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Toxicity Evaluation of Aluminium Chloride on Adult Female Mice

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Abstract: Twelve weeks ingestion of aluminium chloride via drinking water by adult non-pregnant female mice induced adverse effects on reproduction and fertility. Aluminium chloride induced a significant decrease in pregnancy rate and a significant increase in number of females with resorptions and in number of resorptions. Furthermore, histological changes in ovarian sections from adult non-pregnant female mice confirmed the reproductive toxicity of aluminium chloride. Highly congested blood vessels all over the ovary, with large number of atretic follicles at different stages of development have been seen. Also, highly congested blood vessels with necrotic and degenerative area in liver and kidney sections was observed. Intraperitoneal injection of aluminium chloride during day 0-6 of pregnancy caused a significant increase in pregnancy failure, number of females with resorptions and number of resorptions. Besides, a significant decrease in number of viable fetus and number of implantation site were recorded. The results indicated that ingestion of aluminium chloride by adult female mice would cause adverse effects on fertility and reproduction.

Key words: Aluminium chloride, liver, uterus, toxicity, female mice

INTRODUCTION

Oral aluminium (Al) exposure is a consequence of the element presence in food and drinking water and its use in therapeutic preparations administered in large quantities as phosphate binders, antacids and buffered aspirins (Cannata and Domingo, 1989). Until recently, there was little concern about toxic consequences of Al ingestion because it was assumed that Al was not orally biovailable.

Aluminium 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 aluminosilicates associated with dust particles. The most common foods with substantial amounts of aluminium 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 aluminium has been estimated to be 3.5-51.6 mg (Underwood, 1977) and it is estimated that 20% of the daily intake of aluminium comes from aluminium cooking utensils such as pans, pots, kettles and trays.

Aluminium is absorbed through the skin, gastrointestinal tract, lung and nasal mucosa. After absorption, most aluminium is transported in serum by transferrin. Bone, muscle and lung contain the highest aluminium content in the normal human being. Aluminium uptake by the brain is linked to high affinity transferrin receptors (Anane *et al*., 1997).

Aluminium 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; Agarwal *et al*., 1996).

Most foods contain small but variable amounts of Al. it has been reported that 2-3 mg Al daily is probably the lower limit of Al naturally present in western diets (Domingo, 1995; 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 5 g of Al daily from these compounds.

The aim of the present study was to monitor the adverse effects on fertility and reproduction of aluminium chloride ingested with drinking water by female mice. This study was designed to detect the effect of aluminium chloride on reproduction and fertility in the female mice.

MATERIALS AND METHODS

Animals: Sexually mature female Swiss mice (50 days old) were used in these experiments. They were provided by

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the Animal House Unit in the Faculty of Medicine at Jordan University of Science and Technology. Animals were maintained in controlled temperature 21±1°C under a 12 h light: 12 h darkness schedule (light 06:00-18:00 h). Food and tap water were offered ad libitum.

Administration of aluminium chloride: Aluminium 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^{-1}), 1200 ppm (1200 mg L^{-1}) and 1400 ppm (1400 mg L^{-1}). The duration of exposure of female mice was 12 weeks. Control female mice were given tap water. Effect of Aluminium chloride on fertility of adult female mice.

Effect of aluminium chloride on female fertility: Adult female mice were exposed for 12 weeks to 1000, 1200 and 1400 ppm aluminium chloride in drinking water (10 animals per group). Ten control female mice were given tap water.

Treated females and their control counterparts were divided randomly into groups of 2 animals each and housed with a sexually mature untreated male of proven fertility for 10 days. During this period, 2 estrus cycles at least should have elapsed (Rugh, 1968). After the removal of untreated males, the treated females and their control counterparts 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 resorption, number of females with resorptions and fetal body weight.

Effect of aluminium chloride on body and organ weights of adult female mice: Ten treated females for each concentration and 10 control females were sacrificed after 12 weeks of Aluminium chloride ingestion. The ovaries and uteri were excised and weighed. Initial and final body weights were recorded for treated and control females.

Effect of aluminium chloride on pregnancy of female mice
Examination of pregnancy occurrence in female mice: Virgin female mice were left one night with male mice of proven fertility. Males were placed in female cages around 16.00 h. The presence of copulation plug or sperms in the vaginal smears on the following morning was regarded as day 0 of pregnancy.

Administration of aluminium chloride: After mating, pregnant females were randomly distributed to experimental groups (10 females) sequentially; one group was the control. Intraperitoneal injection of aluminium chloride was performed at around 10.00 am from day 0-6 of pregnancy at a total volume of 0.1 mL with a concentration of either 38 mg Kg day^{-1} or 19 mg Kg day^{-1}. The dose for each animal group was selected according to the LD_{50} value for aluminium chloride (LD_{50} for aluminium chloride is 380 mg kg^{-1}) (Cannata and Domingo 1989). Ten control female mice received an equal volume of tap water through the same route and duration.

Dose preparation: The LD50 values for aluminium chloride ($AlCl_3$) following oral administration were 380 mg Kg^{-1} in mice (Cannata and Domingo, 1989):

- Dose 1 was 1/10 of the LD50 = 38 mg kg day^{-1}.
- Dose 2 was 1/20 of the LD50 = 19 mg kg day^{-1}.

Control pregnant female mice were given tap water by intraperitoneal injection.

Effect of administration of aluminium chloride on pre-and post-implantation periods (day 0-6 of pregnancy) in female mice: Effects on pre-and post-implantation were assessed by intraperitonial injection of aluminium chloride from day 0-6 of pregnancy. All animals were sacrificed on day 20 of gestation under light ether anesthesia. During autopsy, the following measurements were recorded: number of implantations, viable fetuses, maternal weight on day 0 and 18 of gestation, fetal body weight and number of resorptions along the whole length of the uterine horns.

Histological evaluation of the effect of aluminium chloride on adult female mouse ovary: The excised ovary was fixed in 10% formalin solution then processed for histology. 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 aluminium exposed groups were analyzed using Student t-test for the mean value, Chi-square and Fisher exact test for the analysis of the number of pregnant females, females with resorptions and the number of resorptions.

RESULTS

Effect of aluminum chloride on fertility of adult female mice: Twelve weeks ingestion of aluminium chloride via

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

Treatment groupdose*	No. of males	No. (%) of pregnant females	No. of implantation site[a]	No. of viable fetuses[a]	Total no. of resorptions/ total no. of implantation site	No. (%) of animals with resorptions
Control tap water	5	10/10 (100.0)	6.70±2.11	6.60±2.17	1/67	10/1(10.0)
Aluminium chloride (1400 ppm)	5	4/9* (44.4)	7.75±0.96	5.50±1.91	9/31***	4/4**(100.0)
Aluminium chloride (1200 ppm)	5	5/10* (50.0)	5.40±2.07	3.40±2.07*	10/27****	5/5**(100.0)
Aluminium chloride (1000 ppm)	5	5/10* (50.0)	7.20±1.92	6.00±1.22	7/36**	4/5*(80.0)

[a]Results are expressed as mean±S.D.; [a]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 aluminium chloride via drinking water on weights of reproductive organs of adult female mice

Treatment group dose*	No. of females	Body weight (B.wt.)(g)[a]	Absolute uterus weight (g)[a] (mg/10 g B.wt.®)	Absolute ovaries weight (g)[a] (mg/10 g B.wt.®)
Control Tap water	10	40.90±1.49	0.032±0.006 (7.82±1.72)	0.18±0.05 (44.14±10.99)
Aluminium chloride (1400 ppm)	9	33.80±1.35**	0.038±0.004* (11.25±1.15**)	0.16±0.03 (47.53±8.34)
Aluminium chloride (1200 ppm)	10	36.70±1.29**	0.032±0.01 (9.00±2.66)	0.16±0.06 (43.69±16.40)
Aluminium chloride (1000 ppm)	10	33.60±1.23**	0.032±0.006 (9.53±1.68)	0.13±0.06* (38.90±16.67)

[a]Results are expressed as mean±S.D. ; ® Relative weights. ;* p<0.05, ** p<0.001 (Student t-test).;* Actual dose consumption is presented in Table 3

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

Treatment group dose	Body weight (g)[a]	Water consumption (mL)[a]	Actual dose consumption (mg kg d^{-1})
Controltap water	36.10±3.54	4.51±0.68	0
Aluminium chloride (1400 ppm)	31.80±1.05***	2.65±0.87****	116.78±3.86
Aluminium chloride (1200 ppm)	34.25±1.41	2.68±0.51****	94.37±3.80
Aluminium chloride (1000 ppm)	30.39±1.58***	2.27±0.48****	74.87±3.75

[a]Results are expressed as mean±S.D.*** p<0.005, **** p<0.0001 (student t-test)

drinking water by adult female mice, revealed a significant reduction in fertility of females ingested 1000, 1200 and 1400 ppm $AlCl_3$ ($p<0.05$). The number of implantation sites was not affected in females ingested $AlCl_3$. The number of viable fetuses was significantly reduced in females ingested 1200 ppm $AlCl_3$ ($p<0.05$). The number of resorptions was increased significantly in females ingested1000, 1200 and 1400 ppm $AlCl_3$ ($p<0.005$, $p<0.0001$ and $p<0.001$, respectively). The number of females with resorptions was significantly increased in females ingested 1200 and 1400 $AlCl_3$ ($p<0.005$) and in females ingested 1000 ppm $AlCl_3$ ($p<0.05$) (Table 1).

Both the absolute and relative ovarian weights was significantly increased in females ingested 1400 ppm aluminium chloride ($p<0.05$ and $p<0.001$, respectively). The absolute uterine weight showed a significant reduction in females ingested 1000 ppm $AlCl_3$ ($p<0.05$) (Table 2).

Average body weight was significantly reduced in females ingested 1200 and 1400 ppm aluminium chloride ($p<0.005$). Water consumption was decreased significantly in females ingested 1000, 1200 and 1400 ppm $AlCl_3$ ($p<0.0001$) (Table 3). Body weight gain expressed in weight difference (final body weight-initial body weight) was significantly reduced in females ingested 1000, 1200 and 1400 ppm $AlCl_3$ ($p<0.0001$) (Fig. 1).

Effect of aluminium chloride on pregnancy in female mice: Pregnant female mice injected intraperitoneally with

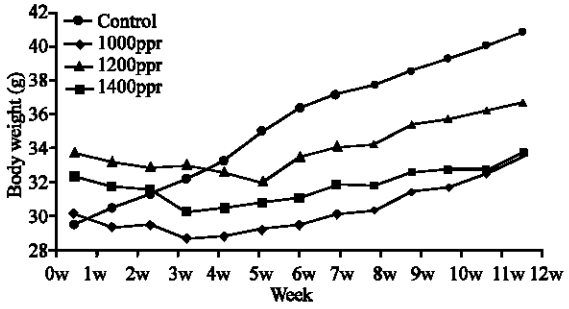

Fig. 1: Effect of 12 weeks ingestion of aluminium chloride via drinking water on body weight of adult female mice. Body weight gain expressed in weight difference (final body weight-initial body weight) was significantly reduced in females ingested 1000, 1200 and 1400 ppm aluminium chloride ($p<0.0001$)

38 mg kg d^{-1} aluminium chloride showed sluggishness, paralysis, decrease in body weight, diarrhea and death.

Effect of aluminium chloride on pre-and post-implantation stage (0-6 day of pregnancy) in female mice: Aluminium chloride was administered by intraperitoneal injection to pregnant females at different doses after successful mating from day 0-6 of pregnancy. This period corresponds to both pre-and post-implantation stage. In pregnant females injected with 38 mg Al kg d^{-1} a complete absence of implantation sites was found in 5 of the 6

Table 4: Effect of intraperitoneal injection of aluminium chloride on pre- and post-implantation (day 0-6) in adult female mice

Treatment group dose	No. of Mated females	No.(%) of dead females	No. (%) of pregnant females	No. of Implantaion Site[a]	No. of viable fetuses	Total no. of resorptions/total no. of implantation site	No. (%) of animals with resorptions
Control tap water	10	0 (0)	10/10 (100.0)	8.30±3.12	8.30±3.12	0/83	0/10 (0)
Aluminium chloride (38 mg kg^{-1})	10	4 (40.0)	1/6** (16.6)	3.00±0.00**	-	3/3***	1/1 (100)
Aluminium chloride (19 mg kg^{-1})	10	0 (0)	6/10* (60.0)	6.67±1.03	5.17±2.06*	9/40***	5/6** (83.3)

[a]Results are expressed as mean±S.D;*p<0.05, **p<0.0001 (student t-test);*p<0.05, **p<0.005, ***p<0.0001 (fishers exact test)

Table 5: Effect of intraperitoneal injection of aluminium chloride on body weight gain and weight of fetus and placenta in adult female mice

Treatment group dose*	Initial body weight[a]	Final body weight[a]	Body weight difference[b]	Fetal body weight[a]	Placental weight[a]
Controltap water	28.40±3.13	48.05±5.93	18.66±5.40	1.20±0.39	0.167±0.017
Aluminium chloride (38 mg kg^{-1})	28.00±2.49	27.63±2.61***	-1.70±3.27***	-	-
Aluminium chloride (19 mg kg^{-1})	26.40±1.65	34.55±6.65**	8.16±6.09**	1.01±0.37	0.152±0.010

[a]Results are expressed as mean±S.D.; [b]Body weight difference (final body weight - initial body weight)**p<0.001, ***p<0.0001 (student t-test)

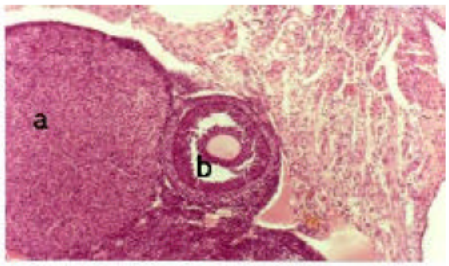

Fig. 2: Ovary section of the control adult female mice showing normal corpus luteum (a) with mature Graafian follicle (b). Mag. (207X). H and E stain

surviving females ($p<0.005$) and in 6 of 10 pregnant females injected with 19 mg Al kg d^{-1} $AlCl_3$ ($p<0.05$) comparable to the control (tap water). The total number of resorptions was significantly increased in pregnant females injected with 38 and 19 mg Al kg d^{-1} $AlCl_3$ ($p<0.0001$). A complete absence of viable fetuses was reported in pregnant females injected with 38 mg Al kg d^{-1} $AlCl_3$ and a significant decrease in the number of viable fetuses in pregnant females injected with 19 mg Al kg d^{-1} $AlCl_3$ ($p<0.05$) (Table 4).

During the period of exposure a significant reduction in final body weight was observed in pregnant females injected with 38 mg Al kg d^{-1} ($p<0.0001$) and in pregnant females injected with 19 mg Al kg d^{-1} aluminium chloride ($p<0.001$).

Body weight difference decreased significantly in pregnant females injected with 38 mg Al kg d^{-1} ($p<0.0001$) and in pregnant females injected with 19 mg kg d^{-1} $AlCl_3$ ($p<0.001$) (Table 5).

Effect of aluminium chloride on ovary of adult female mice: All histological sections of the ovary of treated females ingested 1400 ppm aluminium chloride exhibited some sort of changes compared to the control females.

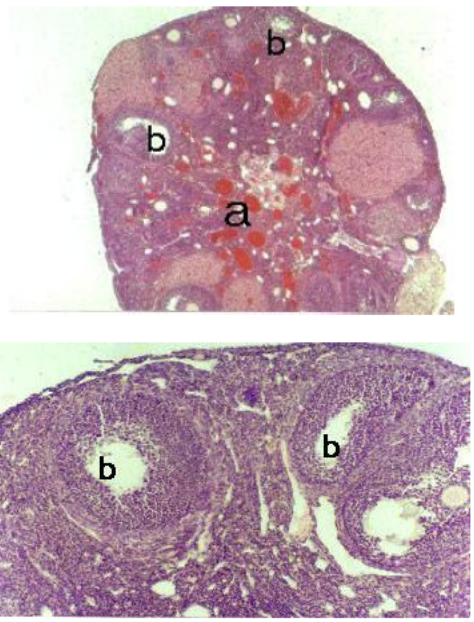

Fig. 3: Highly congested blood vessels all over the ovary (a) with large number of atretic follicles at different stages of development (b) in female ingested 1400 ppm. A. Mag. (82X). B. Mag. (207X). H and E stain

Highly congested blood vessels were seen all over the ovary with large number of atretic follicles at different Highly congested blood vessels were seen all over the ovary with large number of atretic follicles at different stages of development when compared to the control group (tap water) (Fig. 2 and 3).

DISCUSSION

The aim of the present study was to monitor the adverse effects on fertility and reproduction of aluminium chloride ingested with drinking water by female 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 aluminium chloride for 12 weeks by adult female mice had adverse effects on female reproductive system and fertility.

There were, increased numbers of non-pregnant females in females ingested selected doses of aluminium chloride. The number of viable fetuses was significantly reduced while the number of resorptions and the number of females with resorptions were increased.

Embryonal resorptions may indicate transplacental passage of aluminium chloride to the embryo in addition to modifications of the uterine lining function before the arrival of the embryo that restricts the development of implanted embryo. Thus, the results presented in this study emphasis the abortifacient or fetal resorptive properties of aluminium chloride. Increase in resorptions may be attributed to an increase in pre-implantation mortality of zygotes. Maternal toxicity invariably causes increased early resorptions, reduction in fetal body weight or late fetal death.

The deleterious effect of aluminium chloride on female mouse fertility observed in this work suggests a disturbance of reproductive endocrine functions (multiple sites of toxicity along the hypothalamic-pituitary-ovarian-uterine axis are possible). Leonard *et al.* (1986) published evidence suggesting direct ovarian toxicity from lead which decreases the secretion of progesterone responsible for endometrial alteration at the time of implantation. Furthermore, Wide (1980) stated that in addition to possible effects on progesterone secretion, lead also alters uterine estrogen receptors which may have further impact on the maintenance of pregnancy.

Intraperitoneal injection of aluminium chloride from day 0-6 of pregnancy caused mortality with the highest dose used, pregnancy failure, increase in number of females with resorptions and number of resorptions in all selected doses of aluminium chloride.

Data presented in this work are in agreement with previous studies. Subcutaneous injection of manganese chloride from day 6-15 by pregnant female mice revealed significant reductions in weight gain, as well as several treatment related deaths in the high dose group. A significant increase in the number of late resorptions was found in the 4, 8, 16 mg kg d^{+} (Sanchez *et al*., 1993).

The uterus is in a receptive state during the late day 3-4 of pregnancy. Normally, the implantation of conceptus occurs on gestation day 4-5 in rodents (Hodgen and Itskovitz, 1988). Chemical insult prior to completion of the implantation process (day 1-3 of pregnancy) resulted in the decrease in implantation sites. Whereas, insult on (day 4-6 of pregnancy) might result in post-implantation loss of embryos (resorptions of implantation sites). Aluminium chloride was administered between day 0-6 of pregnancy in mice. This corresponds to the period, which begins after fertilization and involves the stages before and after implantation.

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