

Histomorphometry, Stereology of Germ Cells and Hormonal Alterations in Aluminum-Induced Testicular Toxicity of Adult Wistar Rat: The Ameliorative Effect of Composite Teas

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Abstract:- This study aimed at the ameliorating effect of composite teas on aluminum-induced testicular-toxicity of adult male Wistar rats. Thirty adults male Wistar rats of average body weight of 100-180g, divided into 6 groups with Five (5) rats per group were used. Administration of aluminum chloride (AlCl₃) was given for 2 weeks, followed by composite tea administered orally for two (2) weeks. Group 1 received 0.15mg/kg of AlCl₃, Group 2 (control) received feed pellets and distilled water. Groups 3, 4, 5 & 6 received 0.15mg/kg AlCl₃ and 5mg/kg of composite tea respectively. Twenty-four hours after last administration, the animals were weighed, sedated with diethyl-ether in a chamber, and blood was collected. The testes were excised and weighed. There were statistically significant alterations in the percentage of seminiferous tubular and seminiferous ductal diameter within the animals in all the groups (p<0.05). Significant changes in sperm parameters, hormone profiles and defective testicular morphology in Groups 1, 3, 4 when compared to the control group. However significant improvements were noticed in Group 6 after administration of composite tea, with restored sperm characteristics, restored hormonal assessments and preserved testicular structures. Conclusively, the ameliorating effect of the composite teas were revealed on aluminum-induced toxicity of adult Wistar rat.

Keywords:- Male Infertility, Histomorphometry, Composite Teas, Aluminium, Testicular Toxicity

I. INTRODUCTION

Aluminium (Al) is ubiquitous in our surroundings. As a result, under physiological settings, food is the primary source of aluminum intake (Yokel et al., 2001). Because of the pervasive availability of aluminum in the environment and in food, avoiding exposure to this metal ion is nearly impossible (Hewitt et al., 1990). Aluminium is naturally found in conjunction with other elements to form compounds such as aluminum sulphate and chloride due to its reactivity (Oteiza et al., 2008). Aluminum overexposure has been linked to negative effects on testicular tissues (Pizent et al., 2012). In rats and humans, metals have been proven to impair spermatogenesis, which can lead to reduced sperm counts. Green tea includes catechin called epigallocatechin-3-gallate (EGCG), which has anti-cancer, antioxidant, anti-diabetic,

anti-hypertensive, anti-microbial, and anti-metabolic syndrome benefits (Zhang et al., 2016), as well as reducing infertility in people and animals (Wang et al., 2018). Catechins are natural antioxidants that aid to prevent cell damage and give a variety of other advantages (Qian Yi Eng et al., 2017). These chemicals can protect cells and molecules from damage by reducing the generation of free radicals in the body.

This study is aimed at the ameliorating effect of composite teas on Sperm parameters, Hormone profile, Testicular Histomorphometric, and Stereology of Germ cells of adult male Wistar rats.

II. MATERIALS AND METHODS

At Oluwatedo quarter, Ipinsa, Akure, Ondo State, Nigeria, fresh leaves from Moringa plants (*Moringa oleifera*), tumeric root plants (*Curcuma longa*), and *Camellia sinensis* plants (Family Theaceae) were gathered. ELISA kits (Monobind Inc, CA 92630, ab10866, SE120087 USA) were obtained from Nums Diagnostic Centre in Suleja, Niger State, and Aluminum Chloride crystals (Lot No: 20150321, Guangdong Sci-Tech, China) were obtained from PASCAL in Ondo State. Microtome (Leica RM 2125 RTS), Rotary evaporator (Union Laboratories England RE-52A), Vacuum Pump, Centrifuge (Denly, Model BS 400), sensitive weighing balance (Mettler Toledo, Mg 126), Automatic Tissue processor, 96-microplate reader (model SM 600, China), Water bath (model MH-8504), Adjustable pipettes (Surepette RS 16013), Electric oven (Model: DHG-9030A, Searchtech instrument).

A. Preparation of Extracts

The samples were identified at the Federal University of Technology, Akure's Department of Forestry and Wood Technology, Faculty of Agriculture. Foreign elements were eliminated from all plant materials after a thorough inspection. After that, the samples were washed with tap water. All of the samples were air dried in a residence at 350°C. The samples were milled using an electric Binatone Blender after drying (China, Model BLG401). The milled material was sieved (425m) and placed in a container with tight lids and labels. Tea blends (2g) were produced and packaged. All of the bag samples were maintained in glass jars at temperatures ranging from 350 to 400 degrees

Fahrenheit, away from direct sunlight, and were labelled accordingly.

Table A: Herbal tea formation

Tea Name	Peppermint (%)	Moringa leaf (%)	Tumeric leaf (%)	Sunflower (%)	Mango leaf (%)
Pep tea	30	25	20	15	10
Mang tea	25	20	15	10	30
Sun tea	20	15	10	30	25
Tum tea	15	10	30	25	20
Mor tea	10	30	25	20	15

B. Breeding of Animals

This study required thirty (30) adult male Wistar rats weighing between 100 and 180 grams. The rats were maintained in cages (made of plastic, wire gauze, and net) in the animal house of the Department of Human Anatomy, Federal University of Technology Akure, under laboratory settings with 12 hour light/dark cycles. The rats were given ad libitum pellets and water and were given 14 days to acclimate. The National Institutes of Health Guide for the Care and Use of Laboratory Animals was followed in this work (NRC, 2010).

C. Experimental Design

The rats were grouped into six (6) groups of five (5) rats each. The mice were given oral doses of distilled water, compound teas, and aluminium chloride (AlCl₃). Group 1 received 150mg/kg body weight (b,w) of AlCl₃ dissolved in distilled water only, Group 2 (control) rats received equivalent volumes of distilled water and feed pellet, Group 3 received 150mg/kg b,w of AlCl₃ and 5mg/kg of Mango tea, Group 4 received 150mg/kg b,w of AlCl₃ and 5mg/kg of Sunflower tea, Group 5 received 150mg/kg b,w of AlCl₃ and 5mg/kg of Peppermint tea and Group 6 received 150mg/kg b,w of AlCl₃ and 5mg/kg of Moringa tea. Based on previous research, the doses, duration of AlCl₃ delivery, and composite teas were chosen (Malekshah et al., 2005; Samuel et al., 2020; Poonam et al., 2014). At the end of the 28-day testing period, the animals were weighed and anaesthetized with diethyl-ether in a confined chamber. The epididymides and testes were removed and washed in a buffered saline solution. Epididymides were used to test sperm parameters. One of the testes was fixed in Bouin's fluid for histological processing and histopathological examination. The removed organs were weighed using a digital scale with a 0.01g precision. The serum was collected, transferred to an EDTA tube, and stored at -20°C for further analysis of blood hormone levels (testosterone, LH, and FSH). The animals' blood was taken by a cardiac puncture and centrifuged at 2000g for 15 minutes.

D. Histological Evaluation

The testes were fixed in buffered paraformaldehyde at a concentration of 4%. After 24 hours, the material was dehydrated with increasing concentrations of alcohol ranging from 50% to 70% to 90% and first and second absolute ethyl alcohol. After that, the specimen was fixed in paraffin, sectioned at 3 mm thickness, and stained with haematoxylin and eosin (H&E). Under a light microscope, testis slices were

examined and photographed. The thickness of seminiferous tubules (ST) was measured using the ImageJ software.

E. Semen Analysis

The testes were rapidly extracted, separated from the fatty tissue, and weighed. The caudal section of the epididymis was cut off and weighed in order to determine the number of sperm per gram. A Light Microscope with a Makler counting chamber was used to examine sperm count and motility at x100 magnification. The caudal epididymis was removed and minced in 10ml of normal saline with a surgical blade/anatomic scissors, then placed in a rocker for 5-10 minutes and incubated at room temperature for 2 minutes. After incubation, the supernatant was diluted with 5g sodium bicarbonate and 1ml formalin (35%) in a 1:100 ratio. The sperm cell concentrations were counted using new improved Neuber's counting chamber (Haemocytometer) or Mackler Sperm counting chamber. 10 l of the aliquot sample was aspirated with a Pasteur pipette and put on the counting chamber for 5 minutes before being examined under a binocular light microscope (Yokoi and Mayi, 2004).

F. Determination of Sperm abnormalities

The original dilution for motility, dilute 1:20 with 10% neutral buffered formalin, was used to assess the morphology of the spermatozoa (Sigma-Aldrich, Oakville, ON, Canada). To measure the rate of teratospermia anomalies, sperm suspension was used to make smears for study of sperm morphology. A drop of sperm suspension was added to an equal volume of 1 percent eosin-y 5 percent nigrosin, which was then mixed and smeared on pre-warmed clean glass slides, which were then air-dried. Two hundred sperm cells per animal were analysed at x400 magnification using an Olympus light microscope to evaluate morphological abnormalities (Rezvanfar et al., 2008). The presence of one or more abnormal features (teratospermia) such as tail defects (short, irregular, coiled or multiple tails); neck and middle piece defects (distended, irregular, bent middle piece, abnormally thin middle piece); and head defects (distended, irregular, bent middle piece, abnormally thin middle piece) were used to categorize sperm cell morphology (round head, small or large size, double or detached head). The morphology was expressed as a percentage (Saalu et al., 2010).

G. Hormone Analysis

Blood samples taken through cardiac puncture were deposited in an anticoagulant bottle containing EDTA and centrifuged at 2000 rpm for 10 minutes, after which the sera

were pipetted into simple sample vials and stored at -20°C until tested within two weeks. Serum samples were often warmed to room temperature according to the kit manufacturer's recommendations.

H. Serum Assay Testosterone Procedure (Tietz and Saunders, 1994)

Serum testosterone was measured after blood samples were spun at 3000 revolutions per minute for 10 minutes in an angle head centrifuge at 25°C. The samples were tested in batches using the Enzyme linked immunoassay (ELIZA) method. The specimens and controls were divided among the coated wells that were used with the ten micron (10l) standard. A hundred micron (100l) of testosterone conjugate reagent was added, as well as fifty microns (50l) of anti-testosterone reagent. Before being incubated at room temperature for 20 minutes, the contents of the microwell were properly mixed. To stop the process, 100 liters of 1M hydrochloric acid were utilized. At 450nm, an automated spectrophotometer (Rayto; RT-2100C, Microplate-Reader) was used to detect absorbance.

I. Luteinizing Assay Procedure

LH was measured quantitatively according to manufacturer instructions using the Wennink et al. (1990). With twenty-five microns (25l) of the standard, the specimens and controls were dispersed into appropriate wells. Pipette 25 microns (25 l) of enzyme conjugate reagents into the wells, mix well for 30 seconds, and incubate at 36°C for 60 minutes. The microtiter wells were rinsed and flicked five times with 300 micron (300l) of washing solution. To drain any remaining water, the wells were slammed with absorbent paper.

TMB substrate solutions of 100 microns (100µl) were added to each well, stirred, and incubated at room temperature for 15 minutes.

A hundred microns (100µl) of stopping solution was used to stop the reactions.

For 30 seconds, the samples were gently mixed until the blue color changed to yellow. A 'Microplate reader' (model SM 600, China) was used to test absorbances at 450nm within 15 minutes (Wennink et al., 1990).

J. Follicle Stimulating Hormone Assay Procedure

With 25microns (l) of the standard, the specimens and controls were divided into appropriate wells. Pipette 50l of enzyme conjugate reagents into the wells, aggressively mix for 30 seconds, and incubate at 36°C for 60 minutes. The microtiter wells were rinsed and flicked five times with 300 microns (300l) of washing solution. The wells were forcibly hit with absorbent paper to remove any remaining water. 100 microns (100l) TMB substrate solutions were added to each well, mixed, and incubated at room temperature for 15 minutes. A hundred micron (100l) of stopping solution was used to stop the reactions. The samples were gently combined for 30 seconds until the blue hue changed to yellow. A microtiter well reader was used to measure the absorbance at 450nm after 15 minutes (Simoni et al., 1997).

K. Statistical Analysis

The GraphPad Prism statistical software tool was used to create the graphs (version 8.0.2). The data were expressed as a mean sem (standard error of the mean) and compared between the experimental groups using the ANOVA test (one way). When the p value ≤ 0.05 , the data was considered statistically significant.

III. RESULTS

A. Effect of composite teas and aluminium chloride on the body weights

Table 1: The Distribution of Mean and Standard error of mean (sem) for the Animal Weights

GROUPS	Animal weight (g) week 0	Animal weight (g) week 1	Animal weight (g) week 2	Animal weight (g) week 3
GRP 1	145.6 \pm 15.1	171.2 \pm 4.7	177.6 \pm 15.1	164 \pm 13.4
GRP 2	114.6 \pm 4.8	199.6 \pm 3.3	180 \pm 7.9	173.3 \pm 8.1
GRP 3	118.2 \pm 10.2	150.2 \pm 10.2	179 \pm 15.6	185.5 \pm 17.3
GRP 4	126.4 \pm 9.7	158.4 \pm 9.7	193.8 \pm 4.8	201.8 \pm 5.7
GRP 5	144.8 \pm 5.1	185.2 \pm 10.9	125.5 \pm 16.7	119.5 \pm 17.5
GRP 6	140 \pm 6.7	172 \pm 6.7	159 \pm 18.6	195.3 \pm 12.8

There were no statistically significant changes in the animal weights difference of the experimental animals in all the groups in week zero and the third weeks ($p > 0.05$) but there were statistically significant changes in the animal weights of the experimental animals in all the groups in first and second weeks ($p < 0.05$). Table 1, shows that the mean and standard error of mean (sem) for the animal weights in the experimental animals in all the groups in weeks two and three were greatly increased as compared to week zero animal weights; there were mild weights changes between second week and third week animal weights across the groups. Table

1 above shows that group 4 (given sunflower tea with aluminium chloride) animal weights increased drastically starting from the first week to the third week of treatment while group 5 (given peppermint tea with aluminium chloride) was greatly reduced throughout the treatment weeks followed by group 1 (given aluminium chloride alone).

B. The Testicular weights

Table 2. The distribution of Mean and Standard error of mean (sem) for the Testicular weights

	Right Testis (g) ± sem	Left Testis (g) ± sem
GRP 1	1.5 ± 0.11	1.5 ± 0.13
GRP 2	1.6 ± 0.03	1.6 ± 0.05
GRP 3	1.8 ± 0.16	1.8 ± 0.14
GRP 4	1.7 ± 0.03	1.8 ± 0.10
GRP 5	1.5 ± 0.33	1.4 ± 0.30
GRP 6	2.2 ± 0.07	2.3 ± 0.15

There were no statistically significant changes in the testicular weight difference of the experimental animals in all the groups ($p < 0.05$). Table 2, shows that group 6 with dosage of Moringa tea extract and $AlCl_3$, had the highest weight across the group; followed by group 3 with dosage of Mango tea extract and $AlCl_3$, but group 5, with medium dose of Peppermint tea extract and $AlCl_3$ having the lowest weight across the group. The control group was moderate with mean (\pm sem) of 1.6 ± 0.03 (g) and 1.6 ± 0.05 (g).

C. Histopathological Findings

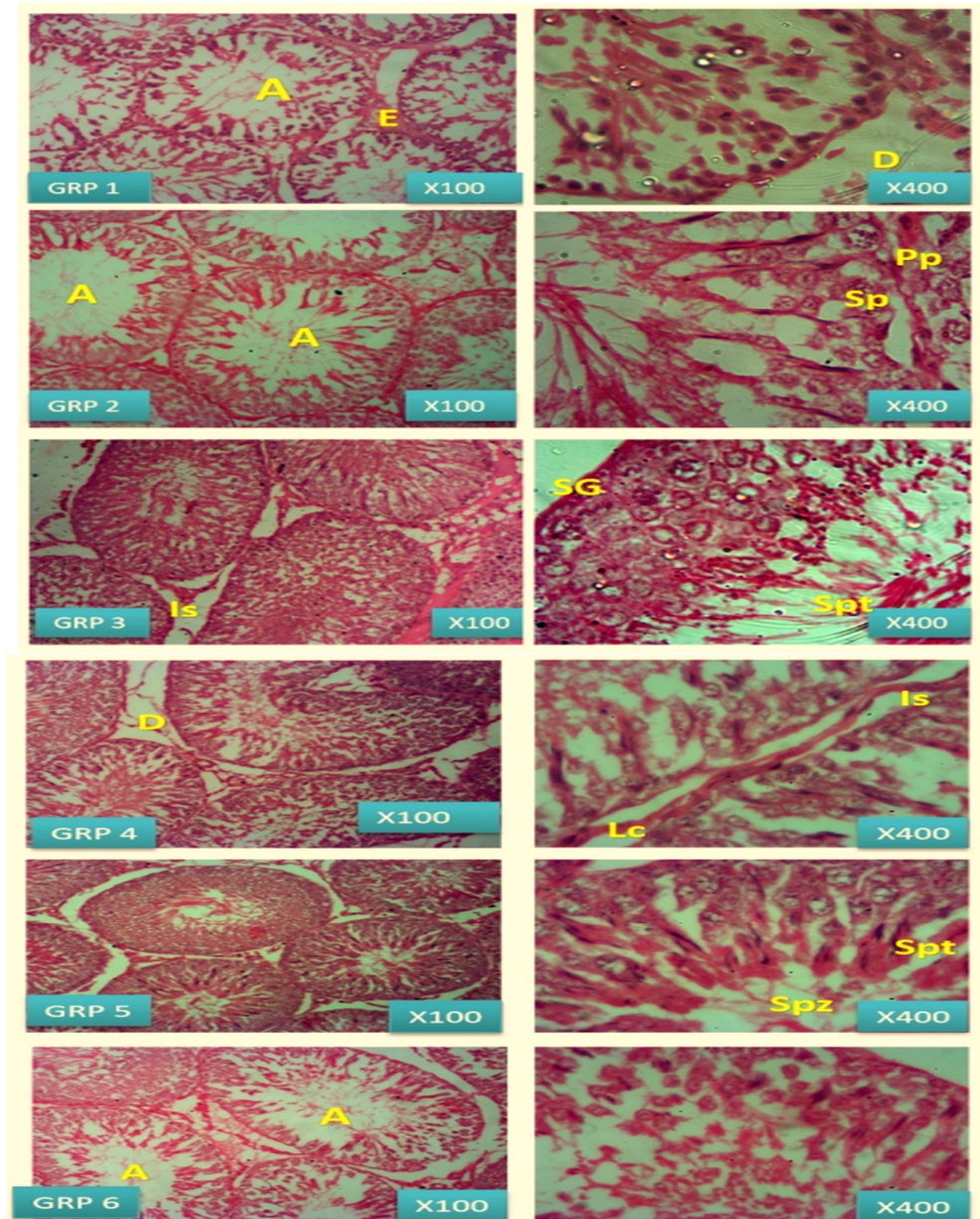


Fig 1. Testicular histopathological changes in group 1($AlCl_3$ alone) and treatment groups 3,4,5 & 6. Elongated spermatids (Spt), Spermatogonia (SG), Primary spermatocytes (Pp), Secondary spermatocytes (Sp), Leydig cell (Lc), showing mild degenerative changes of the seminiferous tubules (A), testicular necrosis (E) and widening of interstitial space (D). H&E stain, MAG X100 & X400.

In group 1, testicular histology shows abnormalities such as cell deformation, degeneration of seminiferous tubules, expanded interstitial spaces, and edematous alterations with mononuclear cell infiltrations near to the basement membrane that separates the underlying layer. Severe hypospermatogenesis or complete spermatogenesis is manifested by a deformed bundle of spermatozoa compared to those seen at baseline. Few germ cells can be seen when the seminiferous epithelium diameter is lowered. There are a few sertoli cells visible, as well as spermatogonia near the basement membrane. There are no secondary spermatocytes migrating to the adluminal compartment that may be seen. Primary spermatocytes with larger nuclei are also not seen, as are no early or late spermatids. Few Leydig cells are seen in

the interstitial space (Figure 1). In the rats treated with Moringa tea (group 6), the portion of seminiferous epithelial diameter reveals germ cells at different spermatogenic phases. Sertoli cells (*Sc*) are observable; including Spermatogonia (*SG*) at the basement membrane. Also visible are Secondary spermatocyte (*Sp*) migrating to adluminal compartment. Primary spermatocyte (*Pp*) with enlarged nuclei are observable too, with early spermatids (*Es*) and Late spermatids. Bundles of spermatozoa (*Spz*) are seen in the lumen of seminiferous tubule indicating spermiation. Clusters of Leydig cells (*Lc*) are also seen in the interstitial space (*Is*).

D. Histomorphometric and Stereological Analysis

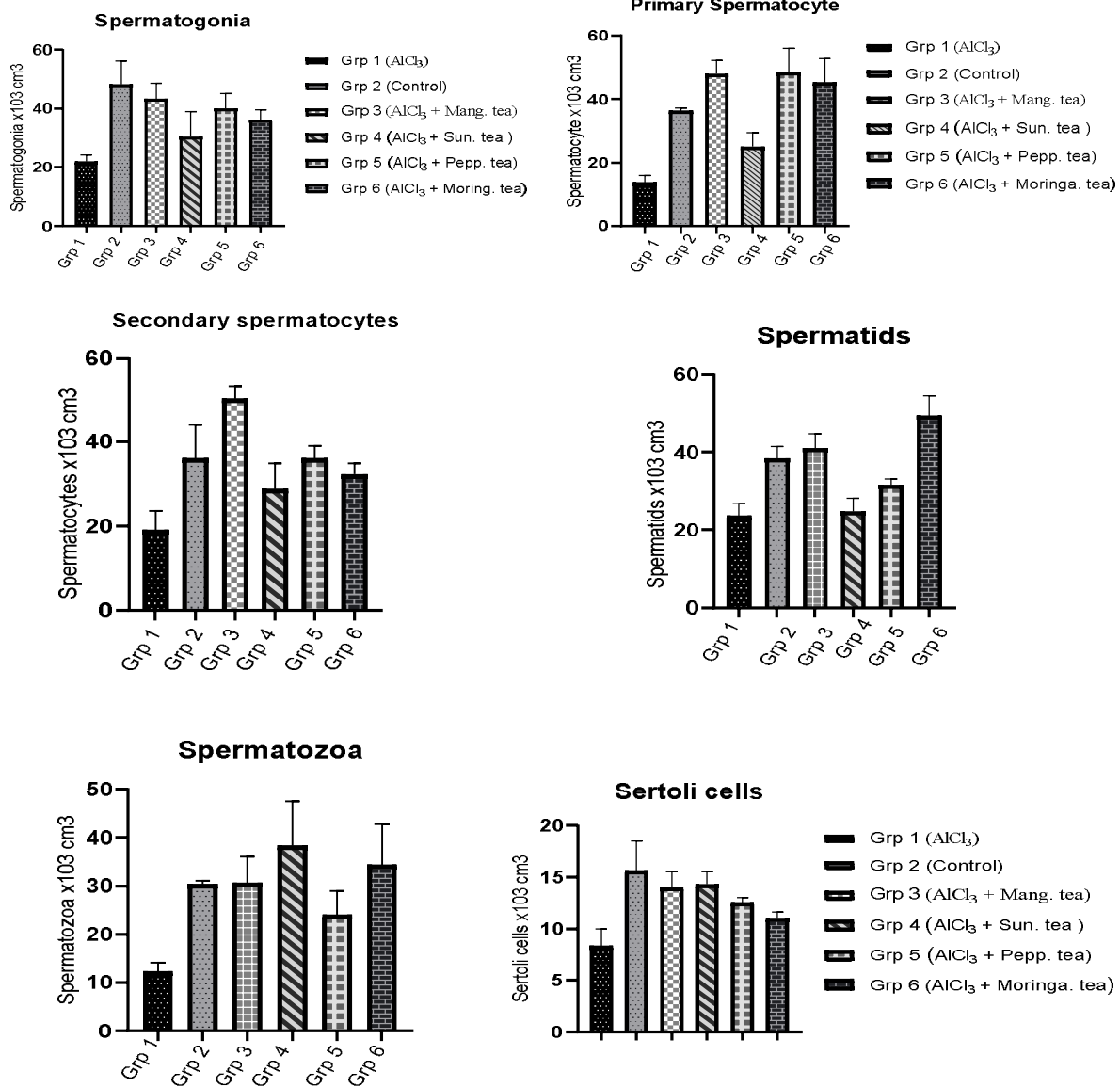


Fig 2. Stereological determination of all germ cell nuclei and Sertoli cell nuclei present at stage VII of the cycle were counted in 10 circulars in micron per cube.

ImageJ software was used to do histomorphometric analysis. Antigen-antibody science is the study of how antigen reacts with antibodies. When compared to the other treatment groups, $AlCl_3$ therapy dramatically reduced body weight, testicular weight, and testis/body ratio. However, in Group 6 (moringa tea) treatment, these three parameters significantly increased compared to the Aluminium group. Also, Group 5 (peppermint tea) treatment increased all the parameters compared to $AlCl_3$ group, but these were not significant.

$AlCl_3$ significantly decreased the number of Spermatogonia, Sertoli cells, spermatocytes, spermatids and spermatozoa compared to the control group. In the rats treated with Peppermint tea, the number of Spermatogonia, Sertoli cells, spermatocytes, spermatids and spermatozoa significantly increased compared to the $AlCl_3$ group. However, treatment with Mango tea only induced a significant elevation in the number of spermatocytes and treatment with Sunflower tea elevated the number of spermatozoa. Finally, the number of spermatids was significantly higher in the group treated with Moringa tea.

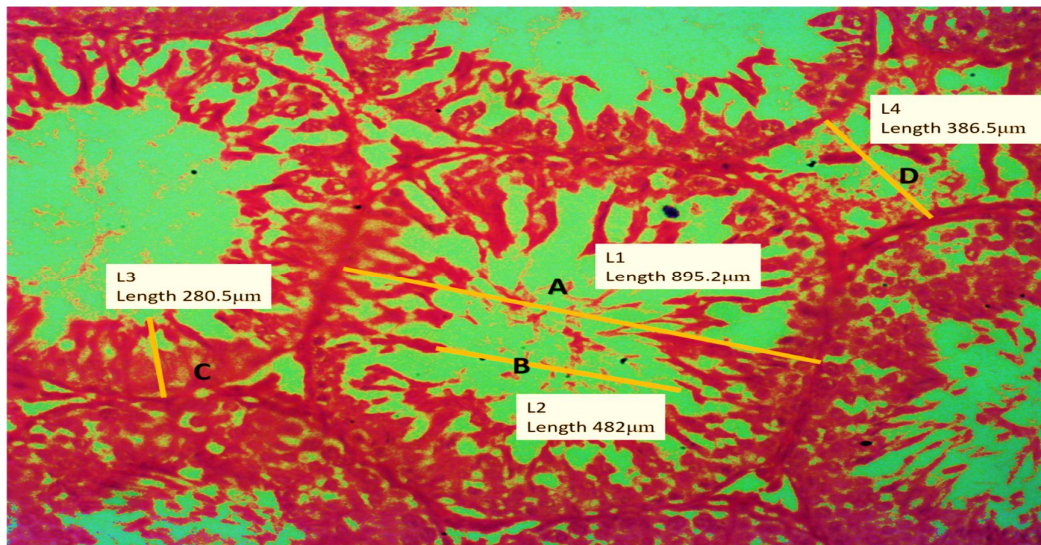


Fig 3. The Seminiferous Luminal diameter 'A', Seminiferous ductal diameter 'B', Seminiferous Epithelial height 'C'. Interstitial space diameter 'D' in microns.

E. Stereological determination of all germ cell nuclei and Sertoli cell nucleoi

Stereological determination of all germ cell nuclei and Sertoli cell nucleoi present at stage VII of the cycle were counted in 10 circular or nearly circular seminiferous tubules cross sections chosen at random, for each animal. The germ cells counted are : Spermatogonia, Primary spermatocyte, Secondary spermatocyte, Spermatids, Spermatozoa and Sertoli cells.

According to table 3, it shows that control rats have the highest spermatogonia standard error of mean (48.3 ± 7.8) $\times 10^3/\text{cm}^3$ while the rats given 0.15mg of aluminium chloride per kg of body weight have the lowest standard error of mean of (22 ± 2.1) $\times 10^3/\text{cm}^3$.

Stereological analysis of primary spermatocytes shows that animals given 5mg/kg of Peppermint tea extract with aluminium chloride have the highest standard error of mean that is: (48.5 ± 7.5) $\times 10^3/\text{cm}^3$; while the rats given 0.15mg of aluminium chloride per kg of body weight were the lowest standard error of mean of: (23.7 ± 2.3) $\times 10^3/\text{cm}^3$. The secondary spermatocytes were highest in the rats given 5mg/kg of Mango tea and aluminium chloride, (50.3 ± 2.9)

$\times 10^3/\text{cm}^3$ and lowest in the rats given 0.15mg of aluminium chloride per kg of body weight, the standard error of mean value is: (19 ± 4.6) $\times 10^3/\text{cm}^3$.

Stereological determination of spermatids reveals highest for control rats (49.3 ± 5.2) $\times 10^3/\text{cm}^3$ also, the rats given 5mg/kg of Mango tea extract and 0.15mg of aluminium chloride, (41 ± 3.8) $\times 10^3/\text{cm}^3$. The lowest standard error of mean: (23.6 ± 3.2) $\times 10^3/\text{cm}^3$ in the rats given 0.15mg of aluminium chloride per kg of body weight alone.

Stereological determination of spermatozoa shows that the rats given 5mg/kg of Sunflower tea extract and 0.15mg aluminium chloride per kg of body weight have the highest standard error of mean that is : (38.3 ± 9.2) $\times 10^3/\text{cm}^3$ while the rats given 0.15mg of aluminium chloride per kg of body weight have the lowest mean (22.3 ± 1.8) $\times 10^3/\text{cm}^3$.

Lastly, according to Table 3 above, the control rats have the highest Sertoli cells standard error of mean that is (15.6 ± 2.9) $\times 10^3/\text{cm}^3$ while the rats given 0.15mg of aluminium chloride per kg of body weight have the lowest standard error of mean of (10.3 ± 1.7) $\times 10^3/\text{cm}^3$.

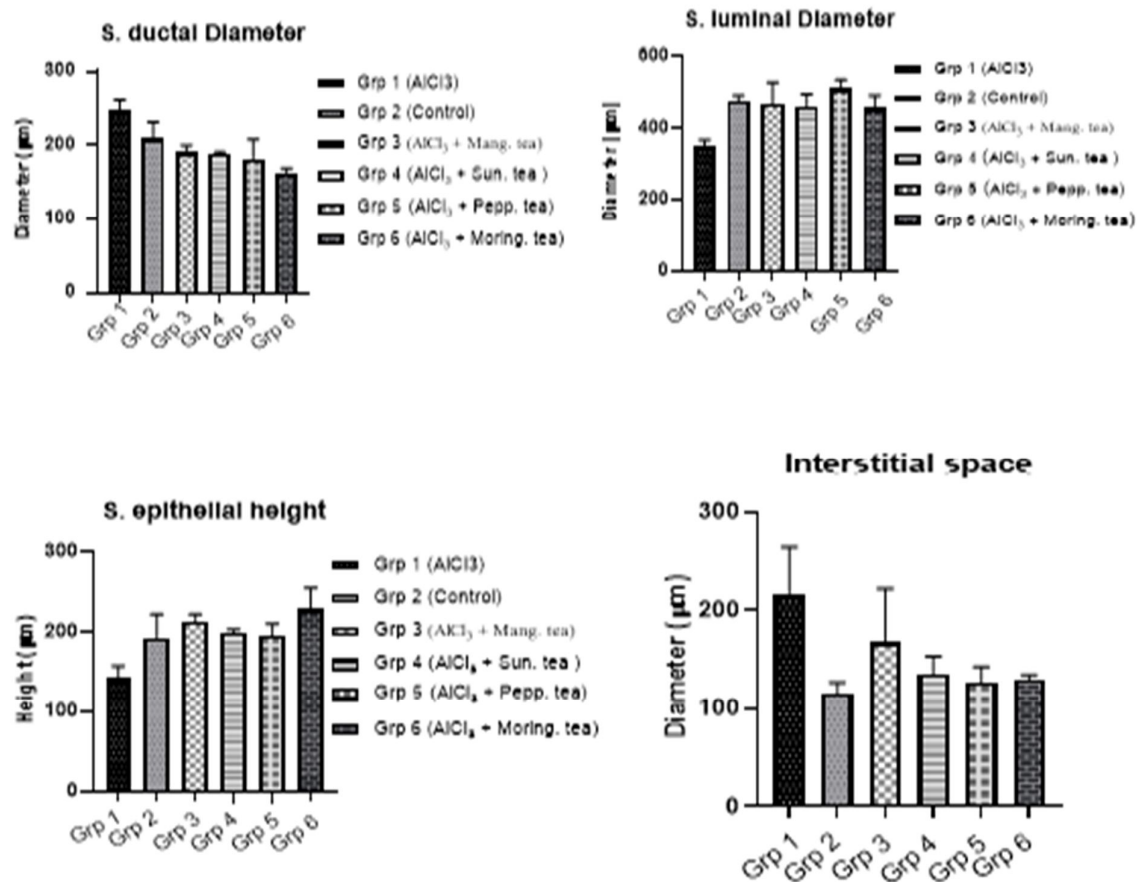


Fig 4. Variations in Seminiferous ductal and Luminal diameters, Seminiferous epithelial height and interstitial space diameter in micron. Statistically significant ($p < 0.05$) comparing AlCl₃ group and the treatment groups.

F. Seminiferous ductal and Luminal diameters, Seminiferous epithelial height and interstitial space diameters

The mean seminiferous ductal diameter within the experimental animals in all the groups ($p < 0.05$) were significantly different ($p < 0.05$). Figure 4, it was found group 6 (moringa tea with aluminium chloride) had the highest mean value ($246.1 \pm 15.4 \mu\text{m}$) followed by group 5 (peppermint tea with aluminium chloride) ($209.2 \pm 21.9 \mu\text{m}$); while the group with the lowest value was group 4 (sunflower tea with aluminium chloride) ($163.5 \pm 5.6 \mu\text{m}$).

There were marked significant statistical difference in seminiferous luminal diameter in the experimental animals' testes in all groups ($p < 0.05$). The mean (sem) for the seminiferous luminal diameter in group 5 (given peppermint tea with aluminium chloride) ($511.6 \pm 24.3 \mu\text{m}$), followed by group 2 (control) ($477.9 \pm 14.4 \mu\text{m}$), see Table 4, while group 1 (given aluminium chloride only) has the least mean value ($349.8 \pm 19.4 \mu\text{m}$).

There were significant statistical differences in seminiferous epithelial height in the experimental animals' testes in all groups ($p > 0.05$). The mean (sem) for the seminiferous epithelial height in group 6 (given moringa tea with aluminium chloride) ($227.9 \pm 27.1 \mu\text{m}$), followed by group 3 (given mango tea with aluminium chloride) ($211.6 \pm 10.2 \mu\text{m}$), (figure 4); while group 1 (aluminium chloride alone) has the lowest mean ($141.6 \pm 16.1 \mu\text{m}$).

Lastly, the interstitial space diameter of the experimental animals in groups 1,2,3,4 are significant to group 6 at ($p > 0.05$). Figure 4, shows that the mean (\pm sem) for the interstitial space diameter in group 1 (given aluminium chloride alone) ($217.8 \pm 47.1 \mu\text{m}$), followed by group 3 (given moringa tea with aluminium chloride) ($167.6 \pm 54.9 \mu\text{m}$), while group 2 (control) has the lowest mean ($116.2 \pm 10.9 \mu\text{m}$).

G. Sperm Parameters

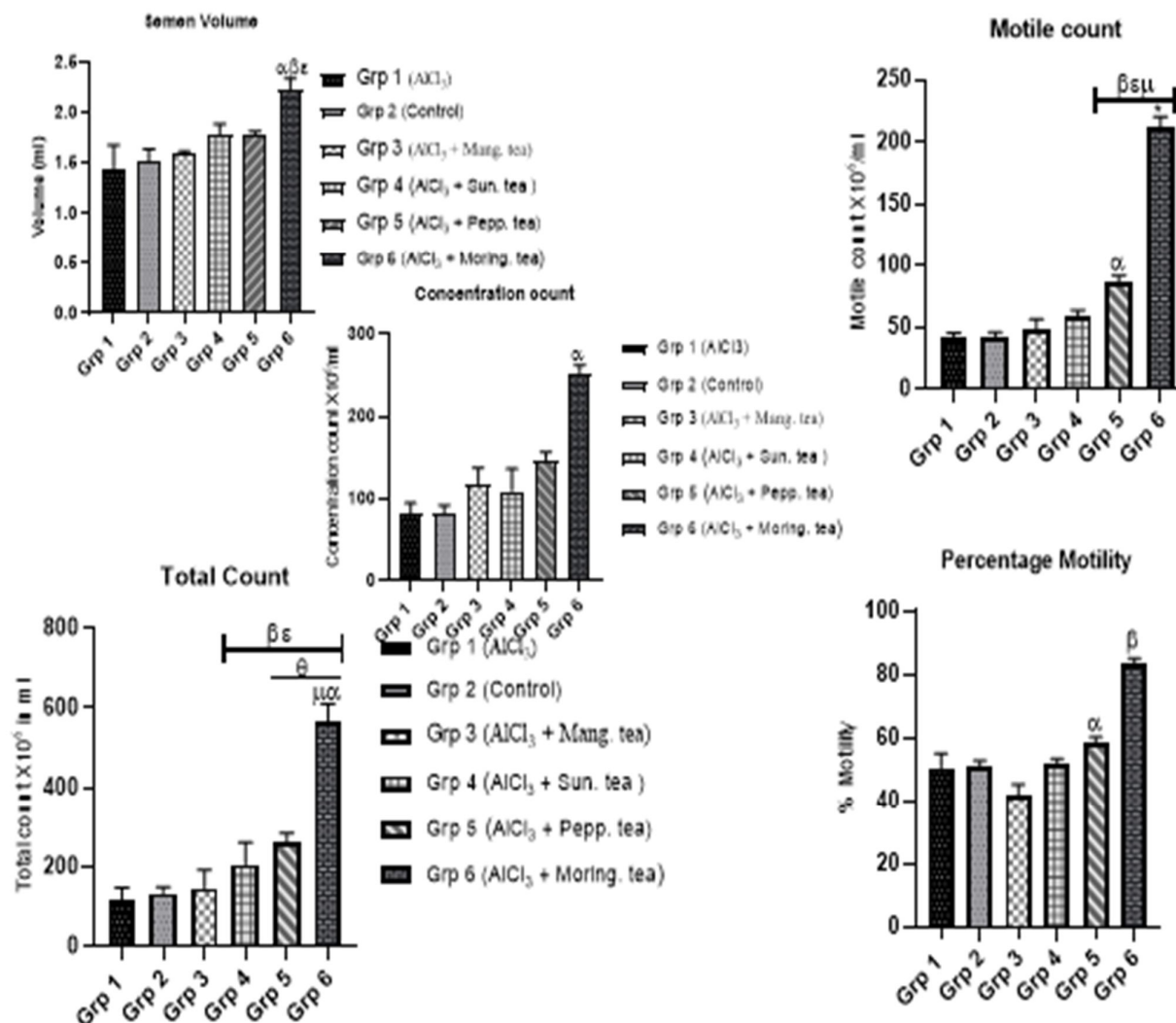


Fig 5. Variation in Semen Parameters, showing the semen volume, motile count, motility, concentration count and total count. Statistically significant ($p < 0.05$) comparing AlCl₃ group and the treatment groups.

There were no statistically significant changes in the Testicular volume across the experimental animals in all the groups ($p > 0.05$). Testicular volume of group 6 was a little bit higher (2.23 ± 0.11) (ml) compared to the others but group 1 treated with AlCl₃ alone were the lowest (1.45 ± 0.23) (ml). The control, group 2 had a moderate value (1.52 ± 0.12) (ml), (Figure 5)

Considering the motile counts, there were marked statistically significant changes ($p < 0.05$). Group 6 with Moringa tea and AlCl₃ was the highest (112.00 ± 8.60 X10⁶/ml), followed by group 5 with Peppermint tea and AlCl₃ (86.25 ± 5.78 X10⁶/ml) and the lowest values are group 1 (with AlCl₃ alone) and group 2 (control group) (41.25 ± 4.29 , 41.67 ± 4.25 X10⁶/ml).

The Semen concentration counts were significantly different ($p < 0.05$). It was clearly shown in the table above; group 5 (with Peppermint tea and AlCl₃) and group 6 (with Moringa tea and AlCl₃) were greatly increased (147.50 ± 9.68 , 253.00 ± 9.70) (X10⁶/ml) but groups 1 (with AlCl₃ alone) and 2 (control group) were greatly reduced (81.67 ± 13.59 , 82.50 ± 10.19) (X10⁶/ml) (figure 5). The percentage motility also were statistically significant ($p < 0.05$), group 5 (with Peppermint tea and AlCl₃) and group 6 (with Moringa tea and AlCl₃) were the highest (86.25 ± 5.78 , 112.00 ± 8.60) (%); while group 3 (with Mango tea and AlCl₃) was the lowest value (50.56 ± 4.55 , 52.00 ± 3.32) (%). Statistically, total count were also marked significantly ($p < 0.05$); with mean and standard error of mean highest in group 6 (with Moringa tea and AlCl₃) (566.00 ± 44.38) (X10⁶/ml) and markedly reduced in group 1 (with AlCl₃ alone) (117.17 ± 30.53) (X10⁶/ml), (figure 5).

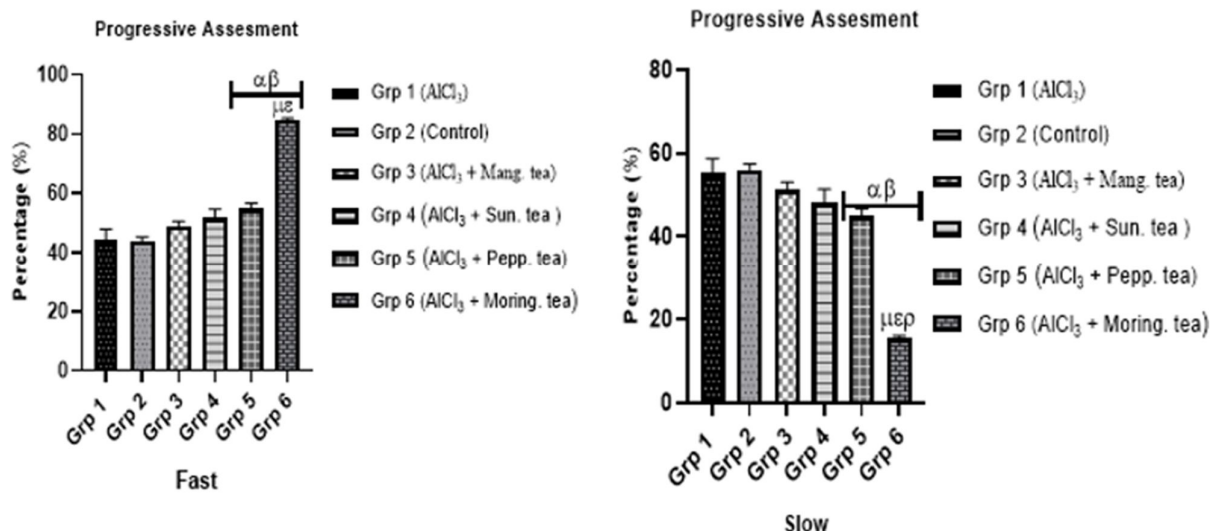


Fig 6. Variation in Semen Progressive Assessment. There were markedly statistically significant changes ($p < 0.05$).

Considering the progressive assessment, there were markedly statistically significant changes ($p < 0.05$). The mean (\pm sem) for forward movement were higher in group 6 (with Moringa tea and AlCl₃) (84.60 ± 0.68) (%) and group 1 (with

AlCl₃ alone) have the lowest value (44.00 ± 1.41), But the slow movement was markedly noticed in group 1 (56.00 ± 1.41 %) and lowest in group 6 (15.40 ± 0.68 %) (figure 6).

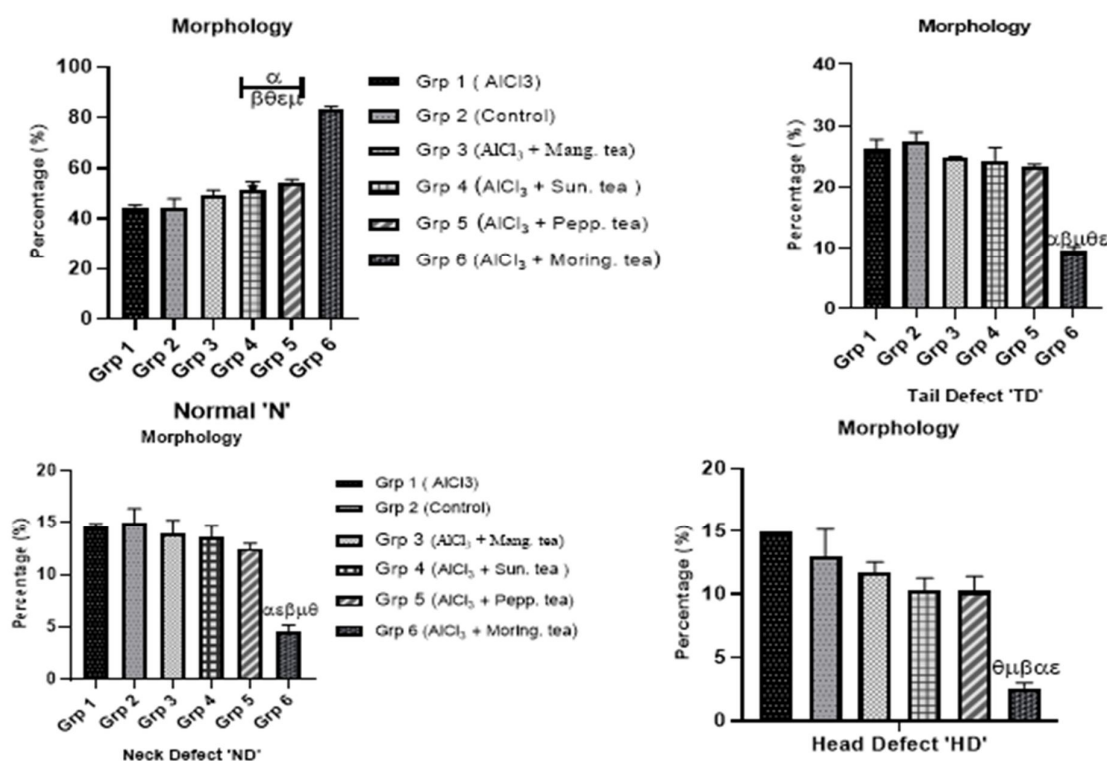


Fig 7. Variation in the Semen Morphology. Statistically significant ($p < 0.05$), the level of teratospermia in group 1 (AlCl₃ alone) was higher compared to other groups (Tail defect 'TD', Head defect 'HD', Neck defect 'ND').

There were statistically significant changes ($p < 0.05$), in the morphology across the groups. The distribution of mean (\pm sem) for morphology (figure 7). Group 6 (Moringa tea with AlCl₃) (83.40 ± 1.08 %), have the highest percentage of

morphologically normal sperm followed by group 5 (Pepp tea with AlCl₃) (54.00 ± 1.52 %) while group 3 (Mango tea with AlCl₃) and group 1 (AlCl₃ alone) had the lowest percentage of the normal sperm morphology; (44.00 ± 1.47 , 44.60 ± 3.33)

(%). The level of teratospermia in group 1 (AlCl_3 alone) was higher in this group compared to other groups (Tail defect 'TD', Head defect 'HD', Neck defect 'ND': 27.40 ± 1.54 , 13.00 ± 2.17 , 15.00 ± 1.38)(%), (figure 7).

H. Hormonal Profile Results

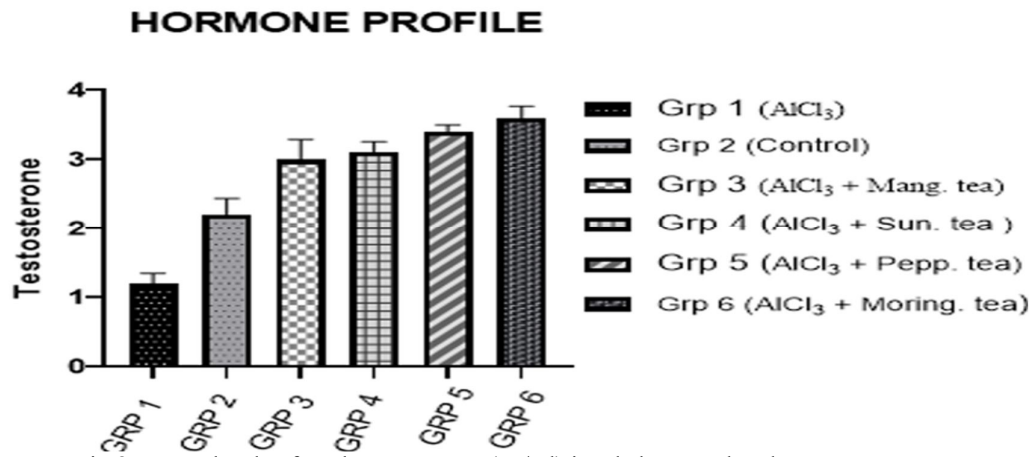


Fig 8. Serum levels of total Testosterone (ng/ml) in AlCl_3 , control and treatment groups.

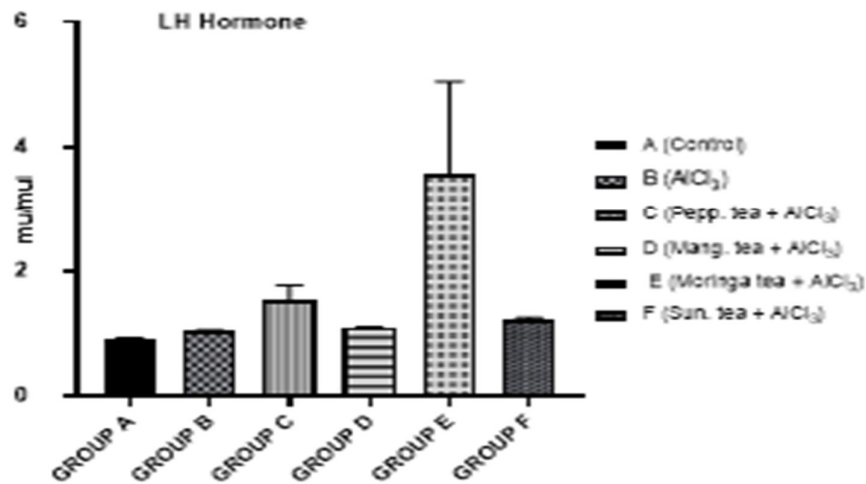


Fig 9. Serum levels of Luteinizing hormonal activity in AlCl_3 , control and treatment groups.

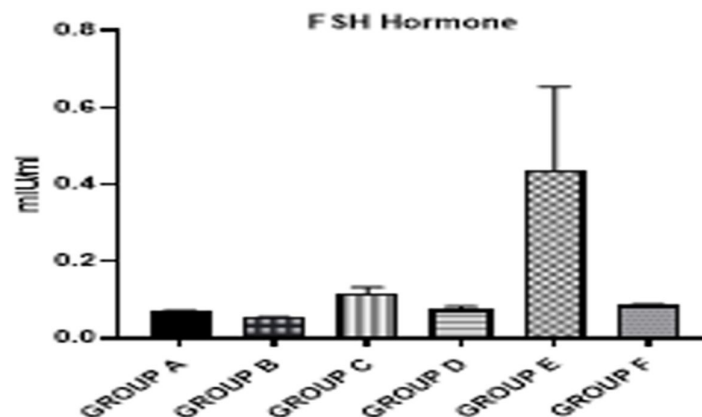


Fig 10. Serum levels of Follicle stimulating hormonal activity in AlCl_3 , control and treatment groups

There were no statistically significant changes in the Testicular volume across the experimental animals in all the groups ($p < 0.05$) but, there were noticeable statistically significant changes for the Testosterone ($p < 0.05$).

Treatment with $AlCl_3$ significantly decreased testosterone levels (1.2 ± 0.15) (ng/ml) compared to the control group (2.2 ± 0.24) (ng/ml). Treatment with Moringa tea (group 6) significantly increased (3.6 ± 0.17) (ng/ml). Other treatment groups when compared to $AlCl_3$ group also increased but these elevations were not significant (figure 8).

There was a statistically significant ($p < 0.05$) decrease in luteinizing hormonal activity of experimental group (A&B) compared to group C (peppermint). The curative group (E) showed statistically higher ($p < 0.05$) luteinizing hormonal activity level when compared to Normal control group (B). Also, there was statistically significant ($p < 0.05$) increase in the Follicle stimulating hormonal activity level of experimental group (E) compared to Aluminum chloride group (B).

There was statistically significant ($p < 0.05$) increase in the Follicle stimulating hormonal activity level of experimental group (E) compared to Aluminum chloride group (B).

IV. DISCUSSIONS

The protective benefits of herbal teas in various formulations on the male reproductive system against Aluminum-induced oxidative stress were investigated in this study. The testes' histopathological characteristics, histomorphometric and stereological analyses, serum hormone levels, and sperm parameters were also studied. $AlCl_3$ resulted in a reduction in body/testis weights, hormone levels, and testis antioxidant capacity. Free radical generation, sperm morphological integrity, and testicular histomorphometric indices were all affected by $AlCl_3$.

Herbal therapy, regardless of the origin of male infertility, has been shown in recent research to help men conceive (Anthony et al., 2006). The potential fertility regulating properties of a vast number of plants have been investigated (Bhatia et al., 2010). Some medicinal herbs are widely used as aphrodisiacs or fertility enhancers to treat sexual dysfunction. They improve sexual function and libido by providing a nutritional boost (Yakubu et al, 2007; Sumalatha et al., 2010).

This current study shows protective effect of composite herbal teas on aluminum-induced oxidative stress in the testis of Adult Wistar rat. There were no statistically significant changes in the animal weight difference of the experimental animals in all the groups in week zero and the third week but there were statistically significant changes in the animal weights in all the groups in first and second weeks. The differences in the weight across the groups were not too much distinct. Nevertheless, group 3 right and left testes with a dose of Mango tea with aluminium chloride and group 6 right and left testes with Moringa tea and aluminium chloride have the

highest weights. This can be inferred from the fact that the extracts have the ability to minimize aluminum toxicity, as well as antioxidant qualities that reduce oxidative stress and contribute to the nutrition of rats with testicular injury. This conclusion is consistent with the findings of Wang et al. (2007), who found that various substances other than enzymes played an antioxidant role in the testis.

In this experiment, the degenerative changes were reversed fully by Moringa tea extract. Dosage of 5ml/kg extract of Peppermint Tea with 150mg/kg of aluminium chloride showed mild recovery from severe histological changes. The reversal is consistent with the findings of Rana and Verna (1996), as well as El-Shahat et al. (2009), who found that green tea had an antioxidant impact on cadmium poisoning.

$AlCl_3$ significantly decreased the number of Spermatogonia, Sertoli cells, spermatocytes, spermatids and spermatozoa compared to the control group. In the rats treated with Peppermint tea, the number of Spermatogonia, Sertoli cells, spermatocytes, spermatids and spermatozoa significantly increased compared to the $AlCl_3$ group. However, treatment with Mango tea only induced a significant elevation in the number of spermatocytes and treatment with Sunflower tea elevated the number of spermatozoa. Finally, the number of spermatids was significantly higher in the group treated with Moringa tea.

Significant alterations were seen in the semen parameter. The concentration count, motile count, progressive assessment, and morphology of the rats treated with Moringa tea all rose significantly, indicating good spermatogenesis and testicular steroidogenesis. The testis relies on a monotonous and predictable reaction characterized by a reduction in spermatogenic production/efficiency for any sort of disruption, according to the findings of this study (Holstein et al., 2003). According to Ghalberg and Brodas (1981), oxidative stress generated by aluminum chloride resulted in asthenospermia, hypospermia, teratospermia, and a decrease in sperm count (1980). Sperm quality is linked to both the testicular and epididymal microenvironments, unlike sperm quantity, which is directly proportional to Sertoli cell efficiency (Makela et al., 2014).

The decreased secretion of reproductive hormones after exposure to aluminum chloride indicates that it may have harmful effects on the male gonads, either directly or indirectly through the anterior pituitary gland, which is responsible for androgen secretion and release (Inass et al., 2005). However, the rise in luteinizing hormone production after treatment with *M. oleifera* extract could be due to the presence of powerful antioxidants in the Moringa tea, particularly flavonoids and carotenoids; these antioxidants could raise the level of reproductive hormone secretion (Inass et al., 2005). The increase in reproductive hormone secretion in *M. oleifera*-treated rats is in line with the findings of Syarifuddin et al. (2017), who found that increased testosterone levels in male rats treated with *M. oleifera* resulted in improved spermatogenesis, sperm motility and morphology, as well as an increase in libido.

Moringa, Sunflower, Mango, and Peppermint were employed in this study, and they have been demonstrated to have a variety of medicinal effects. On aluminum-induced male wistar rats, their varied protective effects were evaluated, and Moringa was found to have the best protective impact in eradicating oxidative stress and its consequences on the functions and size of the testis, as well as hormone levels. Moringa appears to have the ability to protect the testes, pituitary gland, and follicle stimulating hormones from the detrimental effects of aluminum, according to the findings. It also helps to reduce the deleterious effects of aluminum chloride by increasing relative body weight. Administration of Aluminium chloride alone lowered the level of testosterone, follicle stimulating hormone (FSH) and luteinizing hormone, but a combination of aluminium chloride (150 mg/kg) and composite teas (5ml/kg) recovered the hormone level. Many plant-derived compounds, collectively referred to as "phytonutrients" or "phytochemicals," have been well discovered for their antioxidative action, according to Hatch (1995). Flavonoids are phenolic chemicals found in plants that operate as a protective agent against various environmental stresses. Flavonoids are also known to act as biological response modifiers in humans, however their primary therapeutic effects are frequently tied to their antioxidant characteristics (Bendich, 1994).

V. CONCLUSION

In this current study full knowledge has been demonstrated as it affected the testicular architecture where stress impacts were most felt and subsequently translated into drastic reduction in semen parameters which indicated reproductive dysfunction and distortion of spermatogenesis. Equally, hypothalamic-pituitary-testicular (HPT) axis of communication system between the hypothalamus, pituitary, and the testes which regulates male endocrine and reproductive biology was interrupted leading to drastic reduction on level of anterior pituitary hormones: the gonadotropic hormones (FSH and LH) and an anabolic steroid (testosterone). The therapeutic effect of composite herbal teas in detoxifying / ameliorating, was well elucidated in this current study.

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.

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Authors Contributions

OTS and OOO: Conceptualization, Methodology, Validation, Investigation. UOV: Methodology, Project administration, Supervision, Investigation. TJA: Formal analysis, Investigation.

Declaration of competing interest

No conflict of interest.

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