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Mitigating effect of *Annona muricata* Fruit-Extract and *Aloe berbadensis miller* gel on Aluminum-induced Testicular toxicity in Wistar Rats.

Olawuyi T. Solomon
Department of Human Anatomy, School of Health and Health Technology, Federal University of Technology, P. M. B 704, Akure, Ondo-State.

Corresponding Author: Olawuyi TS
Email: tsolawuyi@futa.edu.ng; +2348035020338

ABSTRACT
Male infertility index due to heavy metal poison has increased over the year in Nigeria. *Annona muricata* and *Aloe barbadensis miller* are known for their rich medicinal value. The aim was to investigate the mitigating effect of *annona muricata* fruit-extract and *aloe berbadensis miller* gel with the objectives on histology and semen parameters of aluminum-induced testicular toxicity in wistar rats. Twenty-five male rats weighed (100-150g) with five (5) groups were used for the study. Group A: (control): received rats pellet and water. Group B: Given 100mg/kg/d of AlCl3. Group C: Given 100mg/kg/d of AlCl3 and 3ml/kg/d of *annona muricata* fruit-extract. Group D: Given 100mg/kg/d of AlCl3 and 3ml/kg/d of *aloe barbadensis miller* gel. Group E: Given 100mg/kg/d of AlCl3 with 3ml/kg/d of *annona muricata* fruit-extract and3ml/kg/d of *aloe barbadensis miller* gel. The rats were weighed and sacrificed twenty four hours after the last administration. The results showed that Aluminium treatment induced histological changes in testicular tissue including degeneration of germ cells, lowering of sperm count and increased morphological abnormalities. Animals treated with aluminium along with *annona muricata* fruit-extract as well as *Aloe barbadensis miller* showed recovery of normal testicular architecture and improved semen parameters. It is evident that Aluminum Chloride induced deleterious effect on the health of adult wistar rats as illustrated by the distortion of testis histology and semen parameters. Whereas, Sour-sop (*Annomia muricata*) fruits and aloe Vera gel significantly mitigated the toxic effect of aluminium metal on the reproductive health of male wistar rats.

Keywords: *Aloe barbadensis*, *Annonamuricata*, Antioxidant, Spermatogenesis, Toxicity

INTRODUCTION
World Health Organization[1] report on traditional medication includes all prescriptions that fall under Chinese drug, Indian ayurvola and Arabic unani prescription and to different types of indigenous prescription[2]. Plant-base medicine has been fully or partially a source of medical therapy for about 70% of the instances[3] with an estimated 80% of the world population currently seek therapeutic solution from herbal medicine as primary health care and this has gained recognition in several nations of the world as well as the World Health Organisation 'WHO'[1,3,4].

Infertility is a major issue in numerous social orders[5]. Also, in view of this numerous couples look for restorative assistance so as to tackle infertility[6]. The world Health Organization (WHO) reported that about 80% of the world's population rely on traditional medication[7].

Research on traditional medicinal plants has shown that their potential to improve male fertility is partially due to presence of antioxidants. These antioxidants have been noticed to improve several processes (spermatogenesis, steroidogenesis) of male reproductive function[8] . Medicinal plants are utilized either alone or as combination of a few plants to treat different types of male sexual dysfunctions. Along these lines, a combination of plant varieties has been seen by numerous scientists to treat idiopathic infertility[19].

Aluminium (Al) is the most abundant metal present on the earth's crust. It is said to be the third most enormous metal[2, 10]. Aluminium is mainly mined as bauxite, a solid earth mineral which composes of about 40–60% aluminium oxide (alumina) [11]. Aluminium does rarely occur pure in nature this is because of its high rate of reactivity but is rather found only in combination with other elements[11].

Aluminium ingestion in excessive amount leads to accumulation in target organs and has been associated with damage of testicular tissues of both humans and animals[12]. AlCl3 showed reproductive toxicity on rabbit sperm in vitro[13]. Testicular aluminium accumulation, necrosis of spermatocytes/spermatids and significant decrease in fertility were found in male mice[14].

Annona muricata is a flowering evergreen tree native to Mexico, Cuba, Central America and parts of India. Annona is a genus of tropical fruit trees belonging to the family Annonaceae, of which there are approximately

119 species[15]. It has the largest fruits in the genus. The soursop tree produces dark green, spiny aggregate fruits made up of berries fused together with associated flower parts[16]. The oval or heart-shaped and frequently irregular lopsided composite sour-sop fruit is derived from the fusion of many fruitlets and can weigh more than 4 kg. The fruit pulp consists of white fibrous juicy segments surrounding an elongated receptacle[15].

The sour-sop is astringent, cholagogic and promotes digestion[17]. It also has several medicinal uses such as in the management of diabetes and its complications[17, 18, 19] also as antioxidant and antimutagenic agent[20]. It is usually recommended in cases of constipation, obesity, hypertension and coronary diseases[17]. The white pulp of the fruit is used to make juice, as well as candies, sorbets and ice-cream flavourings. Its flavour is described as a combination of strawberry and pineapple with sour citrus flavour notes contrasting with an underlying creamy flavour reminiscent of coconut or banana. The fruit is rich in B group vitamins, potassium, fructose and vitamin C. The enzymes pectinase, catalase, and peroxidase have been detected in sour-sop pulp[17]. The miracle tree as it is widely known as a natural cancer killer that is 10,000 times stronger than chemotherapy[21]. The use of this plant in medicine has again come to fore as researchers are claiming it to have potential against common pathogen. However, historically ethnomedicine, to an extent has been providing solutions to problems related to human diseases[22].

The sour-sop fruit and other parts of the tree are considered underutilized[22]. Hence, Sour-sop with its miraculous properties was used in this study with an intention to find newer use of these miracle plants.

Aloe is a cactus-like plant that grows in hot, dry climates. The name Aloe vera derives from the Arabic word "Alloeh" meaning "shining bitter substance[23]" while "vera" in Latin means "true." 2000 years ago, the Greek scientists regarded Aloe vera as the universal panacea. The Egyptians called Aloe "the plant of immortality." Today, the Aloe vera plant has been used for various purposes in dermatology. Aloe Vera is a natural product that is now a day frequently used in the field of cosmetology[24].

Aloe produces two substances, gel and latex, which are used for medicines. Aloe gel is the clear, jelly-like substance found in the inner part of the aloe plant leaf. Aloe latex comes from just under the plant's skin and is yellow in colour. Some aloe products are made from the whole crushed leaf, so they contain both gel and latex. The aloe that is mentioned in the Bible is an unrelated fragrant wood used as incense. Aloe medications can be taken by mouth or applied to the skin. People take aloe gel by mouth for weight loss, diabetes, hepatitis, inflammatory bowel diseases, osteoarthritis, stomach ulcers, asthma, radiation-related skin sores, fever, itching and inflammation, and as a general tonic.

MATERIALS AND METHODS

The *Annona Muricata* fruit and *Aloe Barbadensis Miller* used for the experiment was obtained from the Federal university of Technology Akure, Nigeria approved centre. ELISA kits (Monobind Inc, CA 92630, ab10866, SE120087 USA), Aluminium Chloride crystals (Lot No: 20150321, Guangdong Sci-Tech, China). Equipments used are Microtome (Leica RM 2125 RTS), Rotary evaporator (RE-52A from Union Laboratories England), Vacuum Pump, Centrifuge (Denly, Model BS 400), Metler's sensitive balance (Metler Toledo, Mg 126), Automatic tissue processor, 96-microplate reader (Rayto: RT-2100C), Chemistry Analyzer machine (MISPA Excel), Water bath (model MH-8504), Adjustable pipettes (Surepette RS 16013), Electric oven (Model: DHG-9030A, Searchtech instrument).

Preparation and Concentration of *Annona Muricata*Fruit Extract: The *Annona Muricata* fruit used for the experiment was obtained from the Federal university of Technology Akure, Nigeria approved centre. The extraction of *Annona Muricata* pulp is achieved by sieving to separate the pulp from the seeds. In the production of *Annona Muricata* concentrate indicated by Bates *et al*[33].

Plant preparation and extraction of the *Aloe Barbadensis Miller:* Mature, healthy and fresh aloe vera leaves were harvested from the botanical garden of the faculty of Agric and Agricultural Technology, Federal University of Technology, Akure. The leaves were washed with fresh water and the thick epidermis carefully removed. The mucilaginous gel was then homogenized with an electric blender. The homogenate was concentrated by filtration using Whatman paper 2. The thickened concentrated gel and the filtrate were kept at 4°C for use.

Animals and Housing Conditions: The experimental procedures were in conformity with national and international standards on the use of laboratory animals. Also, the study was approved by institutional committee on the care and use of animal for experiments at Federal University of Technology Akure, School of Health and Health Technology.

The Wistar rats were purchased from a disease free stock of Salmonda farms Akure, Ondo State Nigeria. A total of 25 male Wistar rats with weight of (120 ± 10) g were used for the study. The processes of protocols using the experimental animals were in accordance to the Guide for the Care and Use of Laboratory Animals and approved by the scientific committee of the university. The animals were housed in the cages with five per cage and fed *ad libitum*, and they were exposed to a 12 h light: 12 h dark cycle and the room temperature was maintained at (25 ± 2) C.

Experimental Design: The total numbers of animals

were Twenty five (25). They were grouped into one (1) control, four (4) experimental groups with consideration towards size variation. Using a feeding tube (size-6), distilled water, Aloe Vera gel and Sour-sop extracts were administered to the treated animals respectively for a period of three (3) weeks or twenty and one (21) days.

Group 1 (control): (n-5): Received water and rat pellets only;

Group 2 (n-5): Given 100 mg/kg/d of $AlCl_3$ orally;

Group 3 (n-5): Given 3 ml/kg/d Sour-sop (*Annomia muricata*) fruit extract (SFE) simultaneously with 100 mg/kg/d $AlCl_3$ orally;

Group 4 (n-5): Given 3 ml/kg/d aloe Vera gel simultaneously with 100 mg/kg/d $AlCl_3$ orally;

Group 5 (n-5): Given 3 ml/kg/d of Sour-sop fruit extract and aloe vera gel in parallel with 100 mg/kg/d $AlCl_3$ orally.

Animal Sacrifice and Sample Collection: Approximately twenty-four hours after the administration of the last dose, all the overnight-fasted rats of the treatment and control groups were sacrificed under the use of chloroform. Blood samples will be collected from each animal via cardiac puncture. Abdominal cavities were opened by a midline abdominal incision and testis were removed, cleaned off from adherent fat and blood clot and was weighed on a digital electronic balance[25] , while those for semen analysis where weighed using the volumetric method (Archimedes rule).

Sperm motility: Sperm motility was recorded and evaluated immediately after tissue isolation. Cauda epididimis was miced into small pieces and transferred to a clean Petri dishes containing pre-warmed saline. Sperm were allowed to swim out within the 5 min at 37°C. The analysis was carried out under the light microscope magnification of 100 fold. The percentage of sperm motility was calculated using the number of live sperm cells over the total number of sperm cells, both motile and non-motile. The sperm cells that were not moving at all were considered to be non-motile, while the rest, which displayed some movement were considered to be motile.

Sperm Morphology: The morphology of the spermatozoa will be obtained by utilizing the original dilution for motility, dilute/weaken 1:20 with 10% neutral buffered formalin (Sigma-Aldrich, Oakville, ON, Canada).

Sperm suspension was utilized in preparation of smears for assessment of sperm morphology to decide the rate of teratospermia abnormalities.

Histology of Tissues: All specimens were fixed in Bouin's solution, After adequate fixation, the tissues were dehydrated through a graded ethanol series, and cleared in xylene, and then embedded in paraffin wax (Sigma paraplast embedding media, Steinem, Germany). The tissue was sectioned with 5 µm thicknesses, stained with haematoxylin and eosin (H&E) and examined by an expert pathologist under a light microscope (100x and 400x magnification).

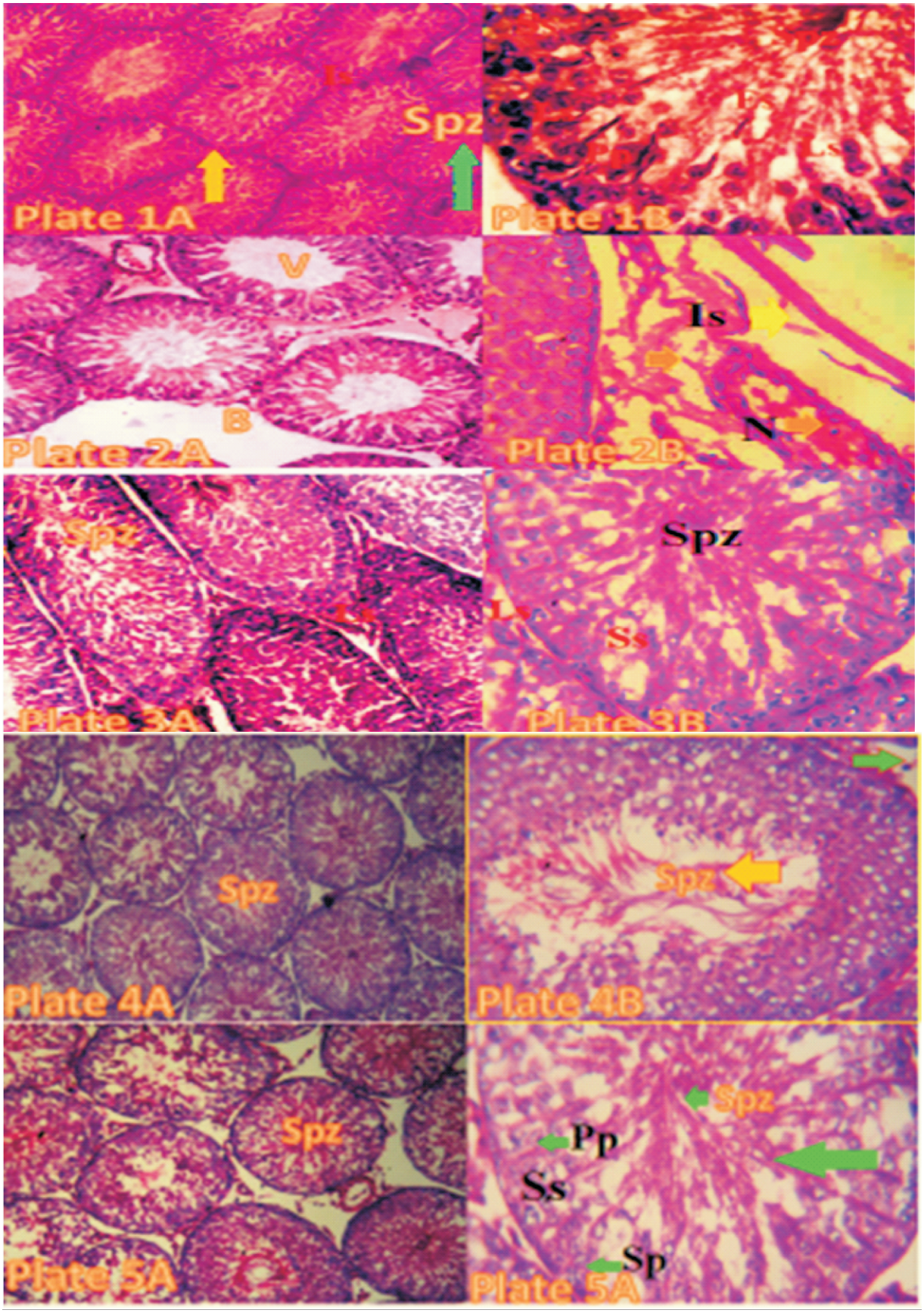
Statistical Analysis: Data was presented as means ± Standard deviation (SD) for the groups. Statistical analyzes were done using graphpad prism version 8.03.

RESULTS

Histology

- ✓ PLATE 1A & B: Photomicrograph of testes of control group=Representative testicular micrograph of wistar rats showing normal "control" structure of seminiferous tubules. *Sc* =Sertoli cells, *Lc* = Leydig cell, *Spt*= spermatogonia, Sp = Secondary spermatocytes, *Pp*= Primary spermatocytes, Ls= Luminal diameter, *ES* = early spermatids, *Spz*= spermatozoa. Stain is H&E and Magnification x100 & x400
- ✓ PLATE 2A & B: Photomicrograph of testes of group 2=Representative testicular micrograph of wistar rats given 100mg of aluminum chloride per kg of body weight, showing abnormal structure of seminiferous tubules. Distortion of most cells and degenerative changes of seminiferous tubules (Is); Widening interstitial space and diffuse edematous changes with mononuclear cells infiltrations besides basement membranes separating from the underlying layers (B). N= Testicular necrosis (E). *V* = Luminal diameter, *Spt*= spermatogonia, *Spz*= spermatozoa. Stain is H&E and Magnification x100, x400.
- ✓ PLATE 3A & B: Photomicrograph of testes of group 3= Representative testicular micrograph of wistar rats given 3 ml/kg/d Sour-sop (*Annomia muricata*) fruit extract (SFE) simultaneously with 100 mg/kg/d $AlCl_3$ showing mild degenerative changes of seminiferous tubules; *Ss*= Sertoli cell, Ld= Leydig Cell, *Spz*= spermatozoa. *Ld* = Luminal diameter, Stain is H&E and Magnification x100 & x400.
- ✓ PLATE 4A & B: Photomicrograph of testes of group 4= Representative testicular micrograph of wistar rats given 3 ml/kg/d Aloe Vera gel simultaneously with 100 mg/kg/d $AlCl_3$ showing normal structure of seminiferous tubules and numerous spermatozoa. *Spz*= spermatozoa, *Spt*= spermatogonia. Stain is H&E and Magnification x100 & x400.
- ✓ PLATE 5A & B: Photomicrograph of testes of group 5 = Representative testicular micrograph of wistar rat given 3 ml/kg/d of Sour-sop fruit extract and aloe vera gel in parallel with 100 mg/kg/d $AlCl_3$ showing structure of normal seminiferous tubules. *Ss* = Sertoli cell, *Spt*= spermatogonia, Pp =primary spermatocyte,

Spz= spermatozoa. Stain is H&E and Magnification x100 & x400.



Semen Morphology

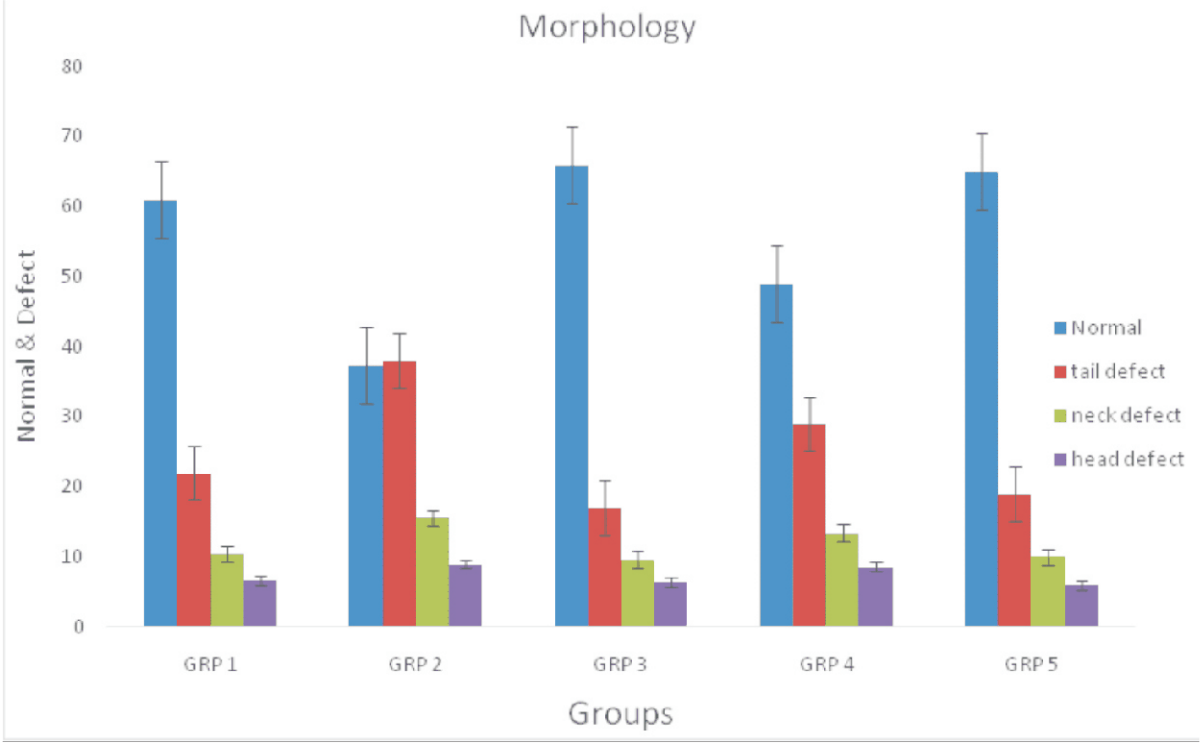


Figure 1: Variation in the Morphology of Semen parameters

Semen Parameters

There were no statistically significant changes in the Testicular volume across the experimental animals in all the groups ($p>0.05$). The mean (±SEM) of the volume for group 2 (aluminum only) however, it is the lowest (1.5 ± 0.03) while group 5 (*annona muricata* and *aloe barbadensis miller* treated) is the highest (1.76 ± 0.11).

The total count of the treated groups was statistically significant to the aluminum only group ($p>0.05$) with the sour-sop fruit-extract treated group having the highest mean (±SEM).

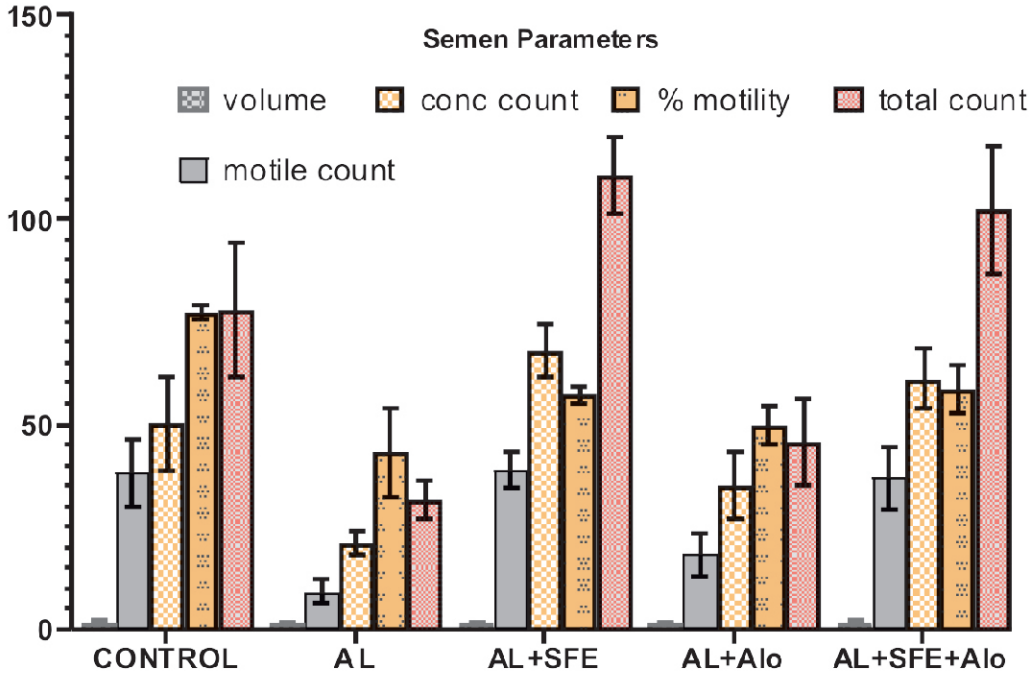


Figure 2: Variations in semen Variations in semen parameters.

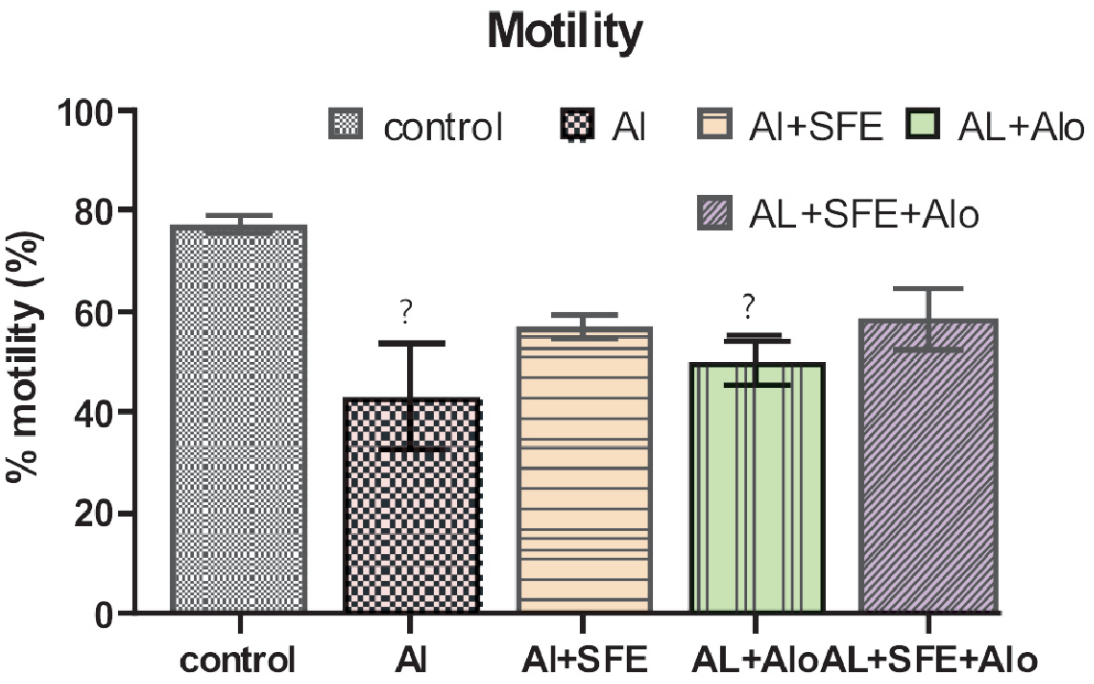


Figure 3: Variation in semen motility count.

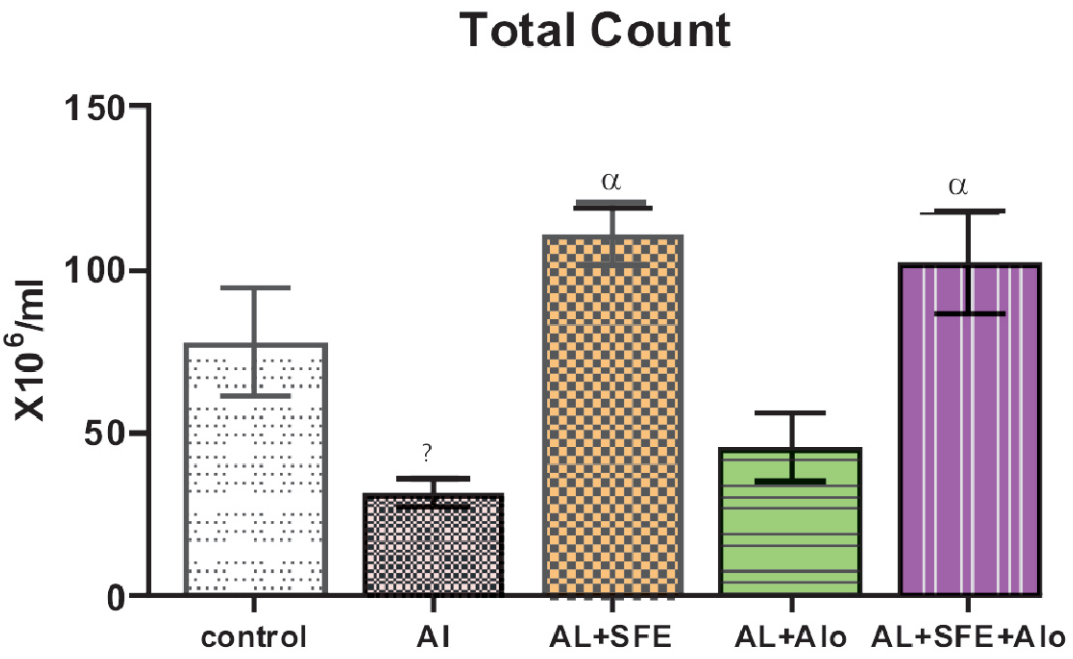


Figure 4: Variations in the Total count
*Statistically significant to the control group at $p<0.05$. α = significant to the aluminium only group ($p<0.05$).

Progressive assessment: Considering the progressive assessment, there were markedly statistical significant changes ($p<0.05$). The mean (±SEM) for forward movement were higher in groups 3 (Sour-sop fruit extract treated) and 5 (treated with aloe-Vera and sour-sop fruit extract). (65.0 ± 2.2, 66.0 ± 3.7)**(%)** and group 2 ($AlCl_3$) have the lowest value (32.0 ± 3.7**%).** (Table 3 Figure 4.4).

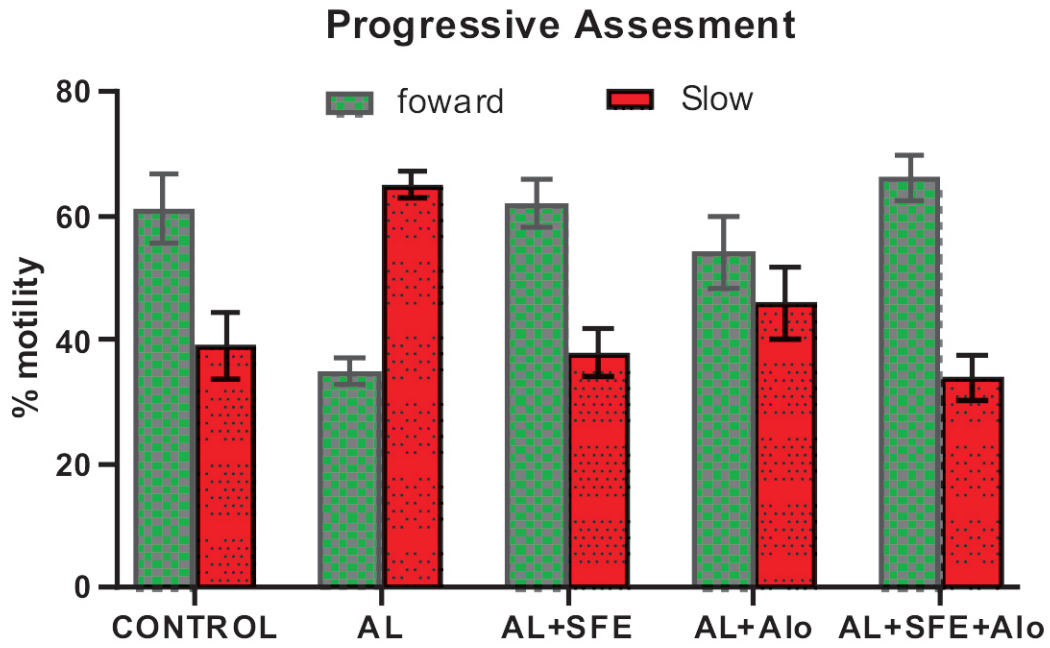


Figure 5: Variation in the Semen Progressive assessment (Forward and Slow).

DISCUSSION

Testicles are specialized gonad organs having two basic functions, to produce germinal cells and to produce steroid hormones. Among the total mineral components of earth, about 8% contains aluminum[26] and this trivalent cation is found in plant tissues as well as in most of the animals. Aluminum has acknowledged as a poison and reported to exert toxic effect particularly when excess amount of aluminum is accumulated in the body through different sources for instance, water, dietary source, environment[1]. The main mechanisms of this toxicity are inhibition of the activity of enzyme and alternation in nucleic acid function through protein synthesis which leads to change in cell membrane permeability. As aluminum toxicity has recently become a matter of concern, several researches have been started to conduct with a view to find natural dietary sources that can reduce this toxic effect of aluminum on health. Therefore, in this study, the mitigating effects of annona muricata fruit-extract and aloe vera gel on aluminium-induced testicular toxicity were investigated.

Annona muricata fruitextracts and *aloe barbadensis miller* on the other hand has anti-oxidant and free radical scavenging activity which is of very significant health effect to humans[27, 28, 29].

In this study, it was observed that *annona muricata* fruit-extract as well as *aloe barbadensis miller* significantly caused mitigative effect on aluminum induced oxidative stress on reproductive function (Plates 3-5).

The semen parameters showed markedly significant changes. The concentration count, the motile count, Progressive assessment and the morphology were greatly increased in the rats co treated with *annona muricata* fruit-extract as well as *aloe barbadensis miller,* (with the *annona muricata* treated group being the most effective); this showed positive spermatogenesis and testicular steroidogenesis (figures 1-5).

Histopathological examination of rats group orally administered AlCl3 showed apparent alteration in the testes, where it induced marked degeneration and necrosis of germ cells lining seminiferous tubules, as well as interstitial odema and marked absence of germ cells (plate 2A &B). Meanwhile, treatment of AlCl3 group with *annona muricata* fruit-extract (Plate 3A &B) and *aloe barbadensis miller* (Plate 4A &B) showed noticeable improvement in histopathological changes induced by AlCl3 in testis sections. The histological changes in testes of rats administered AlCl3 are in agreement with Khattab[30] who studied the effect of AlCl3 on the rat's testes. Also, Guo *et al.*[30] observed deleterious effects and histopathological changes in testicular tissues after 2 weeks of aluminium treatment.

The results of this present investigation clearly indicated that aluminium has deleterious effect on the health of adult wister rats as illustrated by the distortion of testis histology, abnormal semen morphology (increased head abnormality) and semen parameters. Annona muricata fruit-extract significantly mitigated the toxic effect of aluminium metal on the reproductive health of male wistar rats

CONCLUSION

On the basis of the findings from this work and correlation with other works, it is evident that Aluminum Chloride induced deleterious effect on the health of adult wistar rats as illustrated by the distortion of testis histology, abnormal semen morphology (increased head abnormality) and semen parameters. Whereas, Sour-sop (*Annomia muricata*) fruits and aloe Vera gel significantly mitigated the toxic effect of aluminium metal on the reproductive health of male wistar rats.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest

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