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The effect of aluminum chloride on testicular biometry, hormonal profiles, spermatozoa quality, and spermatogenic cell morphology in mice

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Abstract

Background: Infertility is defined as failure to achieve a pregnancy after 12 months or more of regular unprotected sexual intercourse. The prevalence of couples with infertility increases every year. Treatment success for male infertility remains suboptimal despite the advancements of the therapies. Hence, a comprehensive understanding of spermatogenesis is needed to improve existing infertility treatments. Animal models are commonly used in studies regarding male infertility. Aluminum chloride ($AlCl_3$) has been established as an infertility-inducing agent.

Aim: This study investigates the optimal dosage of $AlCl_3$ in infertility mice models.

Method: Male Balb/c mice, aged 3 months and have proven to be fertile with an average body weight of 26, 96, randomly assigned to four groups. The control group received oral gavage with sterile aquadest, while the treatment groups were administrated $AlCl_3$ at doses of 100, 150, and 200 mg/kg BW orally over a 53-day period. Assessment of the sperm motility, concentration, morphology, viability, hormone levels, and testicular histopathology were included in this study.

Results: Administration of $AlCl_3$ did not significantly affect body weight, testicular weight, and hormone levels. However, semen analysis showed a reduction in seminal parameters among treatment groups, supported by testicular histopathology.

Conclusion: Utilizing $AlCl_3$ to induce infertility in mice models is not quite effective and displayed variable efficacy across different dosages. Further investigations are needed to elucidate optimal dosage, route of administration, and timing to establish reliable mice infertility models.

Keywords: Aluminum chloride, Spermatozoa quality, Spermatogenesis, Reproductive health.

Introduction

Infertility is defined as failure to conceive after 12 months of regular unprotected sexual intercourse (Melodie and Christine, 2018). The prevalence of infertility is known to be increased over recent years, with The Global Burden Diseases report indicating a rise of 0.37% in females and 0.29% in males (Sun *et al*., 2019), with male factors solely responsible for 20%–30% of infertility cases (Agarwal *et al*., 2015). The aetiology of male infertility can be divided into three main groups: pretesticular, testicular (primary testicular failure), and post-testicular (Ghuman and Ramalingam, 2018). Treatment modalities for male infertility may vary depending on the underlying causes and may include antioxidants, hormone replacement, surgical interventions, and psychological counseling (Dabaja and Schlegel, 2014; Agarwal *et al*., 2016; Leaver, 2016). Improvement in semen parameters serves as a key indicator of successful treatment in male infertility. However, despite various therapeutic interventions, achieving successful outcomes in the treatment of male infertility can be challenging, prompting a comprehensive understanding of the spermatogenesis process to address existing infertility conditions.

Numerous efforts have been done to investigate male infertility, with animal models which are the most commonly used in this study. The selection of appropriate animal models and substances is important to determine the success of these studies. Various substances, both natural and chemical, have been utilized, among which aluminum chloride ($AlCl_3$) stands out as a well-established infertility agent. Administration of $AlCl_3$ in rodent models has consistently resulted in reduced sperm motility, concentration, morphology, testosterone levels, and offspring numbers (Yousef and Salama, 2009; Soheir and Haya, 2013; Boudou *et al.*, 2020). Histological examinations showed characteristics of germ cell degeneration and necrosis, sperm depletion, exfoliation, seminiferous tubule vacuolation, Leydig cells depletion with increased interstitial space, and

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congestion of interstitial blood vessels (Yousef and Salama, 2009; Khattab *et al.*, 2010; Boudou *et al.*, 2020).

$AlCl_3$ exerts its detrimental effects on the male reproductive tract through several mechanisms, including increased oxidative stress, alterations in membrane function, disruption of cell signaling, enzymatic inactivation or depletion, and perturbation of the blood testes barrier. Elevated levels of reactive oxygen species (ROS) induced by $AlCl_3$ lead to DNA damage, lipid peroxidization, protein modification, and other adverse effect (Geeta and Jain, 2016). Oxidative stress triggers apoptosis in germinal cells, leading to hypo spermatogenesis and decreased adenosine triphosphate (ATP) levels, further compromising sperm motility (Berihu, 2015). $AlCl_3$-induced lipid peroxidization compromises membrane integrity, leading to enzyme inactivation, DNA damage, and cell death (Talwar and Hayatnagarkar, 2015; Geeta and Jain, 2016). Moreover, $AlCl_3$ altered both spermatogenesis and steroidogenesis through two mechanisms. $AlCl_3$ closes the calcium channel on the hypothalamus which will lead to a depletion in GnRH secretion, thereby decreasing follicle-stimulation hormone (FSH) and LH levels. Additionally, $AlCl_3$ may also affect the calcium channel in Sertoli and Leydig cells, thus impairing androgen synthesis (Geeta and Jain, 2016; Zirkin and Papadopoulos, 2018).

Various dosages, administrations, and durations of $AlCl_3$ exposure have been used in animal models of infertility (Berihu, 2015; Geeta and Jain, 2016). Duration of exposure should span at least one spermatogenesis cycle to ensure that spermatozoa have been exposed to the treatment. The choice of administration route, particularly intraperitoneal injection, requires careful consideration due to the prolonged exposure period. Based on the previous research, the aim of the study is to investigate various dosages of $AlCl_3$ which are administered orally in a mouse model of infertility.

Materials and Methods

Chemicals

$AlCl_3$ was obtained from Sigma-Aldrich, Germany. AlC_3 powder was dissolced with water for injection (Ikapharmindo Putramas, Jakarta, Indonesia).

Animals

Male Balb/c mice, aged 3 months and proven to be fertile, with an average body weight of 26.96 g, were sourced from Pusvetma, a certified laboratory animal breeder supplier. The total number of mice was 35, divided into four groups. In the control group (11 mice), oral administration of $AlCl_3$ at doses of 100 (8 mice), 150 (9 mice), and 200 mg/kg BW (7 mice). Mice were housed in standard laboratory animal cages at the experimental animal facility, Universitas Airlangga, under controlled conditions of a 12-hour light/dark cycle in a controlled temperature of 19°–22°C, and humidity ranging from 40% to 65%. A one-week acclimatization period preceded the start of the experiments. Mice were provided ad libitum access to standard commercial laboratory chow and water throughout the experimental period.

Experimental design

Mice were randomly assigned to four groups. The negative control group received oral gavage of sterile aquadest for 53 consecutive days. The second, third, and fourth groups received oral administration of $AlCl_3$ at doses of 100, 150, and 200 mg/kg BW, respectively, also for 53 days. At the end of the study, mice were euthanized in the morning under diethyl ether anesthesia. Dissection was performed, blood samples were collected via cardiac puncture, and the epididymis was carefully processed for evaluation of sperm motility, count, morphology, and viability. Testes were excised for histological examination.

Determination of hormone level

Serum was obtained by centrifugation of blood samples at 2.500 revolutions per minute (rpm) for 15 minutes. FSH and testosterone levels were measured according to the competitive Elisa method (Alhajj *et al*., 2023) and using Ray Biotech, USA, Elisa Analysis Tool.

Sperm parameters

Spermatozoa were collected from the epididymis and homogenized in 1 ml of normal saline in microtubes (Türk *et al*., 2008).

Sperm motility

Diluted sperm samples were placed on object glass covered with a coverslip 22 × 22 mm. The number of motile and immotile sperms of control and treated groups, expressed as a percentage, were observed under a microscope at 400 × (Olympus BX-41) magnification (Soheir and Haya, 2013).

Sperm concentration

Sperm suspension was loaded into an improved Neubauer hemocytometer and counted under a light microscope at a 40 × magnification (Olympus BX-41). Sperm count was expressed in millions per milliliter (10^6/ml) (Kata Shaker, 2013).

Sperm viability

The sperm viability was assessed using eosin and negrosin (Sigma-Aldrich, German). A red color indicated a viable sperm (Yousef and Salama, 2009)

Sperm morphology

Sperm smears were prepared, fixed in methanol, and stained with safranin and crystal violet (Sigma-Aldrich, German). Sperm morphology was evaluated based on head, midpiece, and tail characteristics, with normal and abnormal morphology expressed as percentages (Bernardino *et al*., 2019).

Histological examination

Testicular tissues were fixed in 4% neutral buffered formalin for 24 hours, processed, embedded in paraffin wax, and sectioned at 5 µM thickness using a rotary microtome (Leica 2125, Chicago, IL), and stained with hematoxylin-eosin (Boudou *et al*., 2020). Histological analysis was performed using an Olympus BX-41 microscope at 400X magnification.

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). Statistical analysis were conducted using the GraphPad Prism software (v8.4.3, GraphPad Software, San Diego, CA). One-way analysis of variance (ANOVA) followed by Tukey's post-hoc multiple comparison analysis was performed for parametric data, while non-parametric data were analyzed using the Kruskal-Wallis test. A significance level of $p < 0.05$ was considered statistically significant.

Ethical approval

All experimental protocols were performed in compliance with the ethical guidelines for animal research. Ethical clearance was obtained from the Research Ethics Committee, Medical Faculty, Universitas Airlangga no. 187/EC/KEPK/FKUA/2023

Results

This study showed that the administration of $AlCl_3$ had a noticeable impact on the body weight of male mice. As shown in Table 1 and Figure 1A, the reduction in body weight in the treatment groups was worse than that in the control group. The mice with the worst body weight reduction performance were administered 100 mg/kgBW, although there were no significant differences between the control and treatment groups.

$AlCl_3$ did not influence testis weight, there were no significant differences ($p < 0.005$), as shown in Table 2 and Figure 1A. This study shows that there were significant differences in FSH levels between the control group and treatment with 100 mg/KgBW $AlCl_3$ ($p < 0.005$, Table 2 and Fig. 1B), despite testosterone levels as shown in Table 2 and Figure 1C, were not significantly different ($p < 0.005$) after 53 days.

Sperm analysis as shown in Table 3 and Figure 2 describes no significant difference in all parameters, $p < 0.005$, (motility, concentration, morphology, and viability). Motility in the control group is higher than in all treatment groups. Concentration and morphology in the group administered with a dose of 100 mg/KgBW show higher results than in other groups, although in the control group, the concentration and morphology are slightly higher than the two other treatment groups. The worst viability shown in the group with $AlCl_3$ dose was 150 mg/KgBW.

The evaluation of testicular histology shows no significant difference in all germ cell types, including Sertoli cells, Leydig cells, diameter, and

Table 1. Effect of $AlCl_3$ in body weight.

Groups	Initial weight (mg ± SEM)	Final weight (mg ± SEM)	Weight diffenrence (mg ± SEM)
Control	25.45 ± 0.83	25.00 ± 1.16	0.45 ± 1.16
100 mg/KgBW	25.63 ± 0.88	22.75 ± 0.70	2.87 ± 0.48
150 mg/KgBW	27.89 ± 1.06	25.78 ± 1.64	2.11 ± 1.12
200 mg/KgBW	28.86 ± 1.5	26.86 ± 2.04	2.00 ± 1.07

Each value represents the mean ± SEM for $n = 11$ (control), $n = 8$ (100 mg/KgBW), $n = 9$ (150 mg/KgBW), and $n = 7$ (200 mg/KgBW). Statistical analysis was conducted using one way ANOVA ($p < 0.005$).

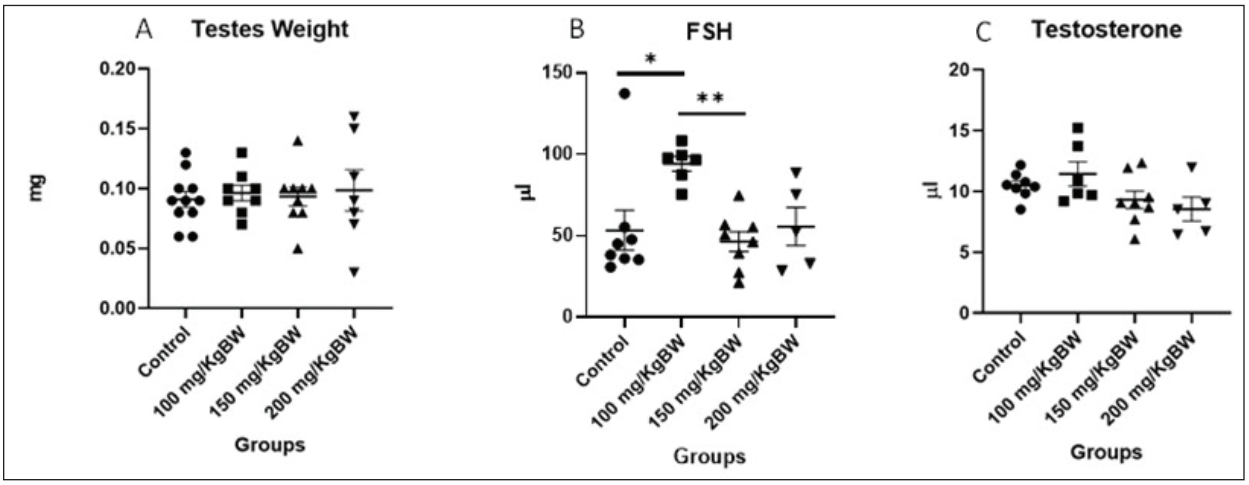

Fig. 1. Testes weight and hormonal levels after 53 days at the end of the experiments (day 53). (control, $n = 11$; 100 mg/KgBW, $n = 8$; 150 mg/KgBW, $n = 9$; 200 mg/KgBW, $n = 7$) in testes weight. $n = 8$ (control), $n = 6$ (100 mg/KgBW), $n = 8$ (150 mg/KgBW), and $n = 5$ (200 mg/KgBW) in hormonal level.

Table 2. Testes weight, FSH level, and testosterone level.

Groups	Testes weight (mg ± SEM)	FSH level (µl ± SEM)	Testosterone level (µl ± SEM)
Control	0.090 ± 0.006	53.21 ± 22[a]	10.48 ± 1.07
100 mg/KgBW	0.096 ± 0.006	46.43 ± 17.05[b]	9.31 ± 2.06
150 mg/KgBw	0.093 ± 0.007	94.17 ± 11.35	11.44 ± 2.46
200 mg/KgBw	0.098 ± 0.002	55.59 ± 26.13	8.54 ± 2.21

Each value represents the mean ± SEM for $n = 11$ (control), $n = 8$ (100 mg/KgBW), $n = 9$ (150 mg/KgBW), and $n = 7$ (200 mg/KgBW) in testes weight. Statistical analysis was conducted using one way ANOVA ($p < 0.005$). Each value represents the mean ± SEM for $n = 8$ (control), $n = 6$ (100 mg/KgBW), $n = 8$ (150 mg/KgBW), and $n = 5$ (200 mg/KgBW) in hormonal level. Statistical analysis was conducted using kruskal wallis ($p < 0.005$).

Table 3. Semen analysis.

Groups	Motility (% ± SEM)	Concentration (10^6/ml ± SEM)	Morphology (% ± SEM)	Viability (% ± SEM)
Control	47.73 ± 6.17	4.95 ± 0.10	15.73 ± 1.20	59.36 ± 4.17
100 mg/KgBW	40.14 ± 8.54	5.63 ± 1.43	16.00 ± 1.90	64.86 ± 4.86
150 mg/KgBw	27.29 ± 8.56	1.84 ± 0.43	11.57 ± 2.13	52.43 ± 3.18
200 mg/KgBw	36.83 ± 10.04	3.75 ± 1.10	10.83 ± 5.56	60.83 ± 3.61

Each value represents the mean ± SEM for $n = 11$ (control), $n = 8$ (100 mg/KgBW), $n = 9$ (150 mg/KgBW), and $n = 7$ (200 mg/KgBW). Statistical analysis was conducted using one way ANOVA ($p < 0.005$).

tubular thickness, $p < 0.005$ (Table 4 and Fig. 3). Spermatogonium, spermatocytes, and spermatid are almost equal in amount (Table 4 and Fig. 3A–C). The somatic cells, Sertoli cells, and Leydig cells, are almost equal in all groups except in the group with a dose of 200 mg/KgBW $AlCl_3$ (Table 4 and Fig. 3D and E). As shown in Table 4 and Figure 3F and G, diameter and tubular thickness are almost equal in all groups.

The testicular histology of the control group showed normal tubulus architecture, shape, size, and characteristic arrangement of all successive germ cell types and somatic cells. The tubular lumen was fully occupied by many healthy spermatozoa (Fig. 4A). Histoarchitecture of the testis in mice treated with 100 mg/KgBW $AlCl_3$ showed a decrease in germ cell amount, loose intertubular spaces, and fewer sperm in the tubular lumen than control (Fig. 4B). Administration of a150 mg/kgBW dose of $AlCl_3$ revealed markedly reduced diameter of seminiferous tubules, disorganized and degenerative germ cells, depletion in all germ cell types, and atrophic changes in Leydig cells (Fig. 4C). Exposure to the highest dose of the $AlCl_3$ (200 mg/KgBW) induced more pronounced disorganization of germ cell, decrease amount of all germ cell types, and luminal spermatozoa (Fig. 4D).

Sperm morphology is shown abnormal in the head and tail. Normal sperm morphology head and tail are shown in Figure 5A. Normal sperm morphology has a hook-like head, a short middle part (neck), and a long tail. Abnormal sperm morphology is shown in Figure 5B and C, the blue arrows show head abnormality, the head without hook and piriform. The red arrow shows sperm abnormality in the head and tail, there is no head and bent tail. The green arrows show the sperm with a bent tail but a normal head. Figure 5C also shows the bent tail.

Discussion

$AlCl_3$ exerts multifaceted effects on the various organs in the body, including the cerebral cortex, lungs, kidneys, liver, testes, epididymis, and could also act as a neurotoxin agent (Adekunle and Adeniyi, 2012; Buraimoh *et al*., 2012; Buraimoh and Ojo, 2013; Al-Otaibi *et al*., 2018; Saad *et al*., 2018). The manifestation of $AlCl_3$-induced effects may not be reflected in all experimental animal phenotypes, potentially influenced by the characteristics of the animal model and route of administration. This study found a higher incidence of body weight reduction in the treatment groups, attributed to the depletion of adipose tissue and body fluid following $AlCl_3$ exposure. The weight loss condition can be further elucidated by factors such as reduced food intake and $AlCl_3$-induced nutritional malabsorption (Iqbal *et al*., 2024). Consistent findings were reported by Pandey and Zhu, showing a decrease in body weight following $AlCl_3$ administration at different dosages (Zhu *et al*., 2014; Geeta and Jain, 2017).

Although no significant differences in testes weights were found in this study, the testis is recognized as a pivotal

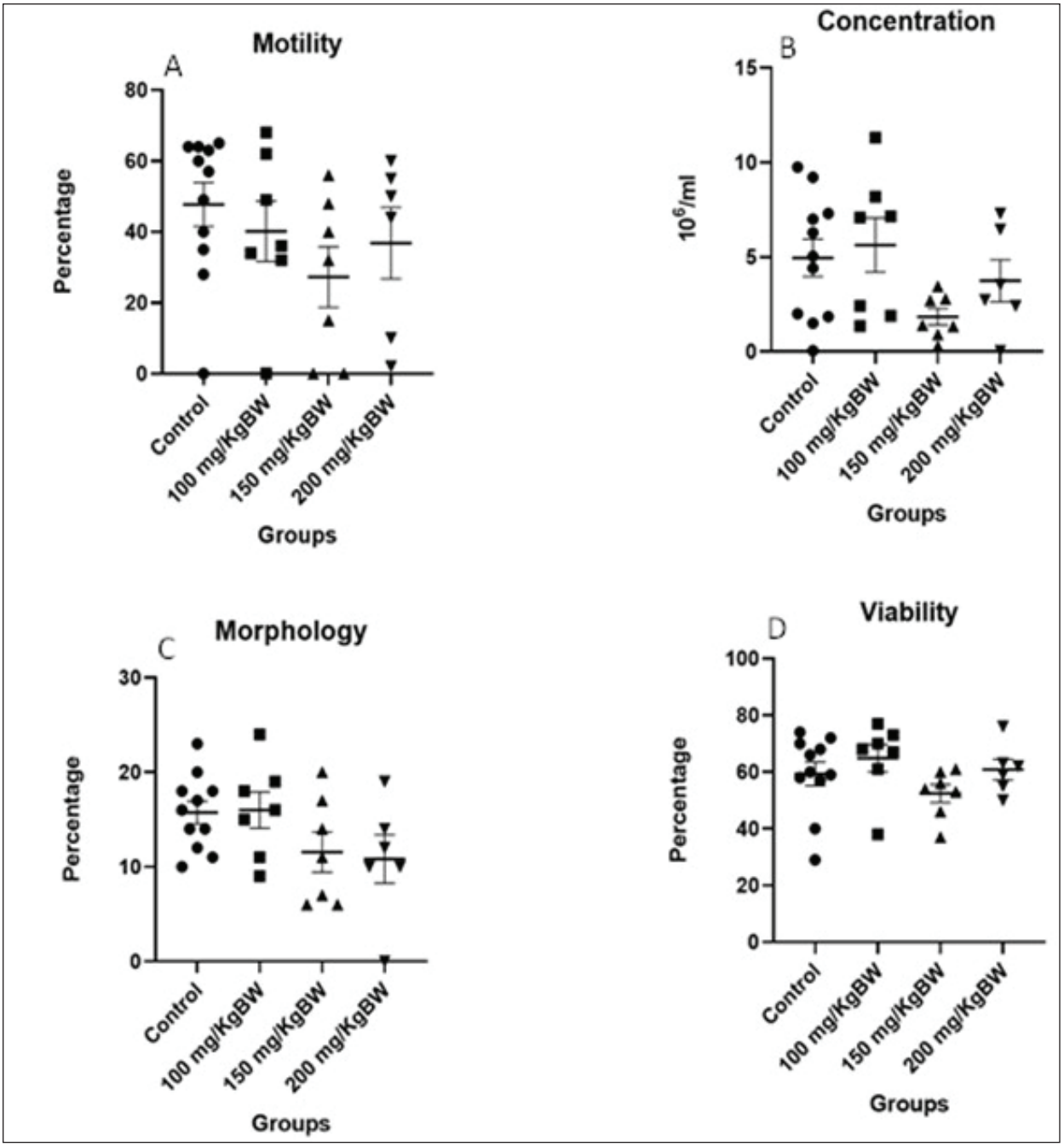

Fig. 2. Semen analysis after 53 days at the end of the experiments (day 53). (A) Analysis of sperm motility showed that mice treated with $AlCl_3$ exhibited a lower motility compared to control group. (B) Sperm morphology in treated groups showed a lower percentage compared to the control group. (C) Sperm concentration was found to be lower in the treatment groups lower compared to the control group, although a slight increase was observed in the group administrated with 100 mg/KgBW $AlCl_3$. (D) Sperm viability remained relatively consistent across all groups, showing no significant differences. (Control, n = 11; 100 mg/KgBW, n = 7; 150 mg/KgBW, n = 7; 200 mg/KgBW, n = 6).

organ within the male reproductive system, responsible for the synthesis of steroid hormones and the production of spermatozoa. Testicular weight serves as a crucial metric in assessing male reproductive health due to its strong positive correlation with sperm production (Geeta and Jain, 2017). Different results were reported by Zhu, where administration of $AlCl_3$ affected testes weights, albeit at higher doses and longer durations compared to

Table 4. Testicular histopathology.

Groups	Spermatogonium (∑± SEM)	Spermatocytes (∑± SEM)	Spermatid (∑± SEM)	Sertoli cells (∑± SEM)	Leydig cells (∑± SEM)	Diameter (µm± SEM)	Tubular thickness (µm± SEM)
Control	32.27 ± 1.08	41.76 ± 1.77	91.09 ± 6.88	13.15 ± 0.45	8.75 ± 0.45	196.1 ± 4.55	66.66 ± 2.42
100 mg/ KgBW	28.68 ± 2.30	41.93 ± 2.42	85.38 ± 6.85	13.53 ± 0.59	8.85 ± 0.38	194.2 ± 4.79	66.36 ± 2.30
150 mg/ KgBw	31.41 ± 1.62	41.78 ± 1.65	94.56 ± 4.79	13.49 ± 0.50	8.78 ± 0.60	196.8 ± 3.33	66.54 ± 1.86
200 mg/ KgBw	29.33 ± 3.82	36.39 ± 5.18	75.57 ± 17.58	14.56 ± 0.64	10.54 ± 0.59	180.9 ± 14.7	62.90 ± 5.84

Each value represents the mean ± SEM for $n = 11$ (control), $n = 8$ (100 mg/KgBW), $n = 9$ (150 mg/KgBW), and $n = 7$ (200 mg/KgBW). Statistical analysis was conducted using one way ANOVA ($p < 0.005$).

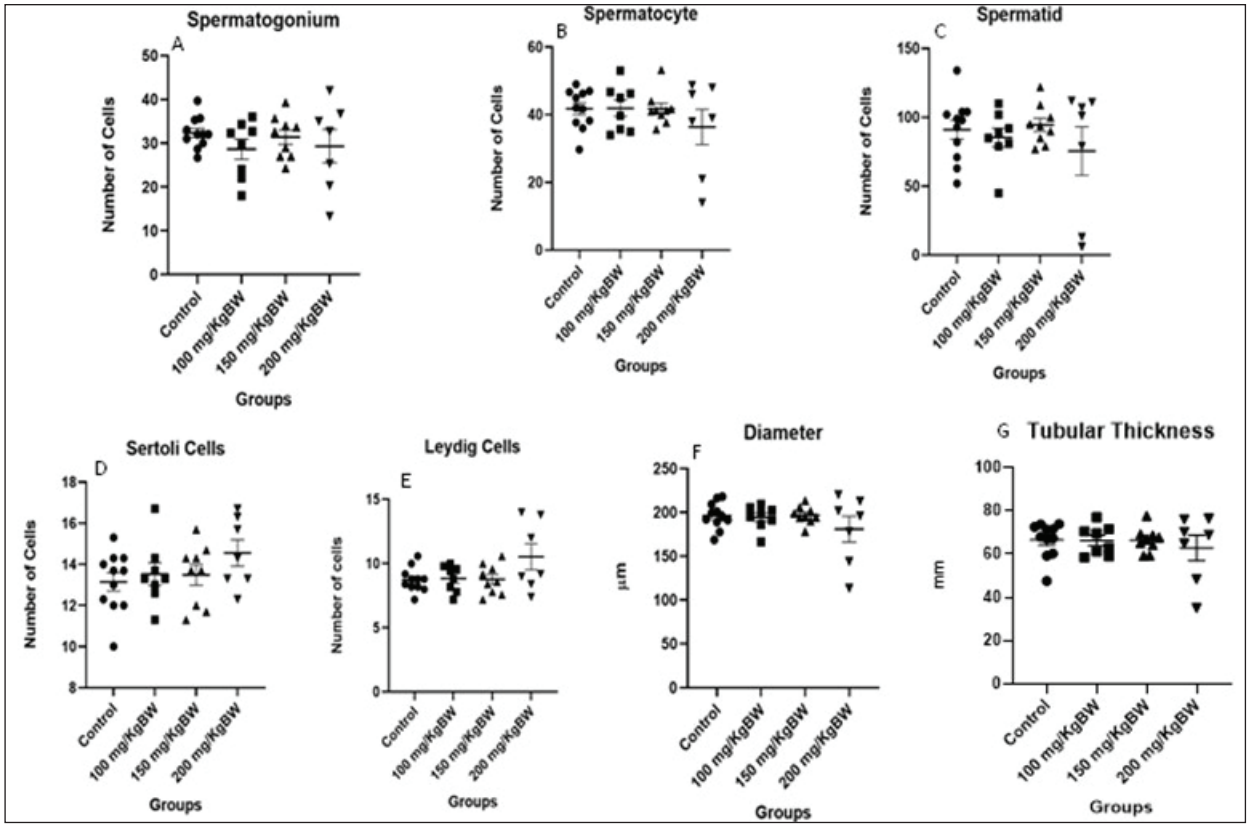

Fig. 3. Testicular histopathology after 53 days at the end of the experiments (day 53). (A)–(C) Displayed the germinal cells in the testes, with all groups were almost equal. (D) and (E) Displayed the somatic cells in the testes, which were almost equal in all groups. (F) and (G) Diameters and thicknesses of seminiferous tubules were almost equal in all groups (control, $n = 11$; 100 mg/KgBW, $n = 7$; 150 mg/KgBW, $n = 7$; 200 mg/KgBW, $n = 6$).

the present study, which utilized rats as a model (Zhu *et al*., 2014). Nevertheless, the testes' weight findings in this study are in accordance with hormone levels, indicating no significant difference. Similar results were found by Al-Eisa, where no significant difference in FSH levels was noted between the control and the treatment groups, although testosterone levels exhibited significant differences (Al-Eisa and Al-Nahari, 2017). Conversely, Rasha reported that FSH, LH, and testosterone levels in the treatment groups are lower compared to the control group with a higher dosage of $AlCl_3$ compared to this study (Nuhair, 2015).

Testes damage resulting from $AlCl_3$ exposure can lead to cellular and functional impairment, particularly

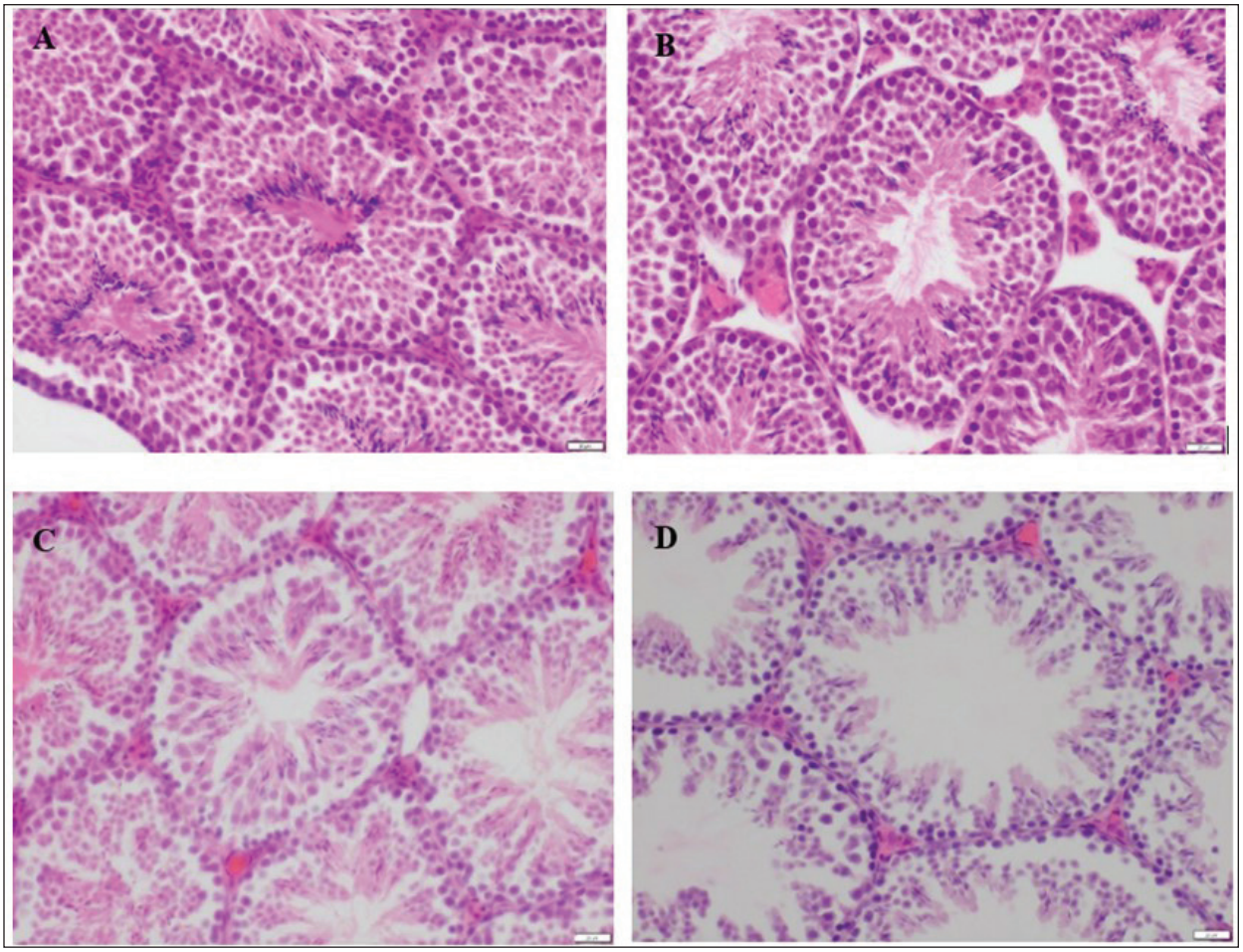

Fig. 4. Testicular Histology. (A) Control group. (B) 100 mg/KgBW. (C) 150 mg/KgBW. (D) 200 mg/KgBW. It was shown that there was a gradual decrease of the tubular thickness and number of germinal cells from (A) to (D). (HE Stain, 400 × magnification).

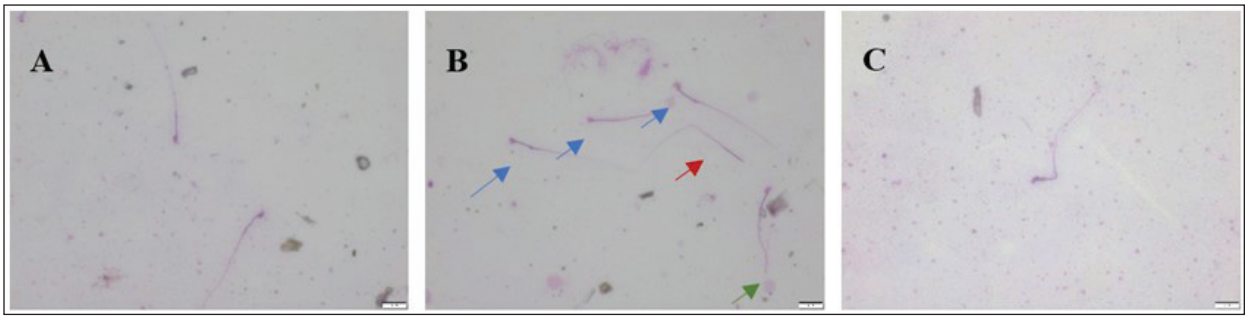

Fig. 5. Sperm morphology. (A) Normal sperm. (B) Head and tail abnormality: blue arrows show head abnormality; red arrow shows head and tail abnormality; and green arrow shows tail abnormality. (C) Tail abnormality (crystal violet stain, 400 × magnification).

affecting Leydig cells responsible for testosterone production. Testosterone depletion adversely impacts spermatogenesis, with $AlCl_3$ potentially inducing this decline by blocking calcium channels, thereby disrupting pituitary gonadotropin secretion (Lokman *et al*., 2022). Consumption of $AlCl_3$ influences pituitary gonadotropin release of LH, leading to decreased testosterone levels in the body. Additionally, earlier studies have demonstrated that $AlCl_3$ inhibits the release of calcium ions (Ca^{2+}), which contributes to its toxicity. Although Ca^{2+} ion levels were not measured in this study, previous investigations have shown that

aluminum increases bound calcium (in conjunction with anions or albumin) while reducing ionized calcium levels. Calcium ions play a crucial role in gonadotropin-releasing hormone (GnRH) exocytosis via synaptotagmin secretory vesicles. Aluminum has the potential to disrupt voltage-sensitive calcium channels in hypothalamic cells, thereby decreasing GnRH secretion, and subsequently lowering FSH and LH levels in the pituitary gland as a result of disrupted GnRH synthesis. This cascade of events adversely affects spermatogenesis, influences androgen synthesis, and impacts testosterone secretion by Leydig cells (Al Murshidi *et al*, 2021).

Aluminum administration induces an increase in ROS and lipid peroxidation (LPO) within the testes, epididymis, and prostate (Martinez *et al*., 2017). Testicular tissue, being lipid-reach, is particularly susceptible to oxidative damage due to elevated ROS levels, leading to increased LPO, nitric oxide (NO), and superoxide dismutase, along with decreased glutathione (GSH) and catalase levels. $AlCl_3$ exposure has been associated with elevated ROS levels (Uzun *et al*., 2004; Jangra *et al*., 2015). Damage to testicular tissue can impair cellular function, including Leydig cells responsible for testosterone production. Depletion of testosterone adversely affects spermatogenesis. Aluminum may exacerbate this testosterone decline by blocking calcium channels, thereby reducing the pituitary gonadotropin secretion (Lokman *et al*., 2022). Current consumption of $AlCl_3$ affects pituitary gland secretion of LH, which in turn decreases the level of testosterone in the body. Additionally, $AlCl_3$ has been shown to inhibit the release of calcium ions (Ca^{2+}), contributing to its toxicity. Although this study did not measure the Ca^{2+} ions, prior studies have demonstrated that aluminum reduces ionized calcium while increasing bound calcium levels (with anions or albumin). GnRH is released through synaptotagmin secretory vesicles, a process facilitated by Ca^{2+} ions. Aluminum has the potential to block voltage-sensitive calcium channels in hypothalamic cells, thereby reducing GnRH secretion. This reduction on GnRH synthesis subsequently leads to decreased FSH and LH levels in the pituitary gland, disrupting spermatogenesis, influencing androgen synthesis, and impacting the testosterone secretion by Leydig cells (Al Murshidi *et al*, 2021).

Semen analysis parameters consistently demonstrated lower values in motility, concentration, morphology, and viability in the treatment groups compared to the control group, although no significant differences were observed. The group administered 100 mg/KgBW showed only slight differences compared to the control group, whereas the other treatment groups exhibited more pronounced differences, indicating that this dosage may be more effective in inducing infertility. In an experimental model using adult mice and an $AlCl_3$ dosage of 150 mg/KgBW, differences were noted in sperm count, morphology, and motility (Mabrouk *et al*., 2021). This finding is consistent with a previous study utilizing $AlCl_3$ at a lower dosage administered intraperitoneally (Cheraghi *et al*., 2017)

Histopathological analysis revealed consistent findings of germinal cells, including spermatogonia, spermatocytes, spermatids, and somatic cells such as Sertoli and Leydig cells, in both the control and treatment groups. Tubular thickness and diameter also exhibited uniformity across these groups. A Previous study showed the same results as this study and indicated that aluminum toxicity in testes is reversible, as demonstrated by improved testicular histopathology and hormonal levels of FSH, LH, and testosterone 60 days after the cessation of aluminum administration (Geeta and Jain, 2017).

The results of semen analysis and testes histopathology in this study did not show a significant difference, suggesting that the dose may not be the primary determinant of aluminum toxicity. Other factors such as exposure conditions, route of administration, individual characteristics, and subsequent distribution and bioavailability throughout the body may play crucial roles (Martinez *et al*., 2017). Further investigation is needed to determine the optimal dose, route, and duration.

Conclusion

In summary, this study shows that administering $AlCl_3$ to induce infertility in mice models may not be universally effective. While body weight, testes weight, and hormone levels exhibited no significant differences between the control and treatment groups, but semen analysis revealed decreased parameters in the treatment group. However, histopathological examination of the testes yielded similar results, further investigations are needed to determine the optimal dose, route, and duration of $AlCl_3$ administration for generating infertile mouse models.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest.

Authors' contributions

Z.F., conceived the idea, designed and performed experiments, analyzed data, and wrote and edited manuscript; H.H., supervised the project, funding management, wrote and edited manuscript. All authors have read and agreed to the published version of the manuscript.

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Data availability
The article includes all data supporting the study's findings. In case additional data is needed, the author can provide it upon reasonable request.

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