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Histomorphology, Hormonal Changes and Redox Imbalance in Aluminum-Induced Testicular Toxicity: The Mitigating Influence of Ethanolic Stembark-Extract of *Prosopis africana*.

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Abstract:- The study aimed at mitigating effect of ethanolic stembark extract of *Prosopis africana* on aluminum-induced testicular toxicity in adult Wistar rats. Twenty-five Wistar rats of an average weight of 200g were grouped into five: Normal control, $AlCl_3$ (200 mg/kg/ d), 200mg/kg/d *P. africana*, $AlCl_3$ (200 mg/kg/ d) + 200mg/kg/ d *P. africana* and $AlCl_3$ (200 mg/kg/d) + 400mg/kg/d *P. africana* groups. All animals were fed orally and allowed access to water *ad libitum* for four weeks. Testicular histology, semen parameters, biochemical assays and testosterone hormone were assessed. The degenerative seminiferous tubule histoarchitecture due to $AlCl_3$ toxicity was improved by *Prosopis africana* extract. Notably, the group with extract alone showed superlative values in all investigated parameters relative to the other groups. This study also showed the significant reduction in testicular mass and parameters. There was improvement in sperm parameters in group co-treated with *Prosopis Africana* extract groups. This study showed reduced antioxidant level of SOD and increased MDA levels in the $AlCl_3$ group relative to the other groups. This study demonstrated the ability of ethanolic stembark-extract of *Prosopis africana* to mitigate aluminium-induced testicular toxicity on reproductive functions and the pro-spermatogenic properties of the compound in boosting andrological parameters.

Keywords:- *Asthenospermia, Semen parameters, Testicular histology, Testicular toxicities.*

I. INTRODUCTION

Infertility management has been a major section of the health services for the past two decades, with testicular toxicity mediating 50% of male-factor infertility (Abarikwu, 2013; Gupta & Verma, 2018). This is in line with a case study that found that after two years of regular unprotected sexual contact, up to 30% of Nigerian couples may have problems having a child (Abarikwu, 2013). The cause of infertility is not far-fetched; increased industrialization and overcrowding have resulted in a proportional increase in environmental contamination in recent years. These pollutants disrupt biochemical and physiological characteristics in humans and laboratory animals, resulting in population-wide reproductive harm (Mathur, Pandey and Jain, 2010; Olawuyi, Paul and Oladipo, 2018; Pandey and Jain, 2013).

It has already been established that success in reproduction is associated with good quality semen. Several authors, as far back as the 1990s till date, have been able to demonstrate that a significant decrease in the sperm quality may consequently result in an increase in the rate of male infertility (Carlsen *et al.,* 1993; Jensen *et al.,* 2008).

Metal-induced toxicity to the reproductive system is a major public health concern around the world. Aluminum, as a metal, now meets many of man's essential needs, and its widespread use in everyday life has aided in the formation of a modern world. It's a trivalent cation that can be found in ionic form in a variety of plant and animal tissues, as well as in all bodies of water. Aluminium is a common metallic element and its known to be the third most abundant element in the crust of the earth (Buraimoh *et al.,* 2012; Camargo, Fernandes, & Martinez, 2009).

Aluminium is a non-redox active metal that is capable of increasing the cellular oxidative environment. It is almost always alloyed due to its reactivity. Aluminium is ubiquitous and exists in mixture with other elements forming compounds such as sulphate, chloride, and silicates (Olawuyi *et al.*, 2019; Verstraeten, Aimo & Oteiza, 2008).Its importance in transportation, packaging, construction, machinery, and household objects, as well as electricity, cannot be overstated. Despite its necessary characteristics, increased exposure to it has recently proven to be harmful. The toxicant aluminum has been found to have repro-neurotoxic effects on the human body (Olawuyi *et al.,* 2018).

Antioxidants and plant phenolics are employed in experimental and epidemiological researches to ameliorate oxidative stress associated with various diseases (Kumar, Dogra, & Prakash, 2009). *Prosopis africana* tree serves several purposes with profound

economic benefits. It belongs to the leguminous spiny pod-bearing family with 45 species. It is commonly found in the tropical as well as the subtropical regions in many African countries including Nigeria (Guinea savanna) (Balogun, Oyeyiola, & Kolawole, 2017). It is a rich source of food and feed for animals (Agboola, 2004). Its various parts have been adopted for use in orthodox and conventional medicinal products. The medicinal values of *Prosopis africana* in treating male/ female infertility have also been reported (Agboola, 2004; Tchacondo *et al*., 2011).

The presence of phenolic chemicals, steroidal compounds, flavonoids, tannins, glycosides, carbohydrates, saponins, and alkaloids in various regions of *Prosopis africana* was discovered using phytochemical screening (Abah, 2014). A flavonoid compound 7,3',4'trihydroxy-3-methoxyflavanone was isolated from the ethylacetate extract of *Prosopis africana*. Based on the spectral data and by comparison with literature, the structure was identified as 7,3',4'trihydroxy-3-methoxyflavanone reported for the first time in the plant *Prosopis africana* (Abah, 2014). The isolated alkaloids from *Prosopis africana* have 2,6-disubstituted piperidin-3-ol. All have a hydroxyl group in the 3-position, an n-C12 side chain in the 6-position, and either a methyl or hydroxymethyl group in the 2 position (Abah, 2014; Chime, Okek and Onunkwo, 2015).

This present study is aimed at investigating the mitigating effect of the ethanolic stembark-extract of *Prosopis africana* on aluminium-induced toxicity on histomorphology, semen parameters, biochemical parameters and testosterone hormone of adult Wistar rats.

II. MATERIALS AND METHODS

➢ *Chemicals and Reagent*

The stem-bark of *Prosopis africana* was gotten from Isanlu Isin, Kwara State, Nigeria; 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), Olympus light microscope, Centrifuge (Denly, Model BS 400), Metler's sensitive balance (Metler Toledo, Mg 126), Automatic tissue processor, 96-microplate reader (model SM 600, China), Chemistry Analyzer machine (MISPA Excel), Water bath (model MH-8504), Adjustable pipettes (Surepette RS 16013), Electric oven (Model: DHG-9030A, Search tech instrument).

➢ *Care and maintenance of animals*

Twenty-five male adult Wistars weighing an average of 200 grams were obtained from the animal house of the Department of Microbiology in the Federal University of Technology Akure (FUTA). The mice were kept in well-ventilated customized plastic cages at room temperature under conventional laboratory settings of a 12-hour light-dark cycle. The animals were provided unlimited food and water. The pelleted feed was procured from the Agro feed sector in FUTA. The acclimation period lasted two weeks.

➢ *Collection and preparation of extracts*

Fresh stem bark of *Prosopis Africana* was procured from Isanlu-Isin, Kwara State, Nigeria. Before the commencement of the study, the ethical approval was sought in Centre for Research and development (CERAD), FUTA Ondo state where it was assigned ethical number: FUTA/ETH/20/27. The stem bark was washed, cut into small pieces, and dried in a cold area before being ground to a coarse powder. Cold maceration in a closed jar with intermittent shaking for 24 hours extracted the powdered plant material (600 g) with absolute ethanol (100%) (Abah, 2014; Chime, Okek and Onunkwo, 2015). Concentration of the filtrate was done with a rotary evaporator and further concentration to dryness by electric oven at 50°c.

➢ *Experimental design*

This study used a total of twenty-five Wistar rats. The animals were divided into five groups of five (n = 5). All of the groups, including the control group, were given distilled water and feedings via a feeding tube (size-6), i.e orally, for a period of four weeks. Groups 1, 2, 4 and 5: (Normal control, $AlCl_3$ (200 mg/kg/d), 200mg/kg/d *P. africana*, $AlCl_3$ (200 mg/ kg/d) +200mg/kg/d *P. africana* and $AlCl_3$ (200 mg/kg/d) +400mg/kg/d *P. Africana)* (Abah, 2014; Chime, Okek and Onunkwo, 2015; Olawuyi, Akinola and Aina, 2020; Olawuyi, 2020). All excepts control and group 2 received ethanolic stembark-extract of *Prosopis africana.*

➢ *Euthanasia and Sample Collection*

Diethyl-ether was used as a sedative, and animals were weighed and sacrificed twenty-four hours after the last dose. Blood samples were taken using needles by cardiac puncture via the mid-clavicular line (i.e. where the animal's heart's apex beat was placed) into a plane bottle as soon as the animals were euthanized. Sera were obtained by spinning samples in a centrifuge (at 2000 rpm) for 15 minutes at 4°C. The reproductive organs (testes) were removed after opening the abdominal cavity using a midline abdominal incision. The wet weights were taken before the administration and after the last dosage (immediately before animals were sacrificed), using an electronic sensitive analytical balance (Sartorious).

➢ *Histopathological examination*

The right testis was assessed for histopathological analysis. The right testis was excised from the animals in each of the five groups and they were immersion-fixed in Bouin fluid for 24 hours, after which each was cut transversely into small (3-5mm thick) slabs and further fixed in a change of the same fixative for another 15 hours. The fixed testis was then subjected into the process of dehydration, clearing and embedding the embedding paraffin blocks were sectioned 5 µ thick with the aid of a rotary microtome and stained using hematoxylin and eosin stains (Gill, Frost, & Miller, 1974). Light microscopic examination of the sections was carried out and photomicrographs of the desired sections were taken using Scope Image software and capture camera of digital image for further observations.

➢ *Sperm Volume*

This was done by mettler method. The weight of the testes were done using an electronic sensitive analytical balance (Sartorious). This can still be carried out by volumetric method i.e When a body is totally or partially immersed in a fluid the up-thrust is equal to the weight of the fluid displaced; i.e. the volume displaced by testis when immersed in water is equal to the volume of the ejaculates (Yokoi and Mayi, 2004).

➢ *Sperm motility*

A modified method of Yokoi and Mayi (2004) was adopted in counting the spermatozoa from the right epididymis. The epididymis was minced with anatomic scissors in the 5 ml of normal saline, packed in a rocker for 10 minutes and allowed to incubate at temperature for 2 minutes. After incubation, the supernatant was diluted at 1: 100 with a solution containing 5 g sodium bicarbonate and 1 ml formalin (35%).

➢ *Epididymal Sperm Concentration*

The epididymis was removed, cleaned of fatty tissue promptly, and weighed. The caudal epididymis was further removed from the epididymis and weighed to determine the number of sperm per gram and sperm motility at ×100 magnification under an Olympus light microscope according to Yokoi and Mayi (2004). Following incubation, the solution was swirled gently so that epididymal tissue could be separated with forceps. The semen analysis evaluated the following parameters: Volume, Epididymal sperm concentration sperm motility, sperm Morphology %motility, progressive assessment total counts.

➢ *Progressive Assessment*

Within a 10-second observation duration per square, sperm motility was assessed across a minimum of five strips of squares. Non-motile sperms were counted first, and only sperms with flagella activity were considered motile. For objective assessment of motility, spermatozoa were classified {based on recommendations of World Health Organization (WHO, 2010) and categorized based on how fast or how slow they appear.

➢ *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 rate of sperm abnormalities. In details, a drop of sperm suspension was added into an equal volume 1% eosin-y 5% nigrosin, which was then mixed together and smeared on pre-warmed clean glass slides and air-dried. Using an Olympus light microscope, two hundred sperm cells were examined at × 400 magnifications per animal to determine the morphological abnormalities. 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 percentage (Yokoi and Mayi, 2004).

➢ *Determination of Plasma Malondialdehyde*

Malondialdehyde levels in plasma were measured according to the protocol outlined by Stocks and Domandy (1971). The reaction mixture contained 100µL of Plasma, 20% Trichloroacetic acid (1.0ml). The above were mixed and centrifuge at 2000rpm for 5mins to obtain the supernatant. 0.5ml of supernatant is mixed with 0.7% Thiobarbituric acid (1.0ml), the tubes were heated in a water bath at 100 ºC for 20 minutes and all tubes were cooled in water. The spectrophotometer was blanked using the reagent blank at 532nm. Absorbance of tests and standards were read and plasma Malondialdehyde level was calculated.

➢ *Determination of Superoxide Dismutase (SOD) Activity*

Superoxide Dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480nm as described by Mistra and Fridovish (1972). The reaction mixture (3ml) contained 2.95ml, 0.05M sodium carbonate buffer initiate the reaction. The reference cuvette contained 2.95ml buffer, 0.03ml of substrate (epinephrine) and 0.02ml of water. Enzyme activity was calculated by measuring the change in absorbance which was read by Chemistry Analyzer machine (MISPA Excel) at 480nm for 5 minutes.

➢ *Serum Assay Testosterone Procedure*

The samples were assayed in batches using the enzyme linked immunoassay (ELIZA) method (Tietz and Saunders, 1994). The microwell kit was from Biotec laboratories Ltd, UK. With ten micron (10µl) of the standard, the specimens and control were dispensed into the number of coated wells to be used. Hundred-micron (100 µl) testosterone conjugate reagent was added and then fifty microns (50 µl) of anti-testosterone reagent. The contents of the microwell was thoroughly mixed and then incubated for 20minutes at room temperature. The reaction was stopped with 100µl of 1M hydrochloric acid. Absorbance was measured with an automatic spectrophotometer (Rayto: RT-2100C, Microplate Reader) at 450nm.

➢ *Statistical analysis*

Experimental data were statistically analysed with a Statistical Package for Social Sciences (SPSS) software for Windows (version 24). Statistical difference in data was represented using Tukey. The statistical probability level of less than 5 % ($p<0.05$) was considered to be significant.

III. RESULTS

➢ *Histological Results*

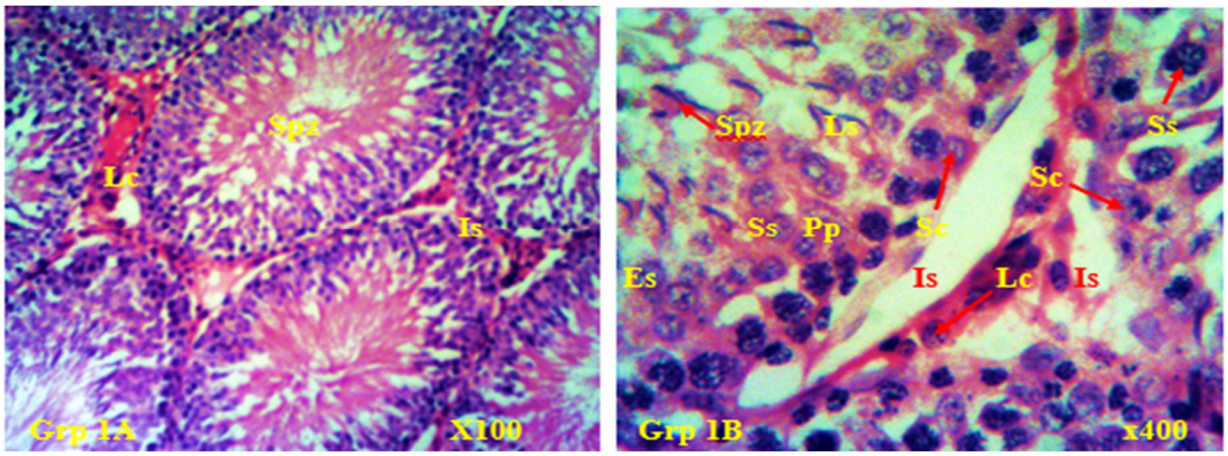

Fig 1: Representative testicular photomicrograph of rat showing normal "control" structure of seminiferous tubules. *Sc*= Sertoli cells, *Is*= Intestitial space, Lc= Leydig cell, *Spt*= spermatogonia, *Pp*= Primary spermatocytes, *Ls*= Late spermatid, *ES*= early spermatids, *Spz*= spermatozoa. Stain is H&E and Magnification ×100 & ×400.

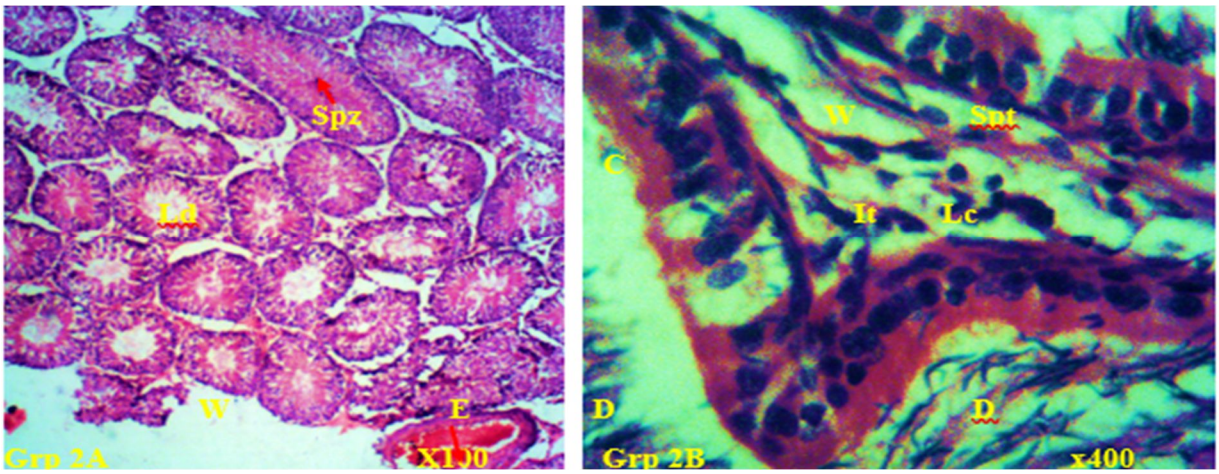

Fig 2: Representative testicular photomicrograph of rat given 200 mg/kg b.wt of aluminium chloride, showing widening interstitial space (W), degeneration of seminiferous tubules (D) and loss of normal distribution in epithelial lining (C) , showing widening lumina diameter (Ld) 'Group 2'. *Lc*= Leydig cells, *Spt*= Spermatogonia; Stain is H&E and Magnification ×100 & ×400.

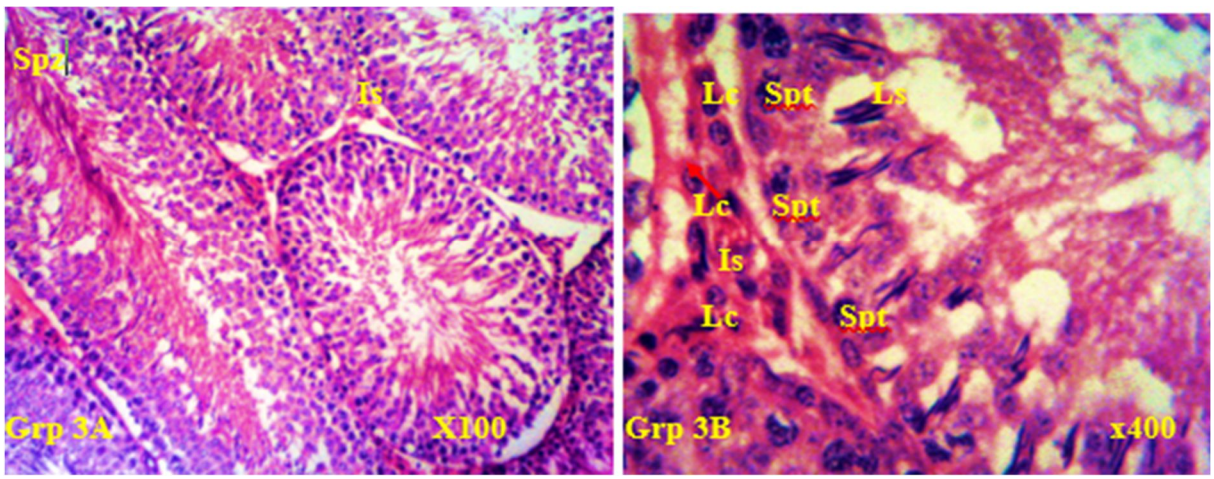

Fig 3: Representative testicular photomicrograph of rat given 200 mg/kg b.wt extract of *Prosopis africana*, showing seminiferous tubules with similar structure compare to control group 'Group 3'. *Sc* =Sertoli cells, *Lc* = Leydig cell, *Spt*= spermatogonia, *Ss*= Secondary spermatocytes, *Pp*= Primary spermatocytes, *Ls*= Late spermatid, *ES* = early spermatids, *Spz*= spermatozoa. Stain is H&E and Magnification ×100 & ×400.

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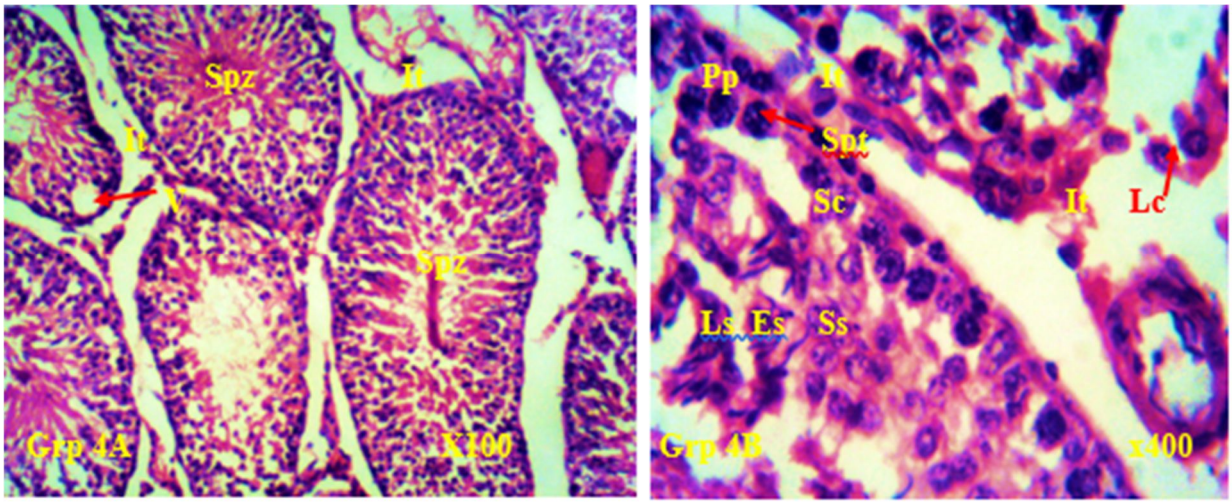

Fig 4: Representative testicular photomicrograph of rat 200 mg/kg b.wt of aluminum chloride and 200 mg/kg b.wt extract of *Prosopis africana*, showing structure of seminiferous tubules 'Group 5'. *Sc* =Sertoli cells, *Lc* = Leydig cell, *Spt*= spermatogonia, *Pp*= Primary spermatocytes, Ls= Late spermatid, *ES* = early spermatids, *Ss*= Secondary spermatocytes, *Spz*= spermatozoa, vacuolation of seminiferous epithelium (V). Stain is H&E and Magnification ×100 & ×400.

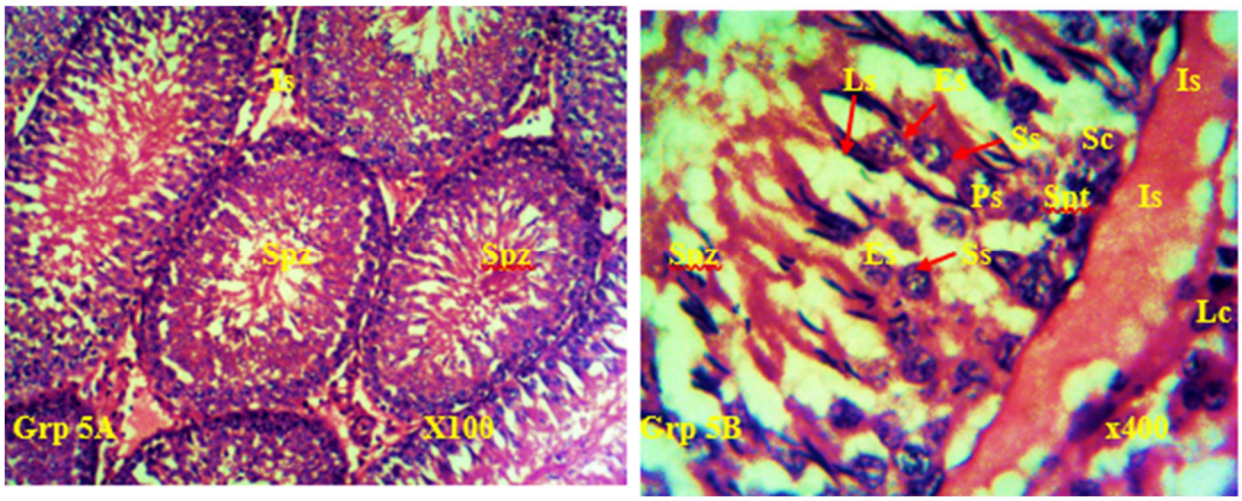

Fig 5: Representative testicular photomicrograph of rat given 200 mg/kg b.wt of aluminum chloride and 200 mg/kg b.wt extract of *Prosopis africana*, showing structure of seminiferous tubules 'Group 5'. *Sc* =Sertoli cells, *Lc*= Leydig cell, *Spt*= spermatogonia, *Ss*= Secondary spermatocytes, *Pp*= Primary spermatocytes, *Ls*= Late spermatid, *ES*= early spermatids, *Spz*= spermatozoa. Stain is H&E and Magnification ×100 & ×400.

➢ *Body weight*

Table 1: The distribution of mean and standard error of mean (SEM) for body weight

Groups (n=5)	Initial Body Weight (g)	Final Body Weight (g)	Body Weight Differences (g)
1(control)	193.4 ± 1.6	198.0 ± 4.9	22.4
2($AlCl_3$)	211.5 ± 2.0	192.0 ± 4.4	19.5
3(200mg/kg *P. Africana*)	182.8 ± 1.7	218.2 ± 3.8	25.2
4($AlCl_3$ + 200mg/kg *P. Africana*)	202.0 ± 1.1	205.2 ± 4.4	3.2
5($AlCl_3$ + 400mg/kg *P. Africana*)	193.0 ± 4.3	212.0 ± 4.0	19.0

Values are expressed as mean ± SEM for n=5, *= Significantly different from control at $p<0.05$. n= Total number of rats within the group

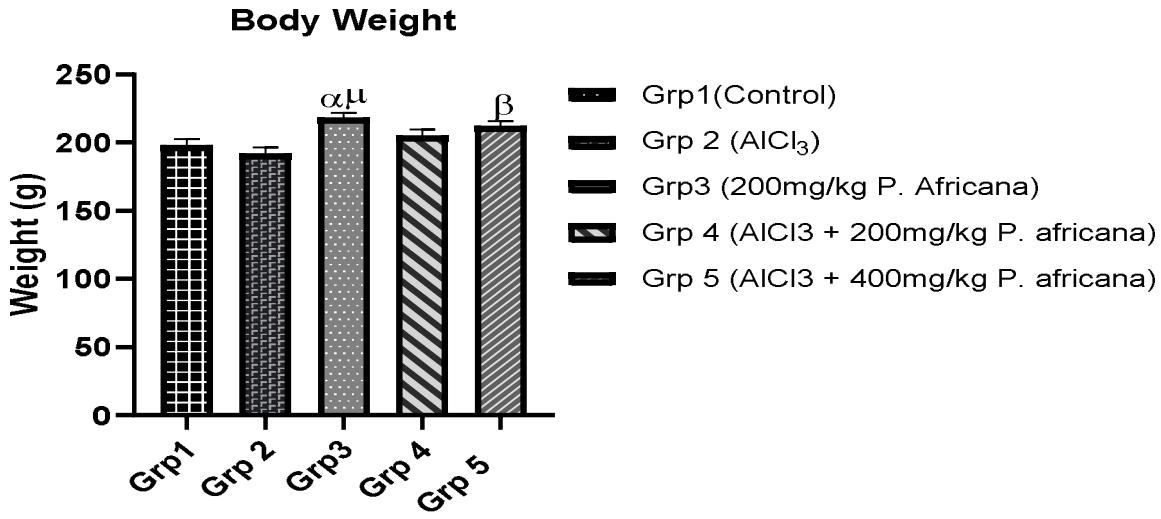

Fig 6A: Variations in mean body weight α = Group 3 was statistically significantly to group 1 (control) at $p<0.05$. **β** = Group 3 was statistically significantly to group 2 ($AlCl_3$ alone) at $p<0.05$. μ = Group 5 was statistically significantly to group 2 ($AlCl_3$ alone) at $p<0.05$.

Data on body weight are expressed in Fig. 6A. The results revealed that groups 2 ($AlCl_3$ alone) (192.0 ± 4.4) body weight significantly decreased ($p<0.05$) when compared with group 3 (extract alone) (218.2 ± 3.8). Group 3 (extract alone) (218.2 ± 3.8) was significantly higher ($p<0.05$) when compared with group 1 (198.0 ± 4.9). Also, group 5 ($AlCl_3$ + 400mg/kg *P. Africana*) (212.0 ± 4.0) was significantly higher ($p<0.05$) when compared with group 2 ($AlCl_3$ alone) (192.0 ± 4.4).

➢ *Testicular Weight Result*

Table 2: The distribution of mean and standard error of mean (SEM) for testicular weight

Groups (n=5)	Right Testis (g) ± SEM	Left Testis (g) ± SEM
1(control)	2.2 ± 0.12	2.0 ± 0.10
2($AlCl_3$)	2.1 ± 0.24	1.8 ± 0.27
3(200mg/kg *P. Africana*)	2.4 ± 0.38	2.1 ± 0.09
4($AlCl_3$ + 200mg/kg *P. Africana*)	2.2 ± 0.20	1.9 ± 0.19
5($AlCl_3$ + 400mg/kg *P. Africana*)	2.3 ± 0.08	2.1 ± 0.19

Values are expressed as mean ± SEM for n=5, *= Significantly different from control at $p<0.05$. n= Total number of rats within the group

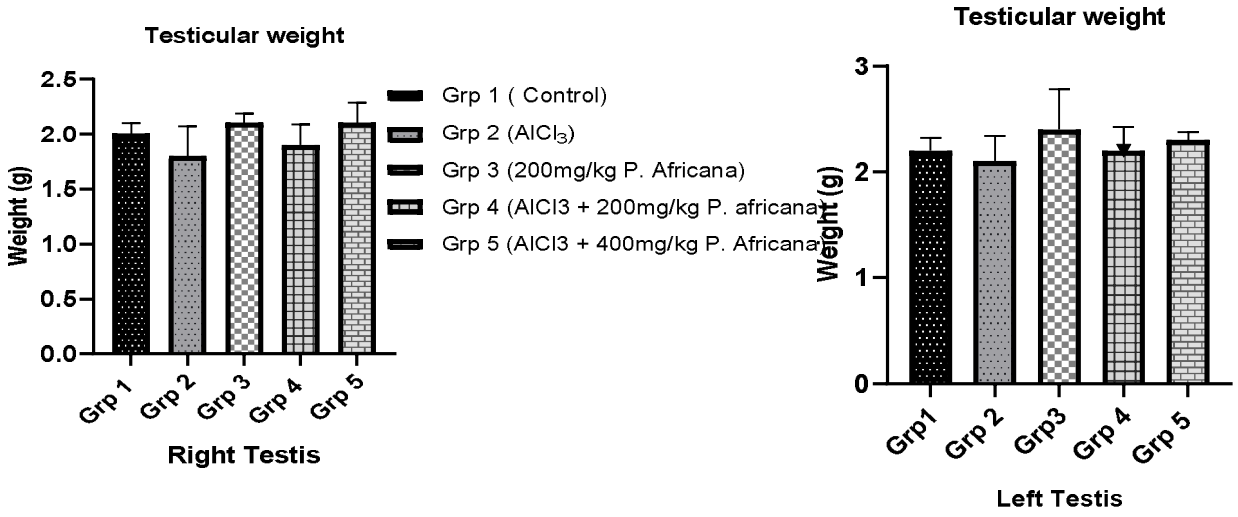

Fig 6B: Variations in mean right and left testicular weight

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Data on testicular weight are expressed in Fig. 6B. The results revealed no statistically significant changes in the testicular weight of experimental animals in all the groups ($p<0.05$). The highest testicular weight on the right testis was group 3 (extract alone) (2.4 ± 0.38) while the highest testicular weight on the left testes were group 3 (extract alone) and group 5 ($AlCl_3$ + 400mg/kg *P. Africana*) (2.1 ± 0.19 & 2.1 ± 0.09). Also, the lowest weights in both right and left testes were group 2 ($AlCl_3$ alone) (2.1 ± 0.24 and 1.8 ± 0.27).

➢ *Semen Parameters Results*

Table 3: The distribution of mean and standard error of mean (SEM) for sperm parameters

Groups (n=5)	Testis volume (ml)	Motile count $X10^6$/ml	Concentration count $X10^6$/ml	%motility	Total count $X10^6$/ml
1	1.8 ± 0.14	50.0 ± 1.5*	85.00 ± 3.2	59.8 ± 1.5	179.0 ± 5.52
2	1.6 ± 0.14*	16.5 ± 3.1***	45.00 ± 3.5	37.8 ± 4.7	125.8 ± 7.63
3	2.10 ± 0.27	74.0 ± 2.9	120.00 ± 3.6	70.9 ± 5.7*	195.2 ± 5.51
4	1.9 ± 0.10	62.0 ± 4.6	144.00 ± 4.2	62.2 ± 4.5	182.8 ± 7.92
5	1.9 ± 0.08	66.0 ± 4.0	128.00 ± 2.2	48.2 ± 4.0	260.6 ± 7.48

Values are expressed as mean ± SEM for n=5, *= Significantly different from control at $p<0.05$, ***= Significantly different from control at $p<0.0001$.

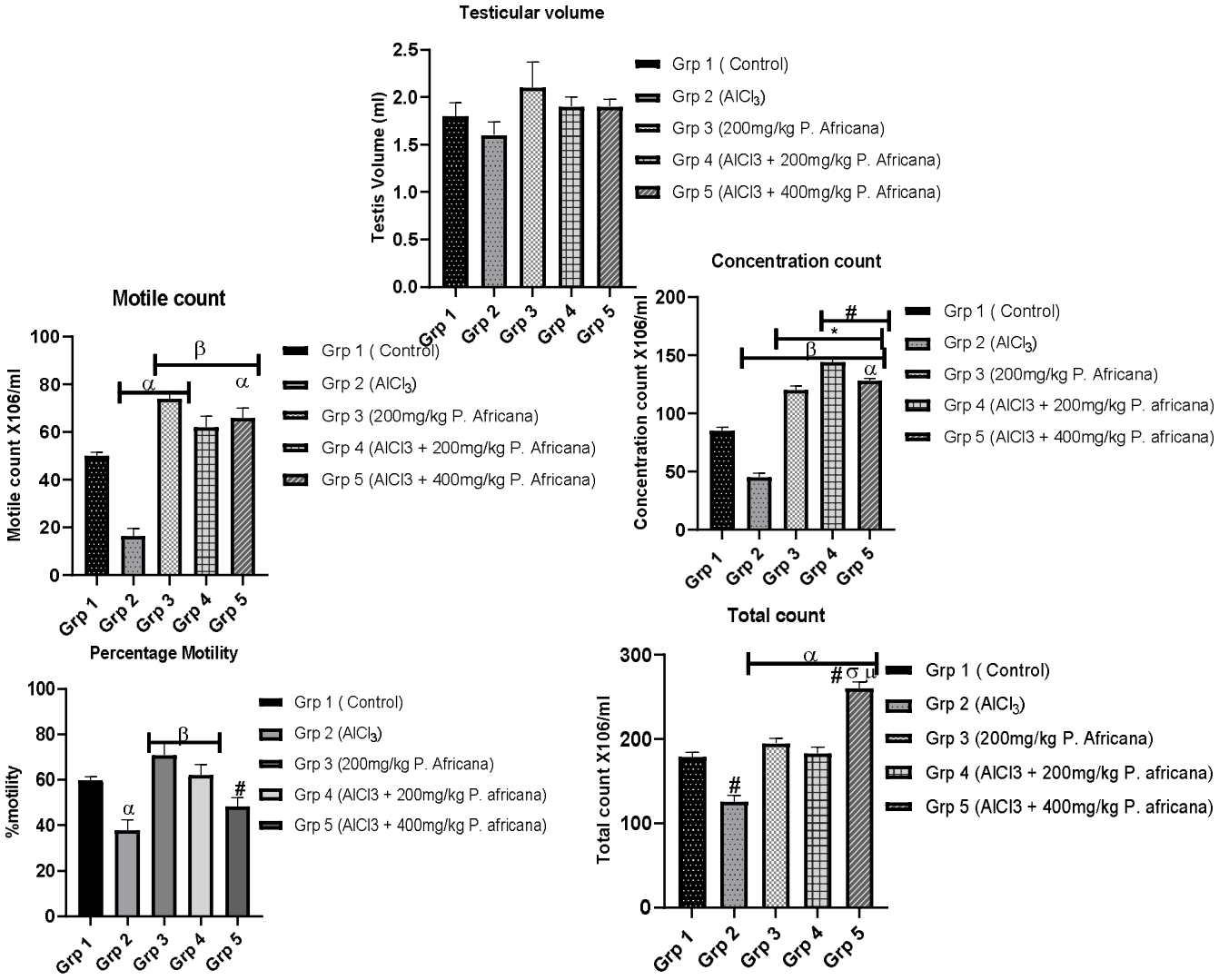

Fig 7: Variations in semen parameters. **Motile count:** α Group 2, 3, 5 were significant ($p<0.05$) when compared with groups 1 (Control); β Groups 3, 4 and 5 were statistically significant ($p<0.05$) when compared group 2 ($AlCl_3$ alone).

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Concentration counts: β Group 2, 3, 4 and 5 were statistically significant ($p<0.05$) when compared with group 1 (Control); * Groups 3, 4 and 5 were statistically significant ($p<0.05$) when compared with group 2 ($AlCl_3$ alone); # Groups 4 and 5 were statistically significant ($p<0.05$) when compared with group 3 (extract alone): α Group 5 was statistically significant ($p<0.05$) when compared with group 4 ($AlCl_3$ + 200mg/kg *P. Africana*).

% motility: α Group 2 ($AlCl_3$ alone) was statistically significant ($p<0.05$) when compared with groups 1; β Groups 3 and 4 were statistically significant ($p<0.05$) when compared with group 2 ($AlCl_3$ alone); # group 5 was statistically significant with groups 3 (extract alone).

Total Count: # groups 2 and 5 were statistically significant ($p<0.05$) when compared with group 1(control); α group 3, 4 and 5 were statistically significant ($p<0.05$) to group 2 ($AlCl_3$ alone); σ group 5 was statistically significant ($p<0.05$ to group3 (extract alone); μ group 5 was statistically significant ($p<0.05$) to group 4 ($AlCl_3$ + 200mg/kg *P. Africana*).

➢ *Testicular volume*

There were no statistically significant changes in the Testicular volume across the experimental animals in all the groups ($p<0.05$). In comparison, testicular volume of group 3 was the highest (2.10 ± 0.27) (ml) while group 2 was the lowest (1.6 ± 0.14) (ml) (Figure 7).

➢ *Motile count*

Data on motility count are expressed in Fig. 7. Group 2 ($AlCl_3$ alone) (16.5 ± 3.1) motile count was significantly lower ($p<0.05$) when compared with the other groups (1, 3, 4, 5) (50.0 ± 1.5) (74.0 ± 2.9) (62.0 ± 4.6) (66.0 ± 4.0). Groups 2, 3 and 5 (16.5 ± 3.1) (74.0 ± 2.9) (66.0 ± 4.0) were statistically significant ($p<0.05$ when compared with group 1 (control) (50.0 ± 1.5).

➢ *Concentration counts*

Data on semen concentration are expressed in Fig. 7. The semen concentration counts revealed statistically significant changes ($p<0.05$). Group 2 $AlCl_3$ alone) (45.00 ± 3.5) was statistical lower when compared with groups (3, 4 & 5) (120.00 ± 3.6, 144.00 ± 4.2, 128.00 ± 2.2). Groups 2, 3, 4 and 5 (45.00 ± 3.5, 120.00 ± 3.6, 144.00 ± 4.2, 128.00 ± 2.2) were statistically significant when compared with group 1 (85.00 ± 3.2). Groups 4 and 5 ($AlCl_3$ + 200mg/kg *P. Africana*) & $AlCl_3$ + 400mg/kg *P. Africana*) (144.00 ± 4.2, 128.00 ± 2.2) were significant ($p<0.05$) increased when compared with group 3 (120.00 ± 3.6). Group 5 $AlCl_3$ + 400mg/kg *P. Africana*) (128.00 ± 2.2) was significant ($p<0.05$) decreased when compared with group 4 (144.00 ± 4.2).

➢ *Percentage (%) motility*

Data on percentage motility are expressed in Fig. 7. The % motility counts revealed significant ($p<0.05$) decreased when group 2 $AlCl_3$ alone) (37.8 ± 4.7) was compared with groups (3 and 4) (200mg/kg *P. Africana*) ($AlCl_3$ + 200mg/kg *P. Africana)* (70.9 ± 5.7 & 62.2 ± 4.5). Group 2 ($AlCl_3$ alone) (37.8 ± 4.7) were significantly lower than group 1 (control) (59.8 ± 1.5). Group 5 ($AlCl_3$ + 400mg/kg *P. Africana*) (48.2 ± 4.0) were significant ($p<0.05$) lower than group 2 (200mg/kg *P. Africana*) (70.9 ± 5.7).

➢ *Total Count*

Data on total count are expressed in Fig. 7. Group 2 ($AlCl_3$ alone) (125.8 ± 7.63) was significantly ($p<0.05$) lower while group 5 *(*$AlCl_3$ + 400mg/kg *P. Africana*) (260.6 ± 27.48) was significantly ($p<0.05$) higher when compared with group 1 (control) (179.0 ± 5.52). Groups 3, 4 & 5 (200mg/kg *P. Africana*) ($AlCl_3$ + 200mg/kg *P. Africana) (*$AlCl_3$ + 400mg/kg *P. Africana*) (195.2 ± 5.51, 182.8 ± 7.92, 260.6 ± 7.48) were significantly higher when compared with group 2 ($AlCl_3$ alone) (125.8 ± 7.63). Group 5 *(*$AlCl_3$ + 400mg/kg *P. Africana*) (260.6 ± 7.48) was significantly higher at ($p<0.05$) when compared with group 3 (200mg/kg *P. Africana*) (195.2 ± 5.51). Also, group 5 *(*$AlCl_3$ + 400mg/kg *P. Africana*) (260.6 ± 7.48) was significantly higher at ($p<0.05$) when compared with group 4 ($AlCl_3$ + 200mg/kg *P. Africana (*182.8 ± 7.92*).*

➢ *Progressive assessment*

Table 4: The distribution of mean and standard error of mean (SEM) for semen progressive assessment

Groups	Slow 'S' %	Forward 'F' %
1(control)	44.0 ± 3.1	56.0 ± 1.1
2($AlCl_3$)	52.5 ± 1.8	47.8 ± 1.2
3(200mg/kg *P. Africana*)	35.2 ± 1.1*	64.8 ± 2.1*
4($AlCl_3$ + 200mg/kg *P. Africana*)	40.6 ± 1.8	59.4 ± 1.8
5($AlCl_3$ + 400mg/kg *P. Africana*)	36.6 ± 1.5*	63.4 ± 1.5*

Values are expressed as mean ± SEM for n=5, *= Significantly different from control at $p<0.05$. n= Total number of rats within the group.

➢ *Semen progressive assessment*

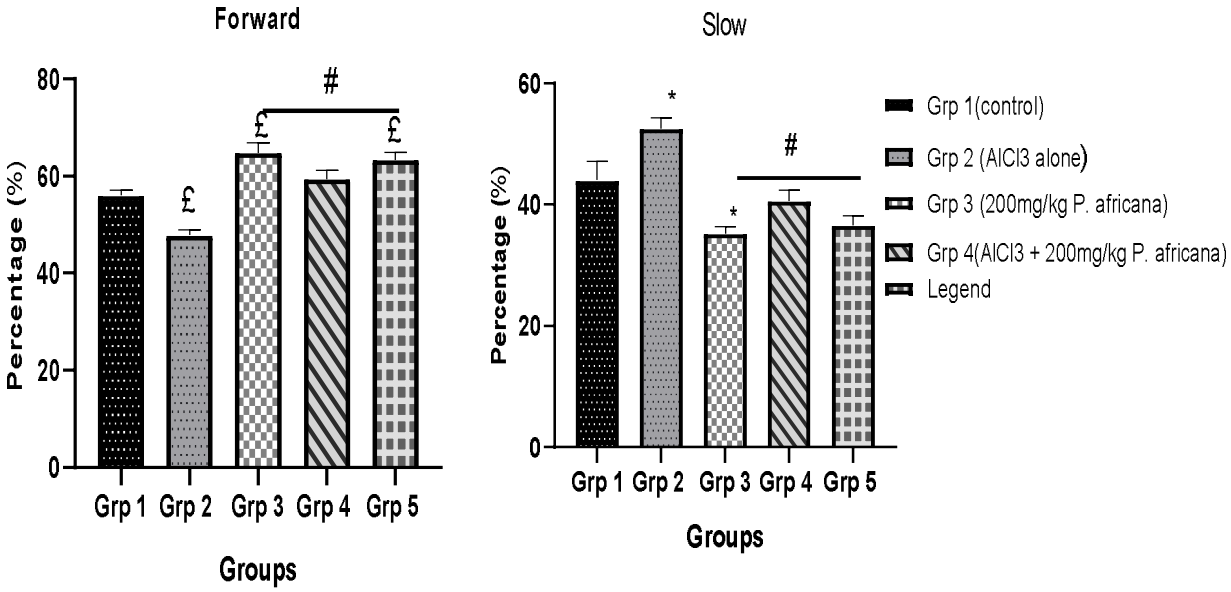

Fig 8: Variations in semen progressive assessment. Forward: **£** Groups 2, 3 and 5 were statistically significant ($p<0.05$) when compared group 1 (control). # Groups 3, 4 & 5 were significant ($p<0.05$) when compared with group 2 ($AlCl_3$ alone). Slow: * Groups 2 and 3 were statistically significant ($p<0.05$) when compared group 1 (control). # Groups 3, 4 & 5 were significant ($p<0.05$) when compared with group 2 ($AlCl_3$ alone).

Data on Progressive assessment are expressed in Fig. 8. The results for forward movement of the sperms revealed that group 3 and 5 (64.8 ± 2.1 & 63.4 ± 1.5) were significantly ($p<0.05$) increased while group 2 (47.8 ± 1.2) was significantly ($p<0.05$) decreased when compared with group 1 (56.0 ± 1.1). The comparison group 2 revealed that groups 3, 4 and 5 (64.8 ± 2.1, 59.4 ± 1.8 & 63.4 ± 1.5) were significantly ($p<0.05$) increased. The results of slow movement revealed significant ($p<0.05$) increased in group 2 (52.5 ± 1.8) and decreased in group 3 (35.2 ± 1.1) when compared with group 1. Also, when group 2 was compared with other groups; groups 3, 4 and 5(35.2 ± 1.1, 40.6 ± 1.8 & 36.6 ± 1.5) were significantly ($p<0.05$) lower.

➢ *Sperm morphology*

Groups	Normal 'N' %	Tail defect 'TD' %	Head defect 'HD' %	Neck defect 'ND' %
1(control)	56.0 ± 2.9	18.80 ± 2.0	15.0 ± 0.9	10.6 ± 0.4
2($AlCl_3$)	43.3 ± 3.1	25.75 ± 1.5	19.8 ± 2.1	11.3 ± 1.3
3(200mg/kg *P. Africana*)	63.6 ± 1.7*	16.20 ± 1.2*	10.8 ± 1.8	9.4 ± 1.1
4($AlCl_3$ + 200mg/kg *P. Africana*)	56.2 ± 1.2	22.60 ± 1.6	14.8 ± 1.2	6.4± 2.7
5($AlCl_3$ + 400mg/kg *P. Africana*)	59.6 ± 1.5	19.00 ± 1.9	11.4 ± 1.6	10.0 ± 1.2

Table 5: The distribution of mean and standard error of mean (SEM) for morphology
Values are expressed as mean ± SEM for n=5, *= Significantly different from control at $p<0.05$. n= Total number of rats within the group

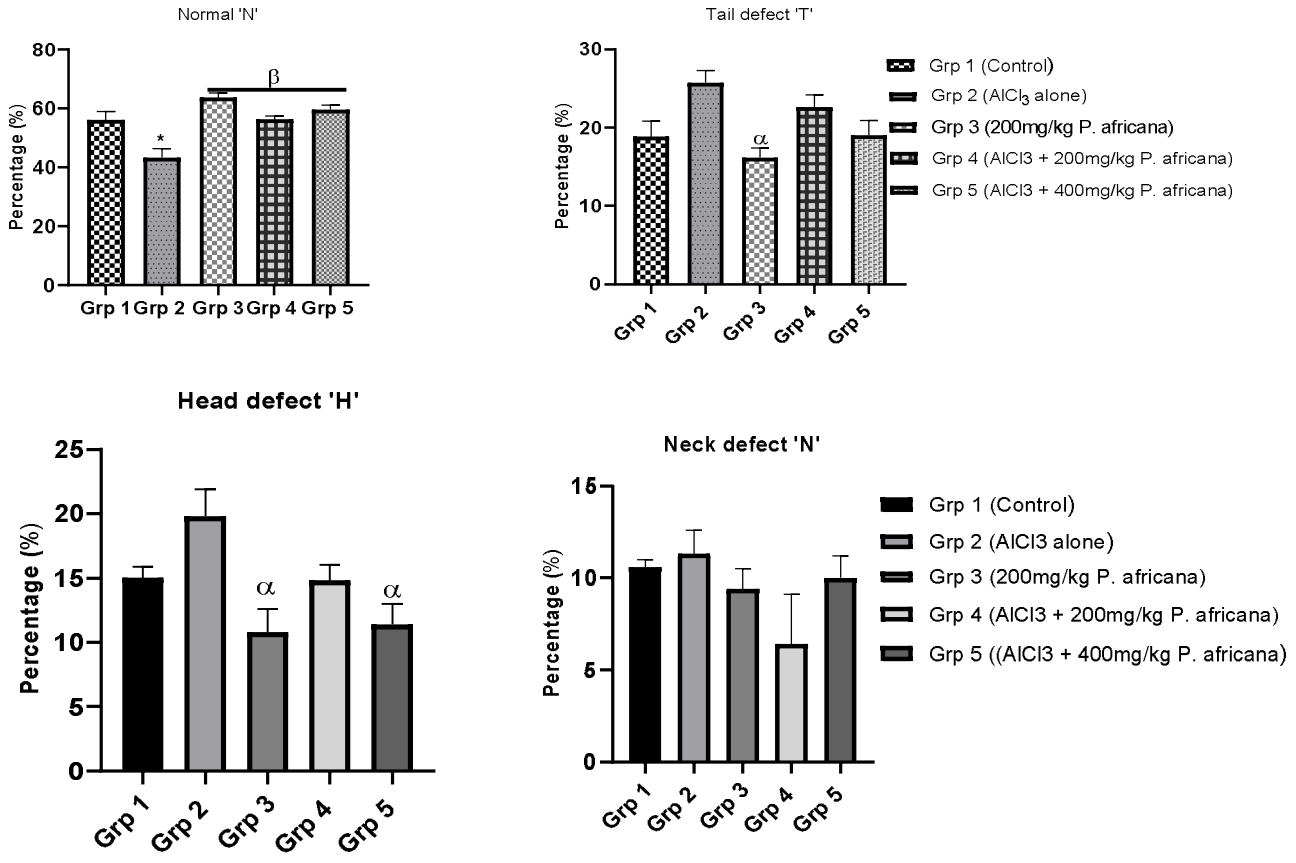

Fig 9: Variations in the morphology of semen parameter. Normal: * Groups 2 was statistically significant ($p<0.05$) when compared group 1 (control); **β** Groups 3, 4 and 5 were statistically significant ($p<0.05$) when compared group 2; Tail defect: **α** Group 4 was statistically significant ($p<0.05$) when compared to other groups; Head defect: Groups 3 and 5 were statistically significant ($p<0.05$) when compared to other groups; Neck defect: Group 4 was statistically significant ($p<0.05$) when compared to other groups

Data on Sperm morphology are expressed in Fig. 9. The results revealed that the normal sperm morphologies were statistically significant ($p<0.05$) in group 3 *(*59.6 ± 1.5*)* group 4 (63.6 ± 1.7). The results for tail defect revealed that group 4 (16.20 ± 1.2) was statistically significant ($p<0.05$) while the highest tail defects among the groups was group 2 The results for head defect revealed that group 2 ($AlCl_3$) was the highest (19.8 ± 2.1) while the lowest was group 4 (10.8 ± 1.8). The neck defect results revealed that group 3 was the highest while group 5 (8.0 ± 1.2) was the lowest.

➢ *Biochemical and Hormone Assay*

Table 6: Malondialdehyde levels (MDA), Superoxide dismutase (SOD) activities and testosterone (TT) levels in experimental groups

Parameters / **Groups (n=5)**	**MDA $\times 10^{-7}$ (ng/ml protein)**	**SOD (u/mg protein)**	**Testosterone (ng/ ml)**
1(control)	2.7 ± 0.23	145.7 ± 8.0	7.3 ± 0.40
2($AlCl_3$)	7.1 ± 0.65*	120.3 ± 9.2	4.9 ± 0.65*
3(200mg/kg *P. Africana*)	2.6 ± 0.14	216.8 ± 13.7	9.6 ± 0.06*
4($AlCl_3$ + 200mg/kg *P. Africana*)	5.3 ± 1.10	148.7 ± 9.4	8.4 ± 0.11
5($AlCl_3$ + 400mg/kg *P. Africana*)	3.7 ±0 .39	155.5 ± 8.0	9.0 ± 0.12

Values are expressed as mean ± SEM for n=5, *= Significantly different from control at $p<0.05$. n= Total number of rats within the group

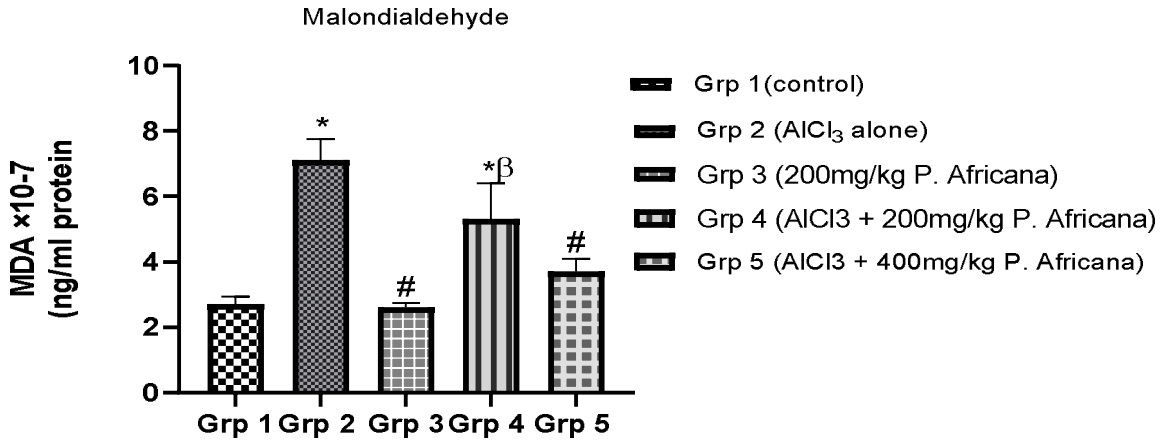

Fig 10: Variations in serum lipid peroxidation (Malondialdehyde: MDA) levels. *= Significantly different from control at p<0.05, #= Significantly different from aluminium induced group at p<0.05, β= Significantly different from extract alone group at p<0.05

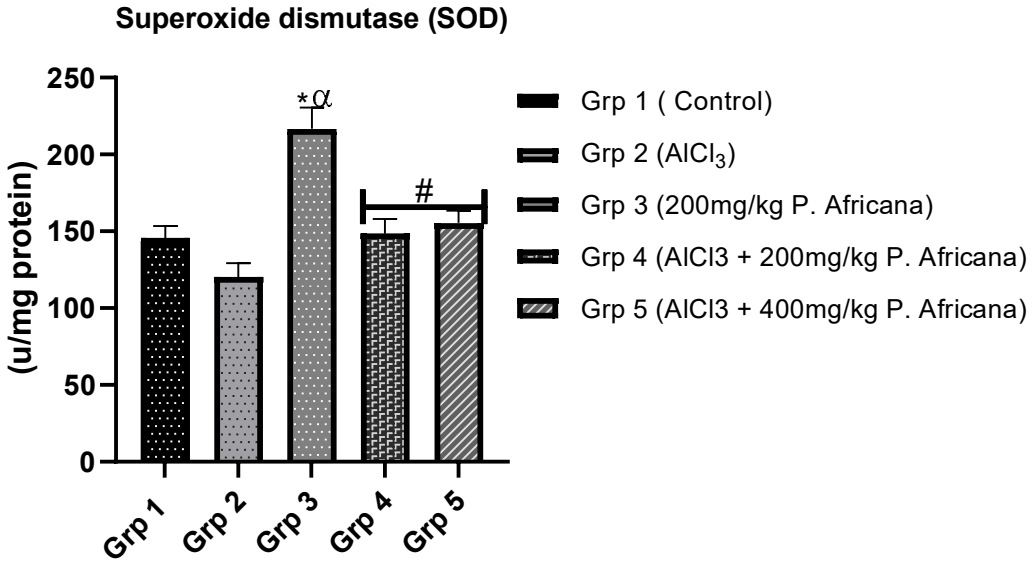

Fig 11: Variations in serum superoxide dismutase (SOD) levels. * Group 3 was statistically significant group 1 (control) p<0.05, α Group 3 was statistically significant to group 2 ($AlCl_3$ alone) p<0.05, # Group 4 and 5 were statistically significant to group 3 p<0.05.

➢ *Malondialdehyde (MDA)*

Data on Malondialdehyde (MDA) are expressed in Fig. 10. When the control group were compared with the other groups, the results revealed that group 2 ($AlCl_3$ only) (7.1 ± 0.65) and group 4 ($AlCl_3$ + 200mg/kg *P. Africana*) (3.7 ±0 .39) were statistically significant (p<0.05). But when group 2 ($AlCl_3$ only) were compared with other groups, group 3 (200mg/kg *P. Africana*) (5.3 ± 1.10) and group 5 ($AlCl_3$ + 400mg/kg *P. Africana*) (2.7 ± 0.23) were statistically significant (p<0.05). Group 3 (200mg/kg *P. Africana*) was compared with other groups and the results revealed statistically significant (p<0.05) in group 4 ($AlCl_3$ + 200mg/kg *P. Africana*) (5.3 ± 1.10).

➢ *Superoxide dismutase (SOD)*

Data on Superoxide dismutase (SOD) are expressed in Fig. 11. The results of comparison of group 1 (control) (145.7 ± 8.0) revealed statistically significant (p<0.05) in group 3 (extract alone) (216.8 ± 13.7) while the results of comparison of group 2 ($AlCl_3$ only) (120.3 ± 9.2) revealed statistically significant (p<0.05) in group 3 (extract alone) (216.8 ± 13.7). Group 3 (extract alone) was compared with other groups and the results revealed that group 4 ($AlCl_3$ + 200mg/kg *P. Africana*) (148.7 ± 9.4) and group 5 ($AlCl_3$ + 400mg/kg *P. Africana*) (155.5 ± 8.0) were statistically significant (p<0.05).

➢ *Testosterone Hormone*

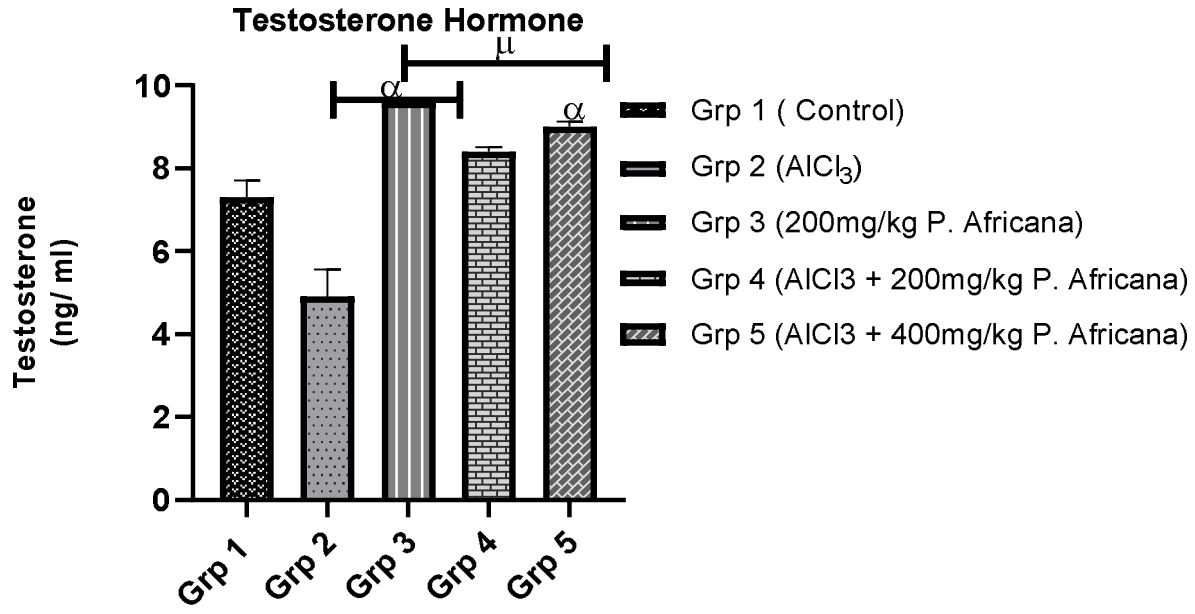

Fig 12: Variations in serum testosterone levels. α groups 2, 3 and 5 were statistically significant when compared with group 1(control) $p<0.05$, μ groups 3, 4 and 5 were statistically significant when compared with group 2 ($AlCl_3$ alone) $p<0.05$

Data on Testosterone hormone are expressed in Fig. 12. When group 1 (control) (7.3 ± 0.40) was compared with other groups there were statistically significant changes ($p<0.05$) in group 2, 3 and 5 ($AlCl_3$ only) (200mg/kg *P. Africana*) ($AlCl_3$ + 400mg/kg *P. Africana*) (4.9 ± 0.65, 9.6 ± 0.06, 9.0 ± 0.12). The result of group 3 (extract alone) (9.6 ± 0.06) was statistically ($p<0.05$) higher when compared with groups 4 and 5 (8.4 ± 0.11, 9.0 ± 0.12).

IV. DISCUSSION

The study focused on mitigating effect of stem-bark ethanolic extract of *Prosopis africana* on aluminium-induced toxicity on the Histology, semen parameters, oxidative stress enzymes and testosterone hormone of adult Wistar rats.

Based on our findings on body and testicular weights, we discovered that despite equal access to food, the treatment with aluminum chloride led to decrease in the body weight of the aluminum chloride alone group. The same results were replicated in the right and left testes. These results were in accordance to Hala, *et al*. (2010); Guo *et al*. (2005, 2009); Khattab *et al.* (2007). Chronic treatment with aluminium chloride was connected with a decrease in total body weight and testicular weights; according to our findings, this weight loss and loss of appetite could be linked to a poor rate of eating and drinking.

As shown in the control micrographs, all features of normal testicular histology are observable in control. The testicular histology of rats in the control group exhibited classical histo-architectural 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 extratubular components are of the classical type described for normal testicular histology. Sertoli cells are observable, including spermatogonia at the basement membrane. 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. Cluster of Leydig cells are seen in the interstitial space. This pattern of normal testicular micro-architecture was also observed in animals that received 200mg/kg *P. Africana* and $AlCl_3$ + 400mg/kg *P. Africana*. There seem to be some positive implications for the observation of normal histological appearance. Bundles of spermatozoa are seen in the lumen of seminiferous tubule indicating spermiation. It shows normal testicular micro-anatomy with full presence of phase of spermatogenesis, well arranged basement membranes, well distributed Leydig cells, and seminiferous tubules filled with sperm cells.

These present findings are in agreement with Hatch (1995) and Olawuyi *et al*. (2019) that many plant-derived substances, collectively termed "phytonutrients," or "phytochemicals," are becoming increasingly known for their antioxidant activity. Flavonoids, for example, are phenolic chemicals that are found throughout the plant kingdom. Flavonoids' extensive medicinal effects are mostly due to their antioxidant capabilities (Bendich,1994). A number of studies have evaluated the roles of *Prosopis africana* but only a few of these studies have suggested a beneficial role for antioxidants therapy in male infertility. These findings proved that *Prosopis africana* aqueous stemback-extract could also perform the role of antioxidant.

The micrographs for the group that received aluminium chloride only showed testicular histology with abnormalities and mild degenerative changes of seminiferous tubules. These changes include: widening of interstitial spaces (W) with infiltration of oesinophilic cells, edematous vacuolated fluids (V), degenerative changes of seminiferous tubules (C) and loss of normal distribution in epithelial lining (D). These findings are in agreement with El-Shahat *et al*. (2009). Few sertoli cells are observable, including spermatogonia at the basement membrane. As well as few visible Secondary spermatocyte and 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.

In these current studies, the degenerative changes were reversed by *Prosopis africana*. Low dose of 200mg/kg *P. Africana* with aluminum chloride showed mild recovery from severe histological changes. This reversal is in agreement with Rana and Verna (1996) also with the report of El-Shahat *et al*. (2009) who reported the antioxidant effect of green tea on cadmium poison.

The semen parameters showed markedly significant changes. The concentration count, the motile count, Progressive assessment and the morphology were greatly increased in the rats treated with *Prosopis Africana* (group 3) stemback-extract alone; this showed positive spermatogenesis and testicular steroidogenesis.

The testis relies on a monotonous and predictable reaction to any sort of perturbation, typified by a decline in spermatogenic production/efficiency, which was examined in this work (Holstein *et al.,* 2003). Semen analysis was supposed to put the discovery of stress-induced attack on testicular biology into right context. All stages of spermatogenesis can be disrupted, and the impact of a single assault can be felt at any or all of them. A marked reduction in the number of spermatozoa, a high percentage of spermatozoa malformation, and/or large numbers of spermatozoa with reduced/inefficient motility are all male factors that can cause subfertility or infertility in any mammalian male, as can be easily detected through semen analysis.

Oxidative stress caused by aluminum chloride led to asthenospermia, hypospermia, teratospermia and reduction in sperm count, which is in agreement with the report of Mathur *et al.* (2010) and Yousef *et al.* (2005). Sperm quality— unlike sperm quantity that is directly related to Sertoli cell efficiency— is associated with both testicular and epididymal micro-environments (Mäkelä *et al.,* 2014). For instance, significant elevation in the production of ROS or significant decrease in anti-oxidant defense capacity in either the testis or epididymis can compromise the cell membrane of spermatozoa.

All groups concormitantly treated with aluminum compound and *Prosopis Africana* stemback-extract (group 4, 5) had their semen parameters improved according to the dosage. High dosage showed marked improvement than the other and significantly lessen the deleterious effect of oxidative stress induced by aluminum compound on spermatogenesis. Hatch (1995) reported that there are many phenolic compounds such as flavonoids.

The biochemical findings revealed that malondialdehyde were markedly significant ($p<0.05$) in group 2 and 4 when control was compared to the other groups. The rats that were given aluminum chloride alone has the highest value while groups 1 and 3 were significantly low.

Exposure to Aluminum chloride induced oxidative stress and leads to increased value of malondialdehyde in the rats given aluminum chloride while rats with low dose of 200mg/kg *P. Africana* and control groups recorded low value of malondialdehyde. Several studies have been done on mechanism of aluminium induced toxicity (Yousef *et al.,* 2005; Khattab and Khattab, 2007). The toxic effect of aluminium expressed through the production of free radicals such as reactive oxygen species (ROS). It also alters the enzymatic antioxidative system (Mathur *et al.,* 2010; Yousef *et al.,* 2005). Although reactive oxygen species (ROS) are produced at a minimal concentration under normal physiological condition but an elevated production and an inhibition of the endogenous antioxidants will result in loss of membrane integrity and ultimately cell death (Mathur *et al.,* 2010).

According to Darley-Usmanr (1995) high generation of malondialdehyde (MDA) indicates Lipid peroxidation. In the same vein, Lucesoli and Fraga (1995) said Oxidative stress is produced by the peroxidation and oxidation of many cell lipids. Oxidative stress in the testes plays a role in a number of disorders that are known to be harmful to male fertility (sex hormones). An imbalance between the creation of reactive oxygen species (ROS) and their effective elimination by available antioxidant mechanisms causes oxidative stress in any tissue.

One of the major mechanisms behind metal toxicity has been attributed to oxidative stress**.** Testicular oxidative stress plays a role in a number of conditions known to be detrimental to male fertility. According to Dandekar. (2002), free oxygen radical cause lipid peroxidation of sperm membrane leading to sperm cells damage and infertility.

The results of superoxide dismutase revealed that aluminium chloride alone was markedly decreased while group 3 and 5 were markedly increased. According to Xia *et al.* (2014), superoxide dismutase is an antioxidant that reduces free oxygen radical to nontoxic products.

Oxidative stress and a failure of antioxidant defense system caused several sperm abnormalities and resulted in infertility. In agreement with Sainath *et al.* (2011) an imbalance between the reactive oxygen species generation and scavenging system might be one of the reasons for aluminum chloride in rats given aluminum chloride alone induces male reproductive toxicity. *Prosopis africana* can indirectly scavenger of reactive oxygen species such as hydrogen peroxide through increased activity of glutathione

peroxides; therefore, it may prevent the testicular injury produce by aluminum chloride.

The results on testosterone hormone, revealed that when the aluminium alone was compared with other groups; they were statistically significantly ($p<0.05$). This is similar to the work of Jensen *et al*. (2008) and Benoff *et al.* (2004) who reported that metals do cause hormonal imbalance and this could result in reduction in quality and quantity of sperm cells production. It could also cause impotence.

According to Wong *et al*. (2000) and Zhu *et al*. (2000) regulation of testosterone secretion is not the exclusive preserve of any single hormone, metabolic factor, micronutrient, or neurotransmitter. Many factors are taken into consideration in looking at the pattern and level of circulating testosterone.

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 and factors— of which LH is the chief regulator— the perturbation of it homeostasis alone can disrupt male reproductive function regardless of whether other reproductive hormones are in balance or not.

The extract of *Prosopis africana* stem bark demonstrated protective activity against aluminium induced oxidative stress in rats. This is evidenced in the significant and increased preventive index with 200 and 400 mg/ kg doses of the extract. Hence, the protective action of *Prosopis africana* extract against aluminium induced toxicity could possibly be attributed to its antioxidant excitatory effect.

V. CONCLUSIONS

This study delineates the protective role of the *Prosopis Africana* stemback-extract on reproductive dysfunction due to aluminum chloride induced toxicity in male Wistar rats. Aluminum chloride results in dysregulation of spermatogenesis, testicular dysfunction, and increased oxidative stress activity in testes. Improved seminal parameters observed in experimental groups administered the *Prosopis Africana* stembark-extract, revealed mitigating effect of the extract. The extract is a good natural phytotherapeutic agent against heavy metal-induced toxicity, according to our findings. Prosopis Africana stemback-therapeutic extract's activity in detoxifying / mitigating was thoroughly elucidated in our current investigation; indeed, the extract has proven to be a powerful antioxidant.

Ethics approval

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. *Proposis africana* was sent for identification at the Centre for Research and Development (CERAD), Federal University of Technology, Akure, where it was given a ethical number FUTA/ETH/20/27.

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Authors contribution

OTS: Conceptualization, Methodology, Validation, Investigation. UVO: Methodology, Project administration, Supervision, Investigation. ABK: Formal analysis, Investigation. IOF: Investigation.

Declaration of competing interest

No conflict of interest.

VI. ACKNOWLEDGEMENTS

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