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Evaluation of Reproductive and Fertility Toxic Potentials of Aluminum Chloride on Adult Male Mice

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Abstract: Sexually mature male mice were exposed to different doses of aluminum chloride in drinking water for twelve weeks. Fertility was significantly reduced in males exposed to aluminum chloride. The number of resorptions and number of females with resorptions were significantly increased in females impregnated by males exposed to aluminum chloride. Ingestion of aluminum chloride significantly reduced testicular and epididymal sperm counts and increase in testosterone and luteinizing hormone (LH) serum levels. Also, absolute weight of the testes, absolute and relative weights of the seminal vesicles and preputial glands were significantly reduced. Furthermore, histological changes were found in testicular sections of adult male mice ingested aluminium chloride. Congested blood vessels, increased amount of interstitial connective tissue and destruction of the seminiferous tubules with large necrotic areas and degenerative cells have been observed. The results indicated that ingestion of aluminum chloride by adult male mice would cause adverse effects on fertility and reproduction.

Key words: $AlCl_3$, Fertility, Toxic potential, Mice

Introduction

Aluminum (Al) is one of the most common elements in the earth's crust. It occurs naturally only in the Al_3^+ valence state. It is non essential, but ubiquitous. Aluminum is found in approximately 300 different minerals, more commonly with silica such as feldspat and micas. It is also present in the air primarily as alumnosilicates associated with dust particles. The most common foods with substantial amounts of aluminum containing additives include some processed cheese, baking powders, cake mixes, frozen doughs, pancake mixes, self raising flours and pickled vegetables. Also, it is commonly found in cookware, cans, tea, beer, antacids and antiperspirants. Daily intake of aluminum has been estimated to be 3.5-51.6 mg and it is estimated that 20% of the daily intake of aluminum comes from aluminum cooking utensils such as pans, pots, kettles and trays.

Aluminum has been shown to be an important central nervous system (CNS) toxin and has been implicated in the development of several neurodegenerative disorders, including Alzheimer's disease, dialysis dementia, Parkinsonism dementia and amyotropic lateral sclerosis of Guam (Alfrey *et al*., 1976 and Agarwal *et al*., 1996). A number of cellular mechanisms by which aluminum is thought to exert its toxicity have been described including increasing the blood-brain barrier permeability, interference with phosphorylation-dephosphorylation reactions, altered iron metabolism with subsequent free radical production and disruption of second messenger systems (Agarwal *et al*., 1996).

Animal models have demonstrated that maternal aluminum exposure during gestation can produce subsequent neonatal effects in the offspring, including skeletal, visceral and other morphological malformations, poor neonatal weight gain, as well as deficiency in locomotor co-ordination (Yokel, 1985; Paternain *et al*., 1988 and Colomina *et al*., 1992). Although Al toxicity was initially recognized as a neurological and/or skeletal disease which occurred in epidemic proportion in some dialysis population (McDermott *et al*., 1978 and Alfrey, 1980, 1986), it has been demonstrated that concentrations of this element that are toxic to many biochemical processes are also found in a number of neurodegenerative disorders affecting nonuremic and nondialyzed individuals (McLachlan *et al*., 1985, 1989; Editorial, 1992). However, in recent years it has been shown that the gastrointestinal tract normally represents a major barrier to Al absorption, however, under some circumstances this barrier can be breached (Alfrey. 1985,1986).

Most foods contain small but variable amounts of Al it has been reported that 2 to 3 mg Al daily is probably the lower limit of Al naturally present in western diets (Domingo , 1995 and Greger, 1993). However, the amount of Al in the diet is small compared to the amount of Al in many antacid products and some buffered analgesics. Some people consume as much as an additional 5g of Al daily from these compounds. The aim of the present study was to monitor the adverse effects on fertility and reproduction of aluminum chloride ingested with drinking water by male mice.

Materials and Methods

Animals: Sexually mature male Swiss mice (50 days old) were used in these experiments. They were provided by the Animal House Unit in the Faculty of Medicine at Jordan University of Science and Technology. Animals were maintained in controlled temperature 21°C $\pm$ 1°C under a 12 h light: 12 h darkness schedule (light 06:00-18:00h). Food and tap water were offered *ad libitum*.

Administration of Aluminum Chloride: Aluminum chloride was obtained from Merck-Schuchardt (Schuchardt, 8011 Hohenbrunn, Munchen, Germany). It was dissolved in drinking tap water at various concentrations namely: 1000 ppm (1000 mg/L), 1200 ppm (1200 mg/L) and 1400 ppm (1400mg/L). The duration of exposure of male mice was 12 weeks. Control male mice were given tap water.

Effect of Aluminum Chloride on Fertility of Adult Male Mice: Adult male mice were exposed for 12 weeks to 1000, 1200, 1400 ppm aluminum chloride in drinking water (ten animals per each group). Ten control males were given tap water.
After 12 weeks of exposure each male was placed in individual cage with two virgin untreated females of the same strain. They were left together for ten days during which two estrus cycles should have elapsed (Rugh, 1968). The aluminum exposed males were removed and the females were killed by cervical dislocation under light ether anesthesia at day 20 of gestation, and their uteri were examined. During autopsy the following measurements were recorded: number of pregnant females, number of viable fetuses, number of implantation sites, number of resorptions, number of females with resorptions and fetal body weight.

Effect of Aluminum Chloride on Body and Organ Weights of Adult Male Mice: Ten treated males for each concentration and ten control males mice were sacrified after 12 weeks of Aluminum chloride ingestion. The following organs were excised and weighed: paired testes, seminal vesicles (stripped of fluid) and preputial glands. Initial and final body weights were recorded for treated males and the control counterparts.

Evaluation of the Exposure Period on the Male Fertility: Other groups of exposed male mice were examined during weeks 3, 6, 9 and 12 for toxic effects of aluminum chloride. Five control and five treated mice, for each concentration (1000, 1200 and 1400 ppm aluminium chloride) were sacrificed by cervical dislocation under light ether anesthesia and the following measurements were recorded: testicular weight; body weight; epididymal and testicular sperm counts for treated and control groups.
The excised left testis and epididymis were weighed. Testis from each mouse was placed in 4 ml of normal saline (0.9% sodium chloride) and homogenized for spermatid counts. Epididymis was placed in 4 ml of normal saline (0.9% sodium chloride) and homogenized for epididymal sperm count.
Sperm count was performed according to method of Amann and Lambiase (1969) epididymal sperm counts were calculated and expressed as number of sperms per gram of epididymis. Testicular spermatid counts were calculated and expressed as number of spermatids per gram of testis. The estimate of daily sperm production (DSP), per testis per day, and per gram of testis per day (efficiency) were calculated based on a factor of 4.84 (Ashby *et al.*, 1999) which is the duration of a seminiferous cycle during which developing spermatozoa are in the spermatid stage.

Effect of Aluminum Chloride on Testosterone, FSH and LH Serum Levels of Adult Male Mice: One ml of blood was collected in non heparinized tubes by cardiac puncture from anesthetized males (five animals for each concentration 1000, 1200 and 1400 ppm and five control) before sacrifice. Blood samples were then centrifuged at 5000 rpm for 4 minutes. The serum was collected and stored in eppendorff tubes at −20 °C until analyzed. Serum testosterone, FSH and LH levels were determined directly by IMMULITE Analyzer, using a solid phase, two-site chemiluminescent immunometric assay, purchased from DPC (USA).

Histological Evaluation of the Effect of Aluminum Chloride on Adult Male Mice Testis: The excised testes were fixed in 10% formalin solution for histological processing. Standard procedure was followed to prepare histological slides which were stained with hematoxylin and eosin stain. Sections of the tissues were examined using Nickon microscope.

Statistical Analysis: Data were analyzed on IBM PC computer using Microsoft Excel 97 and STATMOST program. Differences between control and aluminum exposed groups were analyzed using Student t-test for the mean value, Chi-square and Fisher exact tests were used for the analysis of the number of pregnant females, females with resorptions, and the number of resorptions.

Results

Effect of Aluminum Chloride on Male Reproduction: After three weeks of exposure all treated animals ingested 1000, 1200 and 1400 ppm aluminum chloride via drinking water, started to show signs of weakness, paralysis and sluggishness in a dose dependent manner.

Effect of Aluminum Chloride on Fertility of Adult Male Mice: Twelve weeks ingestion of aluminum chloride via drinking water by adult male mice revealed a significant reduction in fertility of adult male mice ingested $AlCl_3$ at concentration 1400 ppm ($p<0.05$). The number of implantation sites was not affected in females mated by males ingested $AlCl_3$. The number of viable fetuses was significantly reduced in females mated by males ingested 1000 ppm $AlCl_3$ ($p<0.05$). The total number of resorptions was significantly increased in females mated by males ingested 1000, 1200 and 1400 ppm $AlCl_3$ ($p<0.0001$). Also, the number of females with resorptions was significantly increased in females mated by males ingested 1000, 1200 and 1400 ppm $AlCl_3$ ($p<0.0001$) (Table I).

Effect of Twelve Weeks Ingestion of Aluminum Chloride on Weights of Reproductive Organs of Adult Male Mice: The absolute weight of the testes was significantly reduced in males ingested 1200 and 1400 ppm aluminum chloride ($p<0.01$), and in males ingested 1000 ppm $AlCl_3$ ($p<0.05$). The relative weight of testes was not affected in males at all doses. The absolute and relative weights of seminal vesicles was significantly reduced in males ingested 1000, 1200 and 1400 ppm $AlCl_3$ ($p<0.0001$). A significant reduction in the absolute weight of the preputial gland in males ingested 1000, 1200 and 1400 ppm $AlCl_3$ ($p<0.0001$) was noted. Also, the relative weight of preputial gland was significantly reduced in males ingested 1200 and 1400 ppm $AlCl_3$ ($p<0.0001$), and in males ingested 1000 ppm $AlCl_3$ ($p<0.005$) (Table II).

Effect of Twelve Weeks Ingestion of Aluminum Chloride on Average Body Weight and Water Consumption of Adult Male Mice: Average body weight was significantly reduced in males ingested 1400 ppm aluminum chloride ($p<0.0001$). Also, in the same group, average water consumption was decreased significantly ($p<0.0001$) (Table III). Body weight gain expressed in weight difference (final body weight – initial body weight) was significantly reduced in males ingested 1000, 1200 and 1400 ppm $AlCl_3$ ($p<0.0001$) (Fig. 1).

Effect of Aluminum Chloride on Testicular and Epididymal Sperm Counts of Adult Male Mice

Effect of Three Weeks Ingestion of Aluminum Chloride on Testicular and Epididymal Sperm Counts of Adult Male Mice: Testicular sperm counts (total sperm counts) was significantly reduced in males ingested 1200 and 1400 ppm aluminum chloride ($p<0.05$, $p<0.0001$, respectively). Also, total sperm/g testis was significantly reduced in males ingested 1400 ppm $AlCl_3$ ($p<0.0001$). A significant reduction in daily sperm production was noted in males ingested 1200 and 1400 ppm $AlCl_3$ ($p<0.005$, $p<0.0001$, respectively). Sperm efficiency (sperm/mg testis/d) was also reduced significantly in males ingested 1400 ppm $AlCl_3$ ($p<0.005$) (Table IV).

Epididymal sperm counts were significantly reduced in males ingested 1400 ppm aluminum chloride ($p<0.005$), and in males ingested 1200 ppm $AlCl_3$ ($p<0.05$). A significant reduction in sperm/g epididymis was clearly seen in males ingested 1400 ppm $AlCl_3$ ($p<0.01$).

Epididymis weight was significantly decreased in males ingested 1200 and 1400 ppm aluminum chloride ($p<0.005$, $p<0.05$, respectively) (Table V).

Effect of Six Weeks Ingestion of Aluminum Chloride on Testicular and Epididymal Sperm Counts of Adult Male Mice: Testicular sperm counts (total sperm counts) was significantly reduced in males ingested 1200 and 1400 ppm aluminum chloride ($p<0.0001$), and in males ingested 1000 ppm $AlCl_3$ ($p<0.001$). Also, total sperm/g testis was significantly reduced in males ingested 1200 and 1400 ppm $AlCl_3$ ($p<0.01$), and in males ingested 1000 ppm $AlCl_3$ ($p<0.05$). Daily sperm production was significantly reduced in males ingested 1200 and 1400 ppm $AlCl_3$ ($p<0.0001$), and in males ingested 1000 ppm aluminum chloride ($p<0.05$). A significant reduction in sperm efficiency was observed in males ingested 1000, 1200 and 1400 ppm $AlCl_3$ ($p<0.05$, $p<0.005$, $p<0.01$, respectively) (Table IV).

Epididymal sperm counts were decreased significantly in males ingested 1200 and 1400 ppm aluminum chloride ($p<0.0001$), and in males ingested 1000 ppm $AlCl_3$ ($p<0.01$). Also, sperm/g epididymis was significantly reduced in males ingested 1200 and 1400 ppm $AlCl_3$ ($p<0.005$, $p<0.0001$, respectively). Epididymis weight was significantly decreased in males ingested 1200 and 1400 ppm $AlCl_3$ ($p<0.05$) (Table V).

Effect of Nine Weeks Ingestion of Aluminum Chloride on Testicular and Epididymal Sperm Counts of Adult Male Mice: Male mice ingested 1000, 1200 and 1400 ppm aluminum chloride showed a significant reduction in testicular sperm counts (total sperm counts) ($p<0.005$). Furthermore, a significant reduction in total sperm/g testis

was demonstrated in males ingested 1000, 1200 and 1400 ppm $AlCl_3$ ($p<0.005$).
Daily sperm production was significantly reduced in males ingested 1200 and 1400 ppm aluminum chloride ($p<0.005$), and in males ingested 1000 ppm $AlCl_3$ ($p<0.01$). Sperm efficiency was reduced significantly in males ingested 1000, 1200 and 1400 ppm $AlCl_3$ ($p<0.005$) (Table IV).
Epididymal sperm counts decreased significantly in males ingested 1000 and 1200 ppm aluminum chloride ($p<0.0001$), and also, in males ingested 1400 ppm $AlCl_3$ ($p<0.005$). Sperm per gram epididymis was reduced significantly in males ingested 1000 and 1200 ppm $AlCl_3$ ($p<0.0001$), and in males ingested 1400 ppm $AlCl_3$ ($p<0.005$). Furthermore, epididymis weight was significantly decreased in males ingested 1400 ppm $AlCl_3$ ($p<0.005$) (Table V).

Effect of Twelve Weeks Ingestion of Aluminum Chloride on Testicular and Epididymal Sperm Counts of Adult Male Mice: Testicular sperm counts (total sperm counts) were reduced significantly in males ingested 1200 and 1400 ppm aluminum chloride ($p<0.0001$), and in males ingested 1000 ppm $AlCl_3$ ($p<0.005$). Total sperm/g testis was significantly reduced in males ingested 1200 and 1400 ppm $AlCl_3$ ($p<0.01$), and in males ingested 1000 ppm $AlCl_3$ ($p<0.05$). Daily sperm production was significantly reduced in males ingested 1200 and 1400 ppm $AlCl_3$ ($p<0.0001$), and in males ingested 1000 ppm $AlCl_3$ ($p<0.005$).
Sperm efficiency was reduced significantly in males ingested 1200 and 1400 ppm aluminum chloride ($p<0.01$), and in males ingested 1000 ppm $AlCl_3$ ($p<0.05$) (Table IV).
A significant decrease in epididymal sperm counts was recorded in males ingested 1000, 1200 and 1400 ppm aluminum chloride ($p<0.05$, $p<0.01$, $p<0.005$, respectively). Sperm/g epididymis was reduced significantly in males ingested 1400 ppm $AlCl_3$ ($p<0.01$). Epididymis weight was significantly decreased in males ingested 1400 ppm $AlCl_3$ ($p<0.05$) (Table V).

Effect of Twelve Weeks Ingestion of Aluminum Chloride on Serum Levels of Testosterone, FSH and LH Hormones of Adult Male Mice: Adult male mice ingested 1000, 1200 and 1400 ppm aluminum chloride showed a significant increase in the serum level of testosterone ($p<0.01$) (Fig. 2). A significant increase were observed in the serum level of LH in adult male mice ingested 1400 ppm $AlCl_3$ ($p<0.005$)(Fig. 3), while FSH serum level was comparable to the control FSH serum level (Fig. 4).

Effect of Aluminum Chloride on Testis of Adult Male Mice: Histological sections of the testis of adult male mice after ingestion of aluminum chloride were prepared. All sections of the testis of adult male mice ingested 1400 ppm $AlCl_3$ were found to have congested blood vessels, increased amount of interstitial connective tissue, increased number of Leydig cells and changes in the seminiferous tubules. Large necrotic areas covering five tubules in one testis was observed (Fig. 5). Also, the seminiferous tubules in the center of the section had large numbers of spermatids in their lumen while, those in the periphery had less or no spermatids in the testes of males ingested 1400 ppm $AlCl_3$.
Changes in the testis were obvious in all sections examined in adult male mice ingested 1200 ppm $AlCl_3$. Highly congested blood vessels and increase in the interstitial connective tissue were seen. Complete destruction of the seminiferous tubules and replacement by necrotic and degenerated cells were seen. However, the basal area or cell layer was intact (Fig. 6). Also, disruption of the arrangement of normal tubules were seen, especially at the periphery of the testis, while the tubules away from the capsule showed less changes (Fig. 7). Few tubules had been almost completely destroyed compared to the controls (Fig. 8).
Less pronounced changes were observed in the histological sections of the testis of adult male mice ingested 1000 ppm. While, one or two cell layers was seen in the tubules, the rest of the tubule appeared normal, with less connective tissue proliferation.
The epididymis of the treated males ingested 1400 ppm showed complete absence of spermatozoa with no other histological changes in the epithelial wall in comparison to the epididymis of control males which contained large quantities of spermatozoa in their lumen .

Discussion

To our knowledge, there is shortage of data on the effects of long-term ingestion of aluminum chloride on fertility and reproduction in adult male mice. The animal model used in this study has been used previously by several workers to assess the adverse effects of different metals on reproductive functions and fertility in laboratory animals (Johansson and Wide, 1986; Liobet *et al.*, 1993).
The results presented in this study showed that ingestion of aluminum chloride for twelve weeks by adult male mice had adverse effects on male reproductive system and fertility. The mating capability of male mice ingested 1400 ppm aluminum chloride was adversely affected. The results showed that the number of pregnant females impregnated by males exposed 1400 ppm to aluminum chloride was significantly reduced.

Table 1: Effect of twelve weeks ingestion of aluminum chloride via drinking water on fertility of adult male mice

Treatment group Dose*	No. of males	No. (%) of pregnant females	No. of implantation site*	No. of viable fetuses	Total no. of resorptions/ total no. of Implantation Site	No. (%) of animals with resorptions
Control	10	19/20 (95.0)	7.74 ± 1.98	7.74 ± 1.98	0/147	0/19 (0)
Aluminum chloride (1400 ppm)	9	10/18* (55.5)	8.70 ± 0.82	7.20 ± 1.81	15/87****	7/10**** (70.0)
Aluminum chloride (1200 ppm)	10	16/20 (80.0)	9.44 ± 1.90	8.44 ± 2.06	16/153****	10/16**** (62.5)
Aluminum chloride (1000 ppm)	10	15/20 (75.0)	7.33 ± 2.35	6.2 ± 2.11	17/110****	10/15**** (66.6)

*Results are expressed as mean ± S.D.
*P < 0.05 (Student t-test)
*P < 0.05, **P < 0.005, ***P < 0.001, ****P,0.0001 (Fishers exact test)
* Actual dose consumption is presented in Table 3

Table 2: Effect of twelve weeks ingestion of aluminum chloride via drinking water on weights of reproductive organs of adults male mice

Treatment group Dose*	No. of males	Body weight (B.wt.) (G)*	Absolute testes weight (g)* (Mg/10 g B.wt.)	Absolute seminal vesicles weight (g)* (mg/10 gB.wt.)	Absolute preputial gland weight (g)* (mg/10 g B.wt.)
Control	10	40.29 ± 2.7	0.23v0.02 (56.88 ± 6.26)	0.18 ± 0.03 (43.79 ± 6.70)	0.11 ± 0.02 (27.59 ± 4.44)
Aluminum choride (1400 ppm)	9	35.39 ± 3.79**	0.19 ± 0.02** (54.58 ± 8.04)	0.09 ± 0.01**** (25.96 ± 4.59****)	0.058 ± 0.001**** (16.46 ± 3.10****)
Aluminum chloride (1200 ppm)	10	35.56 ± 3.07***	0.20 ± 0.02** (55.09 ± 5.25)	0.07 ± 0.01**** (18.86 ± 2.75****)	0.06 ± 0.02**** (16.36 ± 4.56****)
Aluminum chloride (1000 ppm)	10	32.64 ± 3.50****	0.20 ± 0.02* (62.92 ± 7.95)	0.08 ± 0.02**** (25.53 ± 6.04****)	0.06 ± 0.02**** (18.84 ± 5.64***)

*Resuts for tables 2,3,4 and 5 are expressed as mean ± S.D.
*P < 0.05, **P < 0.01, ***P < 0.005, ****P < 0.0001 (student t-test)
*Actual dose consumption is presented in table 3
Relative weights

Table 3: Effect of twelve weeks ingestion of aluminum chloride via drinking water on average water consumption of adult male mice

Treatment dose	Body weight(g)*	Water consumption(ml)*	Actual dose consumption (mg/kg/d)*
Control	35.28 ± 2.86	6.03 ± 0.43	0
Aluminum chloride (1400 ppm)	31.20 ± 0.81**	3.01 ± 1.07**	135.37 ± 3.48
Aluminum cgloride (1200 ppm)	35.33 ± 0.89	3.64 ± 0.45**	123.72 ± 3.15
Aluminum chloride	33.52 ± 0.70	3.35 ± 0.35**	99.99 ± 2.10

The number of implantation sites was not affected in females impregnated by males exposed to aluminum chloride. However, the number of viable fetuses was reduced in females impregnated by males exposed to 1000 ppm aluminum chloride. The number of resorptions and number of females with resorptions were significantly increased in females impregnated by males exposed to the selected doses of aluminum chloride which may be attributed to an increase in pre-implantation mortality of unhealthy fertilized ova due to alterations in the sperm quality. Similar studies have shown that ingestion of lead acetate by female mice reduced their fertility, this was reflected in reduced pregnancy rate among the females and increased numbers of early embryonic deaths (Varma *et al.*, 1974). Body weight was significantly reduced in males ingested aluminum chloride in a dose dependent manner.

Table 4: Effect of three, six, nine and twelve weeks ingestion of aluminum chloride on testicular sperm counts and daily sperm production (DSP) of adult male mice

Treatment group Dose	Dose (mg/kg/d)	Testis weight (Mg)[a]	Total sperm/testis[a] (x10^6)	Sperm/mg testis[a] (x 10^3)	Sperm/testis/d[a] (x 10^5; DSP)	Sperm/mg testis/d[a] (X 10^3) Efficiency)
3 weeks						
Control	0	113.2 ± 8.4	4.81 ± 0.40	42.58 ± 3.83	9.94 ± 0.08	8.80 ± 0.79
1400 ppm	130.41 ± 3.18	103.6 ± 7.2	2.42 ± 0.16****	23.43 ± 1.41***	5.00 ± 0.03****	4.84 ± 0.29***
1200 ppm	119.76 ± 2.17	97.0 ± 15.1	4.23 ± 0.27*	44.29 ± 5.83	8.74 ± 0.56*	9.15 ± 1.20
1000 ppm	97.93 ± 1.91	93.4 ± 10.1*	4.38 ± 0.51	47.24 ± 6.63	9.06 ± 1.06	9.76 ± 1.37
6 weeks						
Control	0	106.8 ± 5.1	3.95 ± 0.29	37.06 ± 3.02	8.17 ± 0.61	7.66 ± 0.62
1400 ppm	136.80 ± 2.91	96.7 ± 15.0***	2.16 ± 0.09****	22.69 ± 4.17**	4.46 ± 0.18****	4.69 ± 0.86**
1200 ppm	124.27 ± 3.54	108.0 ± 16.2	2.51 ± 0.25****	23.57 ± 3.46**	5.19 ± 0.52****	4.87 ± 0.71***
1000 ppm	99.01 ± 2.13	98.2 ± 9.5	3.18 ± 0.26**	32.41 ± 1.74*	6.56 ± 0.54**	6.70 ± 0.36*
9 weeks						
Control	0	112.5 ± 81	4.82 ± 0.60	42.21 ± 5.09	9.97 ± 1.24	8.87 ± 1.05
1400 ppm	137.13 ± 2.73	102.4 ± 10.8	2.50 ± 0.18***	24.61 ± 3.27***	5.16 ± 0.37***	5.09 ± 0.67***
1200 ppm	127.36 ± 3.07	107.8 ± 13.1	2.61 ± 0.17***	24.35 ± 1.85***	5.39 ± 0.35***	5.03 ± 0.38***
1000 ppm	97.93 ± 1.91	108.2 ± 13.4	2.77 ± 0.23***	25.75 ± 2.33***	5.73 ± 0.48**	5.32 ± 0.48***
12 weeks						
Control	0	112.0 ± 9.4	4.50 ± 0.14	40.42 ± 4.30	9.30 ± 0.31	8.35 ± 0.89
1400 ppm	136.99 ± 3.22	94.2 ± 6.8*	2.75 ± 0.19****	29.41 ± 3.80**	5.69 ± .39****	6.08v0.79**
1200 ppm	122.91 ± 2.89	100.2 ± 5.5	3.18 ± 0.29****	31.77 ± 2.22**	6.58 ± 0.59***	6.56 ± 0.46**
1000 ppm	101.00 ± 2.09	109.6 ± 14.5	3.29v0.36***	30.54 ± 6.20*	6.79 ± 0.74	6.31 ± 1.28*

Table 5: Effect of three, six, nine and twelve weeks ingestion of aluminum chloride via drinking water on epididymal sperm counts of adults male mice

Treatment group dose	Dose (mg/kg/d)	No. of males	Epididymis weight (mg)* (X 10^6)	Total sperm/ epididymis	Sperm/mg epididymis (X 10^3)
3 weeks					
Control	0	5	31.8 ± 3.1	5.78 ± 0.84	184.28 ± 39.10
1400 ppm	130.41 ± 3.18	5	26.0 ± 3.8*	2.68 ± 0.14***	105.10 ± 17.84**
1200 ppm	119.76 ± 2.17	5	21.8 ± 2.17***	4.58 ± 0.29*	211.06 ± 18.047
1000 ppm	97.93 ± 1.91	5	30.0 ± 2.5	4.82 ± 0.31	162.11 ± 15.22
6 weeks					
Control	0	5	31.2 ± 1.8	4.84 ± 0.36	155.45 ± 13.77
1400 ppm	136.80 ± 2.91	4	26.8 ± 2.2*	2.59 ± 0.32****	97.10 ± 12.70****
1200 ppm	124.27 ± 3.54	5	25.8 ± 3.8*	2.97 ± 0.39****	115.80 ± 11.96***
1000 ppm	99.01 ± 2.13	5	29.2 ± 1.8	3.94 ± 0.41**	135.59 ± 17.05
9 weeks					
Control	0	5	32.8 ± 4.1	6.62 ± 0.54	203.90 ± 27.78
1400 ppm	137.13 ± 2.73	5	23.0 ± 1.9***	2.86 ± 0.12***	125.24 ± 12.02***
1200 ppm	127.36 ± 3.07	5	28.6 ± 2.3	2.91 ± 0.22****	102.19 ± 9.04****
1000 ppm	97.93 ± 1.91	5	30.2 ± 5.2	3.25 ± 0.25****	110.54 ± 22.40****
12 weeks					
Control	0	5	33.0 ± 3.7	5.70 ± 0.79	174.14 ± 26.46
1400 ppm	136.99 ± 3.22	5	28.2 ± 0.8*	3.37 ± 0.41***	119.20 ± 11.27**
1200 ppm	122.91 ± 2.89	5	30.0 ± 5.5	4.14 ± 0.43**	141.08 ± 24.12
1000 ppm	101.00 ± 2.09	5	29.4 ± 3.4	4.29 ± 0.46*	148.45 ± 31.08

It appears that this reduction in body weight gain is a clear indication of general toxicity condition. This magnitude of toxicity, which might for example make the animals somewhat lethargic, which might have affected the animals indirectly rather than having any specific and direct effect on reproductive functions.

Chapin *et al.*, (1993) found that even a 30% decrease in body weight gain had only minimum effect on reproduction in rats. They also showed that the reproductive system of male rats was relatively resistant to body weight reduction down to 70% of control body weight.

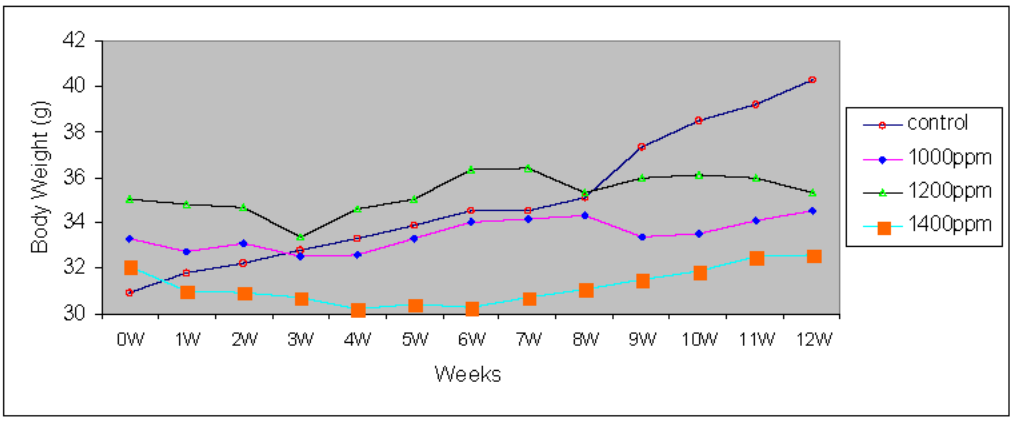

Fig. 1:Effect of twelve weeks ingestion of aluminum chloride via drinking water on body weight of adult male mice. Body weight gain expressed in weight difference (final body weight-initial body weight) was significantly reduce in males igested 1000, 1200 and 1400 ppm $AlCl_3$ (p, 0.0001) comparable to the control

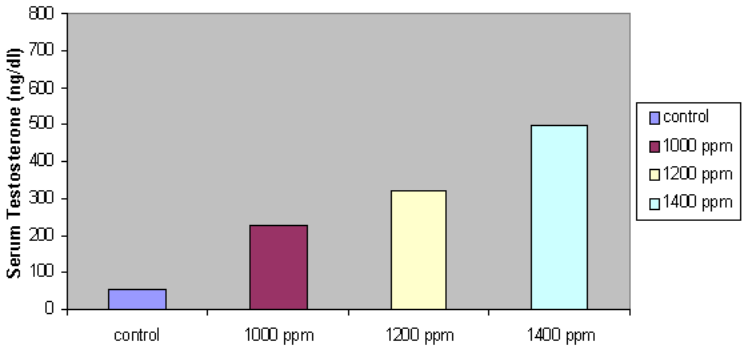

Fig. 2: Effect of twelve weeks ingestions of aluminum chloride via drinking water on serum testosterone levels (ng/dl) of adult male mice. *P< 0.01 vs. Control. Values are expressed as mean ± SD for 5 animals

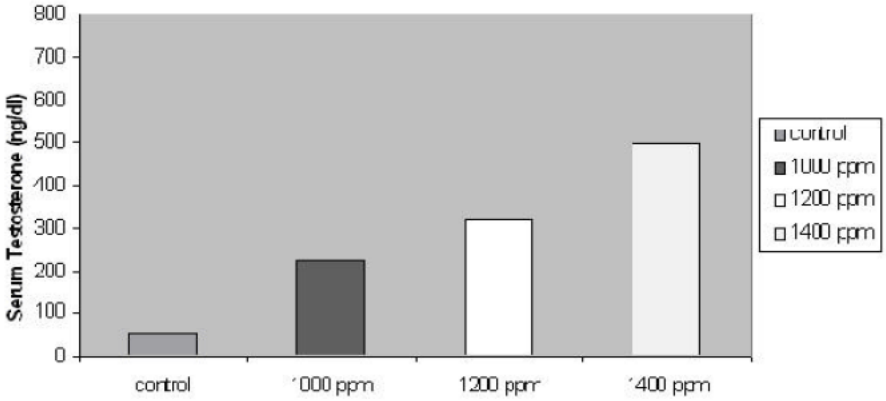

Fig. 3: Effect of twelve weeks in gestiono f aluminum chloride via drinking water on serum LH levels (MIU/ml) of adult male mice. *P<0.005 vs control. Values are expreseed as mean ±S. D. for five animals

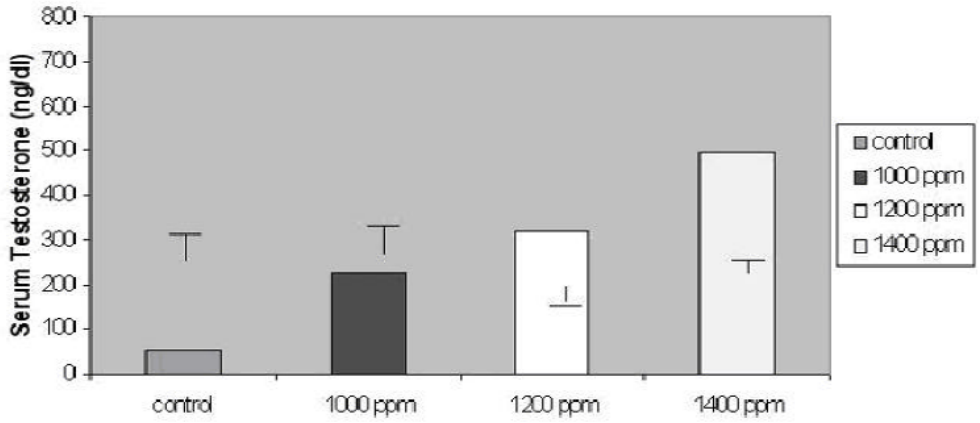

Fig. 4: Effect of twelve weeks in gestion of aluminum chloride via drinking water on serum FSH levels (mIU/ml) of adult male mice. Values are expressed as mean ±SD for 5 animals

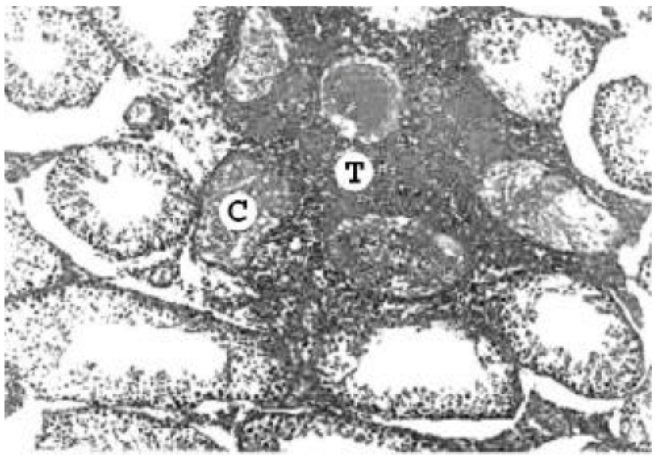

Fig. 5: Relatively large necrotic area convering five tubulse (T) with replacement by connective tissue inside the seminiferous tubules © in male ingested 1400 ppm. Mag. (207X). H and Estain

Fig. 6: Complete destruction of the seminiferous tubules and degnerative cells (a) with congested blood vesssls (b) in male ingested 1200 ppm. Mag. (207X) H and E stain

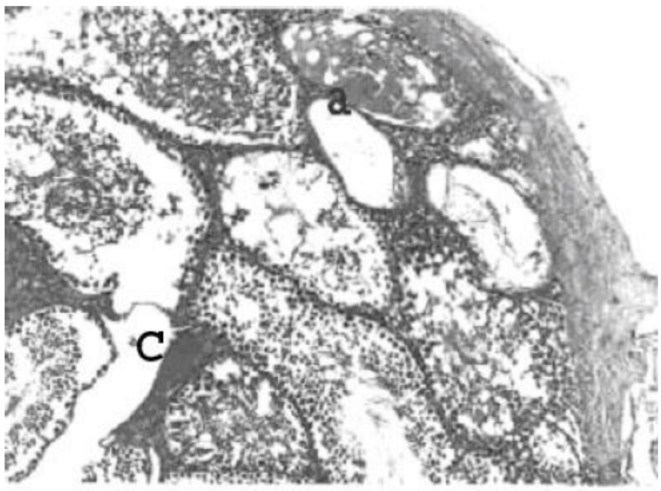

Fig. 7: Layers of cells in the wall of seminiferous tubules completely destroyed (A) with increased interstitial connective tissue in male ingested 1400 ppm. Mag. (207X). H and E stain

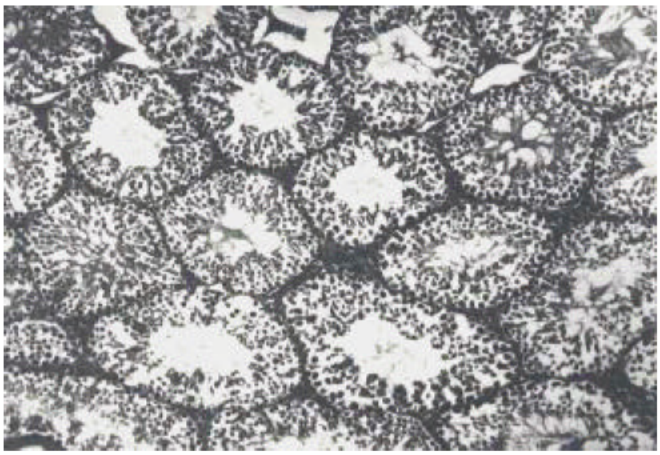

Fig. 8: Seminiferous tubules of the control male mice with little interstitial connective tissue and normal distribution of cellular elements. Mag. (207X) H and E stain

Liobet *et al*. (1995) noticed that short-term exposure to 50-200 mg/kg/day aluminum nitrate reduced body and testicular weights in treated animals.The effect of exposure to aluminum chloride on seminal vesicle and preputial gland weights reflects the effect on sex hormones. The size and activity of the preputial gland in rodents are clearly influenced by a variety of steroid hormones. This gland produces behavior modulating phermones that alter fighting and other behavior (Ebling, 1963).

The results presented in this work are in agreement with the data published by Sokol (1990) on lead toxicity. The reduction in the accessory gland weight might suggest an alteration in the pattern of testosterone secretion.

Data concerning reproductive system presented in this work are consistent with previous work using different metals reported by other researchers (Elbetieha and Al-Hamood, 1997).

In evaluating the time sequence of spermatogenesis and movement of sperm through the male reproductive system (testis, epididymis), the testis was the first to show a change in function as indicated by decreased testicular weight after six weeks of aluminum treatment. Decreased testicular weight is an indication of reduced testicular function (reduced spermatogenesis). After three weeks of aluminum chloride treatment, the reduction in the concentration of epididymal sperm reflected the prior decrease in spermatogenesis. Consequently, the decrease in spermatogenesis resulted in a decrease in fertility.

Ingestion of 1200 and 1400 ppm aluminum chloride by adult male mice caused a significant increase in the serum

level of testosterone and a significant increase in LH serum level in males ingested 1400 ppm while the FSH serum level did not change. The result suggest a direct effect of aluminum chloride ingestion on testicular leydig cells in which testosterone is produced. Also, aluminum chloride ingestion could have affected the function of the hypothalamus. This may result in alteration of gonadotropin releasing hormone (GnRH) levels which may affect LH secretion from the anterior pituitary lobe, which in turn, will affect Leydig and Sertoli cells thus altering testosterone production, which is concordant with the finding of Pedigo *et al.* (1988) on ingestion of cobaltous chloride by adult male mice. Disturbance in testosterone and LH hormone levels after ingestion of aluminum chloride suggested in the premature release of spermatids that may reduce testicular and epididymal sperm counts.

The results of the present study are consistent with those obtained by other studies, which confirmed that aluminum and other metals have obvious adverse effects on adult male reproduction and fertility (Liobet *et al.*, 1995).

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