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Research Article

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Protective effects of curcumin on the concentration of GnRH, FSH and estrogen hormones in adult female rats treated with cadmium chloride

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ABSTRACT

Introduction: *Cadmium, as a major industrial pollutants, has many negative effects on its surrounding area. Curcumin is a strong antioxidant and purifier of free radicals. In this study, Protective effects of curcumin on the concentration of the hormones of GnRH, FSH and estrogen in the rats treated with cadmium chloride were investigated.*

Method: *In this experimental study, 72 adult female Wistar rats were divided into 9 groups of 8 animals, including control group, observant group, experimental group receiving cadmium chloride (5mg/kg), the groups receiving curcumin (at concentrations of 25, 50, and 100 mg / kg) and a group receiving cadmium chloride and different concentrations of curcumin. On 21th day, the serum concentration of FSH, GnRH and estrogen were measured by bleeding from the heart. The data was analyzed at the significance level of ≥ 0.05 by one-way Anova test and Duncan test.*

Findings: *The mean concentration of GnRH hormone in the groups receiving cadmium chloride (alone) and curcumin increased significantly and the concentration of estrogen decreased significantly in compared with control group. And the mean concentration of FSH hormone in the groups receiving cadmium chloride (alone) and curcumin (at the concentration of 25 and 50 mg/kg) increased insignificantly but in the group receiving curcumin with the concentration of 100mg/kg significantly increased in compared with control group.*

The mean concentration of GnRH hormone in the groups receiving cadmium chloride plus curcumin (different concentrations of 25, 50 mg/kg) significantly increased in compared with control group. The mean concentration of GnRH hormone in the groups receiving cadmium chloride plus curcumin (different concentrations of 25, 50 mg/kg) insignificantly increased in compared with control group. The mean concentration of FSH hormone in the groups receiving cadmium chloride (alone) and curcumin (at different concentration of 25, 59 mg/kg) plus curcumin (different concentrations of 25, 50 and 100 mg/kg) didn't show significant change in compared with control group.

Conclusion: *curcumin, due to the antioxidant property, regulates the secretion of GnRH, FSH and estrogen hormones in the rats receiving cadmium chloride.*

Keywords: *curcumin, cadmium chloride, female rat*

INTRODUCTION

Cadmium is one of the most important industrial and environmental pollutants. This toxic heavy metal is easily absorbed by plants, animals and micro-organisms (1). Intracellular concentrations of cadmium lead to create lesions in organs such as the liver, kidney, lung, brain, testes, placenta, ovary, etc. (2, 3). Also, the smoke from forest fires

and volcanic gases are of the major sources of air pollution. Therefore, the inhalation of polluted air and cigarette smoke leads to respiratory infection caused by this metal in humans (4,5). So, food and smoke are known as the biggest pollution sources of cadmium in human (2,3). Half-life of cadmium is long in the body and it was estimated 10-30 years, representing its characteristic of accumulation in cells of the body and the lack of the impact of detoxification on this metal (4).

Ovary is oval shaped object that its surface is covered with cubic epithelium or simple squamous epithelium called germinal epithelium (6). This ovarian germinal epithelium is responsible for making primordial follicles. Primitive eggs are surrounded by granulosa cells and make primordial follicles. Primordial follicle, during cell division, turns to primary and secondary follicles and graph (oocyte). This process is called Oogenesis. During this process, if the follicle gets dysfunction and distinction disorder, that follicle will be called Atresia (Atretic) (7).

The results of different studies on female gender showed that cadmium chloride reduces the levels of the gonadotrope hormones, ovarian steroid hormones and disrupts the ovulation and finally, induces infertility, the number of these studies are referred here. The results of the study performed by Saksena and Salmonsen (1983) showed that injection of cadmium chloride before ovulation stops the process of ovulation and thus, induces infertility in Hamster (8). The results of the study performed by Paksy (1989) showed that in rats, injection of cadmium chloride before ovulation reduces the levels of FSH, LH hormones and disrupts the ovulation process (9). Also, the results of the studies performed by Lienesch et al. (2000), Priya et al. (2004), Zhang et al. (2007) showed that cadmium prevents the proper functioning of the granulosa cells of ovarian in the production of steroid estrogen and progesterone hormones in rats through inhibition of binding of the gonadotrope hormone in these cells (10-12). Wan et al. (2010) have studied on the texture and morphology of ovarian follicles in rats, the results showed that cadmium chloride reduces the amount of normal and mature ovarian follicles and increases the number of atrophy follicles and causes dysfunction in the oocyte maturation (13).

Turmeric is an herbaceous plant that its rhizome is widely used for color and flavor to food. Its rhizome extract is called curcuminoid and it includes curcumin, demethoxycurcumin and bisdemethoxycurcumin (14). Among these three curcuminoids, curcumin is most abundant in turmeric. Curcumin has a good yellow color that can be used as a coloring agent in food industry (15). Curcumin is found pure, crystalline powder and insoluble in water and ether and soluble in some solvents such as alcohol, glacial acetic acid, alkalis and some oils such as olive oil (16, 17). Curcumin has impressive functional characteristics and in many studies, various properties have been reported for it, including anti-cancer anti-tumor activities (15, 18), decrease in cholesterol of blood and liver (15), inhibition of cardiovascular disease (18), increase in immune function (19), prevention of damage of biological membranes against peroxidation (20), anti-inflammatory properties (21) and a decrease in rheumatoid arthritis (22), protection against Alzheimer's disease (23). In addition, curcumin is a well-known antioxidant and also, one of the most powerful purifiers of free radicals that can prevent Reactive Oxygen Species-ROS in vitro and biological environment (16, 24, 25). The results of the studies show that the antioxidant property of curcumin is equal to vitamins C and E (26, 27). In many studies, cadmium-induced oxidative stress as a mechanism for malicious action of this metal on different organs has been raised. Cadmium induces and increases the production of reactive oxygen species in cells (28). On the other hand, it damages the valuable vital molecules, such as enzymes, protein and membrane lipid by reducing the intracellular antioxidant and disrupting the balance between antioxidants and cell oxidizing agents. The best way to deal with it is to increase the antioxidant compounds in cells and also, to increase the lifetime of the cells to cadmium poisoning. It seems that antioxidants inhibit this substance by binding to cadmium in blood and or they reduce the harmful effects of cadmium by blocking its activity inside the cell (29).

Therefore, due to the strong antioxidant properties of curcumin, this study will be conducted with the aim of investigating the protective effects of the antioxidants in the face of cadmium chloride on the concentrations of the GnRH, FSH and estrogen hormones in rats.

Materials and Methods

In this study, total ethical issues about how to work with laboratory animals were considered. To conduct this study, female Wistar rats with an average weight of 180-200 were used. The rats were kept in animal breeding room of Jahrom University of Medical Sciences for a week to be adaptive. Cycle of light and darkness included 12 hours of

light and 12 hours of darkness, the temperature was 23±2 and the humidity was about 50 to 55 %. According to articles published in the field, this study has conducted on healthy adult female Wistar rats in the weight range of 180 to 200 grams. Animals were randomly divided into 9 groups of 8 animals.

The total number of required rats was 72 female rats. According to the previous articles, the injected concentrations of curcumin were determined 25, 50 and 100 mg/kg (30, 31).

Also, according to previous studies, the injected concentration of cadmium chloride was 5 mg/kg which was injected the rats with gavage (32).

Therefore, in this study, the experimental and control groups included following groups.

Control group: this group does not receive any treatment during the experiment (28 days) (n = 8).

Sham group: this group receives 1ml of olive oil by gavage (solvent of curcumin) and 0.2 ml of normal saline intraperitoneally as a solvent of drugs and according to body weight during the experiment (28 days) (n = 8).

Experimental group 1: this group receives 5 mg/kg cadmium chloride dissolved in 0.2 ml normal saline intraperitoneally for 28 days and according to body weight (n = 8).

Experimental group 2: this group receives 25 mg/kg curcumin dissolved in 1 ml olive oil by gavage for 28 days and according to body weight (n = 8).

Experimental group 3: this group receives 50 mg/kg curcumin dissolved in 1 ml olive oil by gavage for 28 days and according to body weight (n = 8).

Experimental group 4: this group receives 100 mg/kg curcumin dissolved in 1 ml olive oil by gavage for 28 days and according to body weight (n = 8).

Experimental group 5: this group receives 25 mg/kg curcumin dissolved in 1 ml olive oil (gavage) plus 5 mg/kg cadmium chloride dissolved in 0.2 ml normal saline intraperitoneally for 28 days and according to body weight (n = 8).

Experimental group 6: this group receives 50 mg/kg curcumin dissolved in 1 ml olive oil (gavage) plus 5 mg/kg cadmium chloride dissolved in 0.2 ml normal saline intraperitoneally for 28 days and according to body weight (n = 8).

Experimental group 7: this group receives 100 mg/kg curcumin dissolved in 1 ml olive oil (gavage) plus 5 mg/kg cadmium chloride dissolved in 0.2 ml normal saline intraperitoneally for 28 days and according to body weight (n = 8).

At the end of the study (29th day), firstly, the animals were weighted and then their hearts were directly bled by a 5 cc syringe (under anesthetioin by ether) and the serum of blood was collected by centrifugation (3000 rpm for 15 minutes). To measure the GnRH, FSH and estrogen hormones, ELISA kits for rats, constructed by Diametra Company, Italy, were used.

Findings

In terms of the mean concentrations of GnRH, FSH and estrogen hormones, there were no significant differences between all experimental groups and control and observant groups ($p \leq 0.05$) (Table1).

Significant increase in the concentration of GnRH hormone was observed in the groups receiving curcumin (at all levels) and also, the group receiving cadmium chloride compared to control and observant groups ($p \leq 0.05$) (Tables 1). Only in the dose of 100 mg/kg, no significant change was observed in the mean concentration of this hormone in the groups receiving cadmium chloride plus curcumin ($p \leq 0.05$) (Table1, Figure 1).

Increase in the concentration of FSH hormone was observed in the groups receiving curcumin (at all levels) and also, the group receiving cadmium chloride compared to control and observant groups that the increase was significant just in the group receiving100mg/kg of curcumin ($p \leq 0.05$) (Table 1). No significant increase in the mean concentration of this hormone was observed in the groups receiving cadmium chloride plus curicum (at all levels) compared to control and observant groups ($p \leq 0.05$) (Table 1, Figure 2).

Non-significant increase in the mean concentration of estrogen hormone was observed in the groups receiving curcumin (at all levels) compared to control and observant groups ($p \leq 0.05$) but significant increase in the mean concentration of this hormone was observed in the groups receiving cadmium chloride compared to control and observant groups ($p \leq 0.05$) (Table 1, Figure 3). No significant difference was observed between the mean concentration of this hormone in the groups receiving cadmium chloride plus curicum (at all levels) and control and observant groups ($p \leq 0.05$).

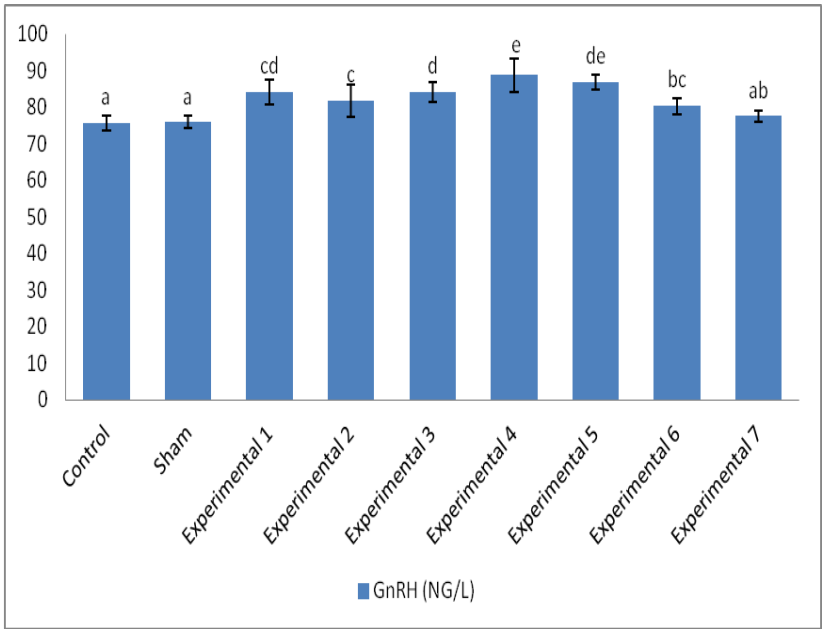
Tabele 1 : Mean comparison of different groups about all parameters

PAREMETERS GROUP	**GnRH (NG/L)**	**FSH (IU/L)**	**ESTROGEN (PG/L)**
Control	75.76 ± .91 **a**	6.88 ± .23 **ab**	283.10 ± 4.56 **bce**
Sham	75.88 ± .76 **a**	6.88 ± .17 **ab**	278.06 ± 3.69 **bce**
Experimental 1	84.10 ± 1.56 **cd**	7.80 ± .11 **bc**	235.96 ± 3.14 **a**
Experimental 2	81.80 ± 1.99 **c**	7.46 ± .12 **bc**	289.68 ± 6.86 **bce**
Experimental 3	84.10 ± 1.19 **d**	7.52 ± .08 **bc**	302.58 ± 3.09 **ce**
Experimental 4	88.70 ± 2.02 **e**	8.14 ± .14 **c**	305.76 ± 8.06 **e**
Experimental 5	86.82 ± .88 **de**	7.12 ± .27 **bc**	277.72 ± 18.20 **bc**
Experimental 6	80.30 ± .94 **bc**	6.04 ± .70 **a**	271.92 ± 5.41 **b**
Experimental 7	77.56 ± .71 **ab**	6.84 ± .52 **ab**	286.46 ± 5.01 **bce**

Based on Duncan's test, means in each column with at least one letter in common are not significantly different at the 5% significance level

- The means are presented in the form of Mean ±SEM
- $P < 0.05$ is considered statistically significant

Figuer 1: The levels of GnRH in different groups

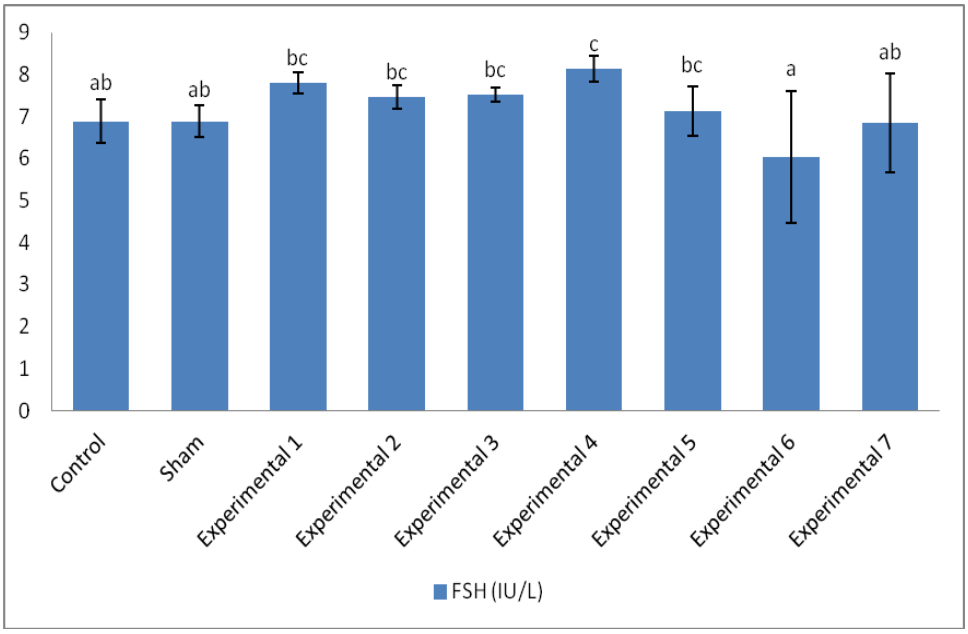


Based on Duncan's test, means in each column with at least one letter in common are not significantly different at the 5% significance level

- The means are presented in the form of Mean ±SEM

- P< 0.05 is considered statistically significant

Figuer 2: The levels of FSH in different groups



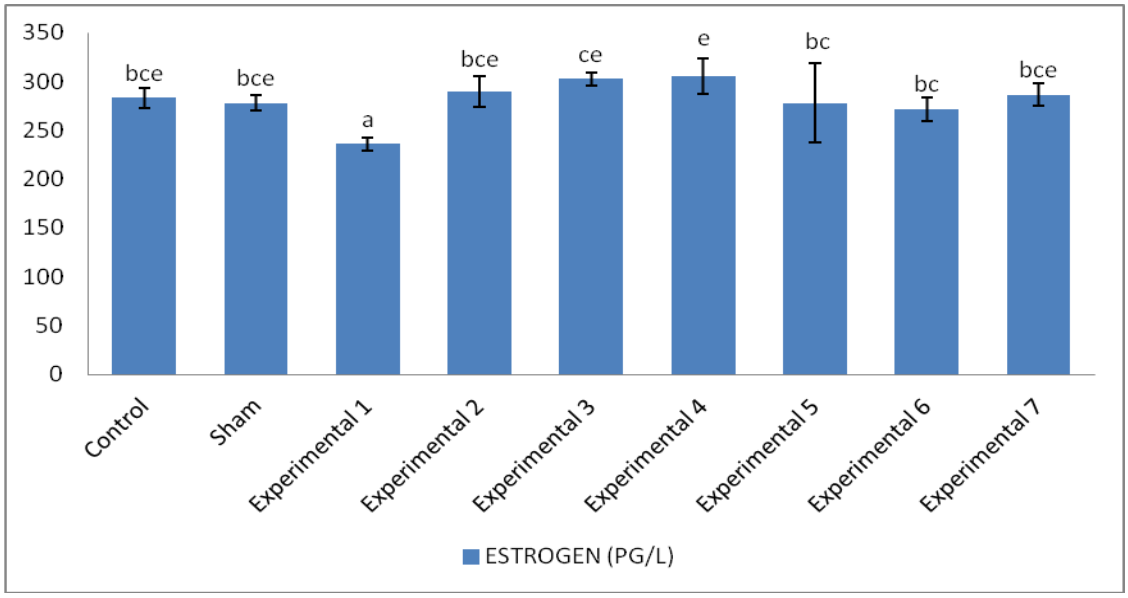
Based on Duncan's test, means in each column with at least one letter in common are not significantly different at the 5% significance level

- The means are presented in the form of Mean ±SEM

- P< 0.05 is considered statistically significant

- P< 0.05 is considered statistically significant

Figuer 3: The levels of Estrogen in different groups



Based on Duncan's test, means in each column with at least one letter in common are not significantly different at the 5% significance level

- The means are presented in the form of Mean ±SEM

- P< 0.05 is considered statistically significant

Discussion and conclusion

As the results showed intraperitoneal injection of cadmium chloride to adult female Wistar rats reduced the levels of gonadotrope hormones, steroid hormones of ovary, disrupted the ovulation process and finally, induced the infertility (10). Cadmium chloride prevents the proper functioning of the granulosa cells of ovarian in the production of steroid estrogen and progesterone hormones in rats through inhibition of binding of the gonadotrope hormone in these cells. As a result of this process and reduction in the level of estrogen hormone, the negative feedback mechanism will be active to compensate the lack of estrogen hormone by producing more GnRH and FSH hormones (11).

Cadmium chloride leads to cellular and vascular changes in the immature rats' ovaries. Medium and large follicles undergo atresia immediately after injection, while small follicles undergo atresia after a period of resistance (33). With the production of Reactive Oxygen Species, cadmium chloride can lead to the destruction of membrane lipids and increase in peroxidation process of membrane lipid and finally, protein degradation. As a result of these actions, destruction of granulosa cells occurs (34).

Curcumin reduces the effects of Reactive Oxygen Species such as superoxide anions and hydroxyl radicals, which play an important role in lipid peroxidation and oxidative stress through increasing the expression of ultra-regulative enzymes such as catalase, glutathione transferase (GST), glutathione peroxidase (GSHPX), superoxide dismutase (SOD) and their related mRNA (35).

Also, curcumin reduces the level of oxidative stress at the cellular level and neutralize free radicals as a cleaner (36). According to a study conducted on pigs in 2012, it was proved that curcumin directly impact on the construction of the steroids (37). According to another study conducted on rats in the same year, proliferative and anti-apoptotic effects of curcumin on ovarian follicles were confirmed. In this study, the rats were irradiated with ionization of curcumin (38). Curcumin inhibits the production of progesterone and 17-beta-estradiol and also increases the granulosa cells of mature follicles in culture. Reduction of FSH or LH with the use of curcumin result in increased progesterone and 17-beta estradiol and more proliferation due to its effect on granulosa cells at all levels (39).

In this study, curcumin acts in a dose-dependent form and showed better effect at the dose of 100 compared to two other doses. Also, more studies are required to study on the effects of other doses.

Therefore, since curcumin is a strong antioxidant, it could probably destroy harmful effects of cadmium chloride. So, it is recommended that additional studies will be conducted to determine the more detailed mechanisms of this process so that curcumin will be used as an antioxidant to protect from the damaging effects of cadmium.

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Conflict of interest

The authors have declared no conflicts of interest.

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