a rocker for 5–10 min, and incubated at  $34 \pm 3$  °C temperature for about 2 min. Five grams sodium bicarbonate and 1 ml formalin (35%) in ratio 1:100 was used to dilute the supernatant after incubation. The concentration count of the sperm cells was done using, new

improved Neubauer's counting chamber (Haemocytometer) and Mackler Sperm counting chamber. Ten microliters from the aliquot sample was aspirated with a Pasteur pipette and mounted on the counting chamber; this was allowed to stand for 5 min and thereafter observed under a binocular light microscope (Yokoi & Mayi, 2004). The equation for sperm concentration/ml = no. of sperms counted  $\times$  1,000,000.

### Sperm motility

The caudal epididymis was excised with a surgical blade. The minced sample was diluted with 0.9 ml of normal saline and was left for 5–10 min. Ten microliters of this solution will be observed under the light microscope at a magnification of  $\times$  100.

### Sperm morphology

The morphology of the spermatozoa was determined by using the original dilution for motility, dilute 1:20 with 10% neutral buffered formalin (Sigma-Aldrich, Oakville, ON, Canada).

Sperm suspension was used to prepare smears for evaluation of sperm morphology to determine the rate of sperm abnormalities. In detail, a drop of sperm suspension was added into an equal volume 1% eosin-y 5% nigrosin, which was then mixed and smeared on pre-warmed clean glass slides and air-dried. Using an Olympus light microscope, two hundred sperm cells were examined at  $\times$  400 magnifications per animal to determine the morphological abnormalities (Rezvanfar et al., 2008). Morphology of the sperm cells was categorized based on the presence of one or more abnormal features (Teratospermia) such as tail defects (short, irregular, coiled, or multiple tail); neck and middle piece defects (distended, irregular, bent middle piece, abnormally thin middle piece); and head defects (round head, small or large size, double or detached head). The morphology was presented in percentages (Yokoi & Mayi, 2004).

### Progressive assessment

Sperm motility was evaluated across a minimum of five strips of squares within a 10 s observation time per square. Non-motile sperms were first counted, and then, only sperms that exhibited flagella activity were taken to be motile. For an objective assessment of motility, spermatozoa were classified based on recommendations of the World Health Organization (World Health Organization, 2010) and categorized based on how fast or how slow they appear.

### Serum assay testosterone procedure

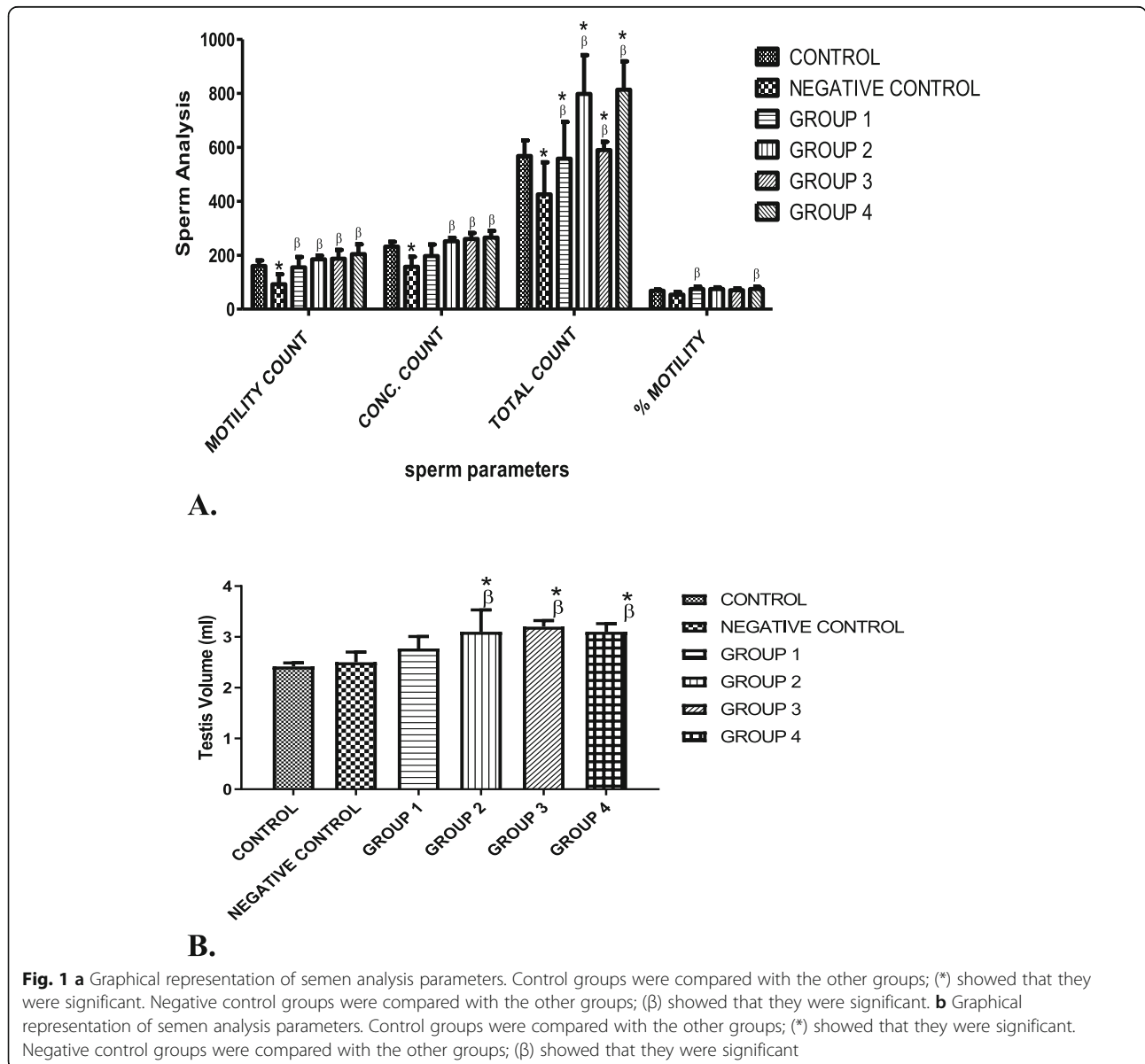
The blood was collected by ventricular puncture into plain redtop venipuncture tubes and spun at 3000 rpm

**Table 1** Semen analysis parameters

Groups	Volume (ml)	Motility count × 10 <sup>6</sup> /ml	Conc count × 10 <sup>6</sup> /ml	Total count × 10 <sup>6</sup> in ml	% motility (%)
Control	2.42 ± 0.07	160.0 ± 20.4	232.0 ± 17.9	561.4 ± 57.5	69.00 ± 4.1
Negative control	2.50 ± 0.20	92.50 ± 36.8	157.0 ± 37.0	392.5 ± 109.1	59 ± 10.6
Group 1	2.77 ± 0.24	155.0 ± 38.6	197.0 ± 42.5	545.7 ± 132.6	79 ± 9.7
Group 2	3.10 ± 0.43	185.0 ± 13.2	252.0 ± 12.5	781.2 ± 141.2	73 ± 6.6
Group 3	2.30 ± 0.12	187.0 ± 32.5	260.0 ± 22.7	598 ± 31.6	72 ± 7.3
Group 4	3.00 ± 0.16	204.0 ± 36.6	265.0 ± 25.3	795 ± 103.3	77 ± 8.4

for 10 min in an angle head centrifuge at 25 °C. The serum obtained was used for testosterone assay. The samples were assayed in batches using the enzyme-linked immunoassay (ELIZA) method. The microwell kit was from Biotec laboratories Ltd, UK. With ten microns

(10 µl) of the standard, the specimens and control were dispensed into the number of coated wells. Hundred-micron (100 µl) testosterone conjugate reagent was added and then fifty microns (50 µl) of anti-testosterone reagent. The contents of the microwell were thoroughly



**Table 2** Representation of sperm morphology

Groups	Normal (%)	Neck defect (%)	Tail defect (%)	Head defect (%)
Control	63 ± 2.5	10.00 ± 0.8	17.50 ± 1.5	9.50 ± 0.5
Negative control	50 ± 3.7*	17.25 ± 2.2	20.50 ± 1.6	12.25 ± 0.9
Group 1	70.75 ± 10.1	9.00 ± 4.3	12.75 ± 4.6	7.00 ± 2.0
Group 2	79.5 ± 4.1*	5.50 ± 1.5	9.75 ± 2.2	5.25 ± 1.0
Group 3	76.25 ± 5.6*	7.00 ± 2.3	10.50 ± 1.1	6.75 ± 2.1
Group 4	84 ± 3.1*	5.25 ± 1.6	8.50 ± 2.2	4.75 ± 1.0

Values are expressed as mean ± SEM; n = 4

\*p < 0.05

mixed and then incubated for 20 min at room temperature. The reaction was stopped with 100 µl of 1 M hydrochloric acid (Tietz & Saunders, 1994). Absorbance was measured with an automatic spectrophotometer (Rayto: RT-2100C, Microplate Reader) at 450 nm.

### Histology analyses

The animals were cut open by abdominopelvic incision after sacrifice, and the testes were taken from each of the six groups and were fixed in 10% paraformaldehyde for 24 h. Then, each specimen was sliced into small slabs (3–5-mm thick) and further fixed in a change of the same fixative for another 15 h. The fixed tissue specimens were trimmed and washed in tap water for 12 h. An alcohol series (methyl, ethyl, and absolute) was used to dehydrate the tissue specimens. The tissue specimens were cleared in xylene and embedded in paraffin. The paraffin blocks were sectioned in 5-micron slices on a rotary microtome. The obtained tissue sections were collected on glass slides and stained with hematoxylin and eosin.

### Statistical analysis

All statistical analyses were done using Statistical Package for Social Sciences (SPSS version 18.0), and some charts were drawn using graph pad prism version 8.03.

Analysis of variance (ANOVA) and post hoc tests were used to analyze the data. Tukey's multiple comparisons were used to test for statistically significant differences between the control and experimental group. Results were presented as mean ± standard error of mean (SEM), and p < 0.05 was considered statistically significant.

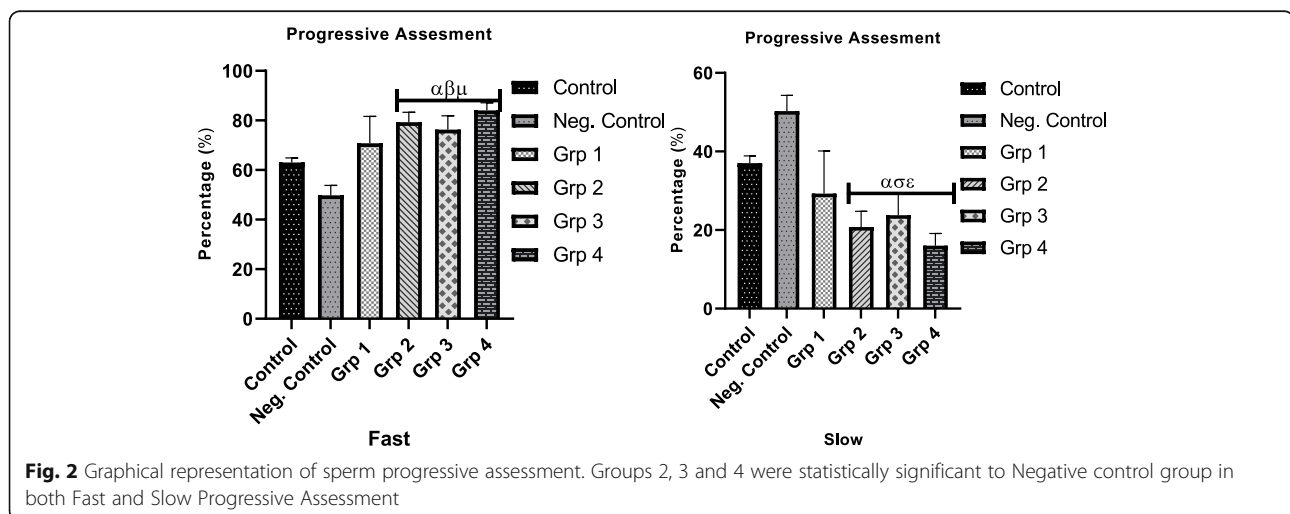
## Results

### Semen analysis parameters

When comparing the control group with other experimental groups (Table 1, Fig. 1b), the volume of sperm increased in various groups to the dose of pineapple juice administered. Experimental group 4 had the highest volume of sperm (3.00 ± 0.16 ml).

### Effect of *Ananas comosus* and aluminum-induced oxidative stress on epididymal sperm concentration, motility counts, and percentage motility

The concentration counts, when compared to the control group, showed an increase that was not statistically significant. The motility count of animals, when compared to the control, increased in various groups except in the negative control group reflecting the dose of pineapple juice administered (Table 1, Fig. 1a). Experimental





**Table 3** Representation of sperm progressive assessment

Groups	Fast progressive (%)	Slow progressive (%)
Control	63 ± 1.9	37 ± 1.9
Negative control	49.75 ± 4.0	50.25 ± 4.0
Group 1	70.75 ± 10.9	29.25 ± 10.9
Group 2	79.25 ± 4.0	20.75 ± 4.0
Group 3	76.25 ± 5.6	23.75 ± 5.6
Group 4	84 ± 3.1	16 ± 3.1

Values are expressed as mean ± SEM; n = 4  
\*p < 0.05

group 1 had the least motility count ( $155.0 \pm 38.6$ ) × 10<sup>6</sup>/ml while experimental group 4 had the highest motility count ( $204.0 \pm 36.6$ ) × 10<sup>6</sup>/ml (Table 1, Fig. 1a). The total count increased in all groups except in the negative control group where only aluminum chloride was administered. Percentage motility was increased in all experimental groups except for the negative control group. The level of increase was not statistically significant (Table 1, Fig. 1a).

**Effect of Ananas comosus and aluminum-induced oxidative stress on sperm morphology**

When comparing the control group with other experimental groups, they each showed lower levels of defect except in the negative control group which showed higher

levels of a defect. The results gotten were statistically significant (p < 0.05) except in group 1 where it was not statistically significant (p > 0.05) (Table 2, Fig. 2).

**Effect of Ananas comosus and aluminum-induced oxidative stress on progressive assessment**

When comparing the control group with other experimental groups, they each showed higher levels of fast progression except in the negative control group (49.75 ± 4.0); however, these were not statistically significant (p > 0.05) (Table 3, Fig. 3).

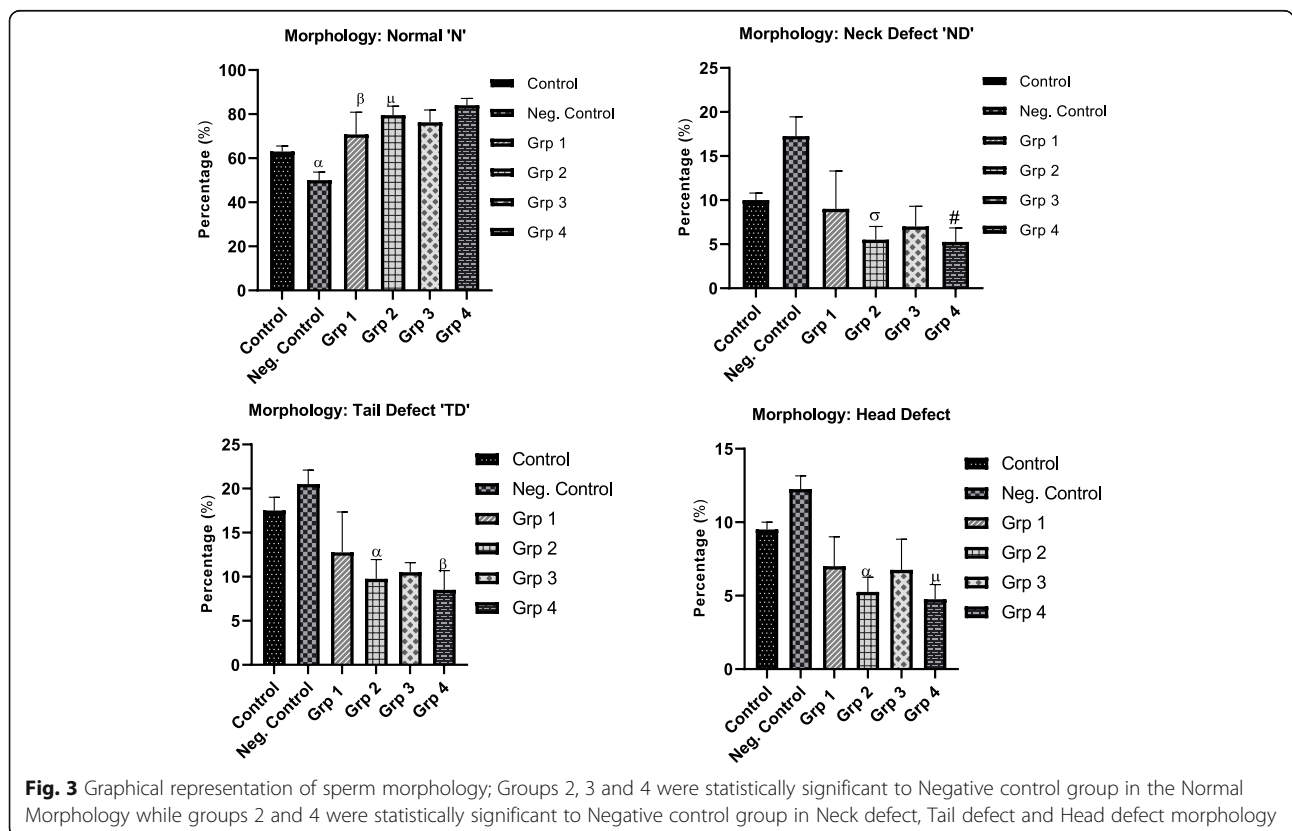
**Effect of Ananas comosus and aluminum-induced oxidative stress on testosterone hormone**

When comparing the control group with other experimental groups, the negative control group was the lowest while the other groups increased according to the dosage, and group 4 was the highest. The results gotten were statistically significant (p < 0.05) (Table 4, Fig. 4).

**Histological evaluation**

**Effect of Ananas comosus and aluminum-induced oxidative stress on the histopathology of the testes**

The testicular histoarchitecture of the control showed normal structure of seminiferous tubules with all the germ cells well represented and zone of spermiation (Fig. 5). Photomicrograph of the testes of the negative



**Table 4** Results showing the effect of aluminum chloride on testosterone hormone in adult male Wistar rats

Groups	Testosterone (ng/ml)
Control	15.25 ± 4.9
Negative control	5.99 ± 0.26*
Group 1	9.13 ± 0.8
Group 2	11.75 ± 0.3
Group 3	15.2 ± 0.6 <sup>β</sup>
Group 4	17.74 ± 0.23 <sup>ββ</sup>

Values are expressed as mean ± SEM

\*Negative control group was significant ( $p < 0.05$ ) when control was compared with the other groups

<sup>β</sup>Groups 3 and 4 were significant ( $p < 0.05$ ) when negative control was compared with other groups

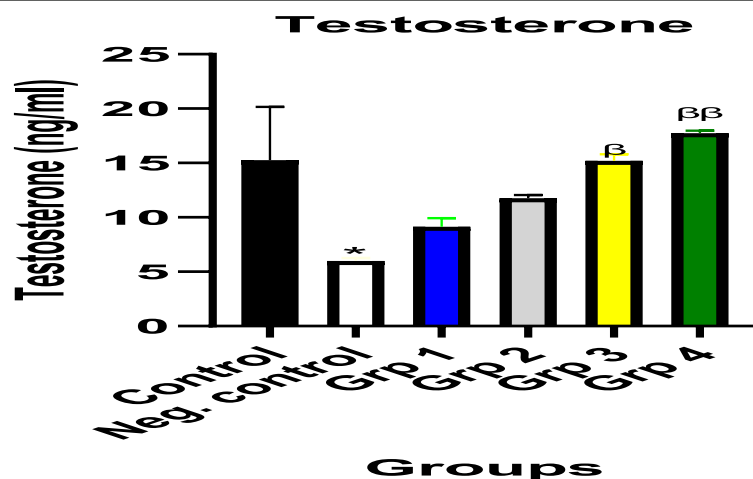
control group (100 mg of aluminum chloride per kg of body weight) showed abnormal structure of seminiferous tubules: distortion of most cells, degenerative changes of seminiferous tubules, widening interstitial space, and diffuse edematous changes with mononuclear cell infiltrations besides basement membranes separating from the underlying layers (Fig. 6). Photomicrograph of the testes of group 1 (Wistar rats given 100 mg/kg/day of aluminum chloride and 1 ml of pineapple juice) showed mild degenerative changes of seminiferous tubules, widening interstitial space, and testicular necrosis (Fig. 7). Photomicrograph of testes of group 2 (Wistar rats given 100 mg/kg/d of aluminum chloride and 1.5 ml of pineapple juice) showed the structure of seminiferous tubules and recovery from degenerative changes (Fig. 8). Photomicrograph of the testes of group 3 (Wistar rats with 100 mg/kg/day of aluminum chloride and 2 ml of pineapple juice) showed structure of normal seminiferous tubules (Fig. 9). Photomicrograph of the testes of group 4 (Wistar rats given 100 mg/kg/day of aluminum

chloride and 2.5 ml of pineapple juice) showed structure of normal seminiferous tubules with all the phases of germ cells well seen (Fig. 10).

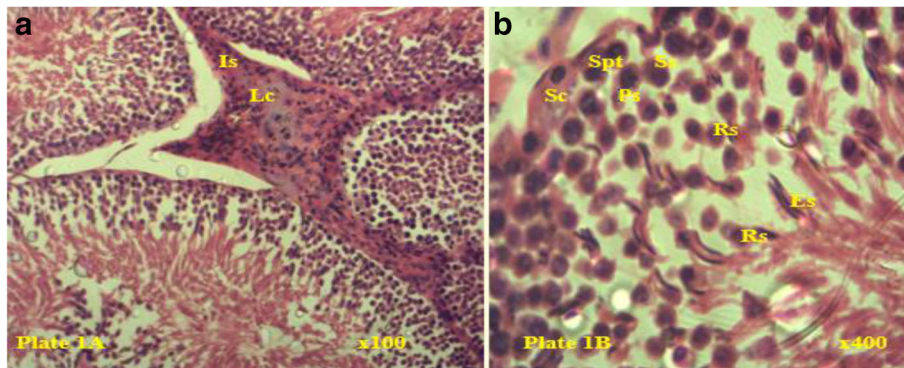
## Discussion

In this study, it was observed that pineapple juice significantly mitigated the deleterious effect of aluminum-induced oxidative stress on reproductive function.

The semen parameters showed insignificant changes. The concentration count, the motile count, progressive assessment, and the morphology were greatly increased in the rats treated with pineapple juice; this showed positive spermatogenesis and testicular steroidogenesis. Assessment of sperm morphology and other sperm parameters in this study revealed that the negative control group receiving only aluminum chloride showed a marked defect in sperm morphology. This was in agreement with the study carried out by Holstein, Schulze, and Davidoff (2003). For any form of perturbation, the testis relies on a monotonous and predictable response characterized by a reduction in spermatogenic production/efficiency (Holstein et al., 2003). Oxidative stress caused by aluminum chloride led to asthenospermia, hypospermia, teratospermia, and reduction in sperm count which is in agreement with the report of Ghalberg and Brodas (1981) and Bell and Thomas (1980). Sperm quality—unlike sperm quantity that is directly related to Sertoli cell efficiency—is associated with both testicular and epididymal micro-environments (Mäkelä, Toppari, Rivero-Müller, & Ventelä, 2014). For instance, a significant elevation in the production of ROS or a significant decrease in antioxidant defense capacity in either the testis or epididymis can compromise the cell membrane of spermatozoa ((Hsu et al., 1997; Koizumi & Li, 1992; Oteiza et al., 1999). All groups concomitantly



**Fig. 4** Representation of testosterone hormone level. (\*) Negative control group was significant ( $p < 0.05$ ), when control was compared with the other groups. (β) Groups 3 and 4 were significant ( $p < 0.05$ ), when negative control was compared with other groups



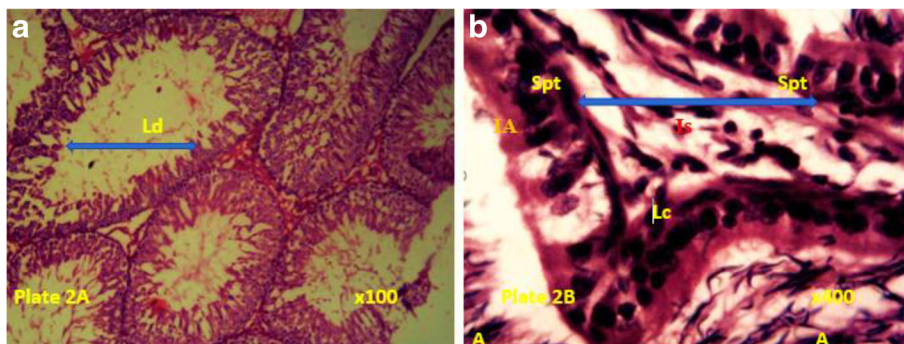
**Fig. 5 a, b** Photomicrograph of testes of the control group: Representative testicular micrograph of Wistar rats showing normal “control” structure of seminiferous tubules. Sc Sertoli cells, Lc Leydig cell, Spt spermatogonia, Ss Secondary spermatocytes, Ps Primary spermatocytes, Es elongated spermatids, Rs Round Spermatid, It Interstitial space, Spz spermatozoa. The stain is H&E and Magnification x 100 and x 400

treated with aluminum compound and pineapple juice had their semen parameters improved according to the dosage. A high dosage showed marked improvement. Pineapple juice markedly lessens the deleterious effect of oxidative stress induced by aluminum compound on spermatogenesis.

In this study, there was a statistically significant difference in the level of testosterone hormone ( $p < 0.05$ ) when comparing the control with the treated groups; the rats that received aluminum chloride and a high dose of pineapple juices recorded the highest value followed by group 3, while the negative control group which received aluminum alone was the least. Testosterone increased in a dose-dependent manner suggesting that pineapple juices enhanced male fertility. A decrease in testosterone hormone causes a decrease in spermatogenesis and hence a reduction in male fertility. This finding is in agreement with McLachlan et al. (2002) who reported that

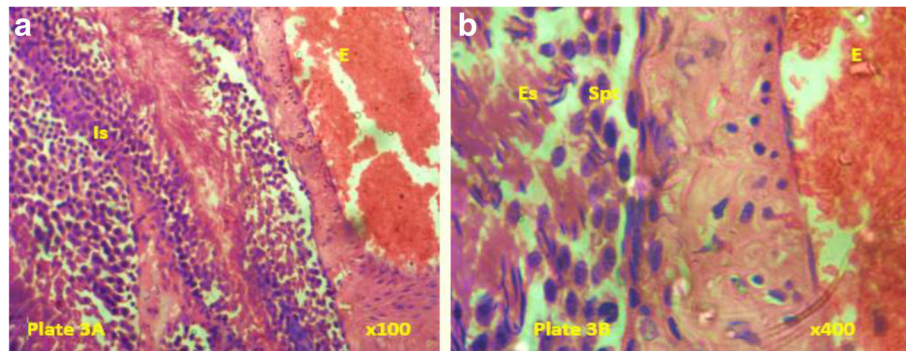
testosterone assists during spermatogenesis (spermatocyte maturation and round to elongated spermatid progression).

According to Wong et al. (2000) and Zhou et al. (2002), regulation of testosterone secretion is not the exclusive preserve of any single hormone; metabolic factor, micronutrient, or neurotransmitter all play major parts. Many factors are taken into consideration in looking at the pattern and level of circulating testosterone. With this in mind, and given the fact that any of these factors can be independently altered by myriads of conditions and stimuli, it is easier to understand why measuring a single factor may not show a very strong correlation with testosterone output under stress. Leydig cells are located in the interstitial space of the seminiferous tubule and it is a very important testosterone secretion. It also clears that the testicular micro-environment is equally



**Fig. 6 a, b** Photomicrograph of testes of the negative control group: Representative testicular micrograph of Wistar rats given 100mg of aluminum chloride per kg of body weight, showing the abnormal structure of seminiferous tubules. Distortion of most cells and degenerative changes of seminiferous tubules (IA); Widening interstitial space and diffuse edematous changes with mononuclear cell infiltrations besides basement membranes separating from the underlying layers (a). Ld Luminal diameter, Spt spermatogonia, It Interstitial space, Lc Leydig cell, Spz spermatozoa. The stain is H&E and Magnification x 100, x 400





**Fig. 7 a, b** Photomicrograph of testes of group 1: Representative testicular micrograph of Wistar rats given 100mg/kg/d of aluminum chloride and 1ml of pineapple juice (PJ) showing mild degenerative changes of seminiferous tubules; Testicular necrosis (E). *Spt* spermatogonia, *Es* elongated spermatids, *Rs* Round spermatids, *It* Interstitial space, *Spz* spermatozoa. Stain is H&E and Magnification x 100 and x 400

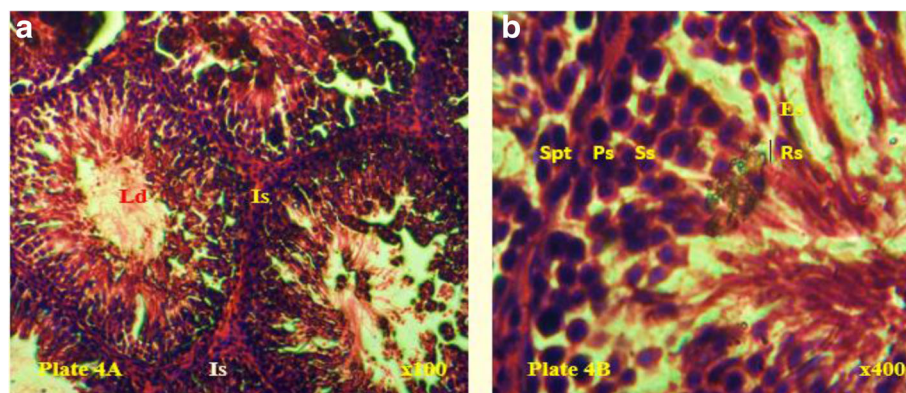
important in determining how well the Leydig cells can respond to testosterone stimulation.

According to Walker (2010), testosterone is the only principal hormone secreted by the mammalian testis and the lead hormonal determinant of male reproductive competence through its classical and non-classical actions. While testosterone is under fine regulation by many other endocrine and paracrine hormones, the perturbation of its homeostasis alone can disrupt male reproductive function regardless of whether other reproductive hormones are in balance or not.

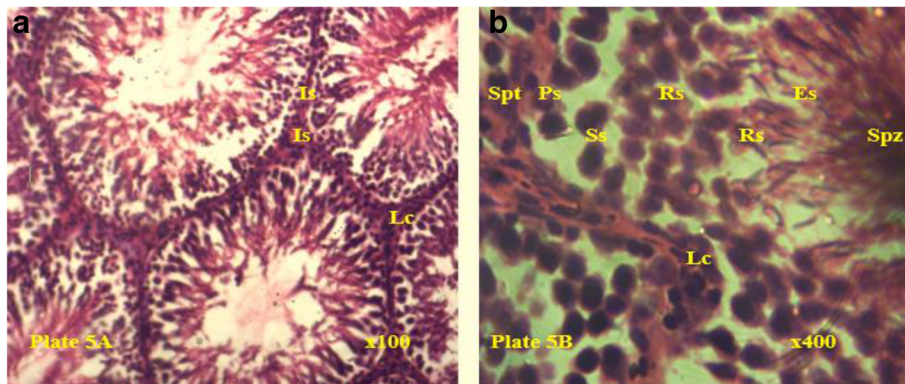
As shown in Fig. 5, all features of normal testicular histology were observable in control. The testicular histology of rats in the control group exhibited classical histoarchitectural appearance with intact seminiferous tubules displaying normal seminiferous epithelia arrangements; also all the germ cells, intact basement membrane of the seminiferous tubules, were all represented. The inter-tubular arrangement of the Leydig cell and other non-Leydig extra tubular components are of the classical type described for normal testicular

histology. Sertoli cells are observable, including spermatogonia at the basement membrane. Also visible are secondary spermatocyte migrating to the adluminal compartment. Primary spermatocytes with enlarged nuclei are observable too, with early spermatids and late spermatids. Bundles of spermatozoa are seen in the lumen of the seminiferous tubule. The cluster of Leydig cells is seen in the interstitial space. This pattern of normal testicular micro-architecture was also observed in Wistar rats given 100 mg/kg/day of aluminum chloride and 2.5 ml of pineapple juice (PJ). Bundles of spermatozoa are seen in the lumen of seminiferous tubule indicating spermiation. It shows normal testicular micro-anatomy in different phases of spermatogenesis, well-arranged basement membranes, well-distributed Leydig cells, and seminiferous tubules filled with sperm cells.

As shown in Fig. 6, testicular histology exhibits abnormalities, slight degenerative changes of seminiferous tubules; these changes include widening of interstitial spaces with infiltration of eosinophilic cells, edematous vacuolated fluids, necrosis, and diffuse edematous; aside



**Fig. 8 a, b** Photomicrograph of testes of group 2: Representative testicular micrograph of Wistar rats given 100mg/kg/d of aluminum chloride and 1.5ml of pineapple juice (PJ) showing the structure of seminiferous tubules *Spt* Spermatogonia, *Ps* Primary Spermatocyte, *Ld* Luminal diameter, *Lc* Leydig cell, *Rs* Round spermatid, *Es* elongated spermatids, *It* Interstitial space Stain is H&E and Magnification x 100 and x 400



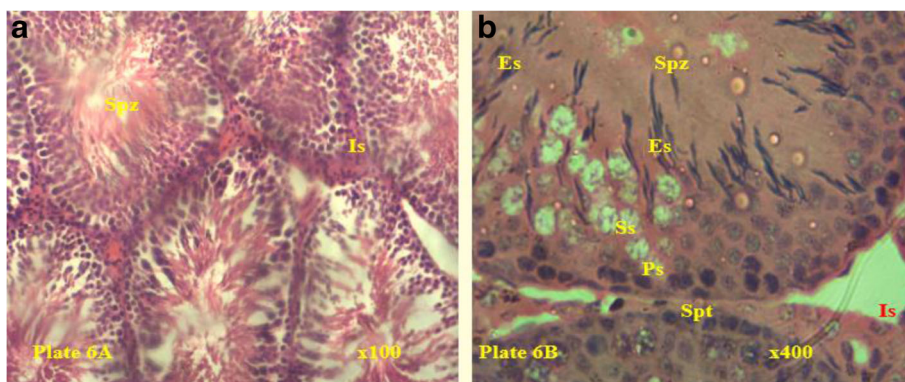
**Fig. 9 a, b** Photomicrograph of testes of group 3: Representative testicular micrograph of wistar rats given 100mg/kg/d of aluminum chloride and 2ml of pineapple juice (PJ) showing structure of normal seminiferous tubules. *Ld* Luminal Diameter, *Lc* Leydig cell, *Spt* spermatogonia, *Ps* primary spermatids, *Ss* Secondary spermatocytes, *Es* elongated spermatids, *Rs* Round spermatids, *It* Interstitial space, *Spz* spermatozoa. Stain is H&E and Magnification x 100 and x 400

from the displacement membrane, there were mononuclear cell infiltrations. Severe hypospermatogenesis or absolute arrest of spermatogenesis as the bundle of spermatozoa seen is distorted compared to those of baseline. These findings were in line with the reports from Olawuyi et al. (2019) and Oteiza et al. (1999). In this current study, we observed swelling, congestion, and few areas of ischemic necrosis. Additionally, the endothelium of small blood vessels was observed to be damaged, with edema and hemorrhagic rete testes. A reduced portion of seminiferous epithelial diameter reveals few germ cells. Few Sertoli cells are observable, including spermatogonia at the basement membrane. Also, there is no visible secondary spermatocyte migrating to the adluminal compartment. Primary spermatocytes with enlarged nuclei are not observable too, with no early spermatids and late spermatids seen. Few Leydig cells are seen in the interstitial space. The observed degenerative changes were reversed by pineapple juice. A low

dose (1 ml of pineapple juice) with aluminum chloride showed mild recovery from severe histological changes. The reversal agrees with the report by Rana and Verma (1996) and El-shahat, Gabr, Meki, and Mehana (2009).

Shown in Figs. 6 and 7 are all features of testicular histology of Wistar rat given 100 mg of aluminum chloride and 1.5 ml and 2 ml of pineapple juice. An enlarged portion of seminiferous epithelial diameter reveals germ cells at different spermatogenic phases. Sertoli cells are observable, including spermatogonia at the basement membrane. Also visible are secondary spermatocyte migrating to the adluminal compartment. Primary spermatocytes with enlarged nuclei are observable too, with early spermatids and late spermatids. Bundles of spermatozoa are seen in the lumen of seminiferous tubule indicating spermiation. A cluster of Leydig cells is seen in the interstitial space.

Testicular histology of Wistar rat given 100mg of aluminum chloride and a high dose 2.5ml of Pineapple



**Fig. 10 a, b** Photomicrograph of testes of group 4: Representative testicular micrograph of wistar rats given 100mg/kg/d of aluminum chloride and 2.5ml of pineapple juice (PJ) showing the structure of normal seminiferous tubules. *Lc* Leydig cell, *Spt* spermatogonia, *Sp* Secondary spermatocytes, *Ps* Primary spermatocytes, *ES* elongated spermatids, *Spz* spermatozoa. Stain is H&E and Magnification x 100 and x 400



juice as shown in Plates 6A and B or Fig. 10, show all features of normal testicular micro-anatomy with full presence of phase of spermatogenesis, well-arranged basement membranes, Cluster of Leydig cells are well distributed in the interstitial space, and seminiferous tubules filled with sperm cells. Sertoli cells are observable, including spermatogonia at the basement membrane. Also visible are secondary spermatocyte migrating to an adluminal compartment. Primary spermatocytes with enlarged nuclei are observable too, with early spermatids and late spermatids. Bundles of spermatozoa are seen in the lumen of seminiferous tubule indicating spermiation.

These present findings are in agreement with Hatch (1995) that many plant-derived substances, collectively termed “phytonutrients” or “phytochemicals” are well identified for their antioxidative effect. Phenolic compounds such as flavonoids are always present in plants that act as a protective agent against various environmentally induced stress, while in humans, flavonoids are known to act as biological response modifiers; however, its primary therapeutic effects are often linked to its antioxidant properties (Bendich, 1994). Therefore, the reproductive benefits of pineapple on aluminum-induced oxidative stress on the testes of male Wistar rats were evidence from this present study.

## Conclusion

Based on the findings from this work and correlation with other works, it is evident that aluminum chloride induces oxidative stress and retards reproduction in males, whereas *Ananas comosus* serves to improve reproduction in males by improving reproductive hormones and other sperm parameters. The result of this study suggests that aluminum chloride deteriorates the histology of the testes while *Ananas comosus* improves the histology.

## Abbreviations

ANOVA: Analysis of variance;  $AlCl_3$ : Aluminum chloride; ELISA: Enzyme-linked immunosorbent assay; PJ: Pineapple juice; SEM: Standard error of mean; SPSS: Statistical Package for Social Sciences; ROS: Reactive oxygen species; WHO: World Health Organization

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## Authors' contributions

The authors STO and LUO designed the study and carried out the experiments. BJL supervised and did the statistical analysis of the data. BJL and LUO wrote the draft of the manuscript. S edited the final draft of the manuscript. All authors approved the final draft of the manuscript.

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## Ethics approval and consent to participate

The experimental procedures conformed with the national and international standards on the use of laboratory animals in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH, 2011). Also, the study was approved by the ethical committee on the care and use of animal experimentation in the animal house of the Department of Anatomy Madonna University, Elele, Rivers State, Nigeria, before the commencement of this study.

## Consent for publication

Not applicable.

## Competing interests

The authors declare no competing interests.

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