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PLASMA GONADOTROPIN AND STEROID HORMONES OF RATS EXPOSED TO ZEARALENONE

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ABSTRACT

Zearalenone is an estrogenic substance produced by *Fusarium* fungi growing on foods or feeds in high moisture environment. This mycotoxin has potent estrogenic activities with a high binding affinity for estrogen receptors and has also been shown to cause diverse toxic effects in animals. In order to define the zearalenone effects in low doses on plasma concentration of gonadotropical and steroidal hormones, this study was conducted in Wistar rats. Twenty newly weaned rats were randomly divided into four groups as control and treatments group that received perionetally 0.5, 1 and 1.5 mg/kg of body weight zearalenone three 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. The lowest concentration was for rats received 1.5 mg/kg body weight zearalenone and the highest concentration was for rats in control group. Rats received 0.5 mg/kg body weight zearalenone had no significant difference for FSH concentration as compared to control group. The decreased in plasma concentration of FSH was observed in rats treated with zearalenone in a dose-dependent manner. The highest concentration of LH was found for rats received control and the lowest one was for those received 1.5 mg/kg body weight zearalenone. A linear decrease ($P < 0.009$) was found in plasma concentration of LH with increases in zearalenone doses. The highest concentration was for control group and the lowest one was related to rats in groups received 1.0 and 1.5 mg/kg body weight zearalenone. The decreased in estrogen concentration was observed in rats treated with zearalenone in a dose-

dependent manner. There were no significant differences among treatments for plasma progesterone concentration. A linear decrease (P<0.034) was found for this parameter as zearalenone doses increased. It was concluded that intraperitoneally injection of zearalenone could affect plasma gonadotropin and steroidal hormones in rats.

Keywords: Gonadotropin, Steroidhormone, Zearalenone, Rat

INTRODUCTION

Zearalenone is a naturally occurring non-steroidal estrogenic mycotoxin produced by *Fusarium* species of fungi which are common in temperate and warm countries, and are regular contaminants of human foods and animal feeds worldwide [1,2,3].This mycotoxin is biologically potent, but it is hardly toxic. It has an estrogenic effect that causes alterations in the reproductive tract of laboratory animals and farm animals [4,5,6]. In rats, the reproductive consequences of zearalenone exposure include decreased fertility, resorption or deformities of fetuses, and abortion at high dietary concentrations [1]. The pituitary gland is also a target for estrogens [7,8] and zearalenone maybe affect its hormone release especially gonadotropin hormones. In the literature, the estrogenic effects of zearalenone have concentrated on effects on peripheral reproductive organs. However, information concerning plasma concentration of gonadotropin and steroidal hormones are scarce.

In addition tothe potential of zearalenone binding to estrogen receptors, this mycotoxin couldgenerate the reactive oxygen species which mediate its toxicity and induces oxidative stress. Plasma corticosterone levels of animals subjected to oxidative stress increased [9]. High plasma corticosterone has negative effect on secretion of gonadotropin and steroidal hormones [10,11]. Moreover, most data concerning estrogenic effects of zearalenone on animals has been obtained using medium to high doses of zearalenone (2 to 90 mg/kg of body weight). Such high dosages are not commonly consumed or found in animal feeds. Therefore, we examined the effects of zearalenone under low doses in rats as mammalian model by determining its effects on gonadotropin and steroidal hormones.

MATERIAL AND METHODS

Chemicals

Zearalenone (CAS Registry No: 17924-92-4) used in this assays was provided by Cayman Chemical Company (USA). The toxin was dissolved in DMSO (Merck, Darmstadt, Germany) to a concentration of 10 mg/ml and

then further dilutions were made in phosphate buffer saline.

Animals and experimental design

Twenty newly weaned female Wistar albino rats (150-155 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. All rats were provided pure water and rodent diet ad libitum. All rats were handled in accordance with the standard guide for the care and use of laboratory animals. After one week of acclimatizationto the laboratory conditions, rats were equally randomized to four groups After 1 week of acclimation, rats were divided into four groups (two or three per each cage) by randomization of body weight (BW). Rats in the control group received intraperitoneally sterile saline solution, and treatment groups were administered with 0.5, 1 and 1.5 mg zearalenone per kg BW intraperitoneally three times per week for 4 week, respectively.

Blood sampling and hormonal measurements

At the end of the experimental duration, rats were fasted overnight with free access to water. Rats were anesthetized with diethyl etherand 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 Horse Radish 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 ELISA Kit 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 $P<0.05$. Differences among treatments were separated using polynomial orthogonal contrasts to determine linear, quadratic, and cubic responses.

RESULTS AND DISCUSSION

The purpose of the present study was to investigate the effects of zearalenone under low doses on plasma concentration of gonadotropin and steroidal hormones in rats. The effect of different doses of zearalenone administration on plasma FSH concentration is presented in **Figure1**. Based on Duncan test, there were significant differences among treatments for plasma FSH concentration. The lowest concentration was for ratsreceived 1.5 mg/kg BW zearalenone and the highest concentration was for rats in control group. Rats received 0.5 mg/kg BW zearalenone had no significant difference for FSH concentration as compared to control group. Orthogonal contrast showed a linear decrease ($P<0.005$) in plasma concentration of FSH with increases in zearalenone doses.In agreement to our finding, an interesting study [12] demonstrated that zearalenone like estrogen could decrease basal secretion of FSH and reduce total FSH production *in vitro*. Zearalenone has been labeled as phytoestrogen and phytoestrogens are belongto isoflavones [13]. These substances can produce inhibitory effects on gonadotropin secretion in both animals and humans. It is concluded that phytoestrogen acts centrally to reduce the frequency of the hypothalamic gonadotropin-releasing hormone pulse generator. In addition, the inhibitory effects of coumestrol, a phytoestrogen, on LH pulses occur at the level of the pituitary by reducing responsiveness to gonadotropin-releasing hormone via an estrogen receptor-mediated process[13]. These events finally resulted in decrease of gonadotropins secreted from pituitary gland.

It was demonstrated that neonatal exposure to zearalenone early in development alters post-pubertal pituitary response to gonadotropin-releasing hormone in rats [15]. In agreement with our results, it was reported that

zearalenone could also alter levels of gonadotropins and sex steroids [16].

The effect of zearalenone administration on plasma LH concentration is presented in **Figure2**. There were significant differences among treatments for plasma concentration of LH. The highest concentration of LH was found for rats received control and the lowest one was for those received 1.5 mg/kg BW zearalenone.A linear decrease ($P < 0.009$) was found in plasma concentration of LH with increases in zearalenone doses. In line to our finding, Chen *et al.*[17]reported that zearalenone administration to gilts resulted in significant decrease of serum levels of luteinizing hormonecompared to the control. They report a quadratic effectof zearalenone on serum luteinizing hormone. The effect of Zearalenone administration on plasma estrogen concentration is presented in **Figure3**. There were significant differences among treatments for estrogen concentration of plasma. The highest concentration was for control group and the lowest one was related to rats in groups received 1.0 and 1.5 mg/kg BW zearalenone. There were no significant differences for estrogen concentration between rats received 1.0 and 1.5 mg/kg BW zearalenone. Orthogonal contrast comparison showed a linear decrease ($P<0.018$) in estrogen concentration as zearalenone administration increased. Decrease in estrogen concentration may be related to a decrease in plasma FSH concentration as a result of zearalenone administration.

The effect of zearalenone administration on plasma progesterone concentration is presented in **Figure4**. Based on Duncan test, there were no significant differences among treatments for plasma progesterone concentration. A linear decrease ($P<0.034$) was found for this parameter as zearalenone doses increased.

Several studies both *in vitro* and *in vivo* reported that zearalenone could enhance the formation of reactive oxygen species and caused oxidative damage [18]. Oxidative stress results in damage to cellular structures and it maybe disrupt the pituitary and ovaries function and their sensitivity to releasing factors or gonadotropins, and finally it could decrease secretion of gonadotropin and steroids hormones.

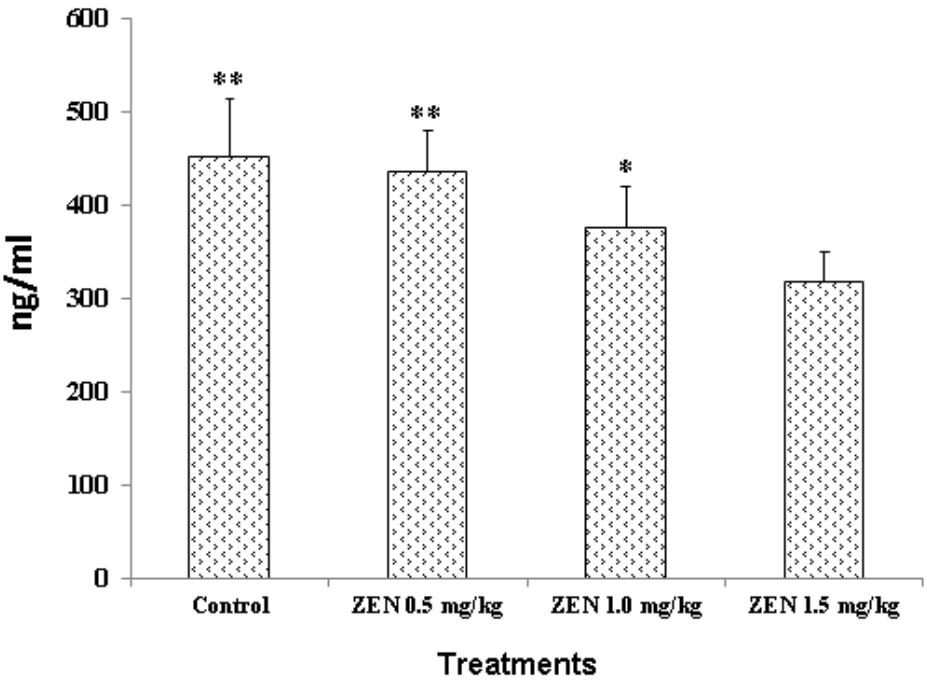

Figure 1: Effect of different doses of zearalenone (ZEN) administration on plasma FSH concentration

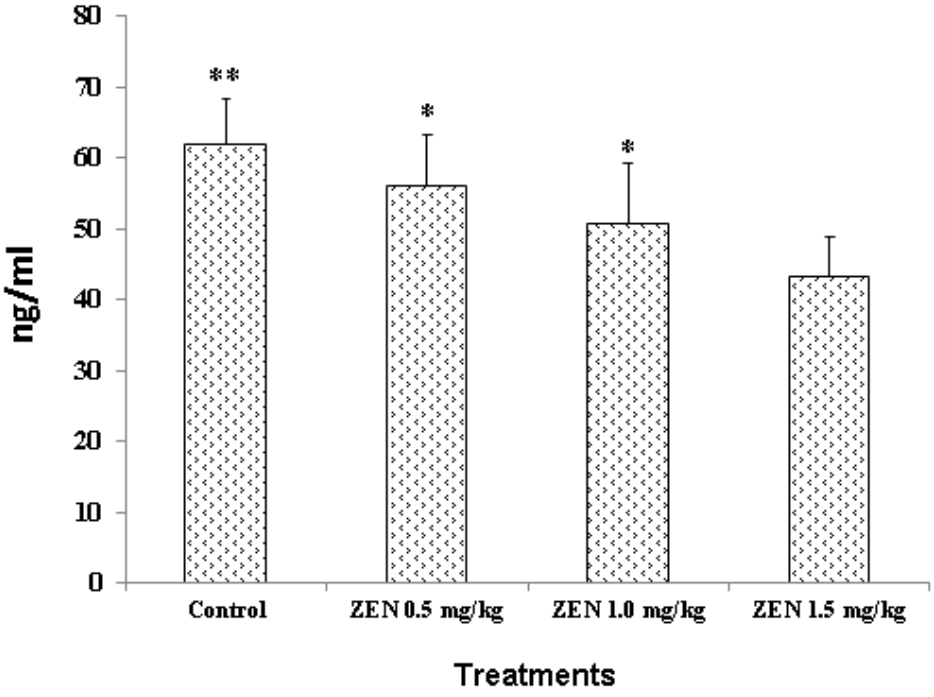

Figure 2: Effect of different doses of zearalenone (ZEN) administration on plasma LH concentration

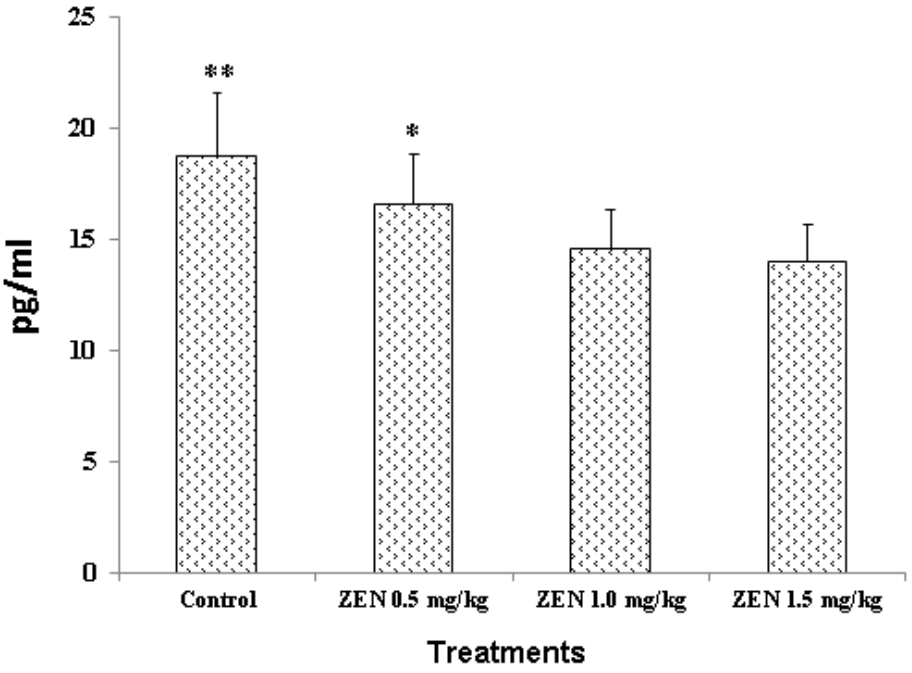

Figure 3: Effect of different doses of zearalenone (ZEN) administration on plasma estrogen concentration

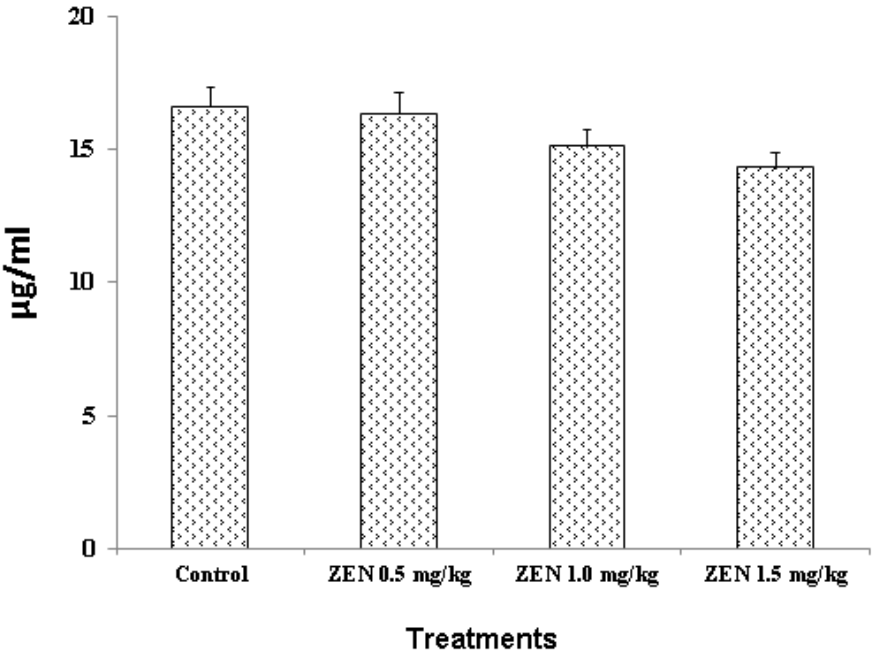

Figure 4: Effect of different doses of zearalenone (ZEN) administration on plasma progesterone concentration

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