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SUBCHRONIC TOXICITY OF ALUMINUM ON MALE FERTILITY OF ALBINO RATS

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ABSTRACT

The present study was carried out to investigate the toxicity of of aluminum chloride hexahydrate administered daily at 20 and 100 ppm through drinking water for 90 consecutive days on male albino rats. The animals were left for 30 days after withdrawal the tested insecticide to allow recovery from toxicity. The spermatozoal examination, hormones assay and histopathological examinations were carried out. Moreover, the weights of testes and accessory glands were detetrmined. Treatment with aluminum chloride hexahydrate caused no effect on testis weight while reduced significantly seminal vesicle and prostate gland weights after 90th day at tested two doses. Sperm motility significantly declined after 90 days of treatment with the two tested concentrations and returned to normal values after recovery periods. Also, sperm concentration showed significant decrease after 90th day of treatment. Oral administration with aluminum chloride hexahyrate decreased pregnancy rate and litter size per female available at the two dose levels. Testosterone level showed no significant changes at the two doses. Also through microscopical examination, testes showed mature seminiferous tubules and complete series of spermatogenesis in treated albino rats.

Key words: Subchronic toxicity-Aluminum- Fertility- Rats.

INTRODUCTION

Normal reproductive function is often assessed by examining the concentration of sperm in semen, as well as the motility and histologic appearance of individual spermatozoons. There are several sites involved in the process of spermatogenesis at which toxicants may act. Because the process is under hormonal control, interference with the secretion of GnRF, LH, FSH, or testosterone could have an impact. Estrogens and progestins block spermatogenesis by suppressing LH and FSH, and thus testosterone secretion, **(Karen and Thomas, 1996).**

Liobet *et al* (1995) studied, i.p. treatment of aluminum nitrate in adult male mice at doses of 0, 50, 100, and 200

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mg/kg/day for 4 weeks before mating with untreated females. Decreased pregnancy rate was observed in the females mated with males previously treated with 100 or 200 mg/kg/day of aluminum nitrate. High-dose male mice showed significantly decreased testicular and an epididymal weight, as well as significant decreases in testicular and Spermatid counts and epididymal sperm counts. Spermatid counts were also reduced at 100 mg/kg/day. However the sperm motility was unaffected. Histological changes, including necrosis of spermatocytes/ spermatids, were observed in the testes of male mice treated with 100 and 200 mg/kg.

Batalneh *et al* (1998) investigated the treatment with solutions of industrial metals salts (manganese sulfate, aluminum chloride, lead acetate and copper chloride) in drinking water at a concentration of 1000 ppm for 12 weeks. Fertility was reduced in male rats ingested with lead acetate. The total number of resorption was increased in female rats impregnant by males ingested with manganese sulfate and lead acetate. Body, absolute or relative testes, seminal vesicles weights were dropped in adult male rats ingested with all tested metals.

Earl *et al* (1998) investigated the comparison between semen levels of lead, cadmium, and aluminum in relation to live sperm in semen samples from 64 apparently healthy men. They reported that, presence of these metals in the environment and in seminal plasma exerts a toxic effect on sperm.

Earl *et al* (2000) compared the seminal plasma trace metal levels of hospital workers with groups of industrial workers in a petroleum refinery, smelter, and chemical plant. The seminal plasma levels of the toxic metals lead and aluminum were increased in each of the industrial groups ($P < 0.001$).

Cristian *et al* (2001) reported that the reference values for trace and ultrace elements concentration in healthy human serum, measured by double- focusing inductively coupled plasma- mass spectrometry were showed high levels of Al, Cr, Sr, Mo, Mn, Pb, U, Co, and Cu and low levels of Fe, Zn and Rb in serum samples from hemodialysis patients compared to the corresponding reference values.

Petrelli *et al* (2001) reported the results of study on fertility of couples in whom the man was occupationally exposed to metals caused delay in conception at the time of their wives' first pregnancy.

Ratna and Singh (2001) reported that young male albino mice were exposed to nickel by oral route of 20mg nickel sulfate /kg b.wt. for 5 d/wk for 6 mo. A decrease in normal (testosterone-dependent) proteinuria was shown, and morphological examination of the seminal vesicles revealed a lower weight and smaller size as well as a histological indication of lower secretory activity of the epithelium compared to controls.

Sheiner *et al* (2003) investigated the influence of working conditions and occupational exposures to potential chemical during work on male fertility. Significant associations were reported between impaired semen parameters and some chemical exposures such metals (lead, mercury).

The present study aimed to clarify the subchronic toxicity of aluminum chloride hexahydrate administered daily at 20 and 100 ppm through drinking water on male fertility of albino rats.

MATERIAL AND METHODS

1. Aluminum chloride hexahydrate

Empirical formula: AlCl3. 6H2O.
Molecular weight: 241.43
The salt was obtained from Brixworth. Northats. V.K. (Sigma).

2- Animals and procedures

Thirty six male albino rats of an average weight 130±10g were used in the present study. The animals were acclimatized to the laboratory conditions in stainless cages for 2 weeks. Water provided ad libitum with balanced diet during the treatment. They were allocated into 3 groups of 12 animals each and treated daily through drinking water as follows:

Group A was kept as normal, groups B & C were administered with Aluminum chloride hexahydrate at concentrations of 20 and 100 ppm, respectively. Daily administration was continued for 90 days. After the experimental period, the remaining animals were left for 30 days without treatment. On days 90 and 120, blood samples were collected by orbital sinus vein technique by using heparinized capillary tubes according to Schalm (1986) to assay the testosterone level on plasma using the coat-A-count technique kits purchased from Diagnostic Products Corporation (DPC), Los Angeles, USA.

On day 90 three male rats from each experimental group were taken and kept with mature female for 7 days and separated. Then, the pregnant females and the pups were counted to calculate the pregnancy rate, average litter size per female and average litter size per group. The progressive motility of sperms and sperm cell concentration were measured according to Bearden and Fuquay 1980.

Testes of rats were examined grossly and specimens were taken and fixed in 15% formalin saline for histopathological examination. The paraffin sections were stained by haematoxylin and eosin for histopathological examination (Humason, 1979).

RESULTS AND DISCUSSION

Data in Table (1) indicated that oral administration of aluminum chloride hexahydrate caused no significant decrease in the testes weights after treatment with 20 ppm and no significant increase was proved after recovery period. While the treatment with the higher dose of 100 ppm showed no difference after 90 days or recovery period.

A highly significant decrease ($P<0.001$) occured in seminal vesicle weights (Table 1) after 90 days with 20 and 100 ppm. No significant decrease was shown after withdrawn the tested compound.

Regarding to the effect of aluminum chloride hexahydrate, prostate gland weights were significantly declined ($P<0.001$) with 20 and 100 ppm on 90th day. In this respect, there was no significant decrease after recovery period (Table 1). Similar findings were reported by Juan *et al* (1995) who stated that i.p. treatment with aluminum nitrate for 4 weeks into mouse decreased fertility at 100 and 200 mg/kg/d. Testicular spermatid and epididymal sperm counts indicated that spermatogenesis was depressed at 200 mg/kg/d.

Table 1. Effect of aluminum chloride hexahydrate on weight of testes and accessory glands in male albino rats.

Organs	Concentrations (ppm)	Treated (90 days) MEAN±S.E.	Weight%	Recovered (30 days) Mean±S.E.	Weight %
Testis	Control	5.7500±0.1820	1.016	5.3020±0.1117	0.856
	20	5.5800±0.1906	0.955	5.4075±0.2256	0.891
	100	5.7080±0.1273	0.993	5.3588±0.1180	0.934
Seminal vesicle	Control	3.5628±0.0627	0.390	4.0179±0.0322	0.481
	20	2.8724***±0.1045	0.285	3.7275±0.2269	0.428
	100	2.9512***±0.0890	0.293	3.8672±0.0802	0.430
Prostate gland	Control	1.2060±0.0304	0.033	1.0448±0.0343	0.035
	20	0.9180***±0.0440	0.027	0.9800±0.0981	0.029
	100	0.9100***±0.0414	0.027	0.9964±0.0064	0.030

***P<0.001

Table 2. Effect of aluminum chloride hexahydrate on sperm motility and concentration in male albino rats.

Parameters	Concentrations (ppm)	Treated (90 days)	Recovered (30 days)
sperm motility	Control	75.946±1.097	77.272±0.192
	20	54.382***±1.474	77.533±0.277
	100	65.536***±0.862	78.996±1.173
sperm concentr-ation (10^6/ml)	Control	46.60±1.077	45.25±1.462
	20	40.44*±1.665	46.50±2.559
	100	41.50**±0.802	45.88±1.078

*P<0.05 **P<0.01 ***P<0.001

Data in **Table (2)** revealed that aluminum chloride hexahydrate caused highly significant decline ($P<0.001$) in sperm motility after 90 days with 20 and 100 ppm and returned to normal values after recovery periods. A significant decrease in sperm concentration was occurred after 90 days ($P< 0.05$) with 20 ppm and ($p< 0.01$) with 100 ppm. In addition there was no significant increase in sperm concentration with 20 and 100 ppm after recovery periods.

Our findings are in accordance with those of **Wang *et al* (1992)** who recorded that moderate lead exposure in men caused qualitative changes in semen parameters, indicating direct or indirect effects of lead on accessory genital function and sperm maturation. Also, **Kamel *et al* (1993)** found that 100 µg of lead acetate/kg.b.wt. in rats caused significant reduction in relative testicular weights. Also **Pandey and Srivastava (2000)** reported that absolute and relative weights of testes, seminal vesicle and prostate gland were significantly decreased at 20mg nickel/kg treated mice. Dose dependant changes in sperm motility and count were observed at 10 and 20 mg/kg groups.Successive oral administration of aluminum chloride hexahydrate produced marked reduction in pregnancy rate of treated animals at 20 and 100 ppm. Concerning to litter size per pregnant female, a decrease patterns with 20 ppm and an increase with 100 ppm was found. While, litter size per female available was declined after prolonged treatment with 20 and 100 ppm, **(Table 3).**

Table (4) indicated no significant decrease in testosterone level after 90th day of treatment with 20 ppm of aluminum chloride hexahydrate. No significant increase was noticed after recovery period of the same treatment. Concerning testosterone level after 90 days with 100 ppm, no significant increase was recorded.

Table 3. Effect of aluminum chloride hexahydrate on male rats for 90 successive days on pregnancies, litter size per pregnant female and litter size per female available.

Concentration (ppm)	Pregnancy rate (%)	Litter size/ pregnant female	Litter size/female available
Control	83.33	7.00	5.83
20	66.67	6.75	4.50
100	33.33	8.50	2.83

Table 4. Effect of aluminum chloride hexahydrate on testosterone level (ng/ml) in male albino rats.

Concentration (ppm)	Testosterone level (ng/ml)	
	Treated (90 days)	Recovered (30 days)
Control	0.8518±0.2994	1.3324±0.0962
20	0.5868±0.2195	1.3923±0.2601
100	1.6398±0.5765	1.013±0.1839

Microscopically, the testis showed mature seminiferous tubules and complete series of spermatogenesis in rats treated with 20 and 100 ppm of aluminum

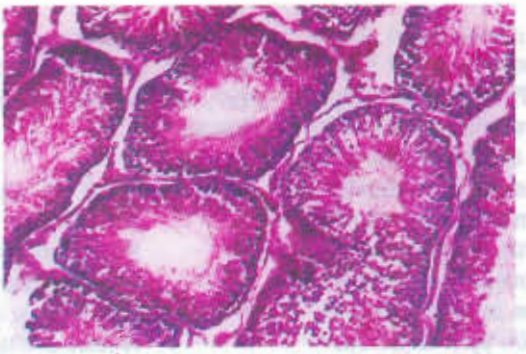

Fig. 1. Cross section in testis of rat treated with aluminum chloride hexahydrate at dose level of 20 ppm for 90 days showed normal mature seminiferous tubules (H & E x 40)

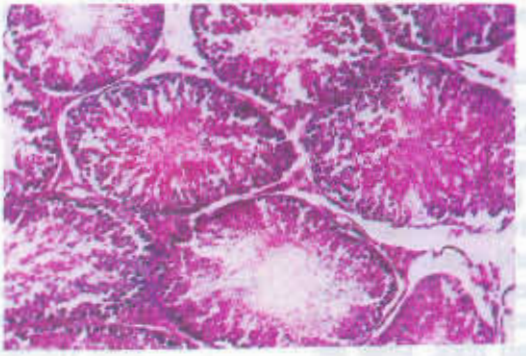

Fig. 2. Cross section in testis of rat treated with aluminum chloride hexahydrate at dose level of 100 ppm for 90 days showed mature seminiferous tubules (H & E x 40)

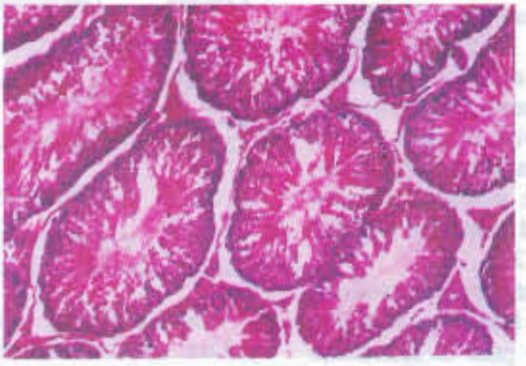

Fig. 3. Cross section in testis of rat kept as control showed normal mature seminiferous tubules (H & E x 40)

chloride hexahydrate for 90 days (**Figs. 1 and 2**) and recovery period compared with the un tested chech (**Fig. 3**).

Our results are in accordance with **El-Massarawy (1997)** who stated that treatments of male rats with lead, zinc, copper and metal mixture have no effect on plasma testosterone level. Similar results were reported by **Nathan *et al* (1992)**, but disagree with **Sokol (1990) and Sokol & Berman (1991)** who noticed that, lead produced suppression in testosterone level.

Also **Seth *et al* (2000)** reported that aluminum treated rats for 20 weeks exhibited a significant decrease in serum testosterone level and sperm counts. Also, they reported that, aluminum caused testicular damage.

The findings in our study are similar to those of **El-Massarawy (1997)** who reported that lead didn't alter plasma testosterone level through 56 days in rats.

The experiment involved prolonged treatment of animals and oral administration of toxic metal in distilled water, both those factors are able to enhance toxicity **(Environmental Health Criteria, EHC (1991).**

Heavy metals are well-known reproductive toxicants **(Karen and Thomas, 1996)**. In general, the effect of aluminum chloride hexahydrate on male fertility wasn't clear. It may due to the degree of protection which appears in testes against toxicants by what is termed the blood testes barrier or tight junctions between the sertoli cells in the seminiferous tubules from a barrier, which prevents many substances from entering the areas where spermatozoa are developing. Also, the testes have metabolic capability in the form of cytochrome P450 activity, and the cells, which will give rise to spermatozoa, have at least some DNA repair capabilities.

REFERENCES

Bataineh, H.; M.H. Al-Hamood and A.M. Elbetieha (1998). Assessment of aggression, sexual behavior and fertility in adult male rat following long-term ingestion of four industrial metals salts . *Hum. Exp. Toxicol. Oct., 17(10): 570-576.*

Bearden, H.I. and J. Fuquay (1980). Applied Animal Reproduction *pp. 158-160.* Resor Published Co.,Inc., Reston, Virgina.

Cristian, S.M.; L.F.M. Jose; M.M.G. Juan; I.G.A. Jose; B.C.A. Jorge and S.M. Alfredo (2001). Reference values for trace and ultrace elements in human serum determined by Double-Focusing ICP-MS. *Biological Trace Element Research, (82): 259-272.*

Earl, B.D.; R. Sherry; A.H. William; R.E. Douglas and C.P. Leslie (1998): Comparison of sperm viability wit seminal plasma metal levels .*Biological Trace Element Research, (64): 215-219.*

Earl, B.D.; R.E. Douglas; A.H. William and C.P. Leslie (2000): Seminal plasma trace metal levels in industrial workers. *Biological Trace Element Research, (74): 97-105.*

El-Massarawy, A.M.D. (1997). Studies on the Effect of Some Heavy Metals on Testis, Thyroid, Liver and Kidney Functions in Albino Rats. *P. 84.* Ph.D. Thesis Fac. of Vet. Med. Cairo Univ., Egypt.

Environmental Health Criteria (Nickel) 108, (1991). Nickel world Health Organization. (Geneva). *Environ. Contam. Toxical. 64: 161-167.*

Humason, G.L. (1979). *Animal Tissue Technique 2nd Ed., 661 pp.* Freeman and Company, New York.

Juan, M.L.; M. Tereso Colomina; J.S. Juan; L.D. Jose and C. Jacinto (1995). Reproduction toxicology of aluminum in male mice. ***Fundamental and Applied Toxicology. 25: 45-51.***

Kamel, E.A.; H.M. El-Tahry; A.M. Ibrahim and Z. Sakkara (1993). Effect of lead acetate on reproductive system of male albino. ***Egyptian Journal of Occupational Medicine. 13: 69-85.***

Karen, E.S. and M.B. Thomas (1996). Reproductive toxicology and teratology, In: ***Principles of Toxicology, Chapter 6: 69-86,*** Lewis Publishers, Boca, Raton New York..

Liobet, J.M.; M.T. Colomina; J.J. Sirvent; J.L. Domingo and J. Corbella (1995). Reproductive toxicology of aluminum in male mice. ***Fundam Appl. Toxicol. Apr.; 25(1): 45-51.***

Nathan, E.; H.F. Huang; L. Pogach; W. Giglio; J.D. Bogden and J. Seebode (1992). Lead acetate dose not impair section of sertoli cell function marker proteins in the adults, Sprague Dawley rat. ***Arch. Environ. Health, 47(5): 370-375.***

Pandey, R. and S.P. Srivastava (2000). Spermatotoxic effects of nickel in mice. ***Bull. Environ. Contam. Toxicol., 64: 161-167.***

Petrelli, G.; L. Lauria and I. Figa-Talmanca (2001). Occupational exposure and male fertility: Results of an Italian multicenter study in an exposed population. ***Med. Lav. Sep.-Oct.; 92(5): 307-13.***

Ratna, P. and S.P. Singh (2001). Seminal toxicity of nickel sulfate in mice. ***Biological Trace Element Research (82): 211-215.***

Schalm, O.W. (1986). ***Veterinary Hematology. 4th Ed., pp. 21-86.*** Lea & Febiger. Philadelphia.

Seth, P.K.; F.N. Jaffery and V.K. Khanna (2000): Toxicology. ***Indian Journal of Pharmacology, 32: S134-S151.***

Sheiner, E.K.; E. Sheiner; R.D. Hammel; G. Potashnik and R. Carel (2003). Effect of occupational exposures on male fertility: literature review. ***Ind. Health Apr.; 41 (2): 55-62.***

Sokol, R.Z. (1990): The effect of duration of exposure on the expression of lead toxicity on the male reproductive axis. ***J. Androl., 11 (6): 521-526.***

Sokol, R.Z. and N. Berman (1991): The effect of age of exposure on lead induced testicular toxicity. ***Toxicology, 69(3): 269-278.***

Wang, L.; C. Ong and S. Foo (1992). LDH-x in human seminal plasma as an indicator of the functional integrity of spermatozoa. ***Journal Plasma American College of Health 41: 115-119.***

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السمية تحت المزمنة للألومنيوم على خصوبة ذكور الفئران البيضاء

[٢٤]

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عند معاملة ذكور الفئران البيضاء بكلوريد الألومنيوم الماني بتركيز ٢٠-١٠٠ جزء في المليون في ماء الشرب لمدة ٩٠ يوم لم يتأثر وزن الخصية بينما حدث انخفاض في وزن الحويصلة المنوية وغدة البروستاتا وانخفضت حركة وعدد الحيوانات المنوية.

كما حدث انخفاض في نسبة الحمل في الإناث غير المعاملة بعد تزاوجها مع الذكور المعاملة بكلا التركيزين لمدة ٩٠ يوما، مع عدم حدوث تأثير في مستوى هرمون التستستيرون. كما أوضح الفحص الهستوباثولوجي عدم تأثر أنسجة الخصية في الذكور المعاملة بكلا التركيزين.

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