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The Effects of Cadmium Pollution on Female Rat Reproductive System

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Abstract

Cadmium is one of the most toxic heavy metals and an environmental and occupational pollutant that endangers human and animal health. In this research we studied the effects of cadmium chloride on adult Wistar rat's female reproductive system. The rats were divided into 5 groups. The experimental groups were fed with 11, 23, 35 and 50 mg/kg of cadmium chloride daily for 10 days (two estrous cycles). Estrous cycle was examined daily by vaginal smear and animals in estrus phase were selected. At the end of the experiment, blood samples were taken from dorsal aorta to assess the blood estradiol and progesterone concentrations. Estradiol was measured by Enzyme-linked immuno sorbent assay (ELISA) and progesterone by radioimmunoassay technique. Then, uteruses and ovaries were removed and after weighting were prepared histologically. Results showed that the length of estrous cycle in animals that were treated with 11 mg/kg of cadmium increased significantly. The serum level of progesterone in groups that were treated with 11 and 23 mg/kg of cadmium increased significantly but only the 35 and 50 mg/kg treated groups showed significant decrease compared with the control group. Histological studies did not reveal any pathological changes, however; there were some atretic follicles in 35 and 50 mg/kg treated groups. Cadmium is a common contaminant of natural environment. It can enter to food chain indirectly and affects the female reproductive system as we showed in rats as an experimental model.

Keywords: Ovary; Uterus; Cadmium; estradiol; Estrous cycle; Progesterone.

1- Introduction

Cadmium (Cd) is a heavy metal posing severe risks to human health (Godt et al. 2006). Cadmium occurs in low concentrations in human diets and in cigarettes. In contaminated areas and in certain occupations, high human exposures occur. Cadmium is widely used in industry such as an anticorrosive agent, stabilizer in PVC products, color pigment, a neutron-absorber in nuclear power plants and in the fabrication of nickel-cadmium batteries. Phosphate fertilizers also contain large amounts of cadmium (Larz 2003). It is reported that Cadmium is capable of distributing through tissues rapidly. Most of the ingested cadmium enters into the liver and the kidneys (Laura et al. 1999). Cadmium has deteriorating effects on the reproduction of women who live near the polluted area (Wu et al. 2004). Chemistry department of Shiraz University reported the presence of cadmium in water pools in the Fars province (south of Iran). Monsefi et al, 2008 reported the effects of cadmium in a concentration similar to that find in Maharloo lake (Shiraz, Iran) on male reproductive system, they showed the level of testosterone decreased significantly in the high dose administered group and also histological studies showed a severe necrosis and atrophy in the high dose group(Monsefi et al. 2008). In this study, we used female rats as an animal model to investigate the effects of different doses (low and high doses) of cadmium base on chemistry department of Shiraz University reports can affect in female reproductive system.

2- Materials and Methods

2-1- Experimental design

Thirty adult Wistar female rats weighting between 120-161 g were obtained from the animal house of Razi Institute in Shiraz. The animals were adapted to the laboratory conditions one week prior to beginning of the experiments. The animals were maintained at standard temperature (22-24 °C) and a period of 12 hours light, and 12 hours darkness. Rats had free access to food and water. The animal experiments were approved by the Institutional Animal Ethics and Health Committee of the Biology Department of Shiraz University. The animals were weighed before and after experiments. Female rats were divided into 5 groups (6 animals in each): control, Low doses group (Ld) that were fed with 11 mg/kg, 23mg/kg and High doses (Hd) with 35 mg/kg and 50 mg/kg of $CdCl_2$ daily. The control group received equal volumes of distilled water under similar condition. Phases of estrous cycle were examined daily by vaginal smear preparation. Animals in estrus phase

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(particular part of the estrous cycle when the female is sexually receptive ("in heat")) were selected. The mentioned doses of CdCl2 were suspended in 1 ml distilled water and administered orally by needle gavages for 10 days (duration of estrous cycle).

2-2- Body and organ weight

At the end of the experiment, animals which were in estrus phase were sacrificed under deep anesthesia and their left ovaries and uteruses were removed and weighed. Then, the standard weights of these organs were calculated using following formula:

Standard weight = [organ weight (g)/ body weight (g)] × 100.

2-3- Hormonal assay

After 10 days, when animals were in estrus phase, they were anesthetized using diethyl ether and blood samples were taken by dorsal aorta puncture (5 ml). The blood samples were centrifuged for 15 minute (4000 rpm) and serums were separated. The estradiol concentrations were measured by ELISA and the progesterone concentration of samples was determined by radioimmunoassay method in the Research Center of Nemazee Hospital.

2-4- Histological studies

Left ovary and uterine tube of each rat was removed and fixed in 10% formalin solution and prepared using routine techniques for histology: Samples underwent dehydration by alcohol, clearing with xylol, embedding in paraffin wax, sectioning under 7 µm thicknesses, and staining with hematoxylin-eosin (Bancroft and Stevens 1991). Finally, photos were taken from the prepared slides under ZEISS microscope.

2-5- Statistical analysis

The data for organ weight, period of estrous cycle, and hormonal assays were analyzed using one-way ANOVA, followed by Tuky test. Statistical analysis was performed using SPSS 11.5 software. $P < 0.05$ was considered as a significant level.

3- Results and Discussion

Body weight (Figure 1), standard weights of ovaries and uteruses (Table 1) and the level of estradiol (Table 3) of the experimental groups did not reveal any significant differences compared to the control group.

The lengths of estrous cycle in animals treated with 11 mg/kg of $CdCl_2$ were significantly longer than the control group (Table 2). Progesterone level was increased in the groups treated with 11 and 23 mg/kg significantly but administration of 35 and 50 mg/kg of cadmium showed significant decrease in progesterone level compared with control group (Table 3). The duration of the estrous cycle in the animals treated with a low dose increased significantly. The duration of each phase of estrous cycle depends on blood level of sex hormones either estrogen or progesterone. In our study, the concentration of progesterone increased in the low dose group that explains the prolongation of luteal phase of estrous cycle (from 7.33 to 11.00 days) because of the high activity of corpus luteum in this group. Serum level of progesterone decreased significantly in the high dose groups and the duration of estrous cycle also shortened, however, these changes were not significant statistically. These results confirmed the previous reports by Piasek et al. 1999, in that study they suggest that Cd may interfere directly with hormone production in steroid producing ovary cells (Piasek et al. 1999). In our study estradiol level increased in all groups. This led to some change in the duration of estrous cycle and also in the some phases such as proestrus and estrus; however, none of these observations was significant statistically. It is demonstrated that Cd disrupts progesterone synthesis via steroidogenic acute regulatory protein (StAR) and P450 cholesterol side-chain cleavage (P450scc), which play important roles in progesterone synthesis. The expression of StAR and P450scc *in vivo* or *in vitro* were inhibited when treated with $CdCl_2$.

The mechanisms were mainly controlled by the cAMP-dependent pathway (Zhang and Jia 2007). Nampoothiri et al. 2005 reported that lead and cadmium are known as reproductive toxins, which accumulate in granulosa cells of the ovary and cause a significant reduction in gonadotrphin binding which altered steroidogenic enzyme activity of these cells (Nampoothiri et al. 2005).

Table 1. Standard weights of ovaries and uteruses (g) in female rats fed with cadmium chloride.

groups	Right ovary	Left ovary	Uteruses and oviducts	Total reproductive system
Control	0.06 ±0.001	0.05 ±0.001	0.24 ±0.003	0.36 ±0.018
Ld(11mg/kg)	0.05 ±0.001	0.06 ±0.008	0.26 ±0.005	0.39 ±0.003
Ld(23mg/kg)	0.05 ±0.002	0.05 ±0.001	0.25 ±0.008	0.37 ±0.007
Hd(35mg/kg)	0.05 ±0.001	0.05 ±0.001	0.25 ±0.001	0.38 ±0.009
Hd(50mg/kg)	0.06 ±0.001	0.05 ±0.00	0.28 ±0.0061	0.40 ±0.008

Values represent mean ± S.D.

Table 2. The duration of the proestrus, estrus, diestrus phase and the estrous cycle (day) in female rats fed with cadmium chloride.

groups	Proestrus phase	Estrus phase	Diestrus phase	estrous cycle
Control	1.33 ±0.082	3.00 ±0.00	7.33 ±1.063	11.7 ±1.20
Ld(11mg/kg)	0.33 ±0.086	3.33 ±1.20	11.0 ± 2.028	16.8 ±2.30*
Ld(23mg/kg)	0.16 ±0.041	2.33 ±0.082	11.2 ±3.043	13.7 ±0.94
Hd(35mg/kg)	0.43 ±0.053	2.43 ±0.079	11.3 ±4.053	14.0 ±0.20
Hd(50mg/kg)	0.66 ±0.082	2.00 ±0.00	11.2 ±1.060	12.3 ±1.021

*significant difference with control group (P<0.05)
Values represent mean ± S.D.

Table 3. The serum levels of estrogen and progesterone in rats fed with cadmium chloride.

groups	Estradiol concentration (pg/ml)	Progesterone concentration (ng/ml)
Control	13.1 ±3.33	27.9 ±0.30
Ld(11 mg/kg)	17.0 ±1.3	40. 0 ±0.56*
Ld(23 mg/kg)	13. 0 ±1.38	43.5 ±1.88*
Hd(35 mg/kg)	15.0 ±1.83	15.3 ±0.20*
Hd(50 mg/kg)	20.6 ±2.55	14.1 ±0.38*

*significant difference with control group (P<0.05)
Values represent mean ± S.D.

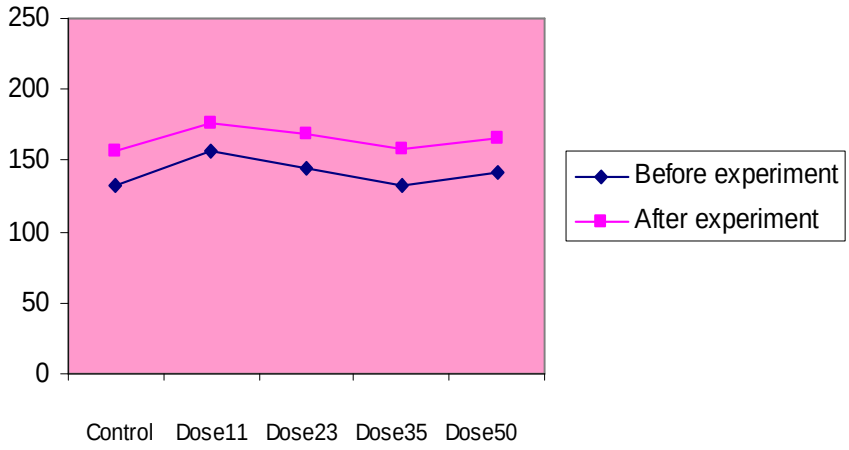

Figure1. Comparison of the body weight between the low and high dose administered groups and the control group.

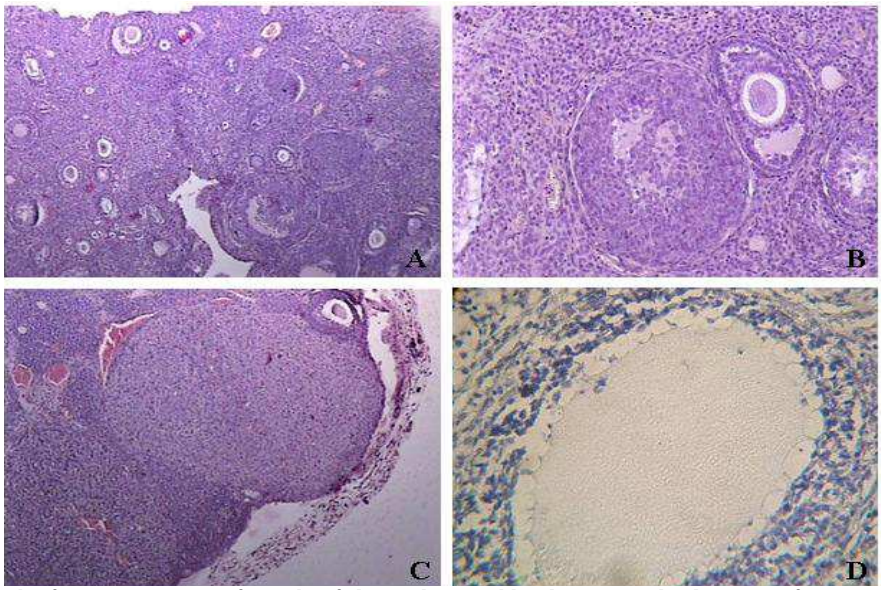

Figure 2- Photograph of ovarian sections of rats that fed to cadmium chloride. Longitudinal section of ovary of the control group, ×4 (A); vesicular or secondary follicles of low dose (11mg/kg) administered group, ×10 (B); corpus luteum of low dose (23mg/kg) administered group, ×10 (C); and graffian follicle of high dose (50 mg/kg) administered group, ×40 (D). There are no histolopathological changes in ovarian follicles and corpus luteum of experimental groups compared to the control group. Hematoxyline and eosin staining.

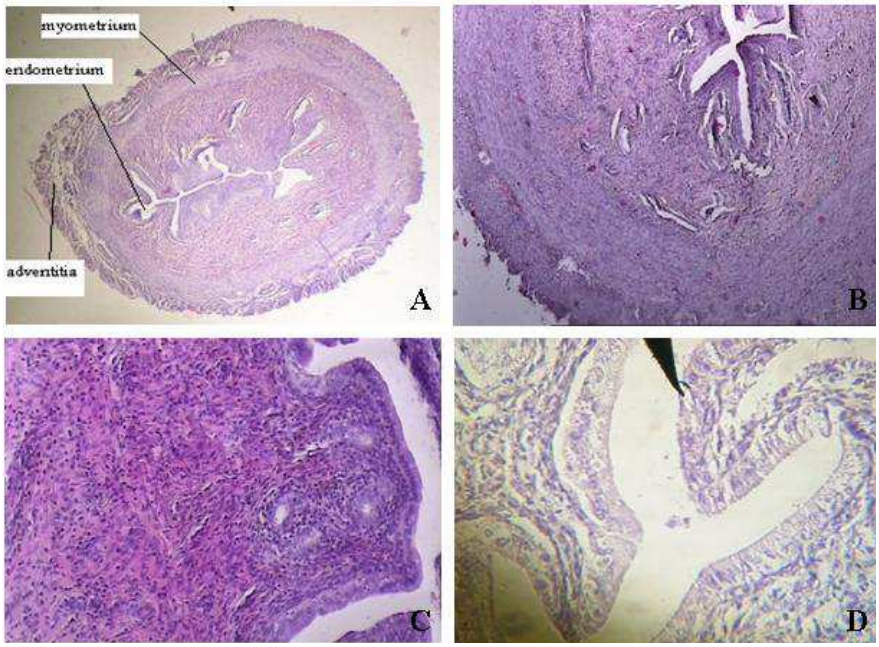

Figure 3- Photograph of uterus sections of rats that fed to cadmium chloride. Transverse section of uterus of the control group, ×4 (A); transverse section of uterus of low dose (23mg/kg) administered group, ×10 (B); transverse section of endometrium of low dose (35mg/kg) administered group, ×40 (C); and endometrial epithelial cells of high dose (50 mg/kg) administered group, ×100 (D). There are no histolopathological changes in different layers of uterus of experimental groups compared to the control group. Hematoxyline and eosin staining.

Ovaries of 11 and 23 mg/kg treated groups were similar to the control group with normal tissues. There were no pathological changes in these groups, however limited edema was observed in the connective tissue of ovarian medullae and granulosa lutein cells of the corpus luteum. The numbers of corpus luteums and atretic follicles were

increased in 35 and 50 mg/kg treated groups. Some atretic stromal cells were also observed (Figure 2 A-D). In the control group and other experimental groups except for the 50 mg/kg group, the epithelium and the glands of the uterus were normal. Myometrium consisted of smooth circular and longitudinal muscles. Inner circular muscles were thicker than outer longitudinal muscles. Connective tissue cells and matrix was observed in the endometrium. No pathological changes were observed in the experimental groups except for the 50 mg/kg group in which endometrial connective tissues showed more density than the control group (Figure 3 A-D). The histological changes in ovarian follicles and uterus after administration of cadmium may also cause hormonal changes. It is well established that cadmium affects the plasma level of pituitary hormones. In previous study Massányi et al., 2007 reported Cd can cause vacuolation, congestion and necrosis in the ovary and uterus (Massányi et al., 2007). Cadmium differentially affects the secretory mechanisms of the pituitary hormones depending on the received dose (Lafuente et al. 2003). In this study we could show that cadmium with different doses can affect hormonal and pathological changes in female reproductive system.

Conclusion

This study evaluated possible cadmium alternations on serum estrogen and progesterone, as well as on the estrous cycle induced by cadmium exposure. We conclude that the cadmium in pool waters around Shiraz is dangerous for mammals and rats as an animal model. Given the relatively high concentration of this element in Maharloo Lake of Fars province and the above-mentioned potential bioaccumulation, this poses a threat to female fertility among mammal in that area.

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Declaration of interest

The authors report no conflict of interest. The manuscript does not have a direct financial relation with the commercial identity. The authors alone are responsible for the content and the writing of the paper.

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