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Assessment of Six Weeks Exposure to Cadmium Chloride on Fertility of Adult Male Mice

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Abstract

This study investigates the effect of six weeks exposure to cadmium chloride ($CdCl_2$) on the fertility and reproduction of Swiss albino adult male mice. Three groups of ten mice each were administered $CdCl_2$ intraperitoneally at the concentrations of 0, 0.25 or 0.5 mg/Kg body weight, weekly for a period of six weeks. Control and exposed mice were allowed to mate with sexually mature unexposed females for ten days and their fertility was assessed 9 days later. Fertility was significantly reduced in males injected with $CdCl_2$. The number of pregnant females was significantly reduced in females mated with males that had been injected with cadmium chloride at a concentration of 0.25 or 0.5 mg/Kg B.wt $CdCl_2$. The total number of resorptions out of the total number of implantation sites was significantly increased. Exposed males showed significant decreases in the seminal vesicle weights. Testicular sperm counts were significantly reduced only in males exposed to 0.5 mg/Kg B.wt $CdCl_2$. Moreover, a significant decrease in the serum testosterone (T) level and a significant increase in both luteinizing (LH) and follicular stimulating hormone(FSH) levels was observed. Mild histopathological changes were observed in testicular sections of adult male mice injected intraperitoneally with cadmium chloride at both concentration of 0.25 or 0.5 mg/Kg B.wt for six weeks. These changes included congested blood vessels, increased amount of interstitial connective tissue and mild destruction of the seminiferous tubules with few necrotic areas and degenerative cells. These results suggest that exposure of adult male mice to cadmium chloride had adverse effects on fertility and reproduction.

Keywords: $CdCl_2$; fertility; male mice.

INTRODUCTION

Heavy metals are natural components of the earth's crust that can't be degraded or destroyed and they tend to accumulate in living tissues (Water Pollution at Lowermoor North Cornwall, 1989). Cadmium (Cd), a natural element of the earth's crust, is considered one of the commonest environmental metal poisons, especially with the dramatic increase in its use during the last 50 years (Baldwin et al., 1999). Cadmium is present in almost everything we use in our daily lives. The most significant use of cadmium is in nickel/cadmium batteries, aerospace applications, in phosphate fertilizers, detergents and refined petroleum products (Baldwin et al., 1999). Cadmium is a proven developmental toxicant in animals, causing fetal malformations and other defects, but no conclusive evidence has been found in humans (ATSDR, 1997).

Although human-based studies are limited, there is some evidence to suggest that maternal cadmium exposure may result in decreased birth weights (ATSDR, 1997). On the other hand, animal-based studies provide some evidence of serious reproductive effects including decreased reproduction and severe testicular damage following oral exposure to cadmium (ATSDR, 1997). Rodent testes are more susceptible to cadmium toxicity than rodent liver. McKenna et al. (1996) examined metallothionein gene expression in testicular interstitial cells of rats treated with cadmium (4.0 μmol Cd/kg). The study showed that testicular interstitial cells accumulated Cd at levels that were 4 times greater than controls. Furthermore, increases in the expression of metallothionein mRNA (MT-I: 1.9, MT-11: 1.4 folds, respectively) were observed in comparison to controls. Similar heavy metal studies showed serious effects on male fertility. Elbetieha et al. (2008) reported that exposure to cobalt chloride for twelve weeks significantly decreased the absolute epididimal and testicular weights and significantly increased the number of resorptions in females mated with male mice that had ingested $CoCl_2$ at concentrations of 200,400 and 800 ppm.

The wide incorporation of cadmium in almost every thing we use in our daily lives suggests that it may cause adverse effects on various body systems including the neural, reproductive and renal systems.

In previous work, we noted serious effects on fertility and reproduction of male mice orally exposed to cadmium chloride for a period of sixteen weeks (Tbeileh et al., 2007). The main aim of this study is to further investigate the effects of cadmium chloride administered intraperitoneally for a period of six weeks on fertility and reproduction of adult male mice.

MATERIALS AND METHODS

Animals

Thirty adult male Swiss albino mice, at day 60 of age, weighing approximately 30 g were used in this study. The study was conducted in the period between September 2005 and July 2006. Animals were raised in the animal house unit in the Faculty of Medicine at Jordan University of Science and Technology under a controlled temperature of 21±1°C on a 12 h light, 12 h darkness schedule (lights on 06.00-18.00h). Food (manufactured by the Faculty of Veterinary Medicine at Jordan University of Science and Technology, Irbid, Jordan, according to standard recipes) and water were available ad libtium. Permission from the animal ethical committee at Jordan University of Science and Technology was obtained before performing the study on the mice.

Tested material: Cadmium chloride ($CdCl_2$) was obtained from Sigma-Aldrich (Pf, D-89552 Steinheim, Germany). Doses were prepared according to previous studies.

METHODS

Exposure of Adult Male Mice to Cadmium Chloride

Thirty male mice were assigned into three groups of ten animals each. Group one and two received intraperitoneal injections of 0.25 and 0.5 mg/kg $CdCl_2$ dissolved in normal saline (0.9 % Sodium Chloride) respectively, while group three received normal saline only. The exposure protocol was applied once a week for a total of six weeks.

Effect of Cadmium Chloride on Body and Organ Weights of Adult Male Mice

Body weights of the animals in all groups were measured before the beginning of treatment and at the end of each week of treatment. At the end of treatment, the males in each group were sacrificed by cervical dislocation under light ether anesthesia and the following organs were excised and weighed: paired testes, seminal vesicles (stripped of fluid) and preputial glands. One of the excised testes of each male was placed in 0.9 % sodium chloride for the sperm counts; the other testis was placed in 10% Formaldehyde for further histological processing.

Evaluation of Male Fertility

Fertility Test

After treatment, each male in each treatment group was placed in an individual cage with two virgin females for ten days, during which two estrus cycles should have elapsed (Rugh, 1968). The treated male mice and the control males were removed at day ten of mating and sacrificed for further evaluation. Nine days later, the mated females were also sacrificed and during autopsy the following measurements were recorded: number of pregnant females, number of viable fetuses, number of implantation sites, number of resorptions, and number of females with resorptions and the fetal body weights.

Sperm Counts

After excision, the testes of each mouse were placed in normal saline (0.9 % NaCl). Sperm counts were performed according to the method of Amann and Lambiase (Amman and Lambiase, 1967) as follows: Testis from each mouse was sectioned by a disposable blade in 4 ml of normal saline and placed into a Petri dish, then minced using a manual glass homogenizer. The homogenate was then placed in a screw-cap tube and mixed with a vortex mixer. Sperm counts were performed using a compound Olympus light microscope and hemocytometer chamber. The total number of sperms per ml was calculated according to the following equation:

Total sperms/ml = Counted sperms/ [1/4 x 1/400 x 1/10 x 1/1000 x 400], where:

1/4: is the dilution of the sample.

1/400: is the hemocytometer capacity measured in mm^3.

1/10: is the height of the chamber measured in mm.

400: is the smallest counted square.

Testicular spermatid counts were calculated and expressed as number of spermatids per gram of testis. The estimates 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 which is the duration of a seminiferous cycle during which developing spermatozoa are in the spermatid stage (Ashby et al., 1999

Effects of Cadmium Chloride on Testosterone, FSH and LH Serum Levels

One ml of blood was collected for hormonal analysis in non heparinized tubes by cardiac puncture from anesthetized males before sacrifice. Blood samples were centrifuged at 5000 rpm for 4 minutes.

The serum was collected and stored in eppendorff tubes at -20 °C. Serum testosterone, FSH and LH levels were quantitatively determined using solid phase Enzyme-Linked Immunosorbent Assay (ELISA) as described in the kit instruction leaflets provided by the manufacturer.

ELISA Test Procedure

All reagents were brought to room temperature, then 25-100 µl of each standard control and samples were dispensed followed by the addition of 200 µl of enzyme conjugate Horseradish peroxidase (HRP) into each well of a 96-well plate.

The solutions were mixed and incubated for 2-3 hours at room temperature to allow sufficient time for antigen antibody complex formation. The plate contents were emptied and rinsed 5 times with washing buffer solution. Then, 200 µl of substrate solution Trimethylbenzidine (TMB) was added to each well and incubated for 15-20 minutes at room temperature. The enzymatic reaction was stopped by adding 100 µl of stop solution (2N HCl) to each well. The optical density (OD) was measured on a calibrated microtiter plate reader at a wavelength of 450 nm. The concentration of serum testosterone, FSH and LH were determined according to the standard curve that was drawn by blotting the absorbance against the standard sample concentrations provided by the manufacturer.

Testosterone ELISA kit was purchased from DRG Diagnostic incorporation 18, D-35039 Marburg, Germany. Both LH and FSH kits were obtained from Endocrine Technologies Incorporation, 35325 Fircrest Street, Newark, CA 94560-1003. U.S.A. (Catalog No. for LH: ERK R 7010, for FSH: ERK R 7007).

Histological Evaluation of The Testers

Histological slides were prepared as follows: The excised testes were fixed immediately in 10% formaldehyde solution for histology. The fixed tissues were dehydrated serially in graded ethanol concentrations (70 %, 80 %, 95 %, 95 %,

and 100 %) followed by two steps of xylene clearance using Rrichert-Jung Histokinette 2000 automatic processor. The tissues were infiltrated in melted paraffin (60 °C), embedded on paraffin blocks and sectioned perpendicular to the long axis of the tissues at a thickness of 3 µm on a microtome apparatus. The tissues then were rehydrated and stained with the basic dye hematoxylin and the acidic dye eosin. Stained sections were then mounted on glass slides with dextran plasticizer xylene (DPX) and covered with a cover slip. Morphometric changes of the tissues and the general histological appearance of the testes, kidneys and associated tubules were examined using Olympus light microscope and photographed using a digital camera.

Statistical Analysis

Data are expressed as mean ± S.D. Differences between control and test groups were analyzed using either Student's t-test or Fisher's exact test using StatMost 2.5 Windows software/ DataMost Corporation. P-values less than 0.05 were considered statistically significant.

RESULTS

Effect of Six Weeks Exposur to Cadmium Chloride on Body Weights of Adult Male Mice

Table 1 presents the data on average body weights of adult male mice exposed to $CdCl_2$. No significant differences were found in body weights of the two treated groups and the mice gained weight at almost the same rate. The actual intraperitoneal doses per mouse per week are also presented in (Table 1).

Effect of Six Weeks Exposur to Cadmium Chloride on Reproductive Organ Weights of Adult Male Mice

Table 1 summarizes the effects of cadmium chloride on weights of some reproductive organs of male mice. The absolute and relative weights of the seminal vesicles were significantly decreased in males ingested 0.25 and 0.5 mg/Kg B.wt $CdCl_2$ ($p<0.05$ and 0.005, respectively). However, no significant changes were observed in the absolute and relative weights of the testis and the preputial gland in control and treated mice (Table 1).

Testicular Sperm Counts of Adult Male Mice Injected With Cadmium Chloride for Six Weeks

Table 2 shows the effects of short-term exposure to cadmium chloride on sperm counts. Testicular sperm counts (total sperm counts per testis and total sperm/mg of testis), were significantly decreased only in males injected with 0.5 mg/ Kg B.wt cadmium chloride ($p<0.05$ and 0.005, respectively). Furthermore, a significant reduction in daily sperm production (sperm/testis/d) and sperm production efficiency (sperm/mg testis/d) was also observed in males treated with $CdCl_2$ at the concentration of 0.5 mg/Kg B.wt ($p<0.005$).

Fertility Effects of Six Weeks Cadmium Chloride Exposure

The data presented in Table 3 show the toxic effects of $CdCl_2$ treatment on the fertility of male mice. A significant reduction in the number of implantation sites were observed only in females mated with males treated with 0.5 mg/Kg B.wt $CdCl_2$ ($p<0.005$).

Furthermore, the total number of resorptions out of the total number of implantation sites was significantly increased in females mated by males that had been exposed to $CdCl_2$ at both concentrations of 0.25 and 0.5 mg/Kg B.wt. ($p<0.05$ and 0.005, respectively). However no significant differences were observed in the number of pregnant females, number of viable fetuses per pregnant female or in the number of females with resorptions between control and cadmium chloride treated groups (Table 3).

Table.1. Effect of six weeks administration of cadmium chloride via intraperitoneal injections on body weights and reproductive organs weights of adult male mice.

Treatment Group Dose	No. of Males	Actual Dose (mg/mouse /week)	Body weight (B.wt.) (g) [a]	Absolute paired testes weight (g) [a] (mg/10 g B.wt.®)	Absolute paired seminal vesicles weight (g) [a] (mg/10 g B.wt.®)	Absolute paired preputial gland weight (g) [a] (mg/10 g B.wt.®)
Control (0.9%NaCl)	10	0	24.85 ± 4.03	0.196 ± 0.052 (70.75 ± 19.1)	0.286 ± 0.045 (92.76 ± 14.5)	0.09 ± 0.012 (32.42 ± 4.07)
Cadmium chloride (0.25 mg/Kg)	8 [b]	0.06	26.49 ± 3.46	0.211 ± 0.02 (73.1 ± 7.06)	0.225 ± 0.026 * (77.79 ± 9.33)	0.081 ± 0.015 (28.167 ± 5.2)
Cadmium chloride (0.5 mg/Kg)	10	0.015	29.09 ± 2.56	0.206 ± 0.076 (66.52 ± 24.79)	0.18 ± 0.056*** (64.98 ± 70.21)***	0.085 ± 0.021 (27.44 ± 6.75)

[a] Results are expressed as mean ± S.D.

[b] Animals died during the exposure period.

® Relative weights.

P<0.05, ** P<0.01, *** P<0.005, as compared to the control group (Student t-test).

Table.2. Effect of six weeks administration of cadmium chloride via intraperitoneal injections on testicular sperm counts and daily sperm production (DSP) of adult male mice.

Treatment group Dose	Actual Dose (mg/mouse /week)	Testis weight (mg) [a]	Total sperm/ testis [a] (X 10^6)	Sperm/mg testis (X 10^3) [a]	Sperm/testis/d [a] (X 10^6; DSP)	Sperm/mg testis/d [a] (X 10^3; Efficiency)
Control Normal saline (0.9% NaCl)	0	196 ± 52.94	40.03 ± 12.58	410 ± 55	9.45 ± 3.26	84.71 ± 17.18
Cadmium chloride (0.25 mg/Kg)	0.06	211.3 ± 20.41	42.34 ± 14.59	398 ± 63	8.26 ± 2.81	82.23 ± 9.47
Cadmium chloride (0.5 mg/Kg)	0.015	206.8 ± 76.35	22.49 ± 9.42*	179.8 ± 45.6 ***	5.02 ± 2.10 *	37.14 ± 8.62***

[a] Results are expressed as mean ± S.D.

* P<0.05, *** P<0.005, as compared to the control group (Student t-test).

Table.3. Effect of six weeks administration of cadmium chloride via intraperitoneal injections on fertility of adult male mice.

Treatment Group Dose	No. of males	No. (%) of pregnant females[+]	No. of Implantation Sites per pregnant female [a]	No. of viable fetuses per pregnant female [a]	Total no. of resorptions/ total no. of implantation sites[+]	No. (%) of females with resorptions[+]
Control Normal Saline	10	16/20 (80.0)	9.58 ± 1.8	8.9 ± 2.4	7/163	4/16 (25.0)
Cadmium chloride (0.25 mg/Kg)	8 [b]	12/16 (75.0)	8.25 ± 2.6	7.54 ± 2.6	12/100*	8/12 (66.67)
Cadmium chloride (0.5 mg/Kg)	10	14/20 (70.0)	7.46 ± 2.07***	7.3 ± 2.25	15/99***	6/14 (42.86)

[a] Data for implantation and viable fetuses are expressed as means ± S. D.

[b] Animals died during the exposure period.

[+] Results are calculated by Fisher's exact test.

* P<0.05, *** P<0.005, as compared to the control group (Student t-test or Fisher's exact test).

Table.4. Effect of cadmium chloride administration via intraperitoneal route on fetal body weights.

Intraperitoneal Administration			
Treatment group	Control Normal saline (0.9% NaCl)	Cadmium chloride 0.25 mg/Kg/week	Cadmium chloride 0.5 mg/Kg/week
Fetal body weights (g) [a]	0.7 ± 0.38	0.54 ± 0.12	0.28 ± 0.2*

[a] Results are expressed as mean ± S.D.

* P<0.05, as compared to the control group (Student t-test).

Table.5. Effect of six weeks administration of cadmium chloride via intraperitoneal injections on Testosterone, FSH and LH levels in adult male mice.

Treatment group Dose	Actual dose consumption (mg/mouse/week) [a]	Testosterone (ng/ml) [a]	FSH (ng/ml) [a]	LH (ng/ml) [a]
Control Normal Saline	0	4.35 ± 0.38	1.1 ± 0.66	0.075 ± 0.0164
Cadmium chloride (0.25mg/Kg)	0.06	2.25 ± 1.92*	0.97 ± 0.32	N.D
Cadmium chloride (0.5 mg/Kg)	0.015	1.8 ± 0.06***	1.7 ± 0.027*	0.13 ± 0.004**

[a] Results are expressed as mean ± S.D.

*p< 0.05, ** p<0.01, *** p<0.005, as compared to the control group (Student t-test).

N. D: not detectable value.

Table 4 shows the effects of cadmium chloride administration via intraperitoneal route on fetal body weights. It is clear that intraperitoneal administration of $CdCl_2$ resulted in a significant decrease in the fetal body weights only at the dose of 0.5 mg/Kg B.wt. ($p<0.05$) (Table 4).

Effect of Six Weeks Exposure to Cadmium Chloride on Serum Levels of Testosterone,FSH and LH Hormones In Adult Male Mice

Table 5 summarizes the effect of six weeks intraperitoneal administration of $CdCl_2$ on serum levels of Testosterone, FSH and LH hormones in adult male mice. Significant decreases in the Testosterone serum levels were observed in mice that were exposed to $CdCl_2$ at the doses of 0.25 and 0.5 mg/Kg B.wt ($p<0.05$, and 0.005, respectively) (Table 3.5). FSH and LH levels were significantly increased only in male mice exposed to 0.5 mg/Kg B.wt cadmium chloride ($p<0.05$ and 0.01, respectively). However, no detectable serum levels of LH had been obtained in the male mice group exposed to 0.25 mg/Kg B.wt (Table 5).

Effect of Six Weeks Exposure to Cadmium Chloride on The Testes of Adult Male Mice

Histological sections of adult male mice testes were prepared after the exposure period following a standard procedure, to determine whether the reduction in male mice fertility observed in this study was a result of the damage imposed by cadmium treatment on testicular infrastructure. Microscopic evaluation of the control group testis histological sections (100X) showed the seminiferous tubules (ST) normally arranged with little connective tissue (CT) in the interstitial spaces where Leydig cells (the cells responsible for testosterone production in the testis) are located (Figure 1).

In general, the effects of the short-term exposure to cadmium chloride on adult male mouse testes did not reveal severe changes. However, increased amount of connective tissue (A) was observed in the interstitial spaces of the testis of mice that had been exposed to 0.25 mg/Kg cadmium chloride treated mice (Figure 2). Moreover, testis sections of male mice exposed to 0.5 mg/Kg cadmium chloride showed some congested blood vessels (C) and few necrotic areas (N) inside the seminiferous tubules (Figure 3).

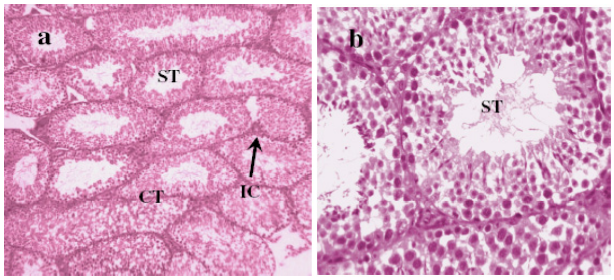

Fig.1. Cross section of control testis showing the seminiferous tubules and small amount of connective tissue (CT) in the interstitial spaces, seminiferous tubule (ST) and interstitial cells (Leydig cells). (a) Magnification (100X), (b) Magnification (400X). H & E stain.

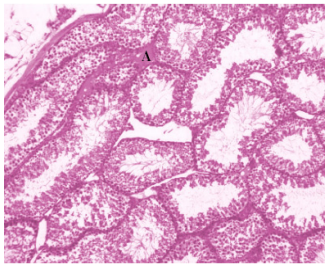

Fig.2. Cross section of the seminiferous tubules in the testis of 0.25 mg/Kg cadmium chloride treated mice, showing increased amount of intertubular connective tissue (A). Magnification (100X). H & E stain.

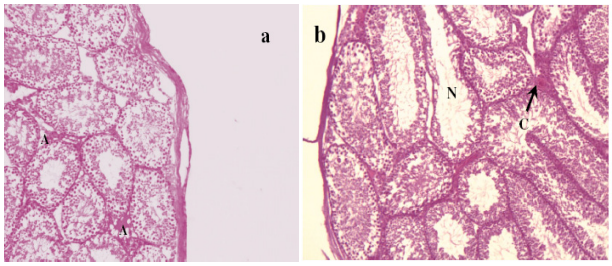

Fig.3. Cross section of the seminiferous tubules in the testis of 0.5 mg/Kg cadmium chloride treated mice. (a) Increased amount of interstitial connective tissue. (b) Necrosis in the seminiferous tubule (N) and a congested blood vessel (C). Magnification (100X). H & E stain.

DISCUSSION

The data presented in this paper provides clear evidence that intraperitoneal exposure to $CdCl_2$ for six weeks had adverse effects on fertility and reproduction of adult male mice. The results show that the number of implantation sites was significantly reduced in females impregnated by treated male mice. Furthermore, the total number of resorptions out of the total number of implantation sites was significantly increased along with a significant decrease in the fetal body weights. These effects may be explained by the increase in prenatal mortality of unhealthy fertilized ova due to alterations in sperm quality (Bench et al., 1999). Moreover, the absolute and relative seminal vesicles weights were significantly reduced in mice exposed to 0.5 mg/Kg B.wt $CdCl_2$. Similar adverse effects have also been reported for other heavy metals. Mayyas et al. (2005) reported reductions in seminal vesicle and preputial gland weights in adult male mice exposed orally to aluminum chloride for a period of three months.

The current study demonstrate that exposure of male mice to $CdCl_2$ resulted in significant decreases in testicular sperm counts as well as daily sperm production, and sperm production efficiency. This reduction could be the result of reduced testicular function (reduced spermatogenesis) due to the internal damage suggested by the histological abnormalities observed in testes of the exposed animals. There are several hypotheses to explain how reduced male fertility may result from incorporation of heavy metals into sperm chromatin (Casswell et al., 1987 and Johansson et al., 1968). One hypothesis suggests that these metals, which bind tightly to free thiols, replace or compete with the zinc that is normally bound to the cysteine residues in protamine, forming more stable metal–SH bond that ultimately prevent proper decondensation of sperm chromatin following fertilization (Casswell et al., 1987). Another hypothesis suggests that the presence of tightly bound cadmium may prevent normal disulfide bond formation within and among protamines during the final stages of sperm maturation (Shelby et al., 1986).

It has been reported that Cadmium may affect several reproductive parameters including manipulation of steroid hormone levels as well as induction of spermiation failure (Aoki and Hoffer, 1978, Hew et al., 1993 and Lafuente et al., 2000). Bench et al. (1999) concluded that cadmium has a detrimental effect on testicular function (It is toxic to the supporting testicular tissue or to the earlier stages of spermatogenesis) that results in reduced sperm production leading to reduced male fertility.

The current study reveals a significant decrease in the serum testosterone levels accompanied with a significant increase in serum FSH and LH levels in adult male mice exposed to $CdCl_2$ at the concentration of 0.5 mg/Kg B.wt. These results suggest that cadmium may have acted directly on the testes and thus affected the androgen biosynthesis pathway. It has been postulated that short-term cadmium exposure may affect steps of signal transduction following cAMP accumulation and/or degradation (REF). In agreement with the results of this study, Laskey and Phelps (1991) found decreased testosterone production in HCG- or cAMP-facilitated cultured mouse Leydig cells exposed to Cadmium. The subsequent increase in serum LH and FSH levels are reported to be a result of the hypothalamus and pituitary gland stimulation by a feedback mechanism to compensate the decrease in testosterone levels.

Spermatogenesis is controlled by two main regulations: endocrine (LH and FSH from pituitary gland) and local intercellular communications mediated through either paracrine effectors such as testosterone, growth factors and cytokines (Weinbauer and Wessels, 1999). Fiorini et al. (2004) reported that testicular toxicants such as cadmium reduce or redistribute specific junctional surface proteins on the Sertoli cell membrane that are necessary for the development and maintenance of spermatogenesis. Such alterations of Sertoli-Sertoli interactions may lead to sterility in males. Furthermore, the disturbance in testosterone and LH hormone levels caused by cadmium chloride exposure may be responsible for the significant reduction in testicular sperm counts and consequently reduced fertility observed in the current study. Moreover, the wide array of abnormalities in histological sections of the testes to provide further evidence for the reduced fertility of exposed animals. Several abnormalities have noticed including congested blood vessels, increased amounts of connective tissue between the seminiferous tubules as well as the degeneration and necrosis of the seminiferous tubules. Similar studies have linked such histopathological abnormalities to cadmium treatment and reported that these effects were also accompanied with reduced male fertility. Lymberopoulos et al. (2003) documented the presence of lesions in testes of the cadmium chloride treated animals at a daily oral dose of 3 mg/kg B.wt for a period of seven months. These lesions were localized in the Sertoli cells, the seminiferous tubules, the primary and the secondary spermatocytes as well as the spermatids, causing several alterations in their functions.

CONCLUSIONS

The results of this study strongly suggest that exposure to the heavy metal cadmium in the form of $CdCl_2$ had severe effects on fertility and reproduction in adult male mice.

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