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Research Article

THE AMELIORATIVE EFFECTOF CATECHIN ON SEXUAL HORMONES INFEMALE RATS EXPOSED TO ARSENICTOXICITY

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ABSTRACT
Arsenic is a well-known toxic metalloid element in the environment that affects human and animal body organs including tissue lipid peroxidation and reproduction system. The present study was aimed to investigate the protective role of catechin on sexual hormones of rats exposed to oxidative damage induced by arsenic. Twenty newly weaned rats were randomly divided into four groups as control group, group received catechin alone, group received sodium arsenate alone and group received catechin with sodium arsenate. Arsenic was administered through drinking water to rats at a concentration of 4 ppm sodium arsenate for 30 days and catechin at rate of 10 mg per kg body weight two times per week for 4 weeks. In the final of experiment, rats were anesthetized with diethyl ether and the blood was collected from heart by heparinized tubes. Rats received sodium arsenate had the lowest concentration of FSH, LH, estrogen and progesterone ($P<0.05$) in the plasma. Catechin administration alone increased ($P<0.05$) plasma FSH, LH, estrogen and progesterone compared to the control group. Rats exposed to arsenic toxicity and received catechin had higher plasma concentrations of gonadotropin and steroidal hormone as compared to those exposed to sodium arsenate alone. In conclusion, arsenic toxicity decreased and catechin had ameliorated effect and increased sexual hormones in female rats.

Keywords: *Sodium Arsenate, Catechin, Gonadotropin, Steroid Hormone, Rat*

INTRODUCTION
Arsenic is an element that raises much concern from the both environmental and human health standpoints. Arsenic is a metalloid found in water, soil, and air from natural and anthropogenic sources and exists in inorganic as well as organic forms (Wang *et al.*, 2002; Valko *et al.*, 2005). Arsenic is a poisonous substance due to its effect on sulphydryl group of cells interfering with cells enzymes, cell respiration and mitosis. Arsenic may also induce oxidative stress by cycling between oxidation states of metals, or by interacting with antioxidants and increasing inflammation, resulting in the accumulation of free radicals in cells (Halliwell *et al.,* 2004). Oxidative stress is currently the most widely accepted and studied mechanism of arsenic toxicity (Ercal *et al.*, 2001).
Oxidative oxidants and its control by antioxidants is one of the important topics in animals' physiology of the female reproductive system. Oxidative stress is one of the factors that cause infertility or recurrent miscarriages, endometriosis, polycystic ovarian syndrome and other disorders related to pregnancy (Ruder *et al.*, 2009).
Catechin, a flavonoid, is present in mainly plant foods and drinks. It has attracted much attention in relation to disease prevention. Its antioxidant activity at least partly accounts for its potential health effect, because oxidative stress leads to a variety of pathophysiological events, especially in reproductive system (Donovan *et al.*, 1999; Terao *et al.,* 1999). Human and animals are exposed to foods, water and air contaminated by arsenate. The effect of catechin on the female gonadotropic and steroidal hormones in human or animals that exposed to arsenic remains unclear. Hence, it is essential to know the effect of arsenate on sexual hormones and also catechin, in order to evaluate its antioxidant activity in females exposed to arsenate (Donovan *et al.*, 1999; Terao *et al.*, 1999). We hypothesized that catechin is capable to prevent the adverse effects of arsenic toxicity on gonadotropic and steroidal hormones in female rats.

Therefore, the present study was carried out to investigate the effects of arsenate, and also the effects of catechin, alone or together with arsenate, on the concentration of gonadotropic and steroidal hormones in female rats.

MATERIALS AND METHODS

Chemicals: Catechin (CAS Registry No: 225937-10-0) and sodium arsenate (CAS Registry No:10048-95-0) used in this assays were provided by Sigma-Aldrich Chemical Company (USA).

Animals and experimental design: Twenty newly weaned female Wistar albino rats (40-45 g body weight) were obtained from the Pasteur Institute (Tehran, Iran). Prior to dosing, they were acclimatized for 7 days to light from 06:00 to 18:00 h alternating with 12 h darkness. The animals were housed in stainless steel cages in an air-conditioned room with temperature maintained at 25± 2 °C. The animals were fed a standard laboratory diet and water *adlibitum*. All rats were handled in accordance with the standard guide for the care and use of laboratory animals. After one week of acclimatization to the laboratory conditions, rats were equally randomized to four groups (one control group and three treatment groups), each consisting of five animals.

Therats in group 2 were injected intraperitoneally with catechin at a dose of 10 mg/kg body weight two times per week for 4 weeks. The rats in group 1 served as control and were allowed *ad libitum* access to tap water. The animals in group 3 were allowed *ad libitum* access to tap water containing 4.0 ppm sodium arsenate (actual concentration, 2.3 ppm arsenic) instead of normal water and maintained for a period of 30 days. A stock solution of 200 ppm arsenic was prepared in distilled water; immediately prior use, it was diluted with filtered tap water to the desired concentration. The fourth groupwas treated with both arsenate and catechin. The doses of catechin were calculated according to the animal's body weight before each injection.

Blood sampling and preparation of serum: At the end of the experimental duration, rats were fasted overnight with free access to water. Rats were anesthetized with diethyl ether and blood was collected into heparinized tubes from heart. The blood was then centrifuged and the plasma was collected and kept at -20 °C for the determination of luteinizing hormone (LH), follicle-stimulating hormone (FSH), estrogen and progesterone.

Measurement of hormones: Hormones of LH, FSH and estrogen were measured using enzyme-linked immunosorbent assay (ELISA) kits. Briefly, this assay employs the competitive inhibition enzyme immunoassay technique. The micro titer plate provided in these kits had been pre-coated with goat-anti-rabbit antibody. Standards or samples were added to the appropriate micro titer plate wells with an antibody specific for hormone and Horseradish Peroxidase (HRP) conjugated hormone. The competitive inhibition reaction was launched between with HRP labeled hormone and unlabeled hormone with the antibody. A substrate solution was added to the wells and the color develops in opposite to the amount of hormone in the sample. The color development was stopped and the intensity of the color measured.

The progesterone ELISAK it for rat is based on the principle of competitive binding. An unknown amount of progesterone present in the sample and a defined amount of progesterone conjugated to horseradish peroxidase compete for the binding sites of progesterone antiserum coated to the wells of a micro plate. After incubation on a shaker the micro plate was washed four times. After addition of the substrate solution, the concentration of progesterone was inversely proportional to the optical density measured.

Statistical Analysis: Data were subjected to analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedures of SAS software. Mean comparison was done using the Duncan`s Multiple Range Test at $P<0.05$.

RESULTS AND DISCUSSION

The main purpose of this study was to assess the effect of arsenic toxicity on plasma concentration of gonadotropic and steroidal hormones and the other purpose was to evaluate the protective effect of catechin on them. In the literature, there is no study concerning the effects of arsenic toxicity and

administration of catechin on hormones concentration in rats especially, there is no report on the effects of these factors on gonadotropic and steroidal hormones.

The effect of Arsenic toxicity and catechin administration on plasma FSH, LH, estrogen and progesterone concentrations is presented in Table 1. There were differences among treatments for plasma concentration of FSH ($P<0.05$). The lowest concentration was for Arsenic toxicity and the highest concentration was for rats received catechin alone. In comparison between treatment 2 and 4, administration of catechin improve the concentration of FSH in rats exposed to sodium arsenate. There were significant differences among treatments for plasma concentration of LH ($P<0.05$). The highest concentration of LH was found for rats received catechin and the lowest one was for those exposed to arsenate alone. Administration of catechin for groups received sodium arsenate (group 4) increased concentration of plasma LH as compared to those received sodium arsenate alone (group 3). In a study (Chattopadhyay *et al.*, 2003) it was observed that ovarian follicular and uterine cell degenerate after arsenic treatment. This was accompanied by increases in dopamine levels in the midbrain and diencephalon, as well as arsenic levels in the ovary, uterus, and plasma. Similar to norepinephrine, low level of dopamine could decrease gonadotropin synthesis and secretion. The low FSH level observed also maybe contributes to the decrease in number of healthy follicles and increase in the number of apoptotic follicles. Arsenic toxicity in the female reproductive system could change in the levels of catecholamines in the brain and this event resulted in decrease of LH, FSH, and estradiol in the plasma, and norepinephrine levels in midbrain and increase in serotonin levels in midbrain and di- encephalon. The elevation in serotonin and decrease in norepinephrine in the midbrain and diencephalon could lower gonadotropin synthesis and secretion. Low gonadotropin levels could in turn decrease activities of ovarian Δ5, 3β-HSD and 17-β-HSD, two important regulatory enzymes for steroidogenesis (Ghersevich *et al.*, 1994a, 1994b; Kaminski *et al.*, 1997; Miro *et al.*, 1995). These observations suggest that low plasma levels of estradiol could be the cause of consistent diestrous. These arsenic-induced ovarian and uterine toxicities and steroidogenic dysfunction were decreased by co-administrations of ascorbic acid orally. Possible mechanisms of ascorbic acid protection included its antioxidant property, facilitating the elimination of arsenic, and influences on hormones.

Regarding hormonal influences, ascorbic acid can enhance endogenous norepinephrine secretion and consequently stimulate gonadotropin releasing hormone release (Miller and Cicero, 1987). The another cause of the reduction in plasma LH and FSH levels of rats received sodium arsenate maybe high plasma corticosterone levels (Artykova *et al.*, 1977) as animals subjected to oxidative stress. High corticosterone can reduce plasma gonadotropin and steroids levels (Hardy *et al.*, 2005; Vreeburg *et al.*, 1988). Catechin as an antioxidant agent may be able to protective pituitary gland against oxidative stress. In our study, catechin administration had positive effect on gonadotropin and steroidal hormones.

Reports concerning the effect of catechin on sexual hormones in animals exposed to arsenic toxicity are scarce.

There is some studies (Prakash *et al.*, 2011; Sinha *et al.*, 2005) about the ameliorate effects of catechin on some clastogenicity and apoptosisin animals exposed to arsenic toxicity.

Table 1: Level of FSH, LH, Estrogen and progesterone hormonesin plasma of rats exposed to arsenic toxicity or received catechin

Items	Control	Catechin	Arsenate	Arsenate + Catechin	SEM
FSH(ng/ml)	377^{ab}	391^{a}	283^{c}	297^{bc}	43.6
LH(ng/ml)	33.9^{a}	37.1^{a}	25.4^{b}	35.2^{a}	2.73
Estrogen(pg/ml)	24.1^{ab}	26.2^{a}	20.0^{b}	22.5^{ab}	2.65
Progesterone(µg/ml)	10.9^{a}	13.3^{a}	6.3^{b}	11.4^{a}	2.37

^{abc} *Means in the same row with different letter are significantly different ($p\leq0.05$)*

Hence, discussion about the ameliorating effect of catechin on of gonadotropin and steroid hormones of rats received sodium arsenate is limited to its antioxidant activity (Donovan *et al.,* 1999; Terao *et al.,* 1999). There were significant differences for estrogen concentration among treatments. Administration of catechin increased ($P<0.05$) and sodium arsenate decreased ($P<0.05$) the concentration of estrogen as compared with the control group. Rats received both sodium arsenate and catechin had no significant difference for estrogen as compared with those received arsenate alone.

There were significant differences among treatments for plasma concentration of progesterone. The effect of treatments on progestrone concentration was followed their effect on plasma concentration of LH. The highest concentration of progesterone was observed in plasma of rats received catechin and the lowest concentration was for those exposed to arsenate alone. Administration of catechin in groups received sodium arsenate (group 4) enhanced ($P<0.05$) concentration of progesterone as compared to those received sodium arsenate alone (group 3).

There are some reports (Chattopadhyay *et al.,* 2001; Navarro *et al.,* 2004; Zhang *et al.,* 2000) that showed administration inorganic arsenic to female mice and rats could suppresses ovarian steroidogenesis, prolongs diestrus, and degenerates ovarian follicular and uterine cells. In a study (Navarro *et al.,* 2004) it was reported that inorganic arsenic could increase meiotic aberrations in oocytes, and decreases cleavage and pre- implantation development.

There is a report (Chattopadhyay *et al.,* 2001) that showed arsenic could induce ovarian and uterine toxicity, and influence neuroendocrine regulation of female sex hormones. In mentioned study a consistent diestrous stage was observed in female rats that were gavaged with 10 ml of 0.4 ppm sodium arsenite daily for 28 days. There were also decreases in relative ovarian and uterine weights, enzyme activities of Δ5, 3-β-HSD and 17-β-HSD in ovary, and the activities of peroxidase in the ovary and uterus. It was reported (Qian *et al.,* 1998) that flavonoids, of which catechin, could increase mean estradiol concentration by inhibiting estrogen sulfotransferase.

A high estrogen sulfotransferase expression, which can be stimulated by progesterone (Falany and Falany, 1996), may result in diminished estrogen hormone levels and a protective effect (Qian *et al.,* 1998). The resulting estrogen sulfates can, however, be hydrolyzed by estrogen sulfatase (Purohit *et al.,* 1998). A recent study indicated that dietary flavonoids may inhibit estrogen sulfatase, suggesting a protective effect of these dietary polyphenols (Huang *et al.,* 1997). Previous studies have demonstrated that flavonoids can be potent inhibitors of a human sulfotransferase (Eaton *et al.,* 1996), which can sulfonate high concentrations of estrogen hormones (Hernandez *et al.,* 1992). Findings of the present research help us to conclude that the catechin in dosages used, has ameliorate effects on concentration of gonadotropic and steroidal hormones in female Wistar rats exposed to sodium arsenate toxicity. These effects may be improved the pregnancy rate in animals or human exposed to arsenic compounds.

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