

Effect of Chronic Administration of Aluminum Trichloride on Testis among Adult Albino Wistar Rats

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Abstract

Different forms of Aluminum are environmental xenobiotics that induce free radical-mediated cytotoxicity and reproductive toxicity. We studied the aluminum trichloride (AlCl₃) toxicity on adult rat testes. Male Wistar rats were treated orally with AlCl₃ at three doses: 100, 200 and 400 mg/kg bw for 6, 12 and 18 months. Body and testes weight were followed. Plasma and testicular metal levels were assayed by atomic absorption spectrophotometry with graphic oven method. Light microscopy observation of testes was also performed. The results showed a significant decrease ($p < 0.05$) of body weight only at 18 months with the highest dose compared to the control group. We also noted a decrease in testicular weight in all treated groups at 12 and 18 months and only for the group treated with 400 mg/kg bw at 6 months. Aluminum trichloride resulted in a significant increase ($p < 0.05$) plasma aluminum concentrations is proportional at doses administered at different periods. In contrast, a significant increase ($p < 0.05$) of the testicular aluminum concentration was observed at 6 months in the group treated with 400 mg/kg bw, at 12 months in all groups and at 18 months only in groups treated with 100 and 200 mg/kg bw. Compared to the control group, a morphological alteration of the seminiferous tubules was observed at 6 months in group treated with 200 and 400 mg/kg bw, at 12 months with 200 and 400 mg/kg bw and at 18 months in all groups. Disruption of spermatogenesis was observed at 6 months with 200 and 400 mg/kg bw and at 12 and 18 months with all doses.

In summary, this study highlights the toxic effect of aluminum on the testes of adult rats. This toxicity is dose and period dependent.

Keywords: Aluminum; Testes; Histopathology; Toxicity

Introduction

Aluminum is the third most abundant element on the Earth crust, widely used for the manufacturing of many everyday products. Recently, Al in drinking water has been suspected as a risk factor for the development of many diseases and many health problems. Aluminum ingestion in excessive amount leads to accumulation in target organs and has been associated with damage of testicular tissues of both humans and animals [1]. According to recent research, Al accelerates oxidative damage to lipids, proteins and nucleic acids. The main toxic effects of aluminum in a chronic exposure were neurological (encephalopathy), (psychomotor functions disturbances), bone (osteomalacia) hematological (microcytic anemia). Aluminum is also responsible for allergic and immunological reactions [2].

Guo et al. [3] and Yousef et al. [4] have studied the toxic effects of chronic exposure to aluminum chloride. They essentially show an abnormality of sperm, of testosterone production and of sexual behavior. These anomalies appear to correlate with the aluminum level in seminal plasma. However, aluminum effects on fertility remain areas of research.

In this context, this study was focused on the chronic exposure to AlCl₃ effects on the seminiferous tubule and germ cells. These effects have been investigated as a function of two variables, dose and period.

Material and methods

Animals and housing conditions

The experimental study was started with 60 adult male Wistar rats (average weight 200-250 g). Animals (Pasteur Institute of Tunis, Tunisia) were high and fed in the Animal Experimentation Unit of the

Medicine Faculty of Tunis. Rats were housed with proper aeration at $25 \pm 2^\circ\text{C}$ and were given feed and water *ad libitum*. After two weeks of acclimation, animals were divided into four equal groups. They were exposed to Aluminum Chloride in drinking distilled water at 0 mg/Kg bw (G_0); 100 mg/Kg bw (G_1); 200 mg/Kg bw (G_2) and 400 mg/Kg bw (G_3) doses, during 6, 12 and 18 months. Rats were weighed regularly. Three sacrifices were performed at 6, 12 and 18 months and every time the testes were weighed. Ethical approval for the study was duly obtained from the responsible Ethical committee.

Chemical and dosing

The test article: Aluminum Chloride (AlCl₃; Sigma Company (St. Louis, MO, USA)) was dissolved in distilled water, and served as drinking water for rats. The dose levels of AlCl₃, used in the present study, were set as: 0.18g/L; 0.72g/L and 3.6g/L, they were reported to be 100, 200 and 400 mg/Kg bw. Control rats were given distilled water alone as drinking water.

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Assay with atomic absorption spectrophotometry with graphic oven

For assays of Al by atomic absorption spectrophotometry, fragments member (0.5g) were placed in polypropylene tubes. To avoid contamination with aluminum, we left all the glassware and bottles use a washing solution (HNO_3 0.01N) for 48 hours, then rinse with ultrapure water. The mineralization was performed in the presence of nitric acid. The fabric is placed in polypropylene tubes and each one puts 2 ml of nitric acid and finally placed in an oven at 50°C for 72h. It is necessary to have an inert or reducing atmosphere inside the furnace to prevent a rapid oxidation of the heating element is used as argon gas. After introduction of the liquid sample (20 μl) in the furnace, heating that leads to the atomization is carried out in an inert atmosphere following steps programmed time and temperature: drying, decomposition, atomizing cleaning and cooling.

Blood samples

The blood collected during the sacrifice is placed in heparin tubes. Then blood samples were centrifuged at 4000 rpm for 15 min. The plasma was collected and stored at -20°C until analysis with atomic absorption spectrophotometry.

Histopathological study

Testes were fixed in a 10% formaldehyde solution, passed through ascending series of ethanol baths, cleared in toluene and embedded in paraffin. Tissues were sectioned at 4 μm and stained with Haematoxylin and Eosin (H&E). The sections were examined by light microscope.

Statistical analysis

Statistical analyses were performed using SPSS 17.0 for Windows (SPSS, Chicago, IL, USA). To determine whether there were differences between all groups, the *Mann-Whitney* test was performed ($p < 0.05$). All data are presented as mean $\pm$ SD.

Results

Effect of AlCl_3 treatment on body and testes weight

Following Table 1, treatment with AlCl_3 showed a significant decrease ($p < 0.05$) in body weight only with 400 mg/kg bw at 18 months compared to the control group and in weight of testes treated with 400 mg/kg bw ($p < 0.05$) at 6 months and 100, 200 and 400 mg/kg bw ($p < 0.05$) at 12 and 18 months compared to the control.

Plasma and Testicular AlCl_3 estimation

Data in Table 2 showed that the plasma aluminum concentration increased significantly ($p < 0.05$) compared to the control. This increase is dose and period dependent. Concerning the testicular aluminum concentration, a significant increase ($p < 0.05$) were noted at 6 months with 400 mg/kg bw, at 12 months with all doses and at 18 months with 100 and 200 mg/kg bw compared to the control.

Histopathological findings

Histopathological observations (Table 3 and Figure 1) revealed compared to the control, a morphological alteration of the seminiferous tubules with 400 mg/kg bw at 6 months, with 200 and 400 mg/kg bw at 12 months and with all doses at 18 months. Disruption of spermatogenesis was observed in the testes of rats treated with 200 and 400 mg/kg bw at 6 months and with all doses at 12 and 18 months compared with control groups that seem to be influenced by the dose and exposure period.

Discussion

Body and testicular weight

The present study showed a significant decrease in body weight at 18 months with only the highest dose compared to the control group. According to the literature, the majority of studies that utilized chronic doses of aluminum reported a significant reduction in the final rat body

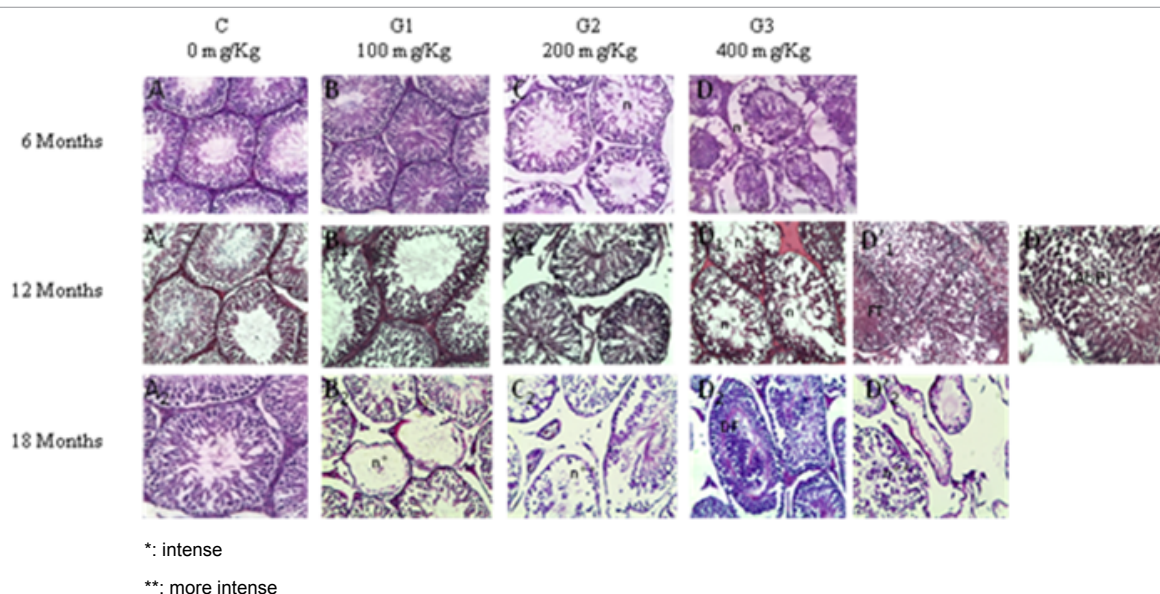


Figure 1: histology of the rat testes (H&E X 200): Necrosis(n); disorganized spermatozoa with flagella on whirlpool form (FT) Abnormal disposition of spz flagella (DF); spermatozoa characterised by elongated head and absence of flagella (AbF)

weight particularly in studies initiated in male animals. Kowalczyk et al. [5] found that during three months observation of rats receiving aluminum chloride, a decrease in water and food intake and transient diarrhea occurred, which resulted in lowering of final body mass of animals compared to controls. The pathophysiological explanation for these results is not clear.

Oral administration of AlCl₃ resulted a significant decrease in testicular weight with 400 mg/kg bw at 6 months and at all doses administered at 12 and 18 months. Bataineh et al. [6] found a decrease in absolute and relative testes and seminal vesicles weights after AlCl₃ ingestion. The decrease in the reproductive organs weights could be due to the decrease in testosterone level that may be due to oxidative damage induced in the rat testes [7,8]. Other study proved that Al can cause endocrinal disorders and interfere with Androgen Receptor expression, which suppresses development and functional maintenance of the testes [9].

Plasma and testicular aluminum concentration

Our study showed an increase in the metal plasma and testes. These findings were in accordance with some studies [6]. In this context, we suggested that the severe reduction in male fertility following aluminum administration might result from excessive aluminum accumulation in the testes. Some studies have shown that aluminum trichloride concentrates preferentially in plasma and testes which explain the spermatotoxicity treated rats [6,1].

Others authors suggested that the severe reduction in male fertility following aluminum administration might result from excessive aluminum accumulation in the testes and low testosterone concentrations [3].

Moreover, there is no significant difference between control group and treated with high dose at 18 months. This could be explained by the destruction of germ cells instead of aluminum accumulation (detailed in the next chapter Aspect).

Histopathological study

The microscopical examination of the testes treated showed several modifications on seminiferous tubules. AlCl₃ at 100 mg/Kg bw at 6 months showed a normal architecture of seminiferous tubules like control testes rats (Figure A-B). In the contrary, with 200 and 400mg/Kg bw doses of AlCl₃, a mild degenerative changes with focal areas of necrosed spermatogenic cells were detected (Figure C-D).

These alterations were more important on seminiferous tubules of rats treated at 12 months. Interestingly, this effect is dose-dependent. In

fact, observation of treated testes with 100 mg/Kg bw revealed necrosis and altered area (Figure B1). Testes treated with 200 mg/Kg bw present an intense necrosis, spermatogenesis disrupts, spermatozoa were absent in the lumen and the germinal epithelium of the seminiferous tubules was degraded (Figure C1). These findings are in agreement with the obtained data by Hala Khattab et al. [10] who mentioned that in the AlCl₃-treated group, histopathological examinations revealed apparent alterations in the testes, where it induced marked lesions in seminiferous tubules. In addition, Chinoy et al. [11] study reported that administration of sodium fluoride and AlCl₃ for 30 days caused disorganized epithelium cell debris in lumen and reduction in sperm density.

The effect of AlCl₃ consumption is more intense with 400 mg/Kg bw dose. At this dose, testes present intense necrosis with altered spermatogenesis. In addition, spermatozoa were disorganized: flagella melted formed a *whirlpool* (Figure D₁). Other area present spermatozoa characterized by elongated head and absence of flagella (fig.D₂); this may be due to a problem in spermatids differentiation especially in flagella differentiation.

Compared to the control, testes of rats treated with different doses of AlCl₃ at 18 months, present an alteration more intense appears to be dose-related. In fact, testes of rats treated with 100 and 200 mg/Kg bw (Figure B₂-C₂) showed interstitial edema, necrosis area and some damaged seminiferous tubules with absence of germ cells. The same changes were observed at 400 mg/Kg bw (Figure D₂), with apparition of some seminiferous tubules where flagella formed a barriers surrounds remains cells in lumen (Figure D₂). The histological changes in testes of rats administered AlCl₃ are in agreement with Atessahin et al. [12] and Khattab et al. [13] who studied the effect of AlCl₃ on testes rats. Also, Guo et al. [14] observed deleterious effects and histopathological changes in testicular tissues after 2 weeks of aluminum treatment, as well as noticeable spermatogenic loss as necrosis in the spermatids and spermatozoa at 5 weeks of aluminum treatment. This damage effect may be explained by Yousef et al. [1] who reported that oxidative stress results from the production of oxygen radicals in excess of the antioxidant capacity of the stressed tissue. Moreover, Turner et al. [15] found over productive of ROS, which can be detrimental to sperm and associated with male infertility, and thus spermatotoxic effect might be due to AlCl₃ induced free radicals.

In the current study, it was concluded that AlCl₃ had a destructive effect on seminiferous tubules of adult rat. The impairment caused by aluminum was accompanied primarily by the prolonged accumulation of aluminum in the rat testes. Interestingly, this effect seems to be dose and period dependent. Our study will be completed by testing the impact of AlCl₃ on different reproductive parameters.

Animal grouping	Body weight (g)			Testicular weight (g)		
	6 months	12 months	18 months	6 months	12 months	18 months
G ₀	373,6 ± 31,20	372,8 ± 37,59	366,66 ± 50,76	2,48 ± 0,02	3,64 ± 0,10	3,55 ± 0,48
G ₁	369,466 ± 29,3	337,4 ± 32,07	302,5 ± 37,74	2,08 ± 0,61	3,25 ± 0,40 ^s	2,57 ± 0,37 ^s
G ₂	354,93 ± 27,07	332,666 ± 34,89	300,00 ± 62,64	2,51 ± 0,14	3,43 ± 0,11 ^s	2,82 ± 0,67 ^s
G ₃	366,93 ± 25,5	349,857 ± 31,76	292,5 ± 10,6 ^s	2,78 ± 0,06 ^s	3,31 ± 0,011 ^s	3,04 ± 0,70 ^s

s: significant in comparison to control (G₀)

Table 1: Body and testes weight at different doses and period of treatment with AlCl₃. Result were expressed as (mean ± S.E.M. value, n=5 rats)

Animal grouping	Plasma aluminium concentrations (g/ml)			Testicular aluminium concentrations (g/gMF)		
	6 months	12 months	18months	6 months	12 months	18months
G ₀	0,009 ± 0,71	0,009 ± 1,17	0,016 ± 0,01	0,015 ± 0,01	0,011 ± 0,01	0,028 ± 0,02
G ₁	0,015 ± 0,50 ^s	0,018 ± 1,72 ^s	0,025 ± 0,02 ^s	0,016 ± 0,01	0,018 ± 0,01 ^s	0,043 ± 0,1 ^s
G ₂	0,023 ± 2,24 ^s	0,029 ± 1,92 ^s	0,025 ± 0,02 ^s	0,021 ± 0,02	0,029 ± 0,02 ^s	0,085 ± 0,08 ^s
G ₃	0,03 ± 5,22 ^s	0,04 ± 2,45 ^s	0,02 ± 0,02 ^s	0,026 ± 0,02 ^s	0,031 ± 0,03 ^s	0,03 ± 0,04

s: significant in comparison to control (G₀)

Table 2: Plasma and Testicular aluminum concentration at different doses and period of treatment with AlCl₃. Result were expressed as (mean ± S.E.M. value, n=5 rats)

Doses/Periods	6 months	12 months	18 months
0 mg/Kg bw	-Normal structure of seminiferous tubules which surrounded by vascularized dense fibrous connective tissue, Tunica albuginea. Each seminiferous tubule appeared normal with orderly arrangement of germ cells at different stages of spermatogenesis		
100 mg/Kg bw	-Normal structure with regular spermatogenesis	-Few alteration of seminiferous tubules	-Some damaged seminiferous tubules -Presence of necrosis zones
200 mg/Kg bw	-Perturbation of spermatogenesis -Few zones of degenerations	-Intense perturbation of spermatogenesis -Intense Necrosis -Absence of spz -Degeneration of Epithelium seminal	-Intense area of necrosis -Perturbation of spermatogenesis -Disorganized spz with flagella on whirlpool form
400 mg/Kg bw	-Several perturbation of spermatogenesis (only presence of spz) -Intense Necrosis -Alteration of adhesive junctions	-Important degeneration of seminiferous tubules -Several Necrosis -Degradation of epithelium seminal (presence of few spermatogonies) -Intacts somatic cells	-More area of necrosis zone -Abnormal disposition of spz flagella -Presence of abnormal cells

Table 3: Histopathological alterations of testes with different doses at different periods

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