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Effects of Long Term Exposure to Cadmium Chloride on Fertility in Adult Male Mice

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Abstract: This study investigated the effect of long term exposure to Cadmium Chloride ($CdCl_2$) on the fertility and reproduction of Swiss albino adult male mice. Four groups of 10 mice each were exposed to $CdCl_2$ (0, 10, 20, 40 ppm) orally every day for a period of sixteen 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. The results showed that females mated with males that ingested 40 ppm $CdCl_2$ showed a significant decrease in the pregnancy rate. Moreover, 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 a concentration of 20 ppm. In addition, exposed males showed significant increases in body weight and significant decreases in testis weights. A significant decrease in the seminal vesicle weight was observed in males ingested 20 ppm $CdCl_2$, while no significant effects were observed on the weights of preputial glands. Testicular sperm counts were significantly reduced. On the other hand, a significant increase in the serum levels of Testosterone (T) and Luteinizing (LH) hormone was observed with no significant differences in the level of FSH as compared to the unexposed controls. Profound histopathological changes were clearly observed in testicular sections of adult male mice ingested cadmium chloride for sixteen weeks. These changes included congested blood vessels, increased amount of interstitial connective tissue and destruction of the seminiferous tubules with severe necrotic areas and degenerative cells. These results strongly suggest that exposure of adult male mice to cadmium chloride has adverse effects on fertility and reproduction.

Key words: Cadmium Chloride ($CdCl_2$), testosterone, FSH, LH, long term exposure fertility

INTRODUCTION

Heavy metals are natural components of the earth's crust and cannot 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 and Marshall, 1999). Cadmium is present in almost everything we use; the most significant use of cadmium is in nickel/cadmium batteries, aerospace applications, in phosphate fertilizers, detergents and refined petroleum products (Baldwin and Marshall, 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 serious reproductive effects such as decreased reproduction and severe testicular damage (ATSDR, 1997). It has been shown that 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^{-1}). The study showed that testicular interstitial cells of the treated animals accumulated Cd at levels that were 4 times greater than in the control group. Furthermore, increases in the expression of metallothionein mRNA (MT-I: 1.9, MT-11: 1.4 folds, respectively) were observed in the treated animals as compared to the control group. There are several hypotheses that explain how reduced male fertility

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may result from incorporation of heavy metals into sperm chromatin (Casswell *et al*., 1987; Johansson and Pelliccian, 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). An alternate hypothesis is 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). Because Cd is widely used in industry and in our daily life, it's likely that many people are exposed to it. The main aim of this study is to further investigate the effects of cadmium administered in drinking water on fertility and reproduction of adult male mice.

MATERIALS AND METHODS

Animals: Forty adult male Swiss albino mice, at day 60 of age, weighing approximately 32 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 ethical committee at Jordan University of Science and Technology was obtained before performing the study on the mice.

Tested material and dose preparation: Cadmium Chloride ($CdCl_2$) was obtained from Sigma-Aldrich (Pf, D-89552 Steinheim, Germany).

For oral administration, $CdCl_2$ was dissolved in tap drinking water at different concentrations, namely: 10 ppm (10 mg L^{-1}), 20 ppm (20 mg L^{-1}) and 40 ppm (40 mg L^{-1}).

The LD_{50} value for $CdCl_2$ for oral administration is 57 mg kg^{-1} B.wt for mice (WHO, 1998).

For oral Administration of $CdCl_2$; The first, second and third doseswere 1/10, 1/20 and 1/40 of the LD_{50} (5.7, 2.8 and 1.4 mg kg^{-1}/day, respectively). The control group received tap water only.

Long-term exposure of adult male mice to cadmium chloride: Forty male mice were assigned into 4 groups of 10 animals each. One group served as the control and animals were given only tap water, the other three groups received 10, 20, 40 ppm $CdCl_2$ dissolved in drinking tap water, respectively. Male mice were subjected to sixteen weeks of treatment, during which daily water consumption and the actual dose of $CdCl_2$ ingestion were recorded.

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 then placed in 0.9 % sodium chloride for the sperm counts. The other testis was placed in 10% formaldehyde for further histological processing.

Evaluation of the exposure period on 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, the 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: Testes from each mouse were sectioned by a disposable blade in 4 mL of normal saline and placed in 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 a hemocytometer chamber. The total number of sperms per mL was calculated according to the following equation:

Total sperms mL^{-1} = Counted sperms/ [1/4×1/400×1/10× 1/1000×400], where:

1/4 : Is the dilution of the sample.
1/400: Is the hemocytometer capacity and is measured in mm^3.
1/10 : Is the height of chamber and is 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 mililitre 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 min.

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 zdispensed 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 h 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 min 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 testes: 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 2 steps of xylene clearance using Rrichert-Jung Histokinette 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 were evaluated using an Olympus light microscope and photographed using a digital camera.

Statistical analysis: Data were 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 ingestion of cadmium chloride for sixteen weeks on body weights and water consumption of adult male mice: The data depicted in Table 1 demonstrate that exposure to $CdCl_2$ significantly increased the average daily water consumption per animal in all treated groups ($p<0.005$, 0.005 and 0.01, respectively), together with an increased average body weight of males exposed to $CdCl_2$ at the doses of 20 and 40 ppm in a dose dependent manner ($p<0.005$ and $p<0.0001$, respectively) (Table 1). The average of the actual doses of $CdCl_2$ that male mice had been exposed to, based on the daily water consumption, per kilogram body weight per day.

Effect of ingestion of cadmium chloride for sixteen weeks on reproductive organ weights of adult male mice: The data presented in Table 2 show the effect of $CdCl_2$ on the absolute and relative weights of some male mouse reproductive organs. The absolute and relative weights of the testes were significantly decreased in males that ingested 20 or 40 ppm $CdCl_2$ ($p<0.05$ and $p<0.01$, respectively). The absolute and relative weights of seminal vesicles was significantly reduced only in males

Table 1: Effect of sixteen weeks ingestion of cadmium chloride via drinking water on average water consumption of adult male mice

Treatment group Dose	Body weight (g)[a]	Water consumption (mL)[a]	Actual dose consumption (mg kg^{-1}/d)[a]
Control	34.07±0.77	3.51±1.09	0
(10 ppm)	33.45±1.08	5.1±0.95***	1.52±0.29
(20 ppm)	35.43±0.85***	4.86±1.00***	2.7±0.57
(40 ppm)	37.00±1.05****	4.71±0.77**	5.16±0.91

[a]Results are expressed as mean±S.D. *p< 0.05, ** p<0.01, *** p<0.005, ****p<0.0001, as compared to the control group (Student t-test)

Table 2: Effect of sixteen weeks ingestion of cadmium chloride via drinking water on body weights and reproductive organs weights of adult male mice

Treatment group dose	No. of males	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^{-1} B.wt.®)
Control	8[b]	34.07±0.77	0.26±0.06 (77.97± 16.43)	0.26±0.05 (76.3±14.96)	0.12±0.05 (38.4±12.06)
(10 ppm)	9[b]	33.448±1.081	0.22±0.05 (59.26±13.03)	0.27±0.06 (71.75±28)	0.11±0.05 (31.04±13.43)
(20 ppm)	10	35.43±0.85***	0.22± 0.03* (59.96±8.913)*	0.23±0.03* (65.84±9.23)*	0.11±0.03 (29.64±9.40)
(40 ppm)	10	37.00±1.05****	0.17±0.07** (49.47± 21.61)**	0.26±0.06 (69.75±16.04)	0.12±0.04 (32.12±10.24)

[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, ****p<0.0001, as compared to the control group (Student t-test)

Table 3: Effect of sixteen weeks ingestion of cadmium chloride via drinking water on testicular sperm counts and Daily Sperm Production (DSP) of adult male mice

Treatment group dose	Dose (mg kg^{-1}/d)[a]	Testis weight (mg)[a]	Total sperm /testis[a](×10^6)	Sperm/mg testis[a](× 10^3)	Sperm/testis/d[a] (×10^6; DSP)	Sperm/mg testis/d[a](×10^3; Efficiency)
Control	0	263±57.7	43.94±12.19	349.28±76.93	9.81±2.72	72.16±17.17
(10 ppm)	1.52±0.29	165.7±72.4**	14.59±6.16***	190.04±62.92	3.26±01.9****	39.26±14.05
(20 ppm)	2.7±0.57	212.2±31.6*	31.95±8.54	283.72±51.77	6.64±2.3*	58.62±11.55
(40 ppm)	5.16±0.91	219.2±48.2	15.56±9.92 ***	126.6±33.3**	3.47±2.213***	26.16±7.4**

[a]Results are expressed as mean±S.D. *p<0.05, **p<0.01 *** p<0.005, ****p<0.0001, as compared to the control group (Student t-test)

ingested 20 ppm $CdCl_2$. Exposure to $CdCl_2$ did not affect the weights of the preputial glands.

Testicular sperm counts of adult male mice ingested cadmium chloride for 16 weeks: Table 3 demonstrates the results obtained on testicular sperm counts of adult male mice after exposure to $CdCl_2$ for sixteen weeks. Testicular sperm counts (total sperm counts per testis) were significantly reduced in males ingested 10 or 40 ppm $CdCl_2$ ($p<0.005$). Moreover, the total number of sperm per mg of testis was significantly reduced in males ingested 40 ppm $CdCl_2$ ($p<0.01$). A significant reduction in the daily sperm production was observed in males that ingested 10, 20 and 40 ppm $CdCl_2$ ($p<0.0001$, $p<0.05$ and $p<0.005$, respectively). On the other hand, sperm production efficiency (sperm/mg testis/d) was significantly reduced only in males that ingested 40 ppm $CdCl_2$ ($p<0.01$) (Table 3).

Fertility effects of cadmium chloride ingestion in adult male mice exposed for 16 weeks: The data depicted in Table 4 reveal the toxic effects of $CdCl_2$ on fertility of adult male mice. The number of pregnant females was significantly reduced only in females mated by males that had been exposed to 40 ppm $CdCl_2$ ($p<0.05$). Females mated with males that were exposed to $CdCl_2$ for sixteen weeks showed no significant differences neither in the number of implantation sites nor in the number of viable fetuses. However, 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 a concentration of 20 ppm ($p<0.05$) (Table 4). No significant difference was found in the fetal body weights between control and $CdCl_2$ treated groups (Table 5).

Effect of 16 weeks ingestion of cadmium chloride on serum levels of testosterone, FSH and LH hormones of adult male mice: Table 6 summarizes the effects of 16 weeks of cadmium chloride ingestion on serum levels of Testosterone, FSH and LH hormones of adult male mice.

A significant increase in the testosterone serum level was observed in males that ingested 10 ($p<0.01$), 20 ($p<0.005$), or 40 ($p<0.01$) ppm $CdCl_2$ (Table 6). No significant difference was observed in the serum level of FSH in adult male mice ingested $CdCl_2$ in all treated groups (Table 6). However, LH serum levels were significantly increased in male mice ingested 40 ppm (Table 6).

Effect of the 16 weeks of exposure to cadmium chloride on the testis of adult male mice: Histological sections of the adult male mice testes were prepared after the exposure period following a standard procedure, to determine whether the reduction in male mouse fertility observed in this study was a result of the damage imposed by cadmium treatment on testicular infrastructure. Microscopic description of the testis in the control group histological sections showed normal 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 (Fig. 1). At the

Table 4: Effect of 16 weeks ingestion of cadmium chloride via drinking water 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		13/16				5/13
	8[b]	(81.25)	7.23±2.77	6.85±3.0	6/94	(38.46)
(10 ppm)	9[b]	14/20				5/14
		(70.0)	7.22±2.91	7.22±2.22	5/74	(35.71)
(20 ppm)		18/19				11/18
	10	(94.73)	7.89±1.60	6.78±2.26	20/142*	(61.11)
(40 ppm)		9/18*				5/9
	10	(50.0)	8.29±1.33	7.86±1.29	6/116	(55.55)

[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, as compared to the control group (Fisher's exact test)

Table 5: Effect of cadmium chloride administration via oral route on fetal body weights

	Long-term administration			
Treatment group	Control	Cadmium chloride 1.52 mg kg^{-1}/d	Cadmium chloride 2.7 mg kg^{-1}/d	Cadmium chloride 5.16 mg kg^{-1}/d
Fetal body weights (g)[a]	0.5±0.32	0.43±0.1	0.35±0.24	0.26±0.07[a]

Results are expressed as mean±S.D

Table 6: Effect of sixteen weeks ingestion of cadmium chloride via drinking water on Testosterone, FSH and LH levels in adult male mice

Treatment group dose	Actual dose consumption (mg/kg/d)[a]	Testosterone (ng mL^{-1})[a]	FSH (ng mL^{-1})[a]	LH (ng mL^{-1})[a]
Control	0	5.3±2.22	083±0.24	0.094±0.042
(10 ppm)	1.52±0.29	12.23±4.35**	0.97±0.39	0.075±0.05
(20 ppm)	2.7±0.57	13.8±2.36***	0.88±0.25	0.09±0.01
(40 ppm)	5.16±0.91	12.46±3.77**	0.94±0.2	0.31±0.2*

[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)

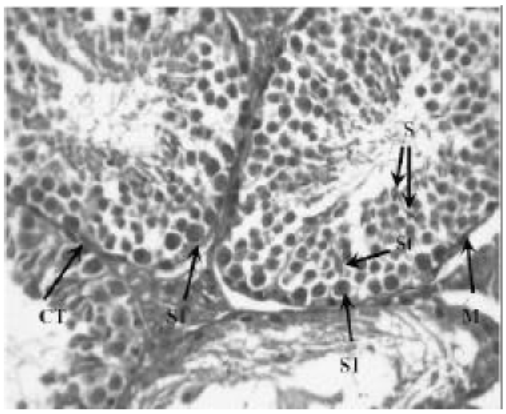

Fig. 1: Cross section in the control testis of the long-term exposure showing seminiferous tubules with spermatocytes and small amount of connective tissue in the interstitial space, Spermatogonial cells (S1), Spermatid (S), Sertoli cells (St), spindle-shaped Myofibroblasts (M) Magnification (400×). H and E stain

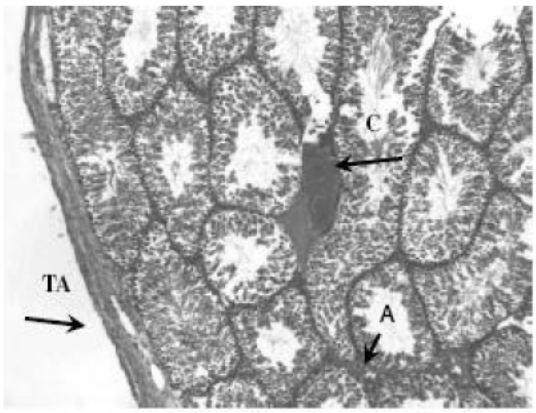

Fig. 2: Cross section of the seminiferous tubules in the testis of 10 ppm cadmium chloride treated mice. Showing increased amount of intertubular connective tissue (A), congested blood vessels (C) and Tunica albuginea (TA). Magnification (100×). H and E stain

magnification of (400×), regularly arranged primary Spermatocytes (S1) were clearly seen in the bases of seminiferous tubules, in addition to some normal Spermatozoa (S) near the lumen of seminiferous tubule, Sertoli cells (St) and spindle-shaped Myofibroblasts (M) (Fig. 1). Histological sections of

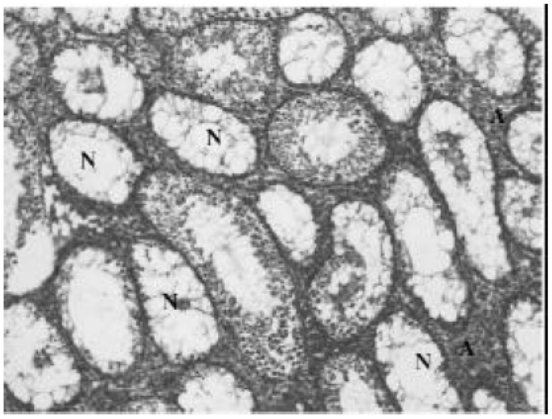

Fig. 3: Cross section of the seminiferous tubules in the testis of 20 ppm cadmium chloride treated mice. Showing increased amount of intertubular connective tissue (A) and severe Necrosis (N) covering large area of the testis. Magnification (100×). H and E stain

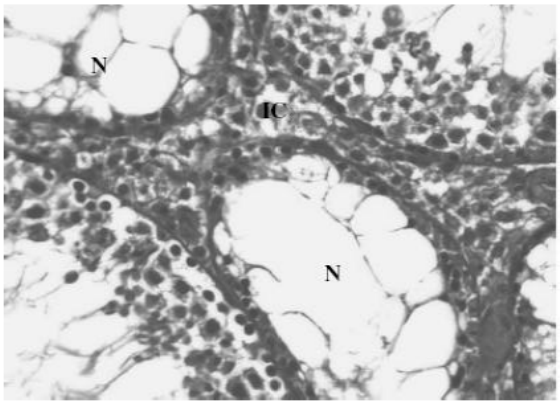

Fig. 4: Cross section of the seminiferous tubules in the testis of 20 ppm cadmium chloride treated mice. Showing increased amount of interstitial tissue (IC) and severe necrosis of the seminiferous tubule (N). Magnification (400×). H and E stain

the testis of adult male mice after ingestion of cadmium chloride for sixteen weeks were similarly assessed. All sections showed Congested blood vessels (C), increased amount of interstitial connective tissue, increased number of Leydig cells and severe Necrotic damage (N) in the seminiferous tubules. However, little necrosis was observed in the seminiferous tubules in male ingested 10 ppm, except for a few congested blood vessels that had started to appear. The most severe effects were observed in the testis sections of the adult male mice that had ingested 20 and 40 ppm $CdCl_2$ (Fig. 2 -5).

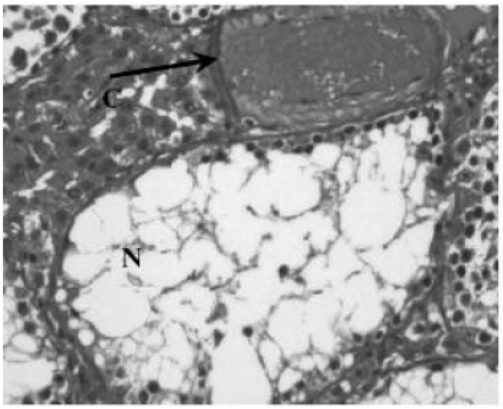

Fig. 5: Cross section of the seminiferous tubules in the testis of 40 ppm cadmium chloride treated mice. Clearly showing a large congested blood vessel (C) and severe tubular Necrosis (N). Magnification (400×). H and E stain

DISCUSSION

The results presented in this study clearly demonstrate that long term exposure of adult male mice to $CdCl_2$ had adverse effects on fertility and reproduction.

The results show that the number of pregnancies in females impregnated by males exposed to $CdCl_2$ for 16 weeks at a dose of 40 ppm was significantly reduced. Also, 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 20 ppm $CdCl_2$. This effect can be attributed to an increase in prenatal mortality of unhealthy fertilized ova due to alterations in sperm quality (Bench *et al*., 1999). Furthermore, it has been reported that Cadmium may affect several reproductive parameters including manipulation of the steroid hormone levels as well as induction of spermiation failure (Aoki and Hoffer, 1978; Hew *et al*., 1993; Lafuente *et al*., 2000).

The current study also found that exposure to $CdCl_2$ resulted in a significant increase in body weight and daily water consumption, in a dose dependent manner. It appears that this increase in body weight gain is a result of excessive salt ingestion due to the treatment. These results are in contradiction to some previous studies that recorded a significant decrease in adult male rat body weight gain when allowed free access to drinking water to which $CdCl_2$ was added at higher concentrations (50, 100 and 200 ppm) for a period of 6 months (Zeng *et al*., 2003).

In the current study, a significant decrease in both the absolute and relative testes weights was observed in

males after ingestion of $CdCl_2$ at both doses of 20 and 40 ppm. The relative and absolute weights of seminal vesicles were decreased only in males exposed to 20 ppm $CdCl_2$. These results are in agreement with the data published by Lymberopoulos *et al.* (2003) who found a significant reduction in testicular weights in animals treated with $CdCl_2$ at a concentration of 3 mg kg^{-1} B.wt for a period of seven months. Moreover, it is well documented by early studies that $CdCl_2$ selectively damages the testes of rats and rabbits and results in degeneration of the seminiferous epithelium, modulation of androgen production in the Leydig cells consequently leading to infertility (Parizek, 1960; Mason *et al*., 1964; Favino *et al*., 1966; Saksena and Lau, 1979).

Similar effects observed with $CdCl_2$ on reproduction have also been reported for many other heavy metals. Bataineh *et al.* (1998) and Elbetieha and Al-Hamood (1997) showed that the exposure of male rats to chromium chloride and potassium dichromate significantly reduced the weights of the testes. Likewise, Mayyas *et al.* (2005) reported similar reduction in testes, seminal vesicles and preputial gland weights in adult male mice exposed orally to aluminum chloride for a period of three months.

The current study also found that exposure to $CdCl_2$ resulted in a significant decrease in: testicular sperm counts (total number of sperms per testis); the total number of sperms per mg of testis; daily sperm production; and sperm production efficiency (sperm/mg of testis/d). This reduction could be the result of reduced testicular function (reduced spermatogenesis) due to the internal damage suggested by the decrease in testicular weights and the histological abnormalities observed in the testes of the exposed animals. These findings are in agreement with several previous studies. 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 result in reduced sperm production leading to reduced male fertility. 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 and these alterations of Sertoli-Sertoli interactions may lead to sterility in males.

The data presented in this work also strongly indicates a serious disturbance in the levels of sex hormones in the exposed groups. A significant increase in serum testosterone level was observed in all treated groups. These results are in agreement with the results of Zeng *et al.* (2004) who reported a significant elevation of the serum testosterone levels in all cadmium exposed groups for a period of three months. Similarly, Lafuente *et al.* (2000) showed an age-dependent cadmium-related increase in plasma testosterone levels in pubertal rats. These results suggest a direct effect of $CdCl_2$ ingestion on testicular Leydig cells that are responsible for testosterone production. Moreover, $CdCl_2$ ingestion could have interfered with the normal function of the hypothalamic-pituitary-gonadal axis (Lafuente *et al*., 2004).

The current study also found a significant increase in the serum LH levels in males expose to 40 ppm $CdCl_2$. These results are consistent those of Pedijo *et al.* (1988) who also reported that exposure to cobalt resulted in a dramatic increase in serum testosterone levels and a slight increase in LH levels in adult male mice. It was suggested that elevated LH levels may not be responsible for increased testosterone production, since LH levels were not elevated in both 10 and 20 ppm cobalt treated animals. These findings suggest that cadmium ingestion may have directly affected testosterone production by Leydig cells and consequently interfering with the local inhibitory feedback mechanisms.

Furthermore, the disturbance in testosterone and LH hormone levels caused by cadmium chloride ingestion may be responsible for the significant reduction in testicular sperm counts and consequently reduced fertility. Moreover, the wide array of abnormalities observed when histological sections of the testes were examined provides further evidence for the reduced fertility of exposed animals. These abnormalities include congested blood vessels, increased amounts of connective tissue between the seminiferous tubules and the severe degeneration and necrosis clearly seen in the two higher doses of 20 and 40-ppm cadmium chloride. Similar studies have linked such histopathological abnormalities to cadmium treatment and 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^{-1} 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.

CONCLUSION

The results of the current study strongly suggest that exposure to the heavy metal cadmium in the form of $CdCl_2$ has adverse, if not, severe effects on fertility and reproduction in adult male mice.

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