

# Aluminium Chloride-Induced Testicular Effects in Rats: A Histomorphometrical Study

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## ABSTRACT

Aluminium (Al) has been considered as an indifferent element from a toxicological point of view. It is present in many manufactured foods, drinking water and medicines. Therefore, the present study was carried out to evaluate the toxic effects induced by aluminum chloride (AlCl<sub>3</sub>) on the testis in rats. Male Wistar rats were randomly divided into four groups. Group I served as control and received vehicle treatment. Rats of groups II, III and IV were orally treated with AlCl<sub>3</sub> at three different doses (30, 60 and 90 mg/kg b.wt/day) for 60 days. A recovery study was also performed after 60 days of treatment withdrawal in the highest-dose group. There was a significant decline in the relative weight of testes in aluminium-treated rats when compared with control rats. Histological analysis of the testis showed dose-dependent, marked degenerative and atrophic changes in the seminiferous germinal epithelium and Leydig cells after the administration of AlCl<sub>3</sub>. A significant dose-dependent decline was also observed in germ cell count, seminiferous tubular diameter and Leydig cell nuclear diameter. The levels of serum testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) also were decreased in a dose-dependent manner in experimental rats when compared with control rats. However, after 60 days of treatment withdrawal in the rats of the highest-dose group, histomorphology of the testis and reproductive hormonal levels showed significant improvement. Based on the results, it can be concluded that AlCl<sub>3</sub> intoxication exerts a partially reversible adverse impact on the testis due to deteriorating steroidogenesis and spermatogenesis in Wistar rats.

Keywords: Aluminium, Histomorphology, Testis, Rats and Reproductive hormones.

## 1. INTRODUCTION

Metals have been used in food, dietary supplements, water, air, alcoholic drinks etc., by humans for thousands of years. Several adverse effects on human health have been reported due to the excessive exposure of humans to these metals. Deterioration of reproductive health due to excessive metal exposure has been raised as a central area of research in the toxicological field today. Metals may have serious effects on the male reproductive system directly, when they target specific reproductive organs, or indirectly, when they act on the neuroendocrine system [1].

Aluminium (Al) is one of the most widely distributed metal in the environment and the third most abundant element in earth's crust. In the environment, aluminium exists in only one oxidation state (+3), and does not undergo oxidation reduction reactions. Humans can be exposed to aluminium *via* consumption of food items, drinking water and inhalation of ambient air [2]. Workers in the aluminium production and user industries, as well as aluminium welders, are widely exposed to aluminium and its compounds. Aluminium compounds are being used in a variety of medicines such as antacids, phosphate binders, buffered aspirins, vaccines and allergen injections as well as in consumer products such as antiperspirants, first-aid antibiotic and antiseptics etc., and food additives [3].

Concomitantly, there has been an increased incidence of exposure of the general population to aluminium, which can cause serious effects on various systems of the body [4, 5]. Although a number of studies have reported disturbed epididymal sperm parameters [6, 7] and disrupted steroidogenesis [8, 9] in aluminium-exposed rats, the testicular

histomorphological effects in aluminium-treated rats and recovery effects after treatment withdrawal are not well reported. Therefore the present study was conducted to evaluate the histomorphological effects of aluminium chloride on the testis, as well as recovery from toxic effects after 60 days of treatment withdrawal in the highest-dose group.

## 2. MATERIAL AND METHODS

### 2.1. Chemicals

Aluminium chloride (AlCl<sub>3</sub>) was procured from Merck, India Ltd., Mumbai, India. All other chemicals used in the study were of analytical-reagent grade.

### 2.2. Experimental animals

Adult, healthy, male albino rats (Wistar strain) of proven fertility were initially procured from IVARI Izzatnagar, Bareilly (U.P.), India and from these a colony was established in the animal house facility of the Department of Zoology, University of Rajasthan, Jaipur.

The subsequent progeny of these rats were used for experimental purpose. The rats were housed in polypropylene cages measuring 12" × 10" × 8" under standard laboratory condition of light-dark cycle (14hr-10hr) and temperature (22 ± 3 °C) and provided water and a nutritionally adequate pellet diet (Aashirwad Food Industries, Chandigarh, India) *ad libitum*.

Animal care and handling were done according to the guidelines set by the Indian National Science Academy (INSA, 1992, New Delhi, India) for the maintenance and use of experimental animals. The study was approved by the

Animal Ethical Committee of the Department of Zoology, University of Rajasthan, Jaipur.

### 2.3. Experiment design

Male rats of proven fertility were divided into four groups, each having eight rats except group IV, which had 16 rats. The first one served as control and received only the vehicle (distilled water, 0.5 ml/rat/day) for 60 days. Rats of groups II, III and IV were administered with aluminium chloride (30, 60 and 90mg/kg b.wt./day, respectively, dissolved in distilled water) for 60 days by gavage tube. Reversibility of the adverse effects was also observed after 60 days of the treatment withdrawal in group IV.

### 2.4. Autopsy schedule

Approximately twenty-four hours after the administration of the last dose, all the overnight-fasted rats of the treatment groups were sacrificed except the group IV, where half of the animals were sacrificed and the remaining half were left untreated for the next 60 days and autopsy was performed on day 120. The testes were dissected out, cleaned off from adherent fat and blood clot and was weighed on a digital electronic balance. The relative weight of testis ((Organ weight/Body weight) × 100) was measured for each rat in the treated and control groups.

### 2.5. Serum testosterone, FSH and LH

Blood samples were collected by cardiac puncture. The blood samples were allowed to clot at 37 °C and the serum was separated by centrifugation and stored at -20 °C for biochemical analysis. Serum samples were examined for serum concentration of testosterone [10], LH (Luteinizing Hormone) and FSH (Follicle Stimulating Hormone) [11] through chemiluminescence assay.

### 2.6. Histopathology

The testes were dissected out from all experimental and control rats and fixed in Bouin's fluid for histological observation. Bouin's-fixed testes were washed in water to remove excess of fixative, dehydrated in graded series of alcohol, cleared in xylene, embedded in paraffin wax and sectioned at 5 µm and counterstained in eosin and hematoxylin. Sections were observed for histopathological effects under light microscope.

### 2.7. Histomorphometry

#### 2.7.1. Seminiferous tubule diameter

Measurements were taken from at least 40 tubular profiles per animal, using a light microscope equipped with an ocular micrometer calibrated with a stage micrometer. Only round seminiferous tubules were randomly selected from various areas of the testicular section. Two diameters perpendicular to each other were measured at x100 magnification, averaged and expressed as seminiferous tubule diameter.

#### 2.7.2. Leydig cell nuclear diameter

The diameters of one hundred Leydig cells were measured on five sections from each testis with the ocular micrometer at x1000. The values were averaged and expressed as mean nuclear diameter of the Leydig cells.

### 2.7.3. Quantitative evaluation of germ cells

Quantitative evaluation of spermatogonia, preleptotene, pachytene spermatocytes and round spermatid cells was performed using 50 round tubules per group selected in stage VII/VIII of the seminiferous tubule cycle at x 400, according to the method described by Leblond and Clermont [12]. The diameters of nuclei of various germ cell types were measured by means of an ocular micrometer. A correction factor was used to obtain the actual numerical density of germ cells [13].

$$\text{True counts} = \text{Crude counts} \times \frac{\text{Section thickness } (\mu\text{m})}{\text{Section thickness } (\mu\text{m}) + \text{Nuclear diameter } (\mu\text{m})}$$

### 2.8. Statistical analysis

All the data were calculated and statistically analyzed with SPSS 10.0 computer software package for Windows (SPSS Inc., Chicago, IL, USA). The data were expressed as mean ± SEM and tested for variance. One-way analysis of variance (ANOVA) was carried out to determine the significance of observed differences. The data was further analyzed using Fisher's least significant difference (LSD) post hoc test. An alpha probability of less than 5% (P < 0.05) was considered as significant.

## 3. OBSERVATIONS AND RESULTS

### 3.1. Body weight

The initial and final body weights recorded at the beginning and at the end of the treatments is represented in table 1. There was a significant (P < 0.001, +16.18%) gain in the mean body weight of control rats when compared with their initial body weight. The rats administered with aluminium chloride showed a significant decline in body weight at 60 mg/kg b.wt./day (P < 0.05, -3.73%) and 90 mg/kg b.wt./day (P < 0.001, -7.14%). After 60 days of treatment withdrawal in the highest-dose group (90 mg/kg b.wt./day), rats of the recovery group depicted a reasonable recovery and there was a significant (P < 0.01, +6.74%) increase in body weight, when compared with their initial weight. (Table 1)

### 3.2. Testicular weight

Table 1 shows a significant decline in the relative weight of the testes in treated rats after administration of aluminium chloride at three different doses, when compared with control rats. However, after 60 days of treatment withdrawal, the relative weight of the testes in the recovery group was found to be significantly increased when compared with group IV rats, but it was still low in comparison to control rats (Table 1).

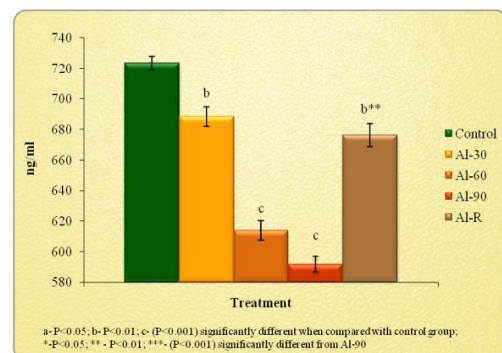


Figure I: Serum testosterone level in aluminium chloride treated rats at different dose levels

### 3.3. Serum testosterone, FSH and LH

A significant decline was observed in the levels of serum testosterone, FSH and LH in rats treated with aluminium chloride (30, 60 and 90 mg/kg b.wt./day), as shown in figures 1, 2 and 3, respectively. After 60 days of treatment withdrawal, levels of serum testosterone, FSH and LH were significantly improved ( $P < 0.01$ ), when compared with group IV (90 mg/kg b.wt./day). However, in comparison to control rats, the recovery in hormonal level was partial and significantly ( $P < 0.01$ ) less (Figures 1, 2 and 3).

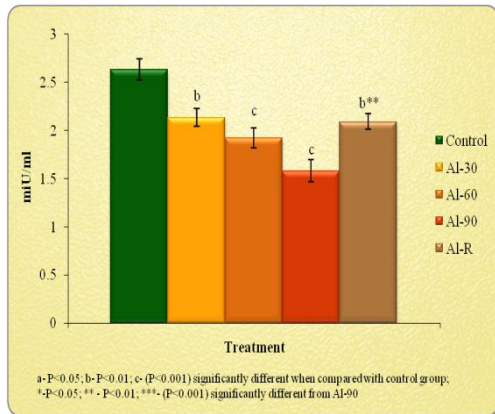


Figure II: Serum FSH level in aluminium chloride treated rats at different dose levels

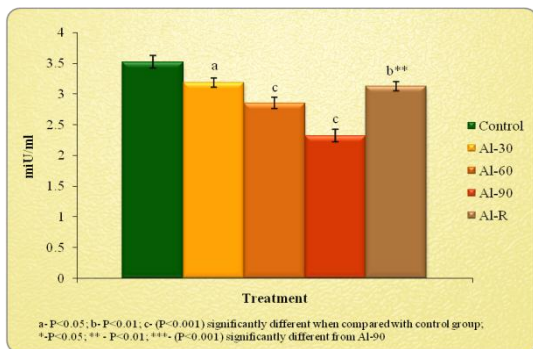


Figure III: Serum LH level in aluminium chloride treated rats at different dose levels

### 3.4. Histology of the testis

The testicular histology of the control rats displayed normal shape, size and characteristic arrangement of all successive germ cell types in seminiferous tubules. The tubular lumen was fully occupied by a large number of healthy spermatozoa (Fig. 4; Table 2). Histoarchitecture of the testis in rats treated with a low dose of aluminium chloride (30 mg/kg b.wt./day) showed marked degeneration of seminiferous epithelium, cytoplasmic vacuolization in germinal epithelium, decline in the number of germ cells and significant reduction in the mean diameter of seminiferous tubules. The Leydig cells also showed a significant reduction in the nuclear diameter (Fig. 5; Table 2). Administration of 60 mg/kg b.wt./day dose of aluminium chloride revealed markedly reduced diameter of seminiferous tubules, disorganized and degenerative germinal epithelium along with atrophic changes in Leydig cells. Quantitative determination of the different stages of germ cells, i.e. spermatogonia, preleptotene and pachytene spermatocytes and round spermatids, showed a further

significant depletion when compared with control. The lumen contains few spermatozoa and exfoliated germ cells (Fig. 6; Table 2). Exposure of rats to the highest dose of the aluminium chloride (90 mg/kg b.wt./day) induced more pronounced degenerative and atrophic changes in the testis, as indicated by shrunken seminiferous tubules with a wavy outline, severe degeneration, necrosis and sloughing of spermatogenic cells in seminiferous epithelium, with reduction in the number of luminal spermatozoa. Some exfoliated spermatogenic cells were also visible in the lumen. Leydig cells also showed degenerative and atrophic changes, with a significant reduction in the nuclear diameter (Fig. 7; Table 2). After 60 days of treatment (90 mg/kg/b.wt./day) cessation, the histological picture of the testis was significantly improved. The relative number of different types of germ cells in the seminiferous tubules was increased and lumen showed presence of moderate number of spermatozoa with some cellular debris (Fig. 8; Table 2).

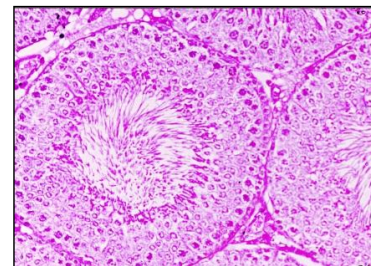


Figure 4. Photomicrograph. (hematoxylin and eosin stain (H.E.), x200) of the testicular section of a control rat, showing normal histoarchitecture.

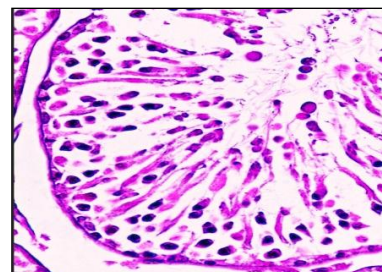


Figure 5. Photomicrograph of the testicular section of an aluminium chloride (30 mg/kg b.wt./day)-treated rat exhibiting marked degenerative changes in seminiferous tubules (hematoxylin and eosin stain (H.E.), x200).

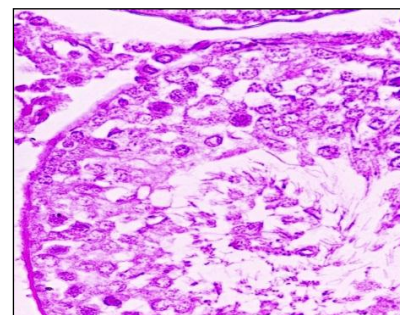


Figure 6. Photomicrograph of the cross section of the testis of an aluminium chloride (60 mg/kg b.wt./day for 60 days)-treated rat, showing moderate degenerative changes and arrest of spermatogenesis (hematoxylin and eosin stain (H.E.), x200).



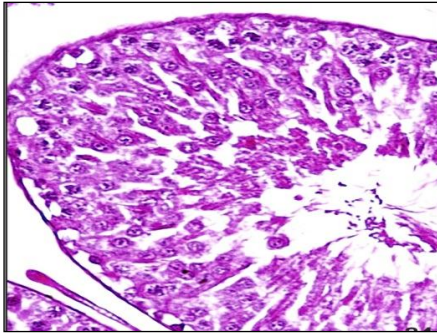


Figure 7. Photomicrograph of the cross section of the testis of an aluminium chloride (90 mg/kg b.wt./day)-treated rat, depicting marked shrinkage of seminiferous tubules, degeneration of germ cells and disruption of spermatogenesis (hematoxylin and eosin stain (H.E.), x200).

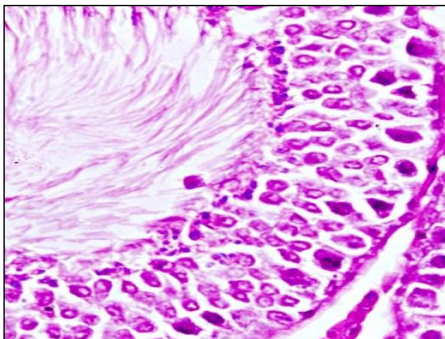


Figure 8. Photomicrograph of the cross section of the testis of a rat after 60 days of withdrawal of aluminium chloride treatment (90 mg/kg b.wt./day for 60 days), exhibiting marked restoration in histoarchitecture (hematoxylin and eosin stain (H.E.), x200).

### 3.5. Histomorphometric study

There was a significant dose-dependent decline in the mean diameter of the seminiferous tubules and Leydig cell nucleus in the rats treated with three different doses (30, 60 and 90 mg/kg b.wt./day) of aluminium chloride when compared with control rats, as shown in table 2. In the recovery group rats, both of these parameters were significantly improved when compared with group IV (90 mg/kg b.wt./day) rats, but were significantly ( $P < 0.01$ ) lower than those of control rats, suggesting partial recovery (Table 2).

### 3.6. Germ cell population dynamics

The testicular germ cell count in control and experimental groups is presented in table 2. Germ cell population, i.e., spermatogonia, preleptotene and pachytene spermatocytes, and round spermatids count in the seminiferous tubules showed a dose-dependent decline in rats treated with three different doses of aluminium chloride (30, 60 and 90 mg/kg b.wt./day), when compared with control. After 60 days of treatment withdrawal, the germ cell count of recovery group was significantly improved when compared with group IV rats (Table 2).

## 4. DISCUSSION

The testis is considered to be the most important organ in the male reproductive system. It is characterized by two main

functions, synthesis of steroid hormones and production of spermatozoa [14]. Testicular weight is an important parameter in the reproductive evaluation of males, owing to its high and positive correlation to sperm production [15]. The decline in testis weight observed in the present study could be correlated with degeneration of germinal epithelium, disruption of spermatogenesis, or inadequate supply of testosterone [16, 17].

Reproductive hormones play an important and complicated role in the regulation of spermatogenesis and sperm development [18]. In the present investigation, the significant decline in the levels of serum testosterone, FSH and LH reflects disturbances in the functions of the anterior pituitary and Leydig cells. Our findings are strengthened by Nuhair [19] and Yakubu *et al.* [20] who also reported that oral administration of aluminium chloride resulted in a significant decrease in the serum testosterone, FSH and LH levels in adult rats. Guo *et al.* [21] suggested that aluminium intoxication induces the production of nitric oxide (NO) which might be a suppressor of testosterone synthesis. Similarly, Dobashi *et al.* [22] also observed that the inhibitory effect of NO on Leydig cells may suppress testosterone synthesis. The decline in the activity of the 17-ketosteroid reductase enzyme that converts androstenedione to testosterone might be another possible reason for decreased testosterone production after aluminium exposure [23]. Shahraki *et al.* [24] suggested that the reduction in the levels of FSH and LH might be associated with the calcium channel blocking effect of aluminium, which leads to impaired secretion of gonadotrophins from the hypothalamus as  $Ca^{2+}$  ions are important for gonadotrophin-releasing hormone (GnRH) secretion in the hypothalamus. Diminished secretion of GnRH might be responsible for decreased FSH and LH levels. Discontinuation of treatment for 60 days in the recovery group induced a significant improvement in the levels of serum FSH, LH and testosterone.

Table 1. Body and relative organ weights of rats treated with various doses of aluminium chloride.

Treatments	Group I Control (vehicle)	Group II Aluminium chloride (30 mg/kg b.wt./day)	Group III Aluminium chloride (60 mg/kg b.wt./day)	Group IV Aluminium chloride (90 mg/kg b.wt./day)	Recovery Group (after 60 days of treatment withdrawal)	
Body weight (g)	Initial	173.5 ± 5.66	172.5 ± 2.84	180.62 ± 2.06	183.75 ± 2.27	170.62 ± 2.20
	Final	201.87 ± 4.33 <sup>c</sup> (+16.18%)	168.37 ± 5.43 <sup>ns</sup> (-2.39%)	173.87 ± 2.18 <sup>a</sup> (-3.73%)	170.62 ± 2.20 <sup>c</sup> (-7.14%)	182.12 ± 3.49 <sup>b</sup> (+6.74%)
Testis (mg/100 g b.wt.)	1297.92 ± 25.01	1191.32 ± 29.01 <sup>b</sup>	1137.91 ± 17.53 <sup>c</sup>	1034.63 ± 28.29 <sup>c</sup>	1177.45 ± 39.16 <sup>ns</sup>	

**Levels of significance:** Values represent mean ± SEM (standard error of mean) (n=8; n: number of animals in each group).

ns- Non-significant; a-  $P < 0.05$ ; b-  $P < 0.01$ ; c-  $P < 0.001$  (aluminium chloride-treated groups compared with control group).

\*\*P<0.01 (recovery group compared with group IV (One-way ANOVA followed by LSD multiple comparison test))

Table 2. Spermatogenic cell count/cross section of seminiferous tubule and morphometric analysis of rats treated with various doses of aluminium chloride.

Treatments	Spermatogonia	Preleptotene spermatocyte	Pachytene spermatocyte	Round spermatid	Seminiferous tubular diameter (µm)	Leydig cell nuclear diameter (µm)
<b>Group-I Control</b> (vehicle)	6.75 ± 0.25	22.12 ± 0.72	23.12 ± 0.74	68.75 ± 2.69	274.5 ± 7.38	6.75 ± 0.25
<b>Group-II Aluminium chloride</b> (30 mg/kg b.wt./day)	6.24 ± 0.27 <sup>ns</sup>	18.68 ± 0.64 <sup>b</sup>	20.25 ± 0.56 <sup>b</sup>	56.5 ± 2.57 <sup>b</sup>	252.62 ± 5.31 <sup>b</sup>	5.87 ± 0.29 <sup>a</sup>
<b>Group-III Aluminium chloride</b> (60 mg/kg b.wt./day)	5.56 ± 0.39 <sup>b</sup>	14.75 ± 0.70 <sup>c</sup>	17.62 ± 0.73 <sup>c</sup>	41.25 ± 2.31 <sup>c</sup>	228.37 ± 4.00 <sup>c</sup>	5.31 ± 0.28 <sup>c</sup>
<b>Group-IV Aluminium chloride</b> (90 mg/kg b.wt./day)	4.74 ± 0.15 <sup>c</sup>	13.12 ± 0.69 <sup>c</sup>	15.12 ± 0.55 <sup>c</sup>	35.87 ± 2.15 <sup>c</sup>	215.25 ± 5.31 <sup>c</sup>	4.07 ± 0.26 <sup>c</sup>
<b>Recovery-Group</b> (after 60 days of treatment withdrawal)	5.90 ± 0.21 <sup>a**</sup>	17.62 ± 1.36 <sup>b***</sup>	19.37 ± 1.13 <sup>b**</sup>	54.75 ± 3.63 <sup>b**</sup>	244.87 ± 5.24 <sup>b**</sup>	5.62 ± 0.26 <sup>b**</sup>

**Levels of significance:** Values represent mean ± SEM (standard error of mean) (n=8; n: number of animals in each group).

ns- Non-significant; a- P < 0.05; b- P < 0.01; c- P < 0.001 (aluminium chloride-treated groups compared with control group).

\*\*P<0.01 (recovery group compared with group IV (One-way ANOVA followed by LSD multiple comparison test))

Histopathological evaluations of animal reproductive tissue play a prominent role in male reproductive risk assessment. The testis is recommended as the most sensitive endpoint in the evaluation of male reproductive toxicity [25]. Histopathological examination of the testis revealed noticeable alterations, including deterioration in spermatogenic cells, shrunken seminiferous tubules, thinner germinal epithelium, exfoliation of germ cells, depletion of spermatozoa from lumina, necrosis and sloughing in germinal epithelium and degeneration of Leydig cells in rats orally administered with aluminium chloride at three dose levels (30, 60, 90 mg/kg b.wt/day). Similar histological changes in testis have been observed in aluminium-exposed rats [17, 19, 26, 27] and mice [9].

Histological alterations in testicular tissue might be resulted from oxidative stress which may cause cell damage and impairment of the steroidogenic activity of Leydig cells. A similar correlation between free radicals induced oxidative stress and testicular damage has been reported by various researchers in experimental animals treated with arsenic [28], cadmium [29], lead [30], mercury [31] and molybdenum [32]. Ige and Akhigbe [6] suggested that penetration of

aluminium through blood-testis barrier could cause degeneration and alteration in spermatogenic cells. Direct influence of aluminium on Sertoli cells might interrupt the intercellular bridges, leading to exfoliation of germ cells. Kim *et al.* [33] suggested that down regulation of a cell adhesion protein, such as cadherin in Sertoli cells, increased sloughing of seminiferous epithelial cells, which leads to tubular atrophy.

The reduced population of spermatogonia, preleptotene and pachytene spermatocytes, as well as round spermatids in the seminiferous tubule corroborates the histological findings which show diminished seminiferous tubular diameter and epithelium height, indicating decreased spermatogenic activity. The observed effects on germ cells are presumably due to increased lipid peroxidation and oxidative stress which leads to an increase in germ cell apoptosis. Similar apoptosis and necrosis of germ cells in the testis has been observed in aluminium-treated animals [26, 34]. Abdel-Moneim [35] suggested that aluminium exposure might induce apoptosis in spermatogonia and primary spermatocytes *via* microtubule targeting and mitotic arrest. Decreased seminiferous tubular diameter could be a sign of defective spermatogenesis, as evidenced by the reduction in the number of various spermatogenic cells in the testis. A similar reduction in seminiferous tubular diameter has been observed in experimental animals treated with other metals like cadmium [36], chromium [37] and lead [30]. A significant reduction in the Leydig cell nuclear diameter was observed in treated rats. Our results are in accordance with Ahmed *et al.* [38] who also found less circular Leydig cell nuclei in cadmium and lead-treated animals.

## 5. CONCLUSION

On the basis of the present study, it can be concluded that aluminium chloride distorted testicular morphometry *via* altered reproductive hormonal level, in a dose-dependent manner. However, the toxic effects induced by aluminium chloride were partially reversed after treatment withdrawal.

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## CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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