Effects of Cadmium on sperm parameters, histological and hormonal changes in testes of mature rats.

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Abstract:

Background:
Cadmium (Cd) is a heavy metal toxicant, present widely in our environment and workplaces. It is well known that cadmium causes adverse effects on male reproductive organs and reproductive functions in experimental animals.

Objective:
This experiment was designed to find out the effect of cadmium on some histological and hormonal parameters of the reproductive system of adult male rats.

Methods:
thirty mature albino male rat were used (Rattus rattus norvegicus albinus), as a mammalian model. these animals were divided into two groups, 15 rat/group after labeling them. The control group (G1) was orally administrated with distilled water during the 4 weeks period of the experiment and the experimental group (G2) was orally administrated with 20 ml cadmium chloride (200 mg/1L body weight) during the 4 weeks. First of all, the rat were anesthetized, after that blood aspiration using anesthetic cardiac puncture and the serum was separated and store at -20ºC until assessment of FSH, LH and testosterone hormones. After that, the weight of reproductive organs (testis,epididymus and seminal vesicle) was recorded. These organs were fixed with 10% formal saline for histological study.

Results:
The statistical analysis shows a significant ($P< 0.05$) decrease in the weight of the testes and seminal vesicles when compared with control group. The results of the sperm parameters show a significant ($P< 0.05$) decrease in sperm concentration and a highly significant ($p\leq0.01$) decrease in sperm motility in treated group as compared to control. The result of testicular sections in treated group shows a decrease in thickness of germ cells layer, widening of the central seminiferous tubules lumen and prominent germ cells population necrosis. Multiple vacuoles were seen within the tubules. Sertoli cells were abnormal in number and shape as compared to control. A peritubular fibrotic change had been seen also in the testicular sections. The result also shows a highly significant decrease in diameters of seminiferous tubules, primary spermatocytes, secondary spermatocytes, spermatids and increase in interstitial space when treated with cadmium compared with control group. The Levels of serum hormones shows a significant ($P<0.05$) decrease in the levels of testosterone and LH hormones mean in the treated group, while highly significant ($P\leq0.01$) increase in the FSH hormone mean in the treated group when compared to the levels mean of the hormone in the control group…

Conclusion:
In conclusion, these findings suggest that cadmium administration with 20 ml cadmium chloride (200 mg/1L body weight) for 4 weeks during the adulthood induced damage in the reproductive organs and lead to impair the functions of sperm parameters and cause histological and hormonal alterations.

Keywords: cadmium, rats, reproductive organs, testis.

Introduction

Cadmium (Cd) is a very toxic heavy metal and an important environmental pollutant which is present in the soil, water, air, food and in cigarette smoke. Cadmium has been released into the environment through human activities and is routinely found as a contaminant in tissues collected from the human population throughout the world (1). It is a toxicant that has a long biological half-life (15–20 years) and accumulates over time within the blood, kidneys, liver, and reproductive organs (2). It caused a poisoning in various tissues of humans and animals. Acute administration of Cd often induces lethal toxicity in mice and rats (3). Cadmium has been classified by the International Agency for Research on Cancer as class I carcinogen in humans and shown to cause various kinds of tumors including pulmonary, testicular and prostatic tumors (4) (5). Cd, like other endocrine disruptors, is able to modify the basal activity of the testes (6). The gonad is considered the main target for environmental toxins and rodent testes are especially sensitive to the toxic effects of Cd exposure. Cd impairs reproductive capacity by causing severe testicular degeneration, seminiferous tubule damage and necrosis in rats (7) (8). Different studies have shown that Cd affects plasma gonadotropin levels (9). The study of the hypothalamic-pituitary-gonadal axis in animals exposed to the metal is of great interest since the levels of cadmium in air, water, soil, and foods have increased several-fold in many parts of the world as a result of emissions from industrial activities (10). The safety limit of cadmium intake for adult humans is of 51 to 71 µg/day in industrialized countries (11) (12). Environmental cadmium (Cd) exposures may contribute significantly to reduced human male sperm concentration and sperm motility (13). Exposure to cadmium has been reported to induce testicular and epididymal damage (14) (15) and may contribute to male infertility by reducing sperm quality in both humans and rodents (16). A most recent study shows that a single dose (sc) of Cd -induced apoptosis in the testes and reduced serum testosterone level (17).

Aim of the study: to detect the histological and hormonal effect of Cadmium on the reproductive organs of mature male rat, as a representative model for human.

Materials and Methods

The study was performed on the albino rat (Rattus rattus norvegicus albinus), as a mammalian model. These animals were divided into two groups (15 rat/group) after labeling them with ear or tail marking and weighting them using an electrical balance, as follows:

1. Control group (G1): This group was orally administrated with distilled water during the 4 weeks period of the experiment.
2. Experimental group (G2): This group was orally administrated with 20 ml cadmium chloride (200 mg/1L body weight) during the 4 weeks period of the experiment.

All treatments were given by intragastric tube daily, separately and continued for 4 weeks. First of all, body weights were recorded then the rats were anesthetized using diethylether. After that blood aspiration using cardiac anesthetic puncture and blood was left in a sterile plastic tube for 20 minutes at room temperature before spin in a centrifuge at 2500 rpm for 5 minutes, the serum was separated using automatic pipette and store at -20ºC until assessment of FSH,LH and testosterone hormones (9). After that each rat was killed by cervical dislocation; incision of rat was done. The weight of all organs was recorded, reproductive organs (testis, epididymes and seminal vesicle) were quickly excised and immersed in few drops of normal saline which was placed in Petri dish to be cleared from surrounding adipose tissue under dissecting microscope using fine surgical scissors.

The organs were blotted dry from normal saline using filter paper and then weighted with electrical balance, while both right and left testes and seminal vesicle were fixed with 10% formal saline for a subsequent histological study. For morphometric analysis, the slides images were captured by using Microns Contain TV-Based computer assisted Morphometry with a 10X and 4X objectives. The actual measurements were done by using the image analyzer software after accurate calibration using a stage micrometer.

The right and left epididymes were put in a small Petri dish contained 1ml of global culture media then the epididymes were minced by microsurgical scissor about 200 times until we got a homogenized solution which contained the spermatozoa.

Results

Daily administration of cadmium chloride (Cd) for 4 weeks to the male rats causes some changes including:

A- Organs Weight Changes: The statistical analysis showed a significant ($P< 0.05$) decrease in weights of testes in comparison with control group (figure 1-a). Similar results were noted in the seminal vesicles weight in comparison with that of the control group (figure 1-b).

B- Sperms parameters: The results of oral treatment of cadmium for 4 weeks duration on sperm parameters are shown in table (1) and showed the following:

1. Sperm concentration: The statistical analysis showed a significant (P< 0.05) decrease in sperm concentration in treated group in compared to control group (figure 2).

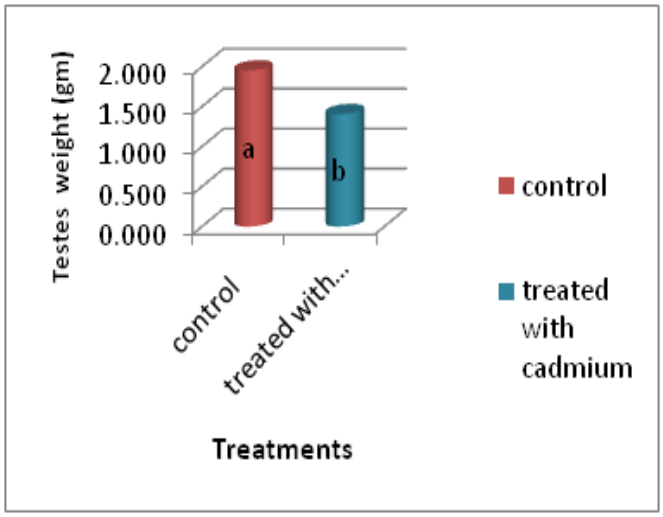
2. Sperm motility: The results of progressive sperm motility percentage revealed a highly significant (p≤0.01) decrease in treated group. Moreover, administration of Cd showed a significant (p≤0.05) decrease in non progressive motility and increase (p≤0.05) in immotile sperm in comparison to control group.

3. Sperm morphology: The results shows a significant (p≤0.05) reduction in normal sperm morphology in treated group when compared to control group.

C- Histological Observations: In Cd treated group, sections showed a decrease in thickness of germ cell layer, widening of the central seminiferous tubules lumen and prominent germ cell population necrosis. Multiple vacuoles were seen within the tubules. Sertoli cells were abnormal in number and shape as compared to control. A peritubular fibrotic change had been seen also in the testicular sections (Figure 3-a). The results also showed a highly significant decrease in diameters of seminiferous tubules (Figure4), primary spermatocytes, spermatids and increase in interstitial space when treated with cadmium compared with control group (figure 5).

Testicular sections of the control group showed seminiferous tubules with normal germ cell population layer thickness with a normal orderly arranged pattern up to mature spermatid. No, malignant or abnormal cell was seen within the germinal epithelium, also no vacuoles is present in the tubules. There were adequate Sertoli cells populations (figure 3-b).

D- Level of serum hormones: the result showed that there was a significant decrease (P<0.05) in the level of testosterone hormone (Testo) in the treated group (figure 6), while highly significant (p≤0.01) increase in the follicle stimulating hormone (FSH) in the treated group when compared to the level of the hormone in the control group (figure 7). On the other hand, a significant (P<0.05) decrease in the level of luteinizing hormone (LH) in the treated group (figure 8).



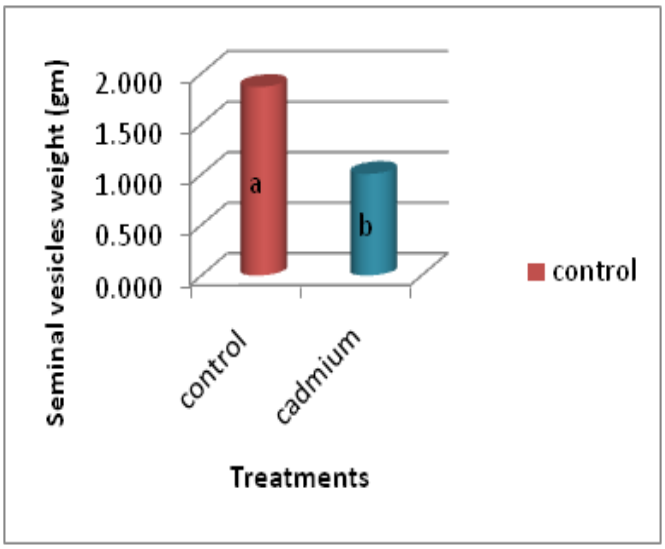


Figure (1): Effects of administration with 20 ml cadmium chloride (200 mg/1L body weight) during the 4 weeks period of the experiment on the weight of the reproductive organs of the male rats, a: testes (gm) and b: seminal vesicles (gm).

Table (1): Effect of administration with 20 ml cadmium chloride (200 mg/1L body weight) during the 4 weeks period of the experiment on certain sperm parameter of the male rats.

treatments sperm parameters	Control (distill water for 4 weeks)	Cadmium chloride (200 mg/1L body weight)
sperm concentration (millions/ mL)	96.750 a ±5.218	42.500 b ±4.233
Progressive sperm motility (%)	40.750 a ±2.175	8.500 c ±1.688
Non progressive sperm motility (%)	32.750 a ±1.109	21.667 b ±2.929
Immotile sperm (%).	34.000 b ±7.246	69.833 a ±4.254
Sperm Morphology (%)	54.500 b ±3.329	1.667 c ±1.667

Differences a, b, c are significant (p<0.05) to compression rows.

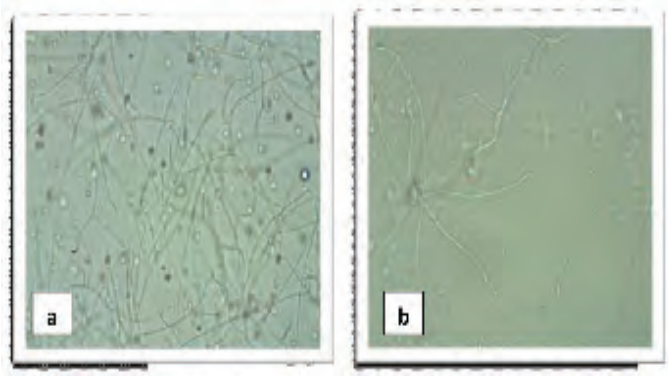
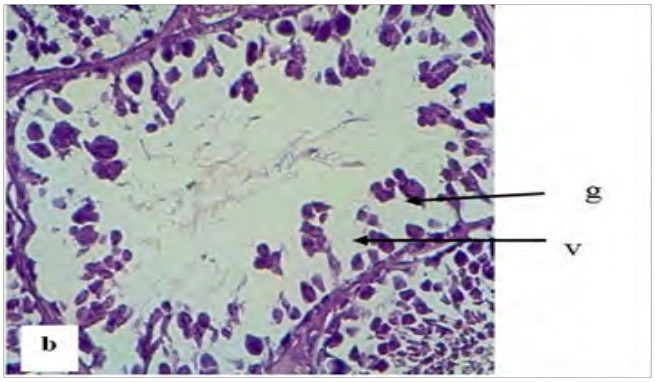


Figure (2): a: Normal sperms concentration from control group (E X40), b: Abnormal sperms concentration from treated (E X40).



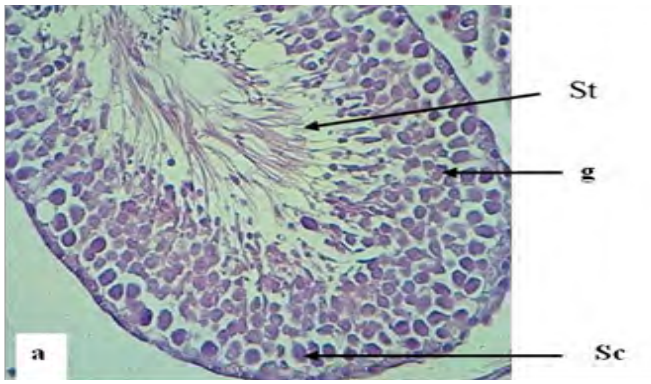


Figure (3): Testicular section of male rat: (a) treated with cadmium. Note the germ cells necrosis (g) and the section also shows vaculation (v) ; (b) control group. Note the normal arranged germ cells layer (g) with and normal Sertoli cells (Sc) contained well organized seminiferous tubules (St). (H&E, 40 x)

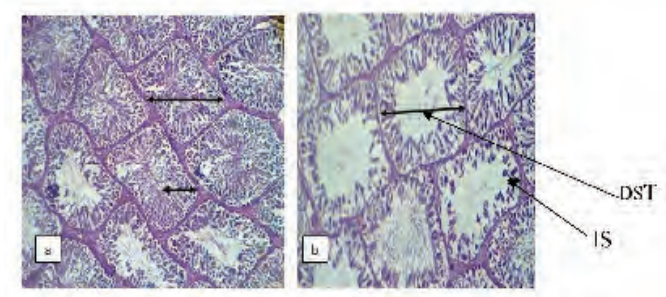


Figure (4): Photomicrograph of testes of rat: (a) control group showing normal structure of seminiferous tubules. (DST- Diameter of Seminiferous tubules, IS- Interstitial space) ; (b) treated group sshowing decrease in diameters of seminiferous and increase in interstitial space. (The actual measurements were done by using the image analyzer software after accurate calibration using a stage micrometer). (H&E, 10 x)

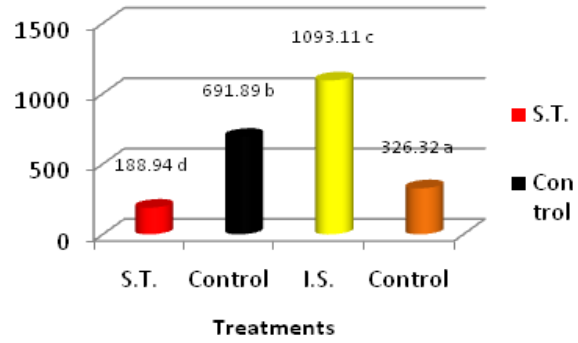


Figure (5): Diameters of seminiferous tubules (um) (S. T.) and interstitial space (um) (I. S.) in both treated and control group.

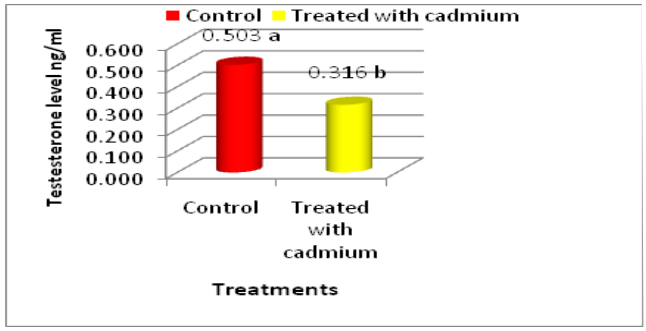


Figure (6): Effect of cadmium (200 mg/1L body weight) on testosterone level (ng/ml) in male rat serum.

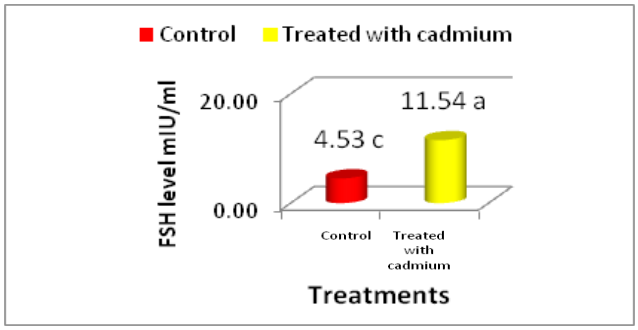


Figure (7): Effect of cadmium (200 mg/1L body weight) on FSH level (mIU/ml) in male rat serum.

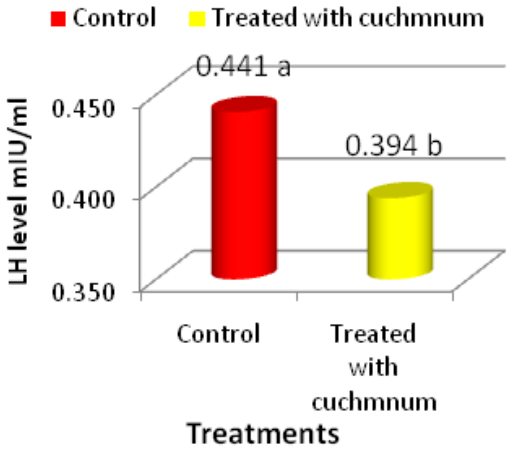


Figure (8): Effect of cadmium (200 mg/1L body weight) on LH level (mIU/ml) in male rat serum.

Discussion:

Cadmium exposure has been reported to be a risk factor for infertility and it is a very dangerous on testicular function (18) [19]. In this study, after Cd administration, we noted weight reductions of accessory sex organs: testes and seminal vesicle after 4 weeks of administration. Probably, this is the natural defenses of this organ required more than 7 days to recover its usual weight after Cd toxicity, as was observed after the longer period (20) (21). Studies of the consequences of Cd contamination have demonstrated that the testis is more sensitive to Cd than other important organs, in addition, Cd can interfere with testis function (22). And these observations agree with our results. The results of this study revealed that cadmium administration decreased sperm count, sperm motility, and sperm morphology of the rats, as in previous reports, which demonstrated that cadmium impairs testicular function (23) (24).

The significant reduction in sperm count, motility and morphology observed in this study following cadmium administration may be associated to impairment of spermatogenesis consequent to reduced secretion of testosterone (25). Cadmium is a potent cell poison known to cause oxidative stress by increasing lipid peroxidation and/or by changing intracellular glutathione levels and to affect the ubiquitin/ATP-dependent proteolytic pathway (26) (27). Acute exposure to large doses of Cd induces gonadal necrosis whereas chronic treatment with low doses has been shown to produce testicular atrophy (28). Cd decreases androgen biosynthesis, possibly by altering progesterone synthesis and metabolism through direct interaction of Cd with DNA and competitive inhibition of essential enzymes (29). Even moderate exposure to Cd can increase abnormal sperm morphology and decrease sperm motility and sperm concentration (30). Different studies have shown that cadmium affects plasma gonadotropin levels (30) (32), and its prominent inhibitory action on testosterone production by interference with hypothalamic-hypophyseal-testis axis (33), this changes found at the hypothalamus, together with those observed in plasma levels of gonadotropins and testosterone (34) (35), suggest a global effect of the metal on the reproductive function and that an interaction exists between the metal and age during cadmium exposure (36).

Our result show increased in plasma levels of FSH after cadmium administration for 4 weeks of life. This finding could be due to the accumulation of cadmium in the testis. In this context, it was shown that cadmium affects Sertoli cell activity by decreasing inhibin synthesis and release. Thus, the increased plasma levels of FSH could be explained, as this peptide is the main inhibitory signal for FSH secretion (37). Our observations were in line with changes observed by De Souza Predes *et al*. (38).

Authors described a marked reduction of seminiferous tubular diameter after the higher dose of cadmium, along with the conspicuous decrease of the tubular volume density, which means that cadmium caused a significant reduction in the relative seminiferous tubule length (39). These data confirm that the severity of cadmium-induced damage at the testicular tissue and the seminal vesicles (40), which lead to decrease in reproductive organ weight and cause changes in the diameter of seminal vesicle and interstial space (41). The decrease in plasma testosterone levels observed after both prepubertal and postpubertal cadmium exposure may reflect direct effects of the metal at the testis as this metal accumulates in this tissue (42). Cd has been considered as an important environmental endocrine disruptor (43), this may lead to variations in plasma LH levels, thus indicating that the metal may act at the hypothalamic level, modifying the activity of the endogenous clock, changing the mean concentration of LH secreted daily by the pituitary gland (44).

In conclusion, these findings suggest that cadmium administration with 20 ml cadmium (200 mg/1L body weight) for the 4 weeks during the adulthood, induce damage in the reproductive organs and impairment of sperm parameter, causing histological and hormonal alterations.

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