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The Impact of Green Tea (*Camellia Sinensis*) on the Amount of Gonadotropin Hormones (LH, FSH) in Immature Female Rats Poisoned with Cadmium Chloride

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ABSTRACT

Cadmium chloride as an environmental and industrial contaminant element duo to the its cumulative nature in body organs, as well as production of reactive oxygen species is considered toxic for many tissues and sexual organs and can disrupt hormonal harmony of these organs. Thus, it is tried in many studies to reduce possible side effects of cadmium chloride using medicinal plants. Green tea is a medicinal plant which is due to its antioxidant properties can be examined in many studies. The aim of the current study is also to investigate the edible effect of green tea (*Camellia sinensis*) on the gonadotropin hormones (LH, FSH), in immature female rats poisoned with cadmium chloride. The used animals were 48 immature female Wistar rats, which were divided into 6 groups of 8 each, including a control group that received no treatment; a blank that received physiological serum or green tea solvent; an experimental group 1 that received 400 mg/lit cadmium chloride; an experimental group 2 that orally received 14 mg/lit green tea by for 21 days; an experimental group 3 that first received 400 mg/lit cadmium chloride for 21 days, then received orally 7 mg/lit green tea for 21 days; an experimental group 4 that first received 400 mg/lit cadmium chloride for 21 days and then orally received 14 mg/lit green tea for 21 days. Then, the rats of different groups were bled to measure FSH and LH hormones. The obtained data were then tested by One-Way Analysis of Variance (ANOVA) were determined at a significant level ($P< 0.05$) by SPSS software version 15. The mean results of FSH and LH hormones in the experimental 1 shows significant reduction compared to the control and blank. While the experimental 2 shows significant increase compared to the control and blank, and these amounts in the experimental 3 and 4 show significant increases compared to the experimental 1. The results of the study show some beneficial effects of green tea against toxicity of cadmium chloride on gonadotropin hormones and its use is recommended to reduce toxicity of cadmium chloride.

Key words: Green Tea, LH, FSH, Cadmium Chloride, Female Rats.

INTRODUCTION

Cadmium is an environmental and industrial pollutant which influences different systems of human and animal bodies by its toxicity. Disorder in functions of liver, kidney, respiratory and nervous systems, as well as damage to reproducing organs caused by infection with the toxin have been reported[1]. Recent studies have shown that the compound even can cause toxicity in male reproducing organs[2]. Cadmium is often applied in battery, color, plastic and tobacco industries and due to its cumulative effect in body's tissues, causes contamination in person[2-3]. Several mechanisms are cited for cadmium toxicity. But different studies have shown that oxidative stress in cells is the main cause of damage by cadmium. Cadmium or cadmium chloride stimulate production of oxygen free radicals in cells, that its result is lipids and proteins peroxidation and DNA damage. On the other hand, by changes of antioxidant system leads to cell death[4-6]. Therefore, use of proper antioxidants is necessary to reduce the effects of cadmium chloride. There are many plants species that are distinct due to their medicinal and antioxidant properties and they have long been used in

traditional medicine. Many researchers have been investigated defects and abnormal conditions of body by doing different experiments and researches and tried to diagnosis and improve the effects by help of medicinal plants. Green tea is an herbal production which is made by leaves or flowers of Camellia (*Camellia Sinensis L*) plant. It is the second most consumed beverage (after water) in the world[7]. The tea plant is a large flower with evergreen leaves. Its origin is China and it has been used for more than five thousand years in the country[8].

The constituents in tea leaves include: cellulosic and resinous materials, dextrin, pectin, fatty substances, wax, starch, sugar, gallic acid, oxalic acid, quercetin, proteins, fiber, minerals, tannins, caffeine, theanine, aromatic compounds and diastase. As it is clear, about 30% of green tea leaves is consisted of flavonoids, which are very useful for health[8]. Also green tea contains polyphenolic compounds includes Epigallocatechin-3-Gallate (EGCG), Epigallocatechin (EGC), Epicatechin Gallate (ECG), and Epicatechin (EC) which are known by generic name Catechin[9]. In general, green tea contains two active components: Catechin polyphenols which inhibit enzymatic activity of catecol-o-methyl transferase and caffeine which inhibits decomposition caused by phosphodiesterase induction that cause norepinephrine to be released[10]. Green tea leaves contain 10-20% of catechins and many studies mentioned their antioxidant and anti-cancer effects[11]. Several reports have shown that daily consumption of catechins can cause reduce of body fat[12], waist circumference size[13], and control of fatness[14]. Epidemiological and laboratory studies suggest positive effects of green tea on human health. Some useful properties of green tea can be mentioned as follows: antioxidant properties[15], lowering blood sugar and cholesterol (16), anti-cancer properties[17], and protection of liver[18]. Also in a study that was performed on women who had consumed green tea and those who had not, was observed that the risk of breast cancer in women who had drunk green tea was 22% less than the others[19].

Given to cadmium chloride effects on different body organs such as reproductive organs and antioxidant properties of green tea, the study will try to evaluate the effect of oral administration of green tea on gonadotropin hormones (LH, FSH) levels in immature female rats poisoned with cadmium chloride.

METHODS

The used animals were 48 immature female Wistar rats with an average weight of 100-150 g and 3-4 weeks of age, which were prepared from Laboratory Animals Breeding Center of Islamic Azad University, Kazeroon Branch. Animals were kept in plastic cages for keeping mice. Dimensions of polycarbonate cages were 40, 20, 15 cm and the floor of cages were covered by sawdust.

Temperature was about 25ÚC during the study. Indirect and uniform light was conducted through laboratory windows and air replaced through air conditioners located in the laboratory. During 21-day period of the experiment, rats were placed in 12 hours of light and 12 hours of darkness. Throughout the experiment, the animals were drank through urban water and they were fed by special food diet for mice. The total number of rats was 48, and 32 and 16 rats were randomly tested during 21 and 42 days, respectively. The rats were divided into 6 groups of 8 each, as follows:

1. The control group: no medical treatment was administered and they were bled after 21 days.
2. The blank group: they received physiological serum or green tea solvent and they were bled after 21 days.
3. The experimental group 1: they orally received 400 mg/liter cadmium chloride for 21 days.
4. The experimental group 2: they orally received 14 mg/liter green tea for 21 days.
5. The experimental group 3: they first received 400 mg/liter cadmium chloride for 21 days, and then orally received 7 mg/liter green tea for 21 days.
6. The experimental group 4: they first received 400 mg/liter cadmium chloride for 21 days and then orally received 14 mg/liter green tea for 21 days.

Administration of cadmium chloride

Cadmium chloride pure powder were obtained from Sigma-Aldrich (USA) and distilled water was used as solvents for preparation of cadmium chloride administrable soluble. Then, the obtained soluble was given orally to the rats in the experimental groups.

Green tea preparation method

First, the required amount of Shahsavand Green Tea by Serial Number of 75216 was prepared from End of Southern Talaash Blvd, Phase 1, Toos Industrial City, Mashhad, Khorasan Razavi, Iran. 14 grams of prepared tea in one liter of water was heated for 15 minutes, and then cooled for 5 minutes at room temperature. After cooling, it was filtered and then 100 ml of solution was brought to a volume of 1 liter with distilled water (1.4mg/ml). Each rat in the experimental groups 2 and 4 received orally 1ml of the obtained solution. After dilution by ratio of 1:1, the rats in the experimental group 3 received 1ml of the extract (about 0.7mg/ml).

Hydra syringes were used for drug injection. Drugs administration was done orally in a certain times during the day.

Table 1: Comparison of mean concentration of LH in serum between experimental, control and blank groups

Different groups under experiment	The number of animals in each group	Standard error of the mean (±) mean of LH hormone (mIU/ml)
Control	8	7.062±0.113
Blank	8	6.77±0.120
Experimental group 1cadmium chloride (400 mg/lit)	8	5.025± 0.261*
Experimental group 2Green tea (14 mg/lit)	8	7.125± 0.265
Experimental group 3(low-dose green tea)	8	5.512± 0.284**
Experimental group 4(high-dose green tea)	8	6.662± 0.129**

Values are given based on standard error of the mean (±).
The number of samples were n=8.

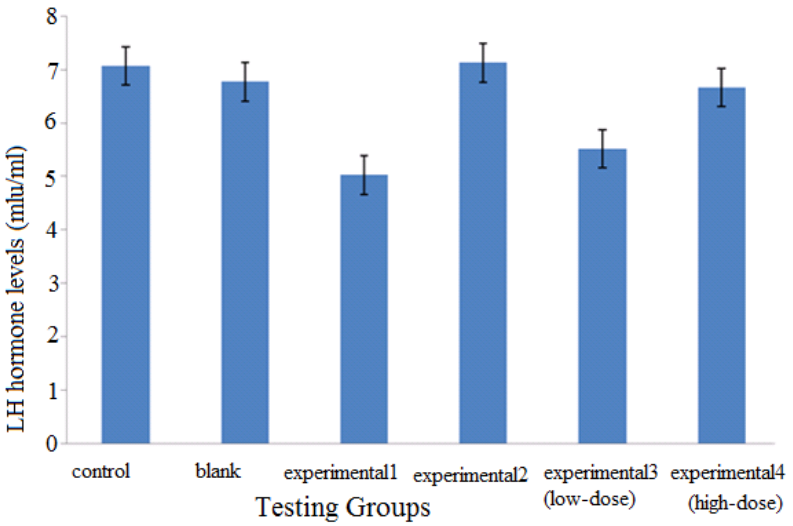


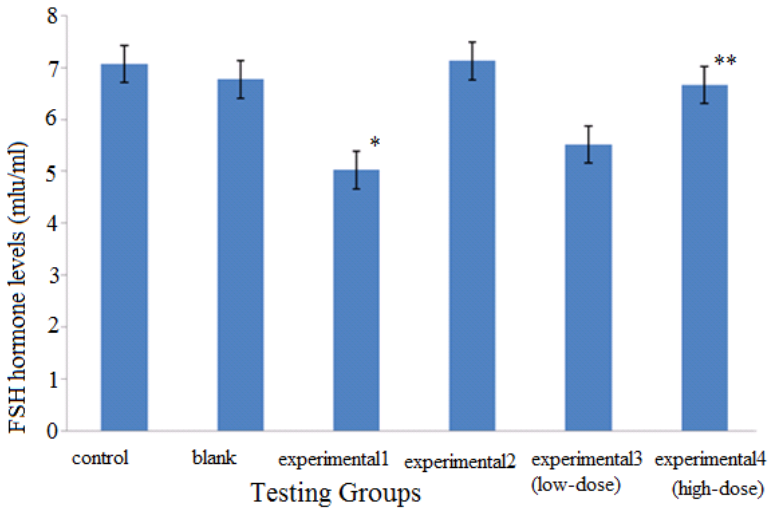
Chart 1: Comparison of mean concentration of LH in serum between experimental, control and blank groups

Blood samples were obtained from the control, blank, experimental 1 and 2 groups at the end of the 21-day period. One day after the last administration of green tea for 42 days, blood samples were taken from the hearts of the rats in the experimental group 3, and the samples were stored in special serum separator tubes. Then, the amounts of LH and FSH hormones were measured using Radioimmunoassay (RIA) method.

Table 2: Comparison of mean concentration of FSH in serum between experimental, control and blank groups

Different groups under experiment	The number of animals in each group	Standard error of the mean (±) mean of FSH hormone (mIU/ml)
Control	8	7.91± 0.206
Blank	8	8.13± 0.235
Experimental group 1cadmium chloride (400 mg/lit)	8	6.18± 0.223*
Experimental group 2Green tea (14 mg/lit)	8	8.375± 0.195
Experimental group 3(low-dose green tea)	8	6.037±0.327
Experimental group 4(high-dose green tea)	8	7.66± 0.147**

Values are given based on standard error of the mean (±).
The number of samples were n=8.
*mark indicates a significant difference at the level (P<0.05) between the experimental groups 1 and 2 with control and blank.
**mark indicates a significant difference at the level (P<0.05) between the experimental groups 3 and 4 with the experimental 1.



The chart is drawn based on standard error of the mean (±) for each group.
*mark indicates a significant difference at the level (P<0.05) between the experimental groups 1 and 2 with control and blank.
**mark indicates a significant difference at the level (P<0.05) between the experimental groups 3 and 4 with the experimental 1.

Chart 2: Comparison of mean concentration FSH in serum between experimental, control and blank groups

The obtained data from calculation of volume by SPSS software were analyzed separately through one-way ANOVA and Ducan test and were compared with each other. The charts were drawn using Excell software based on the obtained information from data analysis by one-way ANOVA.

RESULTS

The obtained results from measurement of LH concentration in serum are shown in Table (1) and Chart (1). LH mean values in the experimental groups receiving 400 mg/liter drug were 5.025±0.261, which has shown a significant reduction with the mean values of control and blank 7.062 ± 0.113 and 6.77 ± 0.120, respectively. The experimental groups receiving 14 mg/lit green tea by mean value of 7.125 ± 0.265 have shown significant increase with mean values of control and blank 7.062 ± 0.113 and 6.77 ± 0.120, respectively. In the experimental groups receiving 7 mg/liter and 14 mg/liter green tea with mean values 5.512± 0.284 and 6.662± 0.129 have shown significant increase at the level of 5% with the experimental group receiving 400 mg/liter drug with mean value 5.025± 0.261.

The obtained results from measuring of FSH concentration in serum are shown in Table (2) and Chart (2). FSH mean values in experimental groups receiving 400 mg/liter drug were 6.18±0.223, which has shown a significant reduction with the mean values of control and blank equal to 7.91 ± 0.206 and 8.13 ± 0.235, respectively. The experimental groups receiving 14 mg/liter green tea by mean value of 8.375 ± 0.195 has shown significant increase with mean values of control and blank equal to 7.91 ± 0.206 and 8.13 ± 0.235, respectively. In the experimental groups receiving 7 mg/liter green tea with mean value equal to 6.037± 0.327 has shown no significant difference at the level of 5% with the experimental group receiving 400 mg/liter drug with mean value equal to 6.18± 0.223. The experimental groups receiving 14 mg/liter green tea by mean value of 7.66 ± 0.147 has shown significant increase at the level of 5% with the experimental group receiving 400 mg/liter drug with mean value equal to 6.18± 0.223.

DISCUSSION

The measuring results of LH concentration in serum are shown in Table (1) and Chart (1). LH values in the experimental groups receiving 400 mg/liter cadmium chloride has shown a significant reduction in compared to control and blank groups. The experimental groups receiving 14 mg/liter green tea have not shown significant difference in compared to control and blank groups. In the experimental groups receiving 7 mg/liter and 14 mg/liter green tea have shown significant increase at the level of 5% in compared to the experimental group receiving 400 mg/liter cadmium chloride. The obtained results from measuring of FSH concentration in serum are shown in Table (2) and Chart (2). FSH values in experimental groups receiving 400 mg/liter cadmium chloride has shown a significant reduction in compared with control and blank groups. The experimental groups receiving 14 mg/lit green tea have shown significant increase in compared to control and blank groups. In the experimental groups receiving 7 mg/liter green tea has shown no significant difference at the level of 5% in compared with the experimental group receiving 400 mg/liter cadmium chloride. The experimental groups receiving 14 mg/liter green tea have shown significant increase at the level of 5% in compared with the experimental group receiving 400 mg/liter cadmium chloride.

Green tea leaves contain 10-20% of catechins. Many studies mentioned their antioxidant and anti-cancer properties (20).

Green tea contains two active components: polyphenol catechin which inhibits enzymatic activity of catechol-o-methyltransferase, and caffeine which inhibits decomposition caused by phosphodiesterase induction that cause norepinephrine to be released[21]. Norepinephrine causes pulsatile secretion of GnRH via pituitary portal veins. This means that the amount of GnRH in blood reach to maximum value every 2 or 3 hours and decrease again. GnRH by binding to its receptors on gonadotropic cells located in anterior pituitary increases calcium levels (by activation of phospholipase C, D and A_2). Calcium entrance into cells with diacetylglycerol activates protein kinase

C. The protein activates the MAPK- messenger cascade. The most important feature of MAPK is entrance to nucleus and activation of transcription various factors. Synthesis of LH and FSH glycolproteins like many hormones do not perform steadily, but occurs in a pulsatile manner. In addition to follicular growth, gonadotropins are necessary for ovulation, luteinization and steroids synthesis by follicle and corpus luteum[22]. FSH mechanism of action is through activation of G protein, cAMP production, and activation of protein kinase A. Then, proteins are phosphorylated by influence of protein kinase A and steroids are produced finally[23].

The main water-soluble components of green tea are polyphenols, particularly catechins. The most important catechines of green tea includes: Epicatechin (EC), Epigallocatechin (EGC), Epicatechin Gallate (ECG), Epigallocatechin Gallate (EGCG). In vivo and in vitro studies over the last decade have shown that green tea and its polyphenols have potent antioxidant effects[24-27]. Green tea catechins are strong scavengers against superoxide, hydrogen peroxide, hydroxyl radicals, and nitric acid which derived from various chemicals. Also catechins due to their catechol structures are attached to metals and inhibit formation of free radicals[28]. And leads to a significant difference in LH and FSH serum levels between experimental and control groups. In addition, green tea catechins have antioxidant properties of urate, â-carotene, vitamin C and E in protection of cells [29].

Many studies have been performed on protective effects of green tea against toxic effects of various chemicals. Jiao *et al*., in 2003 have shown protective effects of green tea polyphenols against toxicity of fenofibrate, a lipid-lowering drug, on HepG2 human cells[30]. Protective effects of green tea polyphenols against SH-SY5Y cells apoptosis-induced by anti-Parkinson drug OHDA-6 are also reported[31].

Green tea due to having of antioxidants ingredients causes a significant difference in levels of gonadotropins (FSH, LH) hormones. This can be due to the presence of antioxidants in green tea that by binding to cadmium in blood inhibits the element and/or by blocking the activity of cadmium inside cells reduces its destructive effects.

CONCLUSIONS

The results of the current study have shown the beneficial effects of green tea against the toxicity of cadmium chloride on gonadotropin hormones. After performing of various tests, observation and comparison of their results, and achievement to positive results, it is found that the plant can be used as a herbal drug with antioxidant properties that can be administered orally, and as supplements and food additives and/or by pharmaceutical industries to prevent gonadotropins hormones damages-induced by cadmium chloride consumption.

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