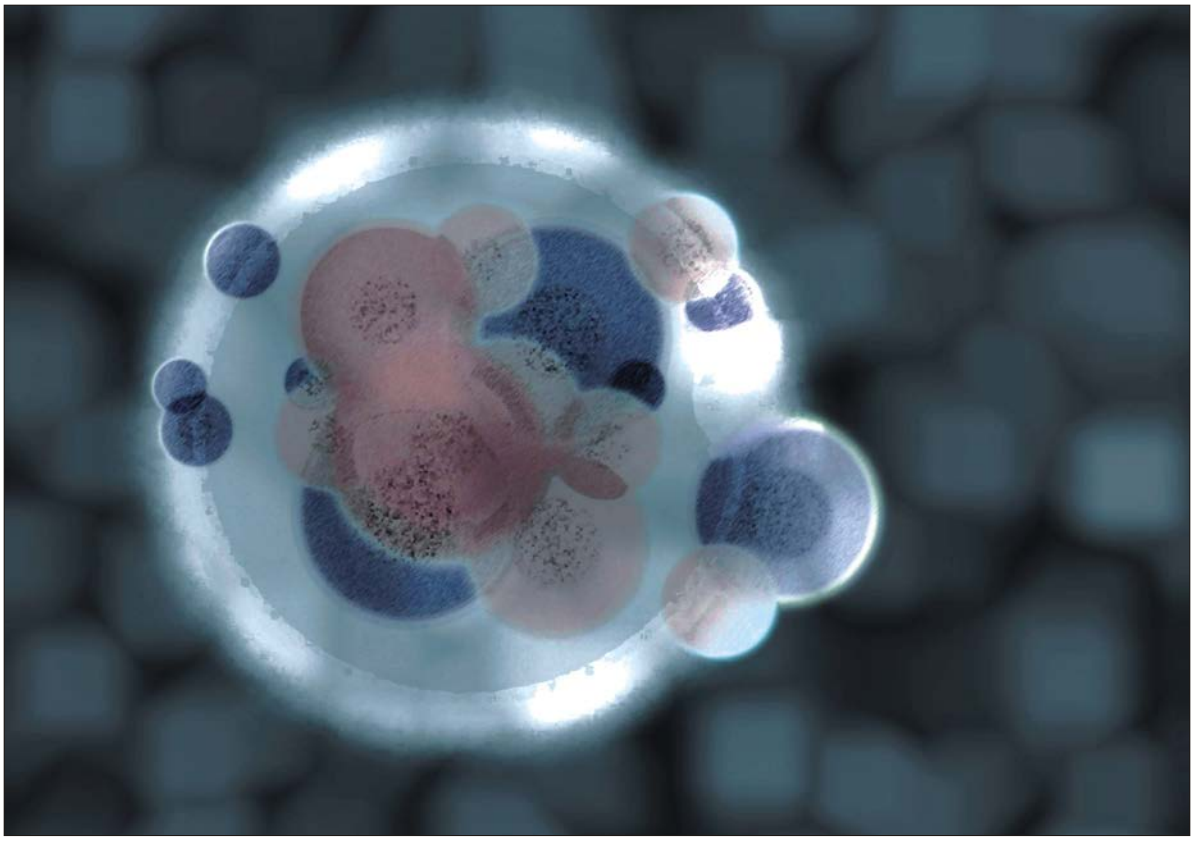

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# Effect of Selenium on Cadmium Induced Gonadotoxicity in Male Rats

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**Abstract:** The present study was conducted to investigate the possible effect of Selenium (Sel) against Cadmium (Cd) toxicity on hormones and gonads of male rats. Male rats were divided into three groups. First group served as control, second group administrated s.c. cadmium chloride (2.5 mg $kg^{-1}$) and third group injected s.c. with Sel (0.35 mg $kg^{-1}$) and Cd. In the last group, the animals were treated with Sel one hour before Cd treatment. Blood samples were collected and eight animals were sacrificed after two, four, six and eight weeks. Plasma testosterone, FSH and LH were assayed using enzyme immunoassay method. The sperm quality was evaluated. Cd in the testes was determined using atomic absorption. The weights of body, testes and accessory sex organs were recorded. The treatment of the rats with Cd results in a significant decrease of testosterone, LH, sperm motility (%), body gain and weights of testes, epididymis, vas deference, seminal vesicle and prostate gland. On the other hand, the sperm abnormalities and FSH concentration increased significantly in Cd treated rats. Cd content in the testes of second group and third group increased in a time-related fashion. Cd concentration in the testes of rats treated with Cd and Sel was less than that of rats injected with Cd alone. Sel was found to reduce the deleterious effects of Cd.

**Key words:** Testosterone, FSH, LH, Sperm

## INTRODUCTION

It is likely that man and animals are exposed to cadmium (Cd) since it is released simultaneously in the environment from a number of natural and man made sources. For example, intoxication in children from ingestion of paint may be associated with Cd exposure because Cd is used as a pigment in yellow and some red paints[1].

Cd is well known for its various adverse effects, e.g. enhancement of lipid peroxidation[2], influence on mitochondrial functions[3] and DNA chain break[4]. Cd has been shown to accumulate in hypothalamus, pituitary and gonads[5,6]. This accumulation leads to different disorders of the endocrine system[7-9]. Cd can cause a number of lesions in testes[10]. Exposure to this heavy metal affects the reproductive function of both males and females[11,12]. It induces testicular toxicity at doses considerably lower than for other organs[13].

Data available suggest the existence of interactions between Cd and gonadotropines[8,14].

In male rats given Cd, necrosis of spermatogonia, spermatocyte and spermatide was observed in some seminiferous tubules[15]. Subcutaneous injection of Cd to adult male rats resulted in a reduction in sperm population in the vas deference and epididymis[16].

Selenium (Sel) is known to have beneficial effects on the reproduction of experimental animals[17]. Moreover, Sel is an important part of the antioxidant system of animal tissues[18]. Sel has been shown to provide protection against cisplatin[19] and nitrite[20]. Sel interacts with Cd in cultured cells in relation to cellular uptake[21]. The present study was conducted to investigate the possible protective effect of Sel against Cd toxicity on hormones and gonads of male rats.

## MATERIALS AND METHODS

**Animals:** Ninety-six male Wister rats used in this investigation. The age of rats was six months and their weight ranged from 250 to 290 g. The animals were maintained under standard laboratory condition (12 h light, temperature 23±1°C). They fed dry ration *ad lib*. The rats were randomly divided into three groups of 32 animals each.

**Experimental design:** The animals were treated four times weekly for 8 weeks as follows: group (1) served as control and was given tap water, group (2) injected s.c. with 2.5 mg $kg^{-1}$ of cadmium chloride[22] and group (3) injected s.c. with Sel at dose level of 0.35 mg $kg^{-1}$[23] and cadmium chloride (2.5 mg $kg^{-1}$). Sel was injected in group 3 subcutaneously 1 h before Cd injection. Both cadmium chloride and Sel were obtained from Merck (Darmstadt, Germany).

**Hormones assay:** Eight blood samples from each group were collected after 2, 4, 6 and 8 weeks from treatment and plasma was separated. Plasma testosterone, LH and FSH were assayed at the four time intervals by enzyme immunoassay (Alfa Scientific Designs, USA). The assay is based on competitive binding technique.

**Evaluation of spermatozoa:** Eight animals from each group were sacrificed at the four time intervals previously mentioned. One epididymis was removed after sacrificing each rat, cleaned from fats and dissected in 10 mL of 0.1 M phosphate buffer which previously incubated at 37°C. Percentage of sperm motility was quantified. Sperm number was calculated using haematocytometer. Smears were prepared from the suspension and stained with 1% eosin solution and examined for abnormalities[24].

**Determination of Cadmium in the testes:** Certain weights of dried samples were transferred to beakers and digested with hot nitric acid near dryness and then diluted to definite volume. The solutions were analyzed by atomic absorption.

**Body and reproductive organs weight:** The weight of body was recorded at 0, 2, 4, 6 and 8 weeks post-treatment in three groups. At the end of time intervals, 8 animals were sacrificed from each group and weights of testes, vas deference, epididymis, seminal vesicle and prostate gland were recorded.

**Statistical analysis:** Significance of the results was evaluated by using the student t-test.

## RESULTS

Figure 1 indicated that the plasma testosterone concentration decreased significantly ($p<0.01$) in the rats treated with Cd by 40, 69, 68 and 94% compared to control at 2, 4, 6 and 8 weeks post-treatment. Testosterone concentration exhibited a significant decrease ($p<0.01$) after 4, 6 and 8 weeks in the rats treated with Cd and Sel in combination by 34, 51 and 71%, respectively. The hormone level of the rats treated with Cd and Sel was significantly ($p<0.01$) higher than that of rats treated with Cd alone at the four time intervals.

LH level (Fig. 2) of the animals subjected to Cd only showed a significant decrease by 18, 29, 32 and 53% during the four time intervals, respectively. Moreover, LH concentration decreased significantly ($p<0.01$) in the rats given Cd and Sel in combination by 18, 24 and 29% after

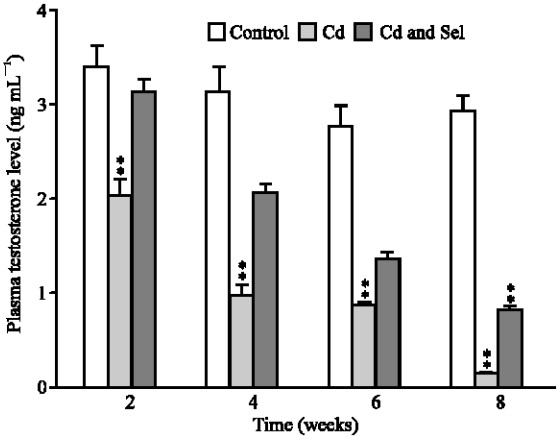


Fig. 1: Effect of Cadmium and Selenium on plasma testosterone level (ng/mL) of male rats
Each value is the mean±SE
**: Significant different from Control group, $p<0.01$

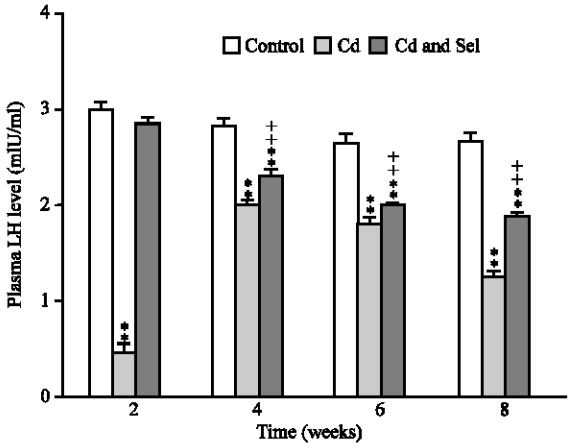


Fig. 2: Effect of Cadmium and Selenium on plasma LH level (mIU/mL) of male rats
Each value is the mean±SE
**: Significant different from Control, $p<0.01$
++: Significant different from Cadmium group, $p<0.01$

4, 6 and 8 weeks, respectively. On the other hand, the hormone level of Cd and Sel group was significantly higher ($p<0.01$) than that of Cd group at all four time intervals.

The results as shown in Fig. 3 indicate a significant elevation of FSH level after 2 ($p<0.05$), 4, 6 and 8 ($p<0.01$) weeks in the rats treated with Cd by 21, 72, 83 and 138%, respectively. FSH level of rats given Cd and Sel together increased significantly after 6 ($p<0.05$) and 8 ($p<0.01$) weeks by 20 and 21% as compared to control. The mean value of FSH level of rats treated with Cd and Sel was

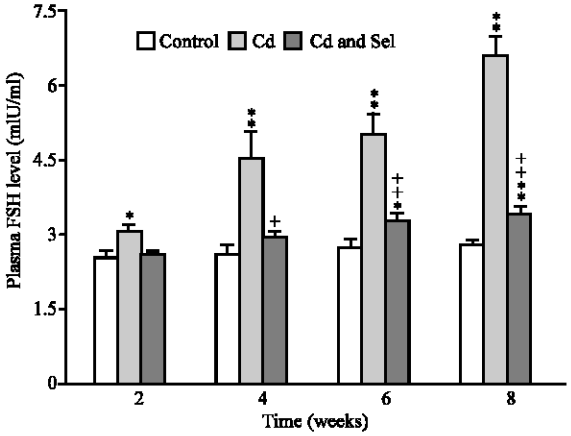


Fig. 3: Effect of Cadmium and Selenium on plasma FSH level (mIU/ml) of male rats
Each value is the mean±SE
*, **: Significant different from Control, p<0.05, p<0.01
+, ++: Significant different from Cadmium group, p<0.05, p<0.01

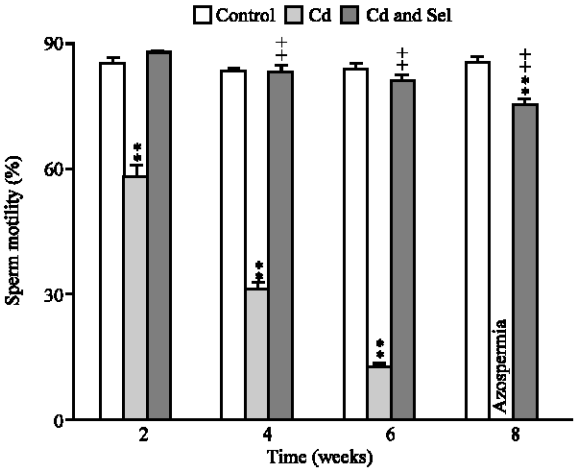


Fig. 4: Effect of Cadmium and Selenium on sperm motility (%) of male rats
Each value is the mean±SE
**: Significant different from Control, p<0.01
++: Significant different from Cadmium group, p<0.01

significantly (p<0.01) less than that of Cd group at all four time intervals.

The sperm motility of rats treated with Cd (Fig. 4) was reduced significantly (p<0.01) by 32, 63 and 86% after 2, 4 and 6 weeks. The epididymis of the rats injected with Cd was devoid of sperms 8 weeks post-treatment. On the other hand the sperm motility decreased significantly (p<0.01) in the rats given Cd and Sel by 12% after 8 weeks.

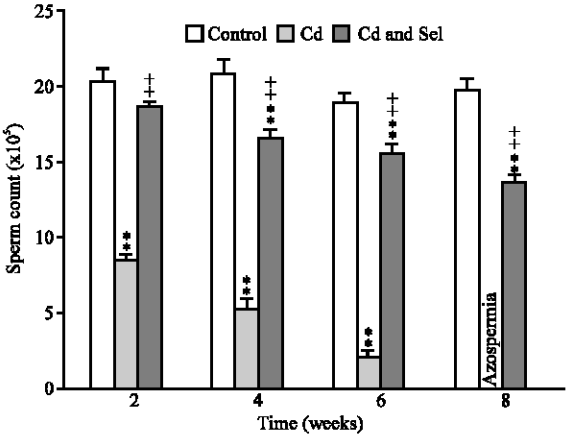


Fig. 5: Effect of Cadmium and Selenium on sperm count ($x10^5$ per epididymis) of male rats
Each value is the mean±SE
**: Significant different from Control, p<0.01
++: Significant different from Cadmium group, p<0.01

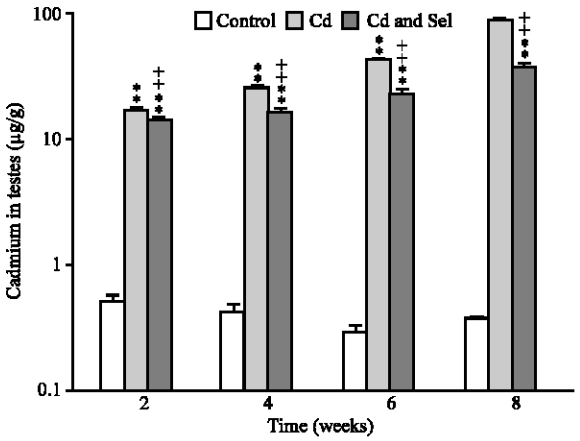


Fig. 6: Effect of Cadmium and Selenium on testicular Cadmium concentration (µg/g) of male rats
Each value is the mean±SE
**: Significant different from Control, p<0.01
++: Significant different from Cadmium group, p<0.01

However, the values of sperm motility of Cd and Sel group were significantly higher (p<0.01) than those of Cd group.

In the rats exposed to Cd, the sperm count (Fig. 5) decreased significantly (p<0.01) by 59, 75 and 89% after 2, 4 and 6 weeks. The sperm count decreased significantly (p≤0.01) by 20, 18 and 32% in the rats injected with Cd and Sel after 4, 6 and 8 weeks. The sperm count of rats treated with Cd and Sel was significantly higher (p<0.01) than that of Cd group.

Injection of Cd either alone or in combination with Sel (Table 1) produced a significant increase (p<0.01) in sperm

Table 1: Effect of Cadmium and Selenium on sperm abnormalities (%) of male rats

| Time (weeks) | Treatments: Control Head | Control Tail | Control Total | Cadmium Head | Cadmium Tail | Cadmium Total | Cadmium and Selenium Head | Cadmium and Selenium Tail | Cadmium and Selenium Total |
|---|---|---|---|---|---|---|---|---|---|
| 2 | 2.59±0.15 | 1.77±0.09 | 4.24±0.22 | 16.05±0.39** | 2.69±0.16** | 18.66±0.38** | 6.41±0.31••** | 1.86±0.20••** | 7.94±0.37••** |
| 4 | 3.21±0.16 | 1.95±0.09 | 5.16±0.19 | 28.74±1.00** | 4.32±0.28** | 32.99±1.01** | 9.92±0.71••** | 2.56±0.21••** | 12.37±0.73••** |
| 6 | 3.67±0.21 | 2.00±0.25 | 5.54±0.15 | 41.00±1.24** | 7.05±0.40** | 48.74±1.76** | 13.74±0.80** | 4.11±0.37••** | 17.55±0.93••** |
| 8 | 3.05±0.17 | 1.49±0.15 | 4.35±0.13 | ___ | ___ | ___ | 16.16±0.70** | 5.30±0.46••** | 21.37±0.90••** |

Each value is the mean±SE, ** : Significant different from Control, p<0.01, •• : Significant different from Cadmium group, p<0.01. ___ : Azospermia

Table 2: Effect of Cadmium and Selenium on the weight (g) of genital organs of male rats relative to body weight

| Time (weeks) | Control Testes | Control Epididymis | Control Vas deferens | Control Seminal vesicle | Control Prostate gland | Cadmium Testes | Cadmium Epididymis | Cadmium Vas deferens | Cadmium Seminal vesicle | Cadmium Prostate gland |
|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 1.41±0.05 | 0.25±0.03 | 0.07±0.002 | 0.45±0.01 | 0.31±0.02 | 0.62±0.02** | 0.15±0.007** | 0.06±0.003* | 0.20±0.004** | 0.16±0.009** |
| 4 | 1.30±0.04 | 0.26±0.01 | 0.08±0.003 | 0.39±0.02 | 0.28±0.02 | 0.49±0.01** | 0.11±0.003** | 0.05±0.004** | 0.16±0.006** | 0.13±0.004** |
| 6 | 1.20±0.09 | 0.27±0.02 | 0.07±0.004 | 0.33±0.02 | 0.27±0.01 | 0.38±0.02** | 0.09±0.003** | 0.04±0.002** | 0.10±0.005** | 0.08±0.005** |
| 8 | 1.10±0.03 | 0.26±0.01 | 0.06±0.001 | 0.40±0.006 | 0.30±0.005 | 0.29±0.005** | 0.08±0.004** | 0.03±0.004** | 0.08±0.004** | 0.07±0.003** |

Table 2: Continued

| Time (weeks) | Cadmium and Selenium Testes | Cadmium and Selenium Epididymis | Cadmium and Selenium Vas deferens | Cadmium and Selenium Seminal vesicle | Cadmium and Selenium Prostate gland |
|---|---|---|---|---|---|
| 2 | 1.15±0.03•• | 0.24±0.01•• | 0.07±0.003 | 0.40±0.03•• | 0.29±0.007•• |
| 4 | 1.04±0.05••** | 0.25±0.01•• | 0.07±0.003•• | 0.37±0.01•• | 0.26±0.005•• |
| 6 | 0.90±0.07••** | 0.22±0.01*•• | 0.06±0.003•• | 0.29±0.03•• | 0.21±0.03•• |
| 8 | 0.89±0.01••** | 0.20±0.003••** | 0.06±0.002•• | 0.30±0.01**•• | 0.20±0.008•• |

Each value is the mean±SE, *, ** : Significant different from Control, p<0.05, p<0.01, •• : Significant different from Cadmium group, p=0.01

Table 3: Effect of Cadmium and Selenium on body weight (g)

| Time (weeks) | Treatments: Control | Cadmium | Cadmium and Selenium |
|---|---|---|---|
| Zero | 249.50±10.43 | 268.00±9.33 | 291.40±8.71 |
| 2 | 280.80±9.15 | 268.30±9.87 | 319.00±8.77 |
| % of change | 12.55 | 0.11 | 9.47 |
| 4 | 310.40±4.70 | 278.50±11.60 | 330.60±9.55 |
| % of change | 24.40 | 3.91 | 13.45 |
| 6 | 343.60±8.92 | 290.30±12.44 | 341.70±10.22 |
| % of change | 37.72 | 8.32 | 17.26 |
| 8 | 360.00±7.41 | 298.40±10.00 | 365.20±8.65 |
| % of change | 44.29 | 11.34 | 25.33 |

Each value is the mean±SE % of change is relative to Zero time

abnormalities. The head abnormalities were higher than tail abnormalities. The sperm abnormalities were less in rats treated with Cd and Sel than in rats treated with Cd alone.

The results as shown in Fig. 6 indicate a significant elevation of Cd in the testes of all treatment groups ($p<0.01$) as compared to control at the time intervals. On the other hand, Cd content of the testes of rats treated with Cd and Sel was significantly lower ($p<0.01$) than that of rats treated with Cd alone.

The weights of testes, epididymis, vas deference, seminal vesicle and prostate gland were reduced (Table 2) significantly ($p<0.01$) in the rats treated with Cd at the four time intervals. On the other hand, Cd had no effect on the weight of vas deference of the rats pretreated with Sel. The seminal vesicle and prostate gland weights of rats treated with Cd and Sel in combination decreased significantly ($p<0.01$) after 8 weeks, but these weights did not change significantly in other time intervals. Moreover, the testes weight decreased significantly ($p<0.01$) after 4, 6 and 8 weeks while epididymis weight reduced significantly after 6 and 8 weeks. The weights of genital organs were higher in Cd and Sel group than in Cd group.

The body gains (Table 3) of rats treated with Cd alone or Cd and Sel in combination were lower than those of control. The body weight of Cd and Sel group was higher than that of Cd group.

## DISCUSSION

The present study shows that administration (s.c.) of Cd to male rats lead to changes in testosterone and gonadotropic hormones. The testosterone and LH levels decreased significantly in rats treated with Cd. It was reported that decreased androgen production by Cd was reflected in reduced nuclear diameter of Leydig cells. Moreover, the increased level of testicular cholesterol in dogs treated with Cd indicate a decreased production of

androgen by testis[25]. The response of Leydig cells to HCG to secrete testosterone was significantly depressed in rats injected with Cd[26]. A marked decrease of activity of hydroxysteroid dehydrogenase as well as a fall of androgen level was observed following Cd administration[27]. Cd exposure through puberty of male rats decreased circulating levels of LH and testosterone and increased FSH[28]. The elevation of FSH level due to Cd treatment in the present study may result from feed back mechanism of testosterone. Zylber-Haran *et al.*[7] showed that a single high dose of Cd affects the circulating levels of gonadotrophins. These changes indicate that the regulatory mechanism of testicular function is damaged.

The sperm motility and sperm count of the rats treated with Cd in the present study decreased significantly. It was reported that the sperm count and sperm motility decreased significantly in rat administrated i.p. Cd. The motility of sperm is an early and sensitive endpoint for the assessment of Cd toxicity on male reproduction[29]. The canine testis exhibited inflammation, hemorrhage, massive necrosis and atrophy of seminiferous tubules due to Cd treatment[30]. Destruction of the spermatogenic elements and general necrosis was observed in rabbits exposed to Cd[31]. The decrease in sperm count of our experiment may attribute to disturbance in seminiferous tubules especially spermatogenic cells. It was observed that daily sperm production was suppressed by Cd in rats[32].

The abnormalities of the sperms of the rats treated with Cd elevated significantly in our investigation. Daily i.p. administration of Cd to rats for three months enhanced lipid peroxidation and inhibited superoxide dismutase activity in testes[33]. Moreover, the activity of glutathione peroxidase was significantly reduced in the rats treated with Cd[34]. In the testes of rats treated with Cd cellular GSH concentration was decreased[35]. The increase of sperm abnormalities induced by Cd may result from oxidative stress. After Cd injection to the present rats, its content in the testes increased. It has been suggested that the reproductive system may be more susceptible to Cd-induced damage than other systems, since even low doses of Cd can produce hemorrhagic necrosis in the testes despite little Cd can reach this organ[36].

The treatment of rats of our study with Cd result in a decrease of testes, epididymis, vas deference, seminal vesicle and prostate gland weights. Zielinka-Psuja *et al.*[37] concluded that the lowering of testes weight of male rats treated with Cd occurred mainly due to the necrosis of seminiferous tubules.

Treatment of rats of our experiment with Sel and Cd improved the gonadal results of the Cd exposed rats. The advantage of Sel treatment during Cd exposure may be due to the participation of Sel in the synthesis of glutathione peroxidase, the enzyme that catalyzes the reactions of reduced glutathione with hydrogen peroxide and organic peroxide[38]. Sel increases the concentration of vitamin E in the rats[39]. This vitamin is an important antioxidant which acts as a scavenger of free radicals and reduces peroxides[40]. Sel reduced lipid peroxidation induced by Cd[41]. When Sel is given, the transfer of Cd to metallothionein is considerably delayed[42].

In conclusion, present results demonstrate that Cd induced changes in the physiological functions of the testes. Sel reduced the deleterious effects of Cd.

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