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Protective Effect of *Aloe Barbadensis Miller* Leaf Gel on the Reproductive System of Adult Male Wistar Rat.

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ABSTRACT
The medicinal and therapeutic effects of Aloe Vera has been widely studied. This study investigated the effects of Aloe Vera leaf gel (AVG) on semen parameters and reproductive hormone of Wister rats. Twenty adult male wister rats weighing 120-150grams were divided into 4 groups. Group A received feed and distilled water, Group B, C and D received oral doses of 100mg/kg b.wt. of Aluminium chloride ($AlCl_3$), 100mg/kg b.wt. of Aluminium chloride and 600mg\kg b.wt. of aloe vera extract, 100mg/kg b.wt. of Aluminium chloride and 5mg/kg b.wt. of vitamin C respectively for 30 days. The rats were sacrificed on the 30th day, the testes excised, and processed for histological and biochemical analysis. The results showed that the administration of AVG tends to ameliorate the testicular cytoarchitectural and histomorphological distortion in rats induced with $AlCl_3$ at the same time significantly enhancing the level of reproductive hormone. Conclusively, AVG offers antioxative effect on the testes by protecting the structural integrity of the testes as well as improving the reproductive hormones.

Keywords: *Aloe Barbadensis Miller,* Reproductive Hormones, Semen parameters, Wistar Rats

INTRODUCTION
The testis is the organ which produces sperm (the male reproductive cell), and androgens, mainly testosterone (the male hormones). The testes exist in humans as a pair of oval-shaped organs found in the scrotal sac, situated immediately behind the penis and in front of the anus. All of the testicular functions are affected by the gonadotropic hormones of the anterior pituitary. Luteinizing hormone (LH) results in the release of testosterone while the presence of both testosterone and Follicle Stimulating Hormone (FSH) is needed to support spermatogenesis [(1)].

The use of metals has been critical to human civilization and this has resulted in their ubiquity despite their suspected toxicity [(2)]. The third most abundant element of the Earth's crust is aluminum, a non-essential and toxic metal in humans [(3)]. Aluminum reaches the human body by medications such as antacids, air, foods, water, aluminum goods and containers and is found in many manufactured foods such as processed cheese, pancake mixtures, cake mixtures, frozen dough, baking powders [(4,5,6)]. Aluminium has a negative impact on human health [(7)]. Several recent studies have shown adverse effects of these metals on certain reproductive parameters such as sperm motility, viability and count, histology of testis and epididymis, as well as reproductive hormone levels at various exposure levels [(8)]. The toxic effects of aluminium appear to be mediated, at least in part, by free-radical generation [(9)].

Plant extracts have been reported to detoxify different types of environmental pollutant [(4)]. One such ancient plant is Aloe Vera, which has a wide range of medicinal applications such as wound healing effects, blood sugar reduction in diabetes, calming burns, digestive relief, ulcer healing and arthritic swellings [(10)]. Aloe vera is a medicinal plant of the *Liliaceae* family with a wide range of therapeutic applications. Aloe vera gel contains anthroquinones (aloin, aloe-emodine) which contain various anti-oxidants that could offer protective function against heavy metal toxicity [(11)].
The goal of this study is to investigate the cytoprotective effect of Aloe Vera gel on the oxidative stress caused by aluminum chloride in adult male Wistar rat semen parameters and reproductive hormones.

MATERIALS AND METHODS
Materials used: Aluminium chloride was obtained from Guangdong Guanghua Sci-Tech Co., Ltd. Vitamin C was obtained from a Pharmaceutical store in Akure. Healthy 20 male adult Wistar rats *Rattus norvegicus* of 10 weeks old and weighing 120-150g was obtained from animal house, School of Health, Federal University of Technology, Akure for the experiments. The rats were kept in plastic cages and allowed to acclimatize to the laboratory conditions for two weeks. Animals were allowed access to feed and water *ad libitum.*

Collection and Preparation of Aloe vera: Aloe vera leaves used for this study were collected in and around Akure. Preparation of Aloe vera leaf was done according to the method of Arunkumar and Muthuselvam, (2009) [12] with slight modifications. The skin of the leaves was pealed and the gel inside was used for extraction. The gel was crushed into a homogenous substance with an electric blender. The homogenous substance was then filtered, the residue and filtrate were collected in a separate container.

Experimental design: All the experimental animals were divided into 4 groups of 5 animals each.
Group A: received normal diet and water serving as control group.
Group B: received 100mg/kg bodyweight (b.wt) of $AlCl_3$ daily orally for 30days.
Group C: received 100mg/kg b.wt of $AlCl_3$ and 600mg/kg b.wt of aloe vera extract daily orally for 30 days.
Group D: received 100mg/kg b.wt of $AlCl_3$ and 5mg/kg b.wt of vitamin C orally for 30 days.
Animals were sacrificed by cervical dislocation at the end of the experiment.

Histological Analysis: Samples of Testis of rats both control and treated with $AlCl_3$ plus AVG were immediately harvested and weighed, fixed in Bouins fluid followed by standard procedures of paraffin embedding and were sectioned at 5 to 6 microns. Sections stained with haematoxylin and eosin, were mounted and examined under light microscope to evaluate histological changes and their photomicrograph was taken at varying magnifications with a Digital Camera.

Semen Analysis: The cauda epididymis of the testes was removed, incised and prepared for semen analysis according to the method reported by Ekaluo et al., (2008) [13]. Sperm parameters such as count, motility, volume, morphology and progression were assessed.

Hormone profile: Hormonal assay was carried out by measuring the activities of serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH) and testosterone (TT). The determination of the concentrations of these hormones was done using commercially available test kits, products of Randox Laboratories (Crumlin, United Kingdom).

Statistical analysis: All data obtained were subjected to statistical analysis. Values were expressed as Mean ± Standard error of mean (SEM) while One way ANOVA was used to test for differences between treatment groups using Graph pad Prism. The results were considered significant at p-values less than 0.05 ($p<0.05$).

RESULTS

Histological Sections of Testes Across the Group: The photomicrograph of the histological section in group C revealed that the administration of AVG tends to ameliorate the cytoarchitectural and histomorphological distortion evident in group B (induced with $AlCl_2$). The observations of the control group (A), showed normal testicular morphology. This is in complete contrast to group B which showed structural distortion typical of testicular toxicity. Group D also shows that Vitamin C is not as potent in ameliorating $AlCl_2$-induced testicular toxicity when compared with the group treated with AVG.

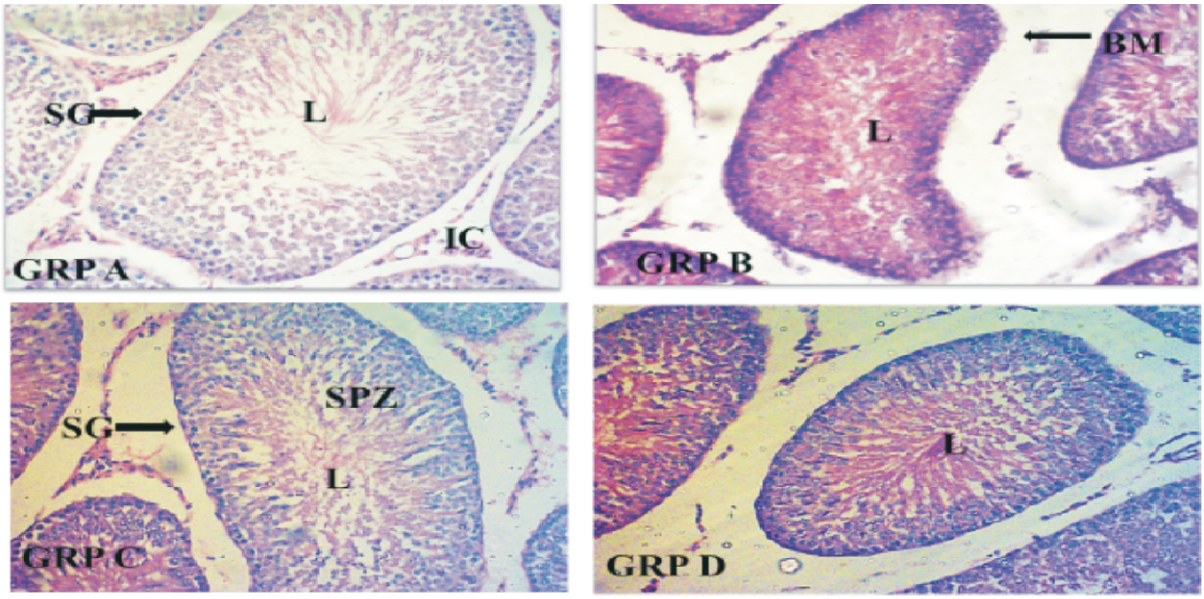

Plate 1: Photomicrograph of testes showing: Group A with normal histoarchitecture characterized by typically organized layers of spermatogenic cells; Group B with distorted tubular architecture and disorganization of the spermatogenic cells in seminiferous tubules and major pathological changes in the lumen (L); Group C with restored microarchitecture of the testicular morphology, distribution of epithelial lining , partially restored lumen (L); Group D with restored microarchitecture of the testicular morphology, distribution of epithelial lining, partially restored lumen (L). Stains:Haematoxylin and Eosin (H&E). Magnification X100.

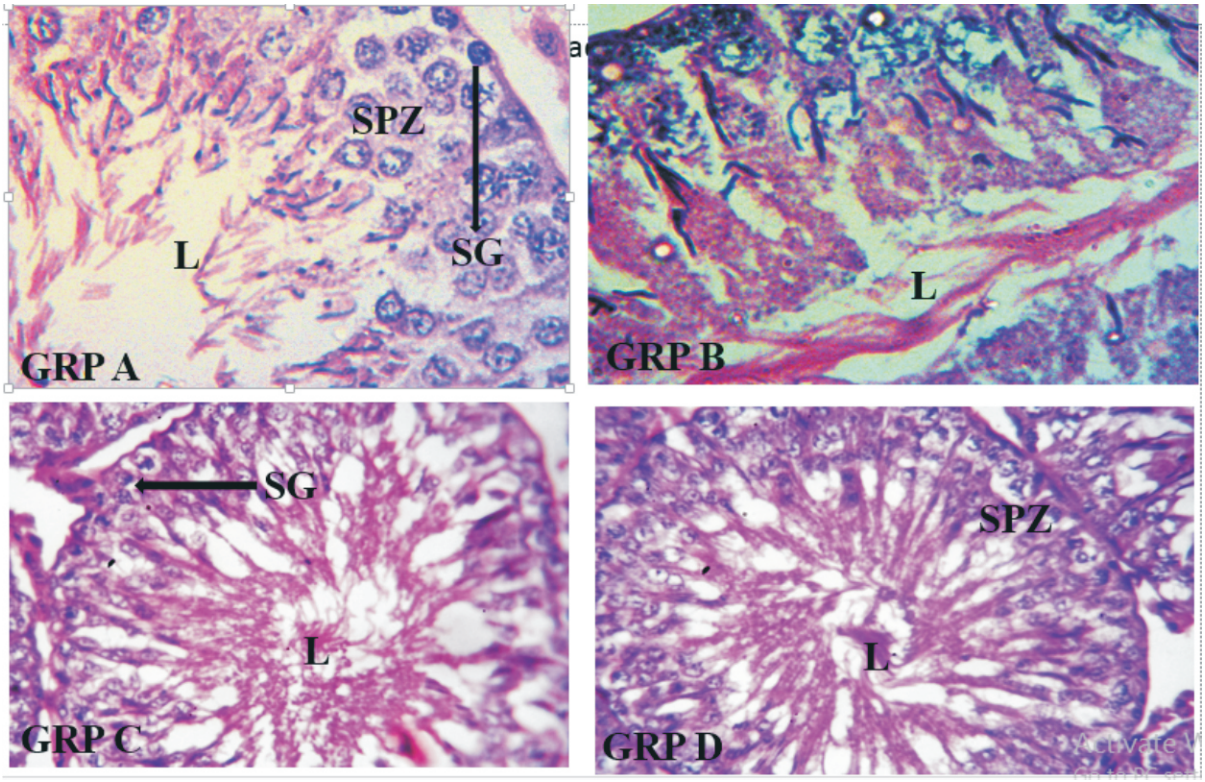

Plate 2: Photomicrograph of testes histoarchitecture showing: Group A with normal histoarchitecture characterized by typically organized layers of spermatogenic cells; Group B with distorted tubular architecture and disorganization of the spermatogenic cells in seminiferous tububules, major pathological changes in the lumen (L); Group C with restored microarchitecture of the testicular morphology, distribution of epithelial lining , partially restored lumen (L); Group D with restored microarchitecture of the testicular morphology, distribution of epithelial lining, partially restored lumen (L). Stains: Haematoxylin and Eosin (H&E). Magnification X400.

Semen analysis: The following semen parameters were assessed;

The percentage motility: This increased significantly ($p < 0.05$) in the group treated with AVG and vitamin C (group C and D respectively) when compared with the control group. The group induced with $AlCl_3$ shows decreased in percentage motility when compared with other groups. (Figure 2).

Sperm volume: This was significantly increased in the group treated with AVG (group C) when compared with the control group. Group B shows decrease in the sperm volume when compared with the control group. (Figure 2).

Total sperm count: This significantly reduced in the group treated with $AlCl_3$ (group B) when compared with the control group. Groups treated with AVG and vitamin C (group C and D) shows significant increase when compared with the control group. (Figure 2).

Progressive Assessment: There were higher fast movement in the group treated with AVG (group C), followed by group D treated with vitamin C. Slow movement was remarkably noticeable in group B induced with $AlCl_3$. (Figure 2).

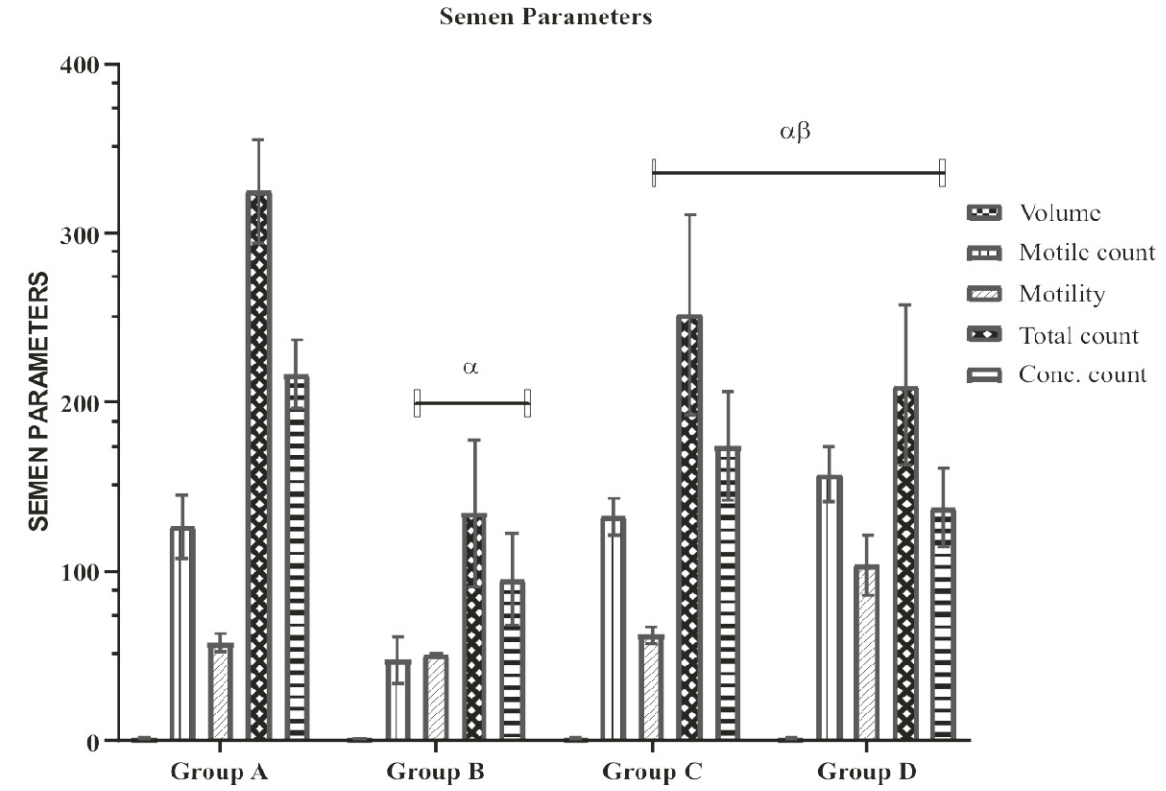

Figure 2: Graph showing semen parameters across group. Each value represented Mean ± SEM, n = 5 readings value of $p < 0.05$ was considered significant. The values with superscript α = significant different from Group A β = different from Group B.

Sperm Morphology: There was a significant difference ($p < 0.05$) in the morphology of the sperm in the treated group (C & D) when compared to the group induced with $AlCl_3$. The recovery in the morphology also appears to be more pronounced in group C. (Figure 3)

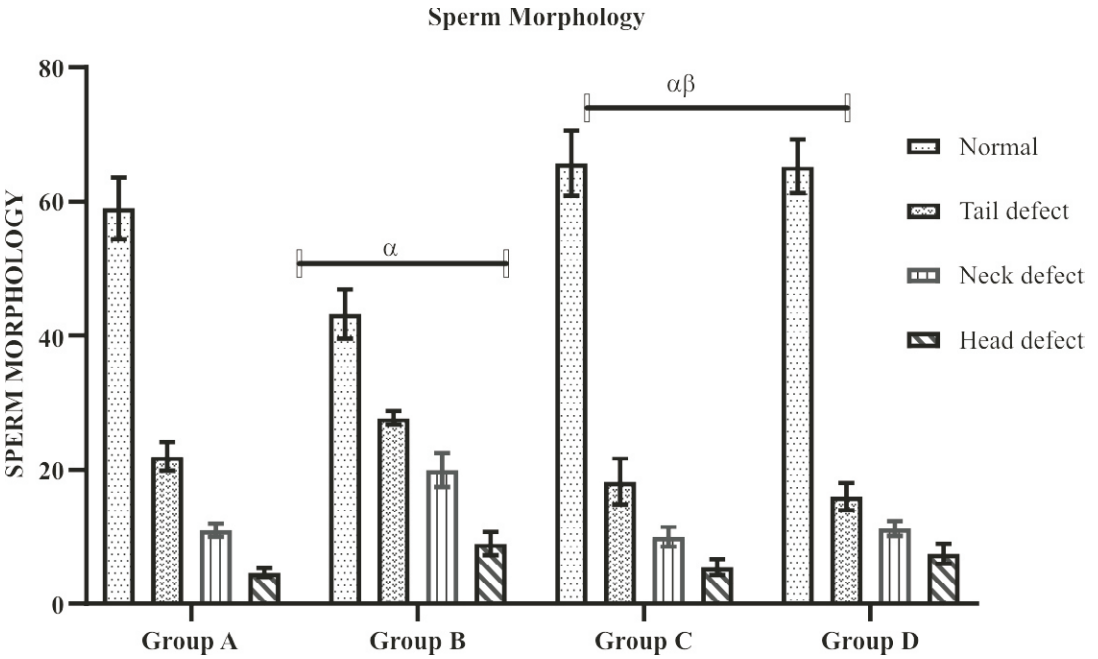

Figure 3: Graph showing the sperm morphology across group. Each value represented Mean ± SEM, n = 5 readings value of $p < 0.05$ was considered significant. The values with superscript α = significant different from Group A, β = different from Group B.

Reproductive Hormone Analysis: Testosterone: There was a significant difference ($p < 0.05$) in the testosterone level in the treated group (C & D) when compared to the group induced with $AlCl_3$. A significant reduction ($p < 0.05$) in the testosterone level was also observed when group B (induced with $AlCl_3$) was compared with the control group. (Figure 4)

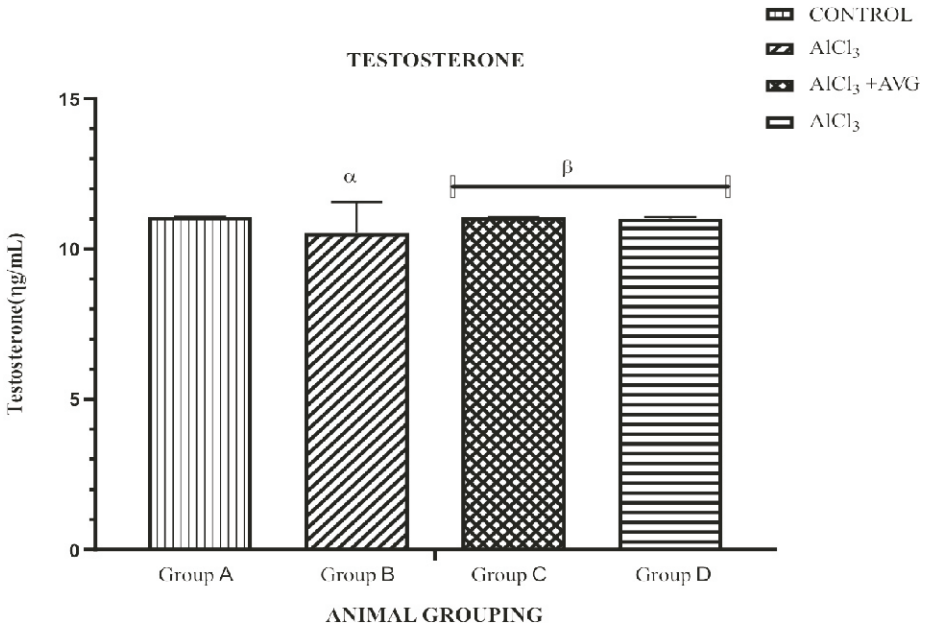

Figure 4: Graph showing testosterone level across the groups. Each value represented Mean ± SEM, n = 5 readings value of $p < 0.05$ was considered significant. The values with superscript α = significant different from group A, β = different from group B.

Follicular stimulating hormone (FSH): There was a significant difference ($p<0.05$) in the FSH level in the treated group(C & D) when compared to the group induced with $AlCl_3$. The increase in the hormone level also appears to be treatment-dependent as group C was found to be higher than group D. Group B induced with $AlCl_3$ decreases significantly when compared with other groups. (Figure 5).

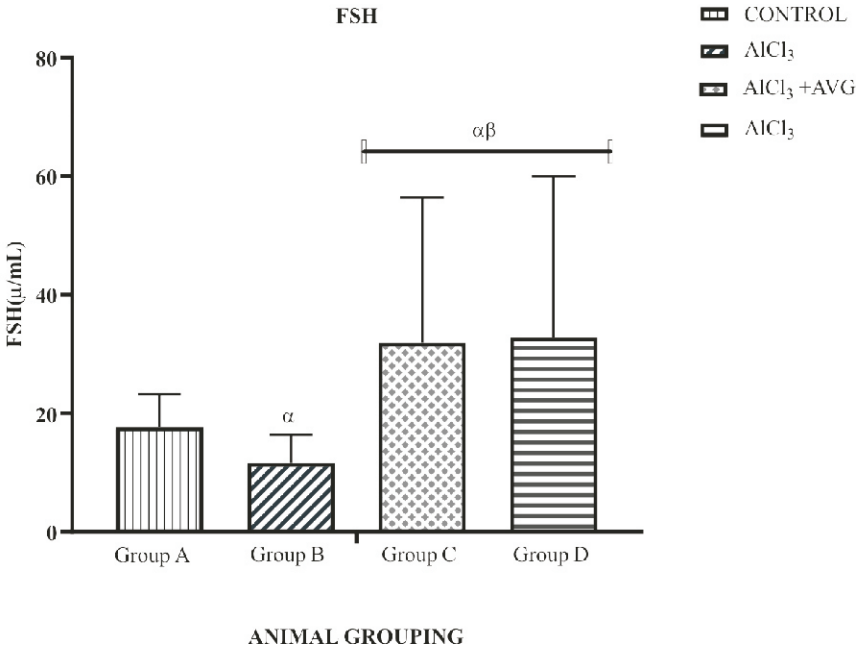

Figure 5: Graph showing FSH level across group. Each value represented Mean ± SEM, n = 5 readings value of $p < 0.05$ was considered significant. The values with superscript α = significant different from Group A β = different from Group B.

Luteinizing hormone (LH): There was a significant difference ($p<0.05$) in the hormone level in the treated group (C & D) when compared to the group induced with $AlCl_3$ (group B). However, it appears group D (vitamin C) enhances the expression of LH more than group C (AVG). (Figure 6).

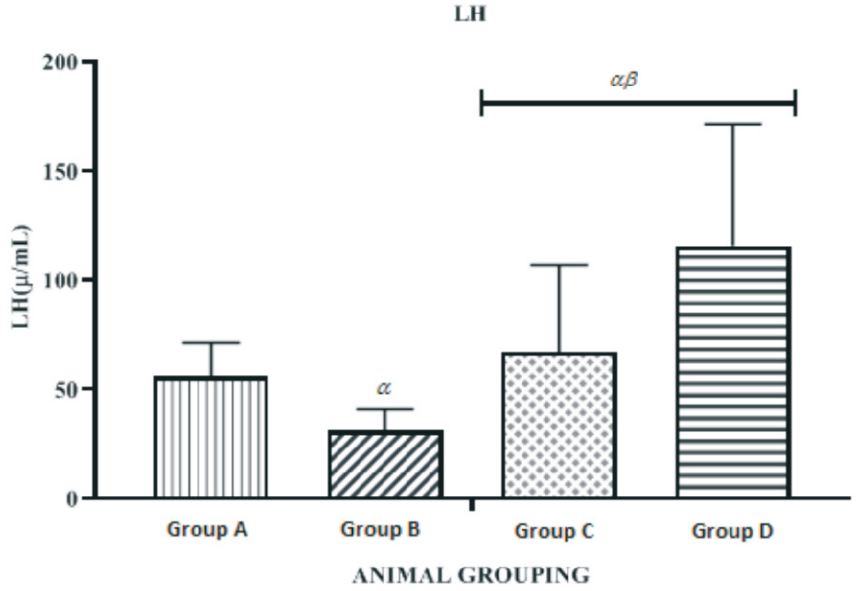

Figure 6: Graph showing LH across group. Each value represented Mean ± SEM, n = 5 readings value of $p < 0.05$ was considered significant. The values with superscript α = significant different from Group A β = different from Group B.

DISCUSSION

Aluminium has been acclaimed to accumulate in target organs when ingested in excessive amount. It has been associated with damage of testicular tissues in both humans and animals [14]. Aluminium has also been reported to cause deterioration in spermatogenesis and sperm quality, enhances free radicals generation and inhibits antioxidant enzymes [15].

Our findings in this study revealed that the administration of Aloe vera gel tends to ameliorate the damaging effects of $AlCl_3$ on the histomorphological features of the testes. It was clearly observed that group D showed near normal testicular morphology and compares fairly well with Vitamin C, a known antioxidant. This is in agreement with the findings of Mohammad *et al*.,[16] who reported that Aloe Vera was effective in ameliorating histopathological changes in the testes following bisphenol A induced testicular toxicity. The high phenolic and flavonoid content of Aloe Vera [17] could explain its potent antioxidant ability which made it effective in decreasing the toxic effect of $AlCl_3$. Histopathological analysis in this study indicated that aluminium has delecterous effect on the testes and could cause male infertility. This is evident in the thinner germinal epithelium and very low spermatid and sperm counts in the lumen of the animals treated with $AlCl_3$ only. This could be caused by oxidative stress imposed by Aluminium on the testes in the rats in this group. This was explained by Mohammadirad and Mohammad[18], stating that oxidative stress results in decrease glutathione levels which cause an increase in the level of reactive oxygen species leading to increase lipid peroxidation or higher lipid content in the testis, causing changes in intercellular stability, damages and detoxification.

The findings from this study on the effect of AVG on semen parameters showed that though $AlCl_3$ could cause significant alteration and aberration in semen parameters and sperm morphology, AVG could reverse these negative changes. The semen parameters (percentage motility, sperm count, sperm volume and progresssion) among rats in group B were significantly decreased when compared with the control group (Figure 2). This is because aluminium elicits toxic pathological changes in the testes by disrupting spermatogenesis and cell differentiation leading to reduction in total sperm count, increase in abnormal sperms, as well as impairing the stability of sperm chromatin thereby causing sperm DNA damage [19,20]. This has led to asthenospermia, hypospermia, teratospermia and reduction in sperm count observed in group B in this study. This is in agreement with the result of Olawuyi *et al*.,[21], who observed oligospermia, teratospermia and hypospermia following aluminium-induced oxidative stress in rats. It was also observed from this study that AVG could significantly improve semen parameters and sperm morphology (Figures 2, 3) . There was a marked improvement in the concentration count, percentage motility, progressive assessment, and the morphology of sperms in the rats in group C treated

with AVG. Aloe vera gel contains anthroquinones (aloin, aloe-emodine) which may have a variety of properties of anti-oxidant agent, including the protective role for heavy metal toxicity [22].

The place of reproductive hormones in the regulation of testicular activity has been well documented. LH stimulates the interstitial cells of the Leydig to secrete testosterone. FSH plays very important role in the maturation and maintenance of spermatozoa. Both LH and FSH are produced in the pituitary gland but exert its effects in the gonads however the testosterone is produced primarily in the testes. This study finds a significant increase in the level of testosterone, Follicular-stimulating hormone, and luteinizing hormone in the treated groups (C & D) when compared to group B ($AlCl_3$ only) however the treated groups showed enhanced reproductive hormones expression when compared with the control group A (Distilled water only) (Figures 4, 5 and 6). This is in agreement with the result of similar study conducted by Samira *et al.,* [23], who reported a dose-dependent response in the level of serum testosterone following 8 weeks of AVG administration in Wister rats. The higher levels of hormone in the rats which received Aloe vera and Vitamin C showed that AVG probably has a stimulating effect on the pituitary hence can influence the hypothalamo-pituitary-gonadal axis indicating a potential ability to enhance fertility however a further research will be needed to unravel the effect of AVG on the pituitary and hypothalamus.

CONCLUSION

Based on the findings from this study, we conclude that Alcl3 is toxic to the testes and reduces spermatogenesis and may cause infertility however AVG exhibits ameliorative effects and may be useful in the treatment of infertility secondary to metal toxicity.

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