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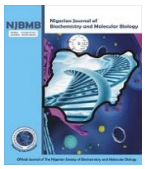

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*Research Article*

# Reproductive Potential of Selenium on Aluminium Sulphate-Treated Prepubertal Male Wistar Rats

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## ABSTRACT

Aluminium sulphate is commonly used for water treatment and excessive exposure to it has an adverse influence on testicular functions. Selenium (Se) is a trace element with a well-known antioxidant property. This study aimed to evaluate the effects of selenium on prepubertal testicular functions of Wistar rats exposed to aluminium sulphate (alum) in drinking water. Twenty prepubertal male rats (6-7 weeks old) were randomized into four groups. Control, Alum, Se, and Alum + Se. The testicular trace elements, oxidative/antioxidant enzyme activities, steroidogenic enzyme activity, inflammatory marker, serum reproductive hormones, epididymal sperm, and testicular morphology were evaluated. Alum exposure increased ($p<0.05$) testicular aluminium level, and reduced Se, iron, and molybdenum levels. The testicular antioxidant enzyme (superoxide dismutase, catalase, glutathione, glutathione-S-transferase) activities and total antioxidant capacity were reduced ($p<0.05$) in the alum group, while total oxidant capacity, nitric oxide, and malondialdehyde and levels increased significantly. Alum also reduced ($p<0.05$) 17β-hydroxysteroid dehydrogenase, luteinizing hormone, follicle-stimulating hormone, testosterone, triiodothyronine, sperm count, and motility. Tumor necrosis factor-alpha (TNF-α) and 8-hydroxydeoxyguanosine were increased ($p<0.05$) in the alum group. Co-exposure to alum and Se reversed ($p<0.05$) testicular Al accumulation, decreased Se, iron, and some antioxidant enzyme activities, and increased TNF-α and 8-Hydroxydeoxyguanosine. Selenium mitigates aluminium-induced testicular damage and spermatogenesis alteration in prepubertal male Wistar rats via a reduction in aluminium accumulation, oxidative, inflammatory markers, and improved testicular antioxidant enzyme activities.

**Keywords**: *Aluminium sulphate, Selenium, Antioxidant, Steroidogenesis, Inflammation*

## INTRODUCTION

The prepubertal stage is crucial in the sexual developmental stage which is marked by changes in the hypothalamic-pituitary-testicular (HPT) axis. The intricate interaction between endocrine, environmental, and genetic factors has been linked to progressive decline in the age of onset of puberty (Ozen and Darcan, 2011). The hastening of industrialization all over the world has contributed massively to environmental pollutants. Some of the pollutants are endocrine disruptors, which can alter the HPT axis. Some metals are part of the major environmental pollutants and endocrine disruptors that have been reported to affect puberty (Ozen and Darcan, 2011). Aluminium (Al) is the third most common element found in nature and one of the major environmental pollutants. Human contact with Al has increased nearly tenfold over the past century, making it an emerging problem of the 21st century (Rahimzadeh et al., 2022). Its

exposure is unavoidable as it is used immensely in industries, pharmaceuticals, food additives, tableware, and water purification (Liu et al., 2019; Krupińska, 2020). It is a toxic metal with no recognised physiological role in human (Liu et al., 2019). It accumulates in different tissues including the testes and leads to testicular dysfunction in both humans and animals (Mouro et al., 2018). Excessive exposure to Al may induce sperm DNA damage, deterioration of spermatogenesis, sperm quality, alteration of testicular steroidogenesis and its regulation by the hypothalamic-pituitary axis (Maghraoui et al., 2023). Studies have suggested that Al exposure causes alteration in the testicular morphology (Leko et al., 2021), increases free radicals' generation and weakens endogenous antioxidant enzymes activities (Obafemi et al., 2021), disturbs testosterone secretion/release and androgen receptor expression (Sun et al., 2011), and negatively affects reproductive organs (Hazmi et al., 2021). Despite the adverse effects of Al on human health, the excessive/unregulated use of aluminium salts for water purification persists in developing countries due to uncontrolled population growth and the mismanagement of water resources.

Selenium is a trace element that is present in several foods such as cereals, meat, fish, onion, garlic, etc. It is necessary for normal body physiology (Kieliszek and Błazejak, 2016). Plants normally accumulate inorganic selenium (selenite and selenate) from the soil and convert it to organic forms (selenocysteine and selenomethionine). Selenium plays essential roles in the formation of DNA, thyroid hormones metabolism, spermatogenesis, and mitigate oxidative damage (Qazi et al., 2019) It has an important role in male reproductive functions, it preserves normal testicular integrity and functions (Reham et al., 2020), improves sperm motility and count (Moslemi and Tavanbakhsh, 2011). Its deficiency has been reported to induce oxidative stress, interrupt spermatogenesis, reduce spermatozoa number in the seminiferous tubules, sperm motility, and degenerate seminiferous tubules (Messaoudi et al., 2010; Xu et al., 2023).

Studies reporting the role of aluminium and selenium exposure on male reproductive functions transition from prepuberty to the adult stage are scarce, hence this study aims to investigate the possible roles of selenium on the reproductive functions of prepubertal male Wistar rats exposed to aluminium sulphate. In this study, the role of selenium on trace elements, antioxidant/oxidative stress, testicular functions, testicular anti-inflammatory, and DNA damage in prepubertal Wistar rats exposed to Alum were investigated.

## MATERIALS AND METHODS

### Materials

Aluminium sulphate and sodium selenite used were products of molychem. All other chemicals and reagents used were of analytical grade.

### Experimental animals

Twenty (20) prepubertal male Wistar rats with a weight range of 60 - 80g were obtained and kept under normal conditions in the Central animals' house, College of Health Sciences, Osun State University, Osogbo, Osun State, Nigeria. The animals were allowed to acclimatize for two weeks. They had free access to standard pellet feed and tap water. The animals' care and handling throughout the study followed the UNIOSUN Health Research Ethics Committee (UNIOSUNHREC 2024/008B).

### Experimental design

The animals were randomly divided equally into four groups. Control; Aluminium sulphate (Alum); sodium selenite (Se); and Aluminium sulphate + Selenium (Alum + Se). The control animals were exposed to tap water. The animals in the Alum group had free access to 500 parts per million (ppm) Alum in drinking water, while the Se group had access to 0.5ppm Se in drinking. The Alum + Se group was exposed to 500ppm Alum and 0.5ppm Se. The doses of alum and Se used in this study were based on the previous reports (Hirata-Koizumi et al., 2010; Burns et al., 2024) to mimic excessive exposure to Alum and therapeutic dose of Se. The period of animals' exposure to the contaminated water was 35 days.

The animals were anesthetized by intraperitoneal administration of 50 mg Ketamine/kg body weight (Konecny, 2021) after 24 hours of the last administration of Alum and Se. The animals' body weights were recorded and blood samples were obtained by cardiac puncture. Sera were obtained from the blood and used for hormone assays. The testes were harvested and weighed. Part of the left testes were homogenized and the supernatant obtained was used for antioxidant/oxidant enzymes, steroidogenic enzyme, pro-inflammatory cytokine (tumor necrotic factor-alpha), and testicular DNA damage (8-Hydroxydeoxguanosine) assays while the remaining part of the left testes were digested and used for metal evaluation. Epididymal sperm were analysed. The right testes were fixed in Bouin's fluid for histology.

### Metal evaluation

The testis (0.2g) was digested in 6 mL of concentrated nitric acid. The digested testes were centrifuged at 3000 g for 12 minutes, the supernatant was made up to 20 mL with deionized water. The inductively coupled plasma optical emission spectrometry (Agilent 5800 ICP-OES) method was used for testicular metal evaluations (dos Santos *et al*., 2018).

### Antioxidant/Oxidant enzyme assays

Antioxidant enzyme activities and oxidative stress markers were evaluated with a spectrophotometric method. The testicular homogenates supernatants were used for superoxide dismutase (SOD), catalase (CAT), Glutathione (GSH), Glutathione-S-transferases (GST), total sulfhydryl (SH), total antioxidant capacity, (TAC), total oxidant

capacity (TOC), nitric oxide (NO), and malondialdehyde (MDA) estimation according to previous reports (Goth, 1991; Wilce and Parker, 1994; Magnani *et al*., 2000; Miranda *et al*., 2001; Riener *et al*., 2002; Rubio, *et al*., 2016).

### Steriodogenic enzyme activity assay

17β-hydroxysteroid dehydrogenase (17β-HSD) was measured using a spectrophotometric method as previously reported (Oyeyemi *et al*., 2020).

### Hormones assays

ELISA technique and Calbiotech ELISA kits were used to assay luteinizing hormone, follicle-stimulating hormone, testosterone, cortisol, and triiodothyronine. The procedures in the manual of the kits were strictly followed and used for the assays.

### Sperm analysis

The conventional microscopic method was used for sperm analysis as previously reported (WHO, 2010).

### Tumor necrosis factor-alpha and 8-hydroxydeoxguanosine

Tumor necrosis factor-alpha and 8-Hydroxyguanosine were evaluated using the ELISA technique with Elabscience ELISA Kit Catalog Number E-EL-0028 and E-EL-R0019 respectively. The procedures in the manual of the kits were strictly followed and used for the assays.

### Testicular histology

The preserved testes in the Bouin's fluid were passed through routine laboratory histological procedures and the slides were stained with Haematoxylin and Eosin (H&E) stain. Testicular micrographs were obtained by Omax 10.0MP digital camera for microscope.

### Statistical analysis

Data collected were analysed using GraphPad Prism 8.0.1. The groups were compared using One-way ANOVA, followed by Tukey's multiple comparisons for significant differences within the groups. The results were presented as mean ± Standard error of mean; the level of significance was set at $p<0.05$. Unbiased and unsupervised multivariate analysis was done with R- programme to consider all tested parameters; principal components analysis (PCA) was used to cluster data into homogeneous groups to show group differences.

## RESULTS

### Relative testicular Weight

The relative testicular weight results of the animals exposed to selenium and aluminium are depicted in Fig. 1. The relative right and left testicular weights for animals in control, alum, Se, and Alum + Se groups are similar ($p>0.05$).

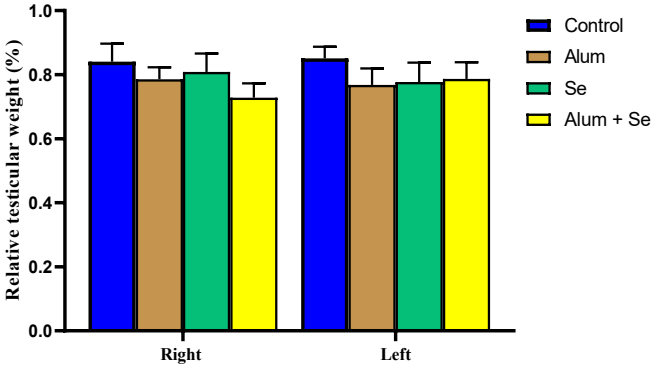


**Figure 1:** Relative testicular weight among the animals exposed to selenium and aluminium

### Testicular trace elements

Table 1 presented testicular trace elements in Wistar rats exposed to Se and Alum in drinking water. The testicular aluminium ions level was significantly higher in the Alum group, while a significant decrease was seen in Alum + Se group relative to the Alum group. Testicular selenium ions level was significantly reduced in Alum and Alum + Se groups relative to the control. Se group shows a significant increase in testicular selenium ions level. The group co-exposed to Alum and Se depicts a significant increase in selenium ions level when compared with the Alum group. Testicular chromium and zinc ion levels were similar across all the groups. Significant reduction in testicular iron ions level was recorded in the Alum and Alum + Se groups, while a significant increase was seen in the Se group relative to the control. Testicular Manganese ions significantly increased in the Se group and Molybdenum ions reduced significantly in the Alum group when compared with the control.

### Testicular antioxidant and oxidant activities

Table 2 shows a significant reduction in testicular SOD, GSH, GST activities, and TAC in Alum and Alum + Se groups, while the CAT only reduced in Alum group when compared with the control. The activities of SOD, CAT, and TAC increased ($p<0.05$) in Alum + Se group relative to the Alum group. The testicular level of SH was reduced ($p<0.05$) in Alum and Alum + Se groups when compared with the control, while Alum + Se group showed a significant increase when compared with the control.

**Table 1:** Testicular Trace Elements in Male Wistar Rats Exposed to Selenium and Aluminium in Drinking Water

| | Control | Alum | Se | Alum + Se |
|---|---|---|---|---|
| Aluminium (mg/L) | 0.14 ± 0.0032 | 0.27 ± 0.019$^{\alpha}$ | 0.13 ± 0.0067 | 0.17 ± 0.0053$^{\beta}$ |
| Selenium (mg/L) | 0.088 ± 0.0045 | 0.040 ± 0.0027$^{\alpha}$ | 0.157 ± 0.0043$^{\alpha}$ | 0.059 ± 0.0042$^{\alpha, \beta}$ |
| Chromium (mg/L) | 0.048 ± 0.0026 | 0.047 ± 0.0024 | 0.048 ± 0.0013 | 0.046 ± 0.0032 |
| Iron (mg/L) | 0.98 ± 0.036 | 0.80 ± 0.023$^{\alpha}$ | 1.25 ± 0.040$^{\alpha}$ | 0.84 ± 0.023$^{\alpha}$ |
| Mn (mg/L) | 0.055 ± 0.0024 | 0.048 ± 0.0016 | 0.075 ± 0.0034$^{\alpha}$ | 0.053 ± 0.0037 |
| Mo (mg/L) | 0.066 ± 0.00024 | 0.0043 ± 0.00017$^{\alpha}$ | 0.0072 ± 0.00022 | 0.0044 ± 0.00033 |
| Zinc (mg/L) | 0.32 ± 0.016 | 0.25 ± 0.023 | 0.42 ± 0.032 | 0.27 ± 0.021 |

Values representing mean±SEM, $^{\alpha}$p<0.05 and $^{\beta}$p<0.05 were significant relative to the control and Alum groups respectively. Key - Alum: Aluminium sulphate; Se: sodium selenite.

Total oxidant capacity and NO increased significantly in the Alum and Alum + Se groups, while MDA increased (p<0.05) only in Alum group when compared with the control. No significantly reduced Se group relative to the control.

The animals exposed to Alum + Se show a significant reduction in TOC, NO, and MDA levels relative to the Alum group.

The testicular levels of SOD, CAT, GSH, GST, SH, TAC, TOC, and MDA were similar in the Se and control groups.

**Table 2:** Testicular Antioxidant Enzyme Activities and Oxidative Markers in Male Wistar Rats Exposed to Selenium and Aluminium In Drinking Water

| | Control | Alum | Se | Alum + Se |
|---|---|---|---|---|
| Superoxide dismutase (U/mL) | 2.01 ± 0.116 | 0.54 ± 0.069$^{\alpha}$ | 2.14 ± 0.097 | 1.06 ± 0.052$^{\alpha, \beta}$ |
| Catalase (U/mg protein) | 19.62 ± 0.764 | 13.48 ± 0.443$^{\alpha}$ | 21.85 ± 0.582 | 18.22 ± 0.783$^{\beta}$ |
| Glutathione (mM) | 0.64 ± 0.042 | 0.41 ± 0.019$^{\alpha}$ | 0.55 ± 0.039 | 0.47 ± 0.050$^{\alpha}$ |
| Glutathione-S-transferase (µmol/min/mL) | 0.05 ± 0.002 | 0.02 ± 0.001$^{\alpha}$ | 0.04 ± 0.003 | 0.02 ± 0.002$^{\alpha}$ |
| Sulfhydryl (mM) | 2.83 ± 0.161 | 2.08 ± 0.087$^{\alpha}$ | 2.95 ± 0.255 | 2.42 ± 0.149$^{\alpha, \beta}$ |
| Total antioxidant capacity (mM) | 11.68 ± 0.219 | 8.62 ± 0.447$^{\alpha}$ | 13.10 ± 0.496 | 10.97 ± 0.394$^{\alpha, \beta}$ |
| Total oxidant capacity (mM) | 11.33 ± 0.494 | 19.67 ± 0.494$^{\alpha}$ | 12.67 ± 0.715 | 15.0 ± 0.730$^{\alpha, \beta}$ |
| Nitric oxide (µM) | 10.17 ± 0.477 | 19.83 ± 1.014$^{\alpha}$ | 6.50 ± 0.764$^{\alpha}$ | 13.67 ± 0.667$^{\alpha, \beta}$ |
| Malondialdehyde (µM) | 0.32 ± 0.027 | 0.76 ± 0.041$^{\alpha}$ | 0.37 ± 0.042 | 0.47 ± 0.0497$^{\beta}$ |

Values representing mean±SEM, $^{\alpha}$p<0.05 and $^{\beta}$p<0.05 were significant relative to the control and Alum groups respectively. Key - Alum: Aluminium sulphate; Se: sodium selenite.

**Testicular 17β-Hydroxysteroid dehydrogenase and hormones**

Fig. 2A Shows decreased (p<0.05) testicular 17β-HSD activity in Alum and Alum + Se groups, while it increased (p<0.05) in Se group relative to the control. The 17β-HSD level was improved significantly in the Alum + Se group when compared with Alum group.

The serum LH (Fig. 2B) and triiodothyronine (Fig. 2F) levels were reduced (p<0.05) in Alum, Se, and Alum + Se groups relative to the control animals.

Fig. 2C shows that serum FSH was significantly reduced in rats exposed to Alum relative to the control. The FSH level in the animals exposed to Se and Alum + Se was similar to the control.

Serum testosterone level (Fig. 2D) was reduced (p<0.05) in Alum and Alum + Se exposed rats when compared with the control. The serum testosterone levels in Se and control were similar.

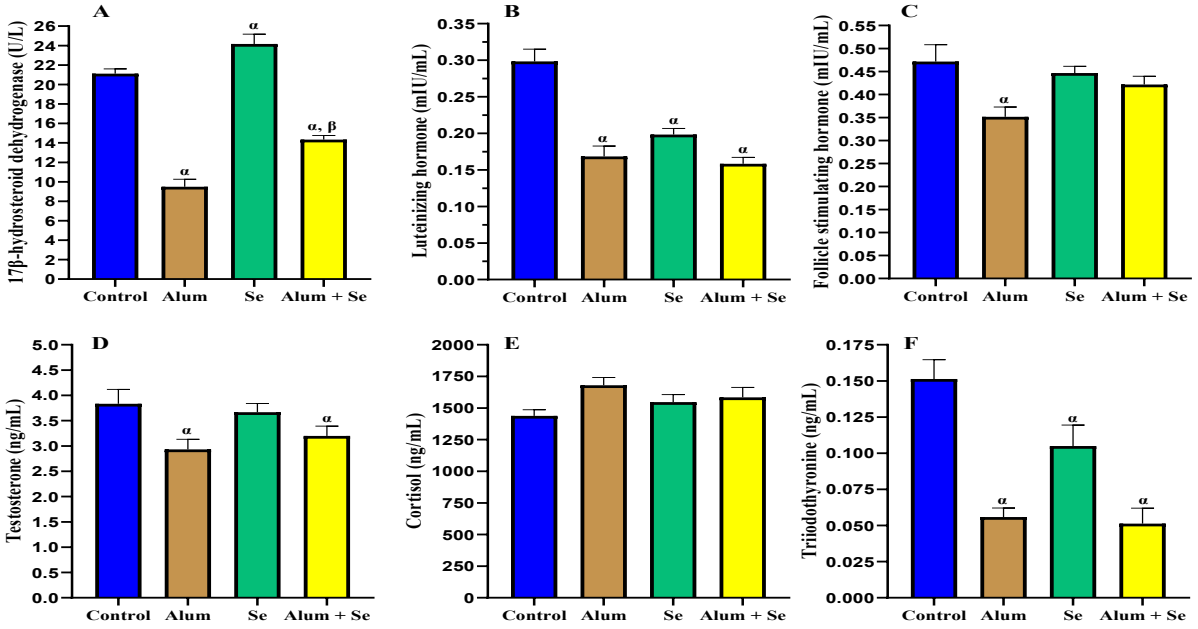


**Figure 2:** Testicular 17β-hydroxysteroid dehydrogenase (A), sera level of luteinizing hormone (B), follicle-stimulating hormone (C), testosterone (D), cortisol (E), and triiodothyronine (F) levels in male Wistar rats exposed to selenium and aluminium in drinking water

Bars represent mean±SEM, $^{\alpha}p<0.05$, and $^{\beta}p<0.05$ were significant relative to the control and Alum groups respectively. Key - Alum: Aluminium sulphate; Se: sodium selenite.

### Sperm parameters

Fig 3 shows that sperm count and motility were significantly reduced in the Alum group relative to the control, while Alum significantly increased abnormal sperm morphology when compared with the control. The sperm count, motility, and abnormal sperm morphology in the Se and Alum + Se groups were similar to the control.

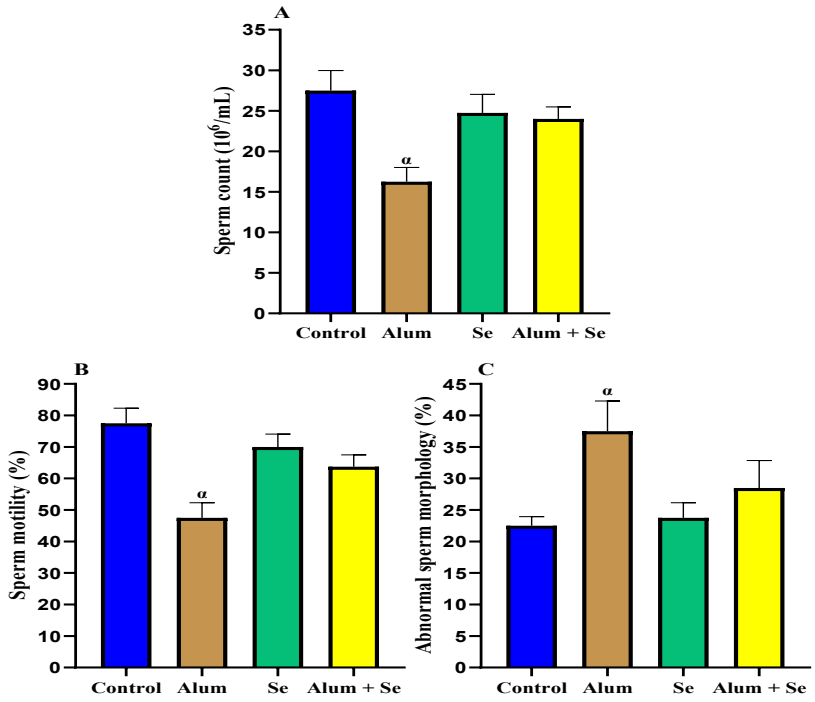


**Figure 3:** Sperm count (A), motility (B), and abnormal sperm morphology (C) in male Wistar rats exposed to selenium and aluminium in drinking water

Bars represent mean±SEM, $^{\alpha}p<0.05$ was significant relative to the control. Key - Alum: Aluminium sulphate; Se: sodium selenite.

**Tumor necrosis factor alpha (tnf-α) and 8-hydroxydeoxyguanosine**

Fig 4 shows that the level of TNF-α and 8-OHdG were increased significantly in the Alum and Alum + Se groups when compared to the control. Their levels were reduced ($p<0.05$) in Alum + Se group relative to the Alum group. Testicular levels of TNF-α and 8-OHdG in Se and control were similar.

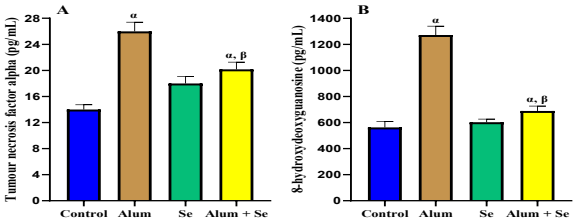


**Figure 4:** testicular tumour necrosis factor-alpha (A) and 8-Hydroxydeoxyguanosine (B) levels in male Wistar rats exposed to selenium and aluminium in drinking water

Bars represent mean ± SEM, $^{\alpha}p<0.05$ and $\beta p<0.05$ were significant relative to the control and Alum groups respectively. Key - Alum: Aluminium sulphate; Se: sodium selenite.

**Testicular histomorphology**

Control (A) shows normal seminiferous tubules (white arrow), the germ cells are normal, and the interstitium shows normal Leydig cells. Alum (B) indicates some normal seminiferous tubules (white arrow), the germ cells are normal, and other seminiferous tubules exhibiting maturation arrest, having wide lumen without spermatozoa (black arrow), and the interstitium shows normal Leydig cells. Se (C) shows a normal seminiferous tubule (white arrow), the germ cells are normal, and the interstitium shows normal Leydig cells. Alum and Se (D) show normal seminiferous tubules (white arrow), the germ cells are normal, few seminiferous tubules exhibit maturation arrest with wide lumen without spermatozoa, the interstitium shows normal Leydig cells.

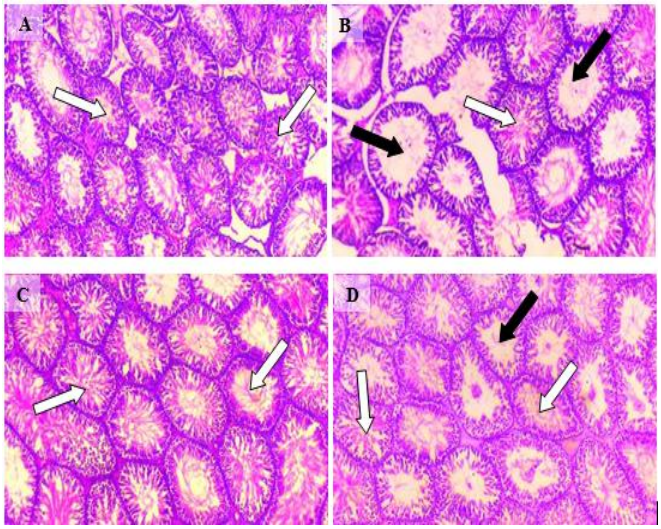


**Figure 5:** Testicular histomorphology of male Wistar rats exposed to selenium and aluminium in drinking water (H&E stain) ×100.

**Multivariate analysis distinguishes evaluated parameters**

Unsupervised multivariate analysis (PCA) was employed to analyse the difference between the control and the animals treated with Alum, Se, and Alum + Se respectively based on metals, oxidative stress, hormones, sperm characteristics, a pro-inflammatory cytokine, and testicular DNA damage marker. PCA score plot (59.61% and 13.33% for PC1 and PC2 respectively) showed that all groups except control and Se clearly show clear demarcation. This implies that control and Se are partially related while Control, Alum, and Alum + Se are different based on all tested parameters (Figure 6).

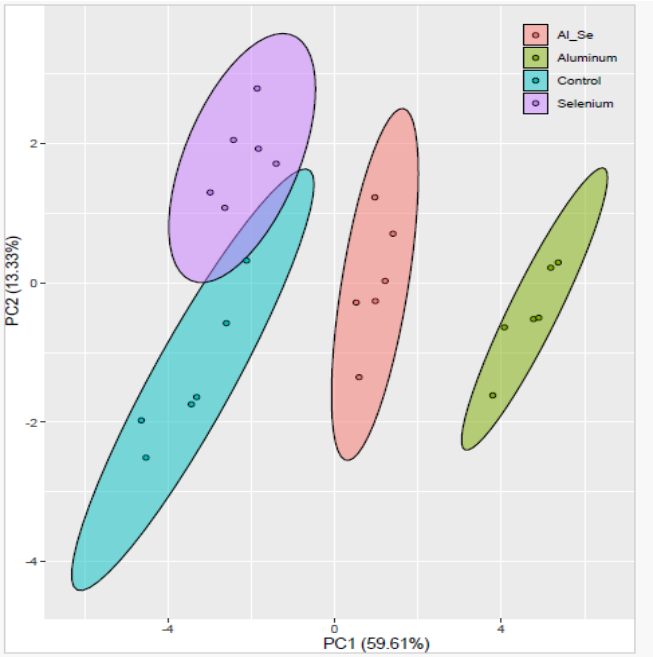


**Figure 6:** Multivariate analysis to distinguish metals, oxidative stress, hormones, sperm characteristics, pro-inflammatory cytokine, and testicular DNA damage marker

Key - Alum: Aluminium sulphate; Se: sodium selenite.

## DISCUSSION

Most previous studies have reported that Al reduced testicular weight (Moselhy et al., 2012; Zhu et al., 2014; Kalaiselvi and Ramalingam, 2016). The high dosage, prolonged duration of exposure, degeneration of germinal epithelium due to oxidative stress, and decline in serum testosterone have been ascribed to testicular weight loss in Wistar rats exposed to Al. The administration of alum and Se in this study did not alter the relative testicular weight of male Wistar rats. The previous reports on the testicular weight of Al-exposed rats are similar to this current work (Ige and Akhigbe 2012; Ulfanov, et al., 2020). This observation may be an indication that alum at a concentration of 500 ppm might not negatively affect testicular growth in pre-pubertal Wistar rats.

Noxious metals including Al have been reported to compete with trace metals, thereby lowering their

bioavailability for various physiological processes (Maciejewski et al., 2022). They have been reported to intervene in trace metal functions such as antioxidant defense, enzyme activity, and protein synthesis via induction of apoptosis and oxidative stress (Maciejewski et al., 2022). However, trace elements can minimize noxious metal absorption, retention, and avert their toxic actions through an antioxidant defense system. Also, they play a crucial role in reproductive functions (Maciejewski et al., 2022). Oral administration of alum in this study increased $Al^{3+}$ and reduced $Se^{4+}$, $Fe^{2+}$, and Mo in the testes. This observation is similar to a previous study (Guo et al., 2009). The increase in testicular aluminium ions following exposure to alum may be associated with elevated oxidative stress markers, reduced antioxidant activity, and declined serum testosterone recorded in this study. The administration of alum in this study does not interfere with the testicular levels of chromium, manganese, and zinc ions.

This study also explored the effects of the co-administration of alum and Se on some testicular elements. Administration of Se alone improved testicular ion levels of selenium, iron, and manganese ions. The co-administration of alum and Se reduced testicular aluminium ions and increased selenium ions, without affecting other trace elements studied. The administration of Se appeared to mitigate testicular accumulation of aluminium ions via ion transport mechanisms which might avert its deposition in the testes. The Se has been proposed to mitigate toxic metal accumulation in serum (Ansar, 2016). The antioxidant properties of Se may be a key mechanism responsible for reduced testicular aluminium accumulation by reducing aluminium-induced oxidative stress and promoting detoxification processes.

Previously, aluminium has been reported to induce oxidative stress through increased ROS accumulation and MDA levels in the testes and epididymis due to high polyunsaturated membrane lipid contents in the testes (Guo et al., 2009; Martinez et al., 2017). In the current study, alum exposure causes a significant reduction in SOD, CAT, GSH, GST, SH activities, and TAC. Our present study shows that alum exposure increased testicular aluminium ion levels which might be responsible for the induction of oxidative stress that may overwhelm endogenous antioxidant enzyme activities, impair the overall antioxidant capacity, and mitigate them from forming the front line of defense against reactive oxidative stress damage. Hence alum exposure may result in testicular damage due to its ability to reduce testicular antioxidant enzyme activities.

In addition, alum increased testicular TOC, NO, and MDA levels. The increase in testicular oxidative markers studies might be ascribed to aluminum sulphate-induced inflammation and oxidative stress via its testicular accumulation. The increased testicular level of NO after alum exposure might be responsible for the testicular reduction in antioxidant enzyme activities since the enzymatic process of nitric oxide synthase contributes to the creation of superoxide radicals.

The co-exposure of Se with alum increased the testicular activities of the antioxidant enzyme, TAC, and reduced oxidative markers levels. The Se antioxidant properties may be responsible for mitigating oxidative stress caused by alum and enhancing testicular antioxidant enzyme activities as previously reported (Ansar, 2016; Maniradhan et al., 2024). Also, the result of the current study shows that Se mitigates testicular Al accumulation which may be responsible for the observed improved antioxidative enzymes and reduced oxidative markers investigated.

The results of the current study indicated that alum exposure inhibits testicular 17β-HSD activity while Se enhances testicular 17β-HSD activity. The action of Se on the testicular 17β-HSD activity is similar to earlier reports (Maniradhan et al., 2024). An increase in the activity of 17β-HSD following the Se administration with alum is a pointer that the trace element can positively influence steroidogenesis. Also, we recorded that Se mitigates Al accumulation, improves antioxidant enzyme activities, and SH which has a positive influence on protein synthesis and thus improves testicular 17β-HSD synthesis and activity.

Luteinizing hormone and FSH play a key role in testicular steroidogenesis and spermatogenesis. The results of the current study show a decrease in the level of serum LH in rats exposed to alum, Se, and alum combined with Se in drinking water. The present result also shows a reduction in serum FSH following alum exposure. The present result of FSH is similar to an early work in which alum decreases the serum level of FSH and testicular androgen receptor expression (Sun et al., 2011). The possible mechanism of alum in reducing LH and FSH levels might be the disruption of gonadotropin-releasing hormone from the hypothalamus, which regulates the release of LH and FSH from the pituitary gland. Also, alum may have an inhibitory effect on LH and FSH by interfering with its receptor binding or signalling. The effect of Se on FSH is similar to the control group. Se may offer opposing effects to the alum alteration of hypothalamic-pituitary-gonadal axis and mitigates interruption of alum with FSH binding site or signalling.

Alum and its co-exposure with Se decreased serum testosterone levels in the current study. This is in line with an earlier study in which alum decreased the level of serum testosterone in male Wistar rats (Maghraoui et al., 2023). One of the possible mechanisms of alum in reducing serum testosterone might be ascribed to reducing the activity of steroidogenic enzyme activities (Maghraoui et al., 2023), especially the 17β-HSD which was reduced by the alum in this study. It has also been reported that alum altered testosterone receptors, signalling pathways and increased testosterone metabolism through cytochrome enzymes such as P450 1A1 (CYP1A1) and P450 2E1 (CYP2E1)2 (Maghraoui et al., 2023). The results of the current study indicated that Se has a similar effect on serum testosterone to the control animals. Although, it has been earlier reported that Se has a positive effect on testosterone levels by enhancing its synthesis and secretion (Zhao et al., 2022), but the inability of Se to improve testosterone in the alum-exposed rats might be linked to reduced LH observed in this study.

Cortisol has been reported to have both inhibitory and stimulatory action on steroidogenesis and spermatogenesis (Tovo-Neto et al., 2020). Al-exposure increased serum cortisol levels (Vasanthan and Joshi, 2018). Although, the serum cortisol level in this study is similar across the groups. The stability of cortisol levels in these studies implies that alum and Se influence may be more pronounced in other endocrine pathways or physiological systems rather than directly affecting serum cortisol levels. Also, triiodothyronine affects almost every physiological process in the body, including reproductive functions. The results of the current study indicated that the alum, Se, and alum with Se decreased serum triiodothyronine levels. Alum and Se could disrupt thyroid hormone metabolism and inhibit the conversion of thyroxine to triiodothyronine, leading to decreased triiodothyronine levels.

The exposure of the prepubertal male Wistar rats to alum reduced sperm count and motility while the level of abnormal sperm morphology increased. This observation is similar to the former studies (Zhu et al., 2014; Muselin et al., 2016). The effect of alum on the sperm parameter may be ascribed to the testicular accumulation of Al and induction of oxidative stress. Also, the recorded decline in the LH and FSH after alum exposure in this study may be responsible for the observed alteration in sperm parameters. Furthermore, TNF-α has been reported to disrupt the testicular cellular basement membrane, allowing the recruitment and migration of inflammatory cytokines to the testes which may negatively affect the spermatogenesis process (Ali et al., 2024). We observed that the sperm parameters resulting in alum with Se are similar to the control group. This may be due to the observed FSH result in this study. The FSH is well known to play a significant role in stimulating spermatogenesis.

Tumor Necrosis Factor-Alpha (TNF-α) mediates inflammation and immune responses to pathogens, toxins, and foreign substances, by activating the nuclear factor-kappa B (NF-κB) pathway (Ali et al., 2024). The present study showed a significant elevation in testicular TNF-α levels after 35 days of alum exposure. The elevation of TNF-α may be due to the disruption of the testicular cellular basement membrane which allowed the recruitment and migration of inflammatory cytokines to the testes (Ali et al., 2024). The present study showed similarity in the testicular TNF-α level of Se exposure rats and the control group. The co-exposure of Se with alum was able to decrease testicular TNF-α in this study. The activity of Se in mitigateing elevation of testicular TNF-α in alum-exposed rats may indicate its anti-inflammatory, antioxidant, and immune properties. Also, the ability of the Se to mitigate testicular Al accumulation may be responsible for the decline of testicular TNF-α levels in the alum group.

8-Hydroxyguanosine is a biomarker of oxidative DNA damage. Many factors especially heavy metals have been reported to alter 8-OHdG. The present study showed a significant elevation in testicular 8-OHdG levels after alum exposure. The observed result is in agreement with the previous study (Leko et al., 2021). This result is an indication of Al-induced testicular oxidative stress and DNA damage which can be supported by the decrease in testicular antioxidant activities such as superoxide dismutase, catalase, glutathione, glutathione-S-transferase, and total antioxidant capacity and an increase in total oxidant capacity, nitric oxide, and malondialdehyde reported in this study. Se co-exposed with alum, reduced testicular 8-OHdG level. The antioxidant properties of Se may mitigate testicular oxidative stress and DNA damage in alum-exposed rats through a reduction of testicular 8-OHdG formation or accumulation and Al accumulation.

The testicular histomorphology results showed normal seminiferous tubules, germ cells, and the interstitium with normal Leydig cells in the control, Se, and alum + Se groups, while the alum-exposed group testes showed normal seminiferous tubules with normal germ cells and few other seminiferous tubules exhibiting maturation arrest meaning that the seminiferous tubules, which are the sites of spermatogenesis in the testes, exhibit a failure of germ cell development beyond a certain stage. Exposure to alum may result in seminiferous tubule degeneration and maturation arrest (Ulfanov et al., 2020). It has been suggested that increased expression of TNF-α which is an inflammatory marker in the testes may contribute to testicular damage (Leko et al., 2021). Maturation arrest observed in the alum group is a pointer that aluminium intervenes with spermatogenesis via its accumulation, induces oxidative damage, and inflammation. The testicular histomorphology of the alum group also showed a wide lumen without spermatozoa, this is in line with previous studies (Ulfanov et al., 2020; Falana et al., 2017). Al-exposed has been recorded to cause degeneration of the seminiferous tubules, reduction of the germinal epithelium, vacuolization of the Sertoli cells, and wide lumen without spermatozoa. Selenium administration with alum was able to mitigate testicular morphology alteration in this study. The action of the Se on the testicular histology may be related to its antioxidant and anti-inflammatory properties recorded in this study.

Unsupervised multivariate analysis showed that all groups except control and Se clearly show clear demarcation. All the parameters investigated in the study are partially related to the control and Se. Although there is a clear separation in the alum combined with Se group relative to the control, Se, and alum groups, the analysis shows that alum combined with Se group is in between the alum and control/Se groups based on all tested parameters.

The outcomes from this study show that excessive exposure to aluminium sulphate via drinking water can lead to testicular toxicity and damage by altering testicular essential metals, inducing oxidative damage, increasing inflammatory markers, altering steroidogenesis, and spermatogenesis. While selenium, an essential trace element that is mainly taken by humans through dietary means, demonstrated a potential to mitigate aluminium-induced testicular toxicity, especially the antioxidant, anti-inflammatory, and sperm parameters. Therefore, there is a need to emphasize and educate the populace of developing countries on the safe usage of environmental exposure to

aluminium sulphate in drinking water, particularly in water purification to prevent alteration in male reproduction, particularly during the adolescent stage. Also, the monitoring bodies should appropriately regulate the sale of the aluminium sulphate and provide adequate information, surveillance, and training on its uses. The populace should be encouraged to the eating cereals, meat, fish, onion, and garlic, which are good sources of the essential element selenium that possess strong antioxidant and anti-inflammatory properties that can mitigate the toxic effects of toxic metals.

## CONCLUSION

Selenium mitigates aluminium-induced testicular damage and spermatogenesis alteration in prepubertal male Wistar rats via a reduction in aluminium accumulation, oxidative, inflammatory markers, and improved testicular antioxidant enzyme activities. However, selenium is unable to lessen testicular steroidogenesis alteration in aluminium-exposed rats.

### Institutional review board

The animals' care and handling throughout the study followed the UNIOSUN Health Research Ethics Committee (UNIOSUNHREC 2024/008B).

### Data availability

Data is provided within the manuscript or supplementary information files.

## AUTHORS' CONTRIBUTIONS

Conceptualization, AWO. And WMO; methodology, AWO., WMO., AOA., BFO., OOD. and LOA.; validation, AWO., WMO., AOA., BFO., OOD. And LOA.; formal analysis, AWO., BFO., OOA. and OSO.; investigation, AWO., WMO., AOA., BFO., OOD., OOA., OSO. and LOA.; resources, AWO., WMO., BFO., OOA. and OSO. data curation, AWO., WMO., AOA., BFO., OOD. and LOA.; writing-original draft preparation, OOA. and OSO.; writing-review and editing, AWO., WMO., AOA., BFO., OOD. and LOA.; supervision, AWO., WMO., AOA., BFO., OOD. and LOA.; project administration, AWO.; funding acquisition, OOA. and OSO. All authors have read and agreed to the published version of the manuscript.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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