Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231– 6345 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jls.htm
2015 Vol. 5 (1) January-March, pp. 149-155/Mostafa and Sadeghi
Research Article

PLASMA GONADOTROPIN AND STEROID HORMONES OF STRESSED RATS FED DIETARY QUERCETIN

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ABSTRACT
Quercetin, a naturally occurring flavonols commonly detected in most fruits and vegetables, has been reported to possess antioxidant and estrogenic properties. Positive results have been reported in numerous antioxidant assays of quercetin, however there is no report concerning its antioxidant activity in stress condition induced by tert butyl hydroperoxide (t-BHP) and its effect on release of gonadotropical and steroidal hormones. In order to further define the antioxidant potential of quercetin and its effects on reproductive hormones in chronic stress condition, this study was conducted in Wistar rats. Twenty newly weaned rats were randomly divided into four groups: control, quercetin (20 mg/kg), tert butyl hydroperoxide(t-BHP) at 0.2 mmol/kg and combination of t-BHP and quercetin for 30-day trial period. Rats were anesthetized with diethyl ether and the blood was collected from heart by heparinized tubes. Oxidative stress induced by t-BHP decreased the plasma level of FSH and LH($P<0.05$), but had no significant effect on estrogen and progesterone ($P>0.05$). Quercetin administration alone increased plasma LH and FSH ($P<0.05$), but had no effect on plasma concentrations of estrogen and progesterone. Therefore, oxidative stress induced byt-BHP reduced, but quercetin administration alone increased the level of gonadotrop in hormones. In rats exposed to oxidative stress and received quercetin, plasma gonadotrop in concentration increased as compared to rats exposed to oxidative stress. Both oxidative stress and quercetin administration had no effect on plasma concentration of steroid hormones.

Keywords: *Gonadotropin, Steroid Hormones, Stress, Quercetin, Rat*

INTRODUCTION
Quercetin is a flavonoid belonging to a group of plant-derived non-steroidal compounds known as phytoestrogens (Moutsatsou, 2007). Nonsteroidal, plant-derived compounds that disrupt or mimic the normal action of estradiol are referred to as phytoestrogens. These chemicals influenced endocrine activity in animals and have been shown to significantly improve the reproductive functions of animals (Dhawan *et al.,* 2002; Korzekwa *et al.,* 2006). Phytoestrogens are able to bind to estrogen receptors thus activating several estrogen responsive genes. In particular, quercetin has been found to exhibit both estrogenic and antiestrogenic actions in vitro thus suggesting different potential effects on reproductive function. In addition it was reported that quercetin can inhibit the action of pregnant serum gonadotropin on ovarian and uterine growth in rats (Gumbinger *et al.,* 1988). There is growing interest in using natural, dietary plant estrogens (phytoestrogens), as a potential chemo preventive regimen of certain hormone-dependent diseases (Castle and Thrasher, 2002). Quercetin has been reported to show significant health promoting activities such as anti-mutagenicity, lipoxygenase inhibition, histamine release inhibition, cell cycle regulation and anti-angiogenic activity (Murota and Terao, 2003). There are few reports that quercetin can increase serum testosterone levels and decrease serum dihydrotestosterone levels in male rats (Ma *et al.,* 2004). However, the effect of quercetin on the female gonadotropinal and steroidal hormones remains unclear.

Oxidative stress occurs as a result of an imbalance between pro-oxidants and antioxidants (Al-Gubory *et al.,* 2010). This imbalance is due to increased levels of reactive oxygen species, nitrogen species or decreased antioxidant defense system occurs (Burton and Jauniaux, 2010; Cindrova-Davies *et al.,* 2007). If the production of reactive oxygen species be more than usual, it can damage the cells, including damage to DNA, lipid membranes, and proteins. Oxidative oxidants and its control by antioxidants is one

of the important topics in animals' physiology of the female reproductive system. The overall reactive oxygen species have an important transitional role in the regulation of ovulation, oocyte maturation, corpus luteum formation, uterine activity, fetal cycle, embryo implantation and development of the placenta and the fetus through diverse signaling and transition pathway, but the imbalance between the production of reactive oxygen species and antioxidants is a reason for the start and spread of damage to the reproductive process. 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). The hypothalamic-pituitary-adrenal axis is a major component of the stress response (Kawabata *et al.*, 2010) and is vital for survival, whereas its abnormal activation by chronic and severe stressful conditions is included as an important risk factor for reproduction disorders.

Quercetin acts as antioxidant and is only one of several dietary flavonols, has been identified to occur naturally in apples, cranberries, blueberries, broccolis, and onions at relatively high concentrations (Harnly *et al.*, 2006). The effect of quercetin as an antioxidant on the female gonadotropinal and steroidal hormones in the stress condition remains also unclear. We hypothesized that quercetin is capable to prevent the adverse effects of oxidative stress on gonadotropic and steroidal hormones in female rats. Therefore, the present study was carried out to investigate the effects of oxidative stress induced by tert butyl hydroperoxide (t-BHP), and also the effects of quercetin, alone or together with oxidative stress, on the concentration of gonadotropic and steroidal hormones in female rats.

MATERIALS AND METHODS

Chemicals: Quercetin (CAS Registry No. 117-39-5) used in this assays was provided by Merck KGaA (Darmstadt, Germany). The purity of the test material was reported to be 103.6% (on dry weight basis; maximum 10% water so adjusted to 93.6%). In the micronucleus assay, quercetin was suspended in 10 ml of a 0.25% aqueous solution of hydroxypropyl methylcellulose (HPMC) (MethocelRK4M Premium; Colorcon, Dartford, Kent, UK) to a final concentration of 20.0 mg quercetin/ml and was serially diluted to obtain lower concentrations. The solvent also was used as the negative control. Tert butyl hydroperoxide (2-Methylpropane-2-peroxol) was purchased from Sigma–Aldrich Chemical Company (CAS Registry No. 75-91-2).

Animals and experimental design: Twenty newly weaned female Wistar albino rats (45-55 g body weight) were obtained from the Razi Institute (Karaj, Iran). The animals were housed in plastic cages, fed a standard laboratory diet and water *adlibitum*. Rats were exposed to a 12 h light/dark cycle, and maintained at 20 ± 2 °C. The animals were quarantined for 10 days before beginning the experiments. 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 randomly divided into four experimental groups (5 rats in each) as follows: The first group served as normal control group and was injected HPMC solution. Rats of the second group were intoxicated with 0.2 mmol/kg body weight t-BHP (Al-Gubory *et al.*, 2010) two times per week for 4 weeks. The third group was treated with quercetin at a dose of 20 mg/kg body weight two times per week for 4 weeks. The fourth group was treated with both t-BHP and quercetin. The t-BHP was dissolved in sterilized distilled water and quercetin was dissolved in sterilized HPMC aqueous solution and both were injected intraperitoneally. Quercetin treatment started one week before t-BHP injection and continued throughout the duration of the experiment. The doses of t-BHP and quercetin 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 aim of this study was to evaluate the effect of oxidative stress on plasma concentration of reproductive hormones and the other aim was to evaluate the protective effect of quercetin. There are some studies concerning the effects of oxidative stress induced by t-BHP and administration of quercetin on hormones concentration in rats (Kawabata *et al.*, 2010), but in the literature, there was no report on the effects of quercetin on gonadotropic and steroidal hormones.

The effect of t-BHP and quercetin administration on plasma FSH concentration is presented in Figure 1. There were differences among treatments for plasma concentration of FSH. The lowest concentration was for control and those exposed to oxidative stress and the highest concentration was for rats received quercetin alone. In comparison between treatment 2 and 4, administration of quercetin could improve the concentration of FSH in rats exposed to oxidative stress.

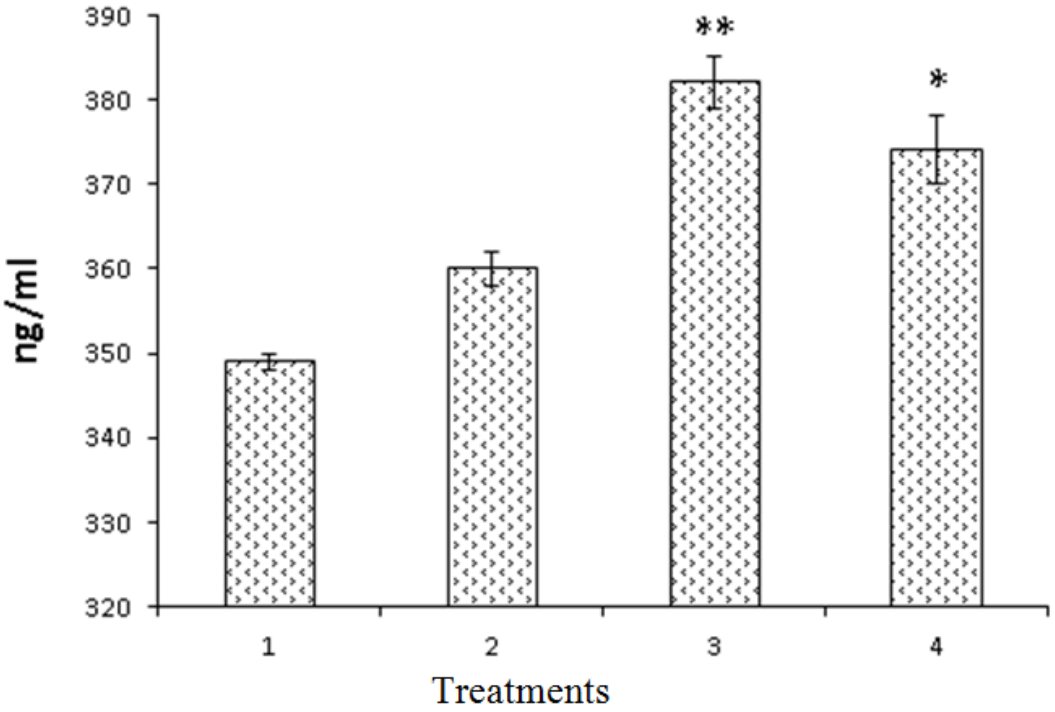

Figure 1: Effect of different treatments (1, control; 2, oxidative stress; 3, quercetin alone; 4, oxidative stress and quercetin administration) on plasma FSH concentration

The effect of t-BHP and quercetin administration on plasma LH concentration is presented in Figure 2. There were significant differences among treatments for plasma concentration of LH. The highest concentration of LH was found for rats received quercetin and the lowest one was for those exposed to oxidative stress alone.

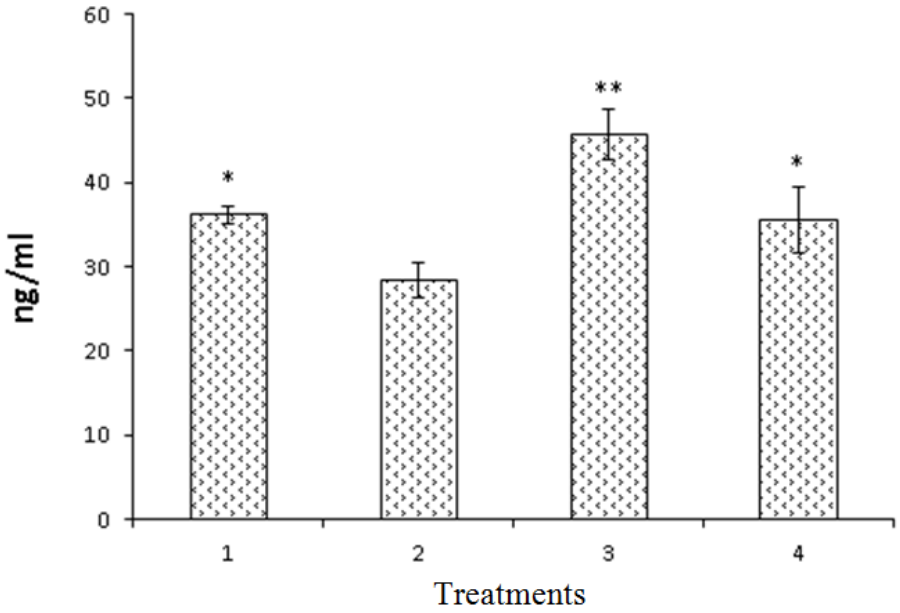

Figure 2: Effect of different treatments (1, control; 2, oxidative stress; 3, quercetin alone; 4, oxidative stress and quercetin administration) on plasma LH concentration

Consistent to our finding, in the study by Gökçe *et al.,* (2011), oxidative stress induced by t-BHP reduced sexual hormones levels that this reaction was significant only about progesterone. It was reported **(**Modaresi and Poor-Naji, 2012**)** that oxidative stress reduced LH and FSH.

Oxidative stress can modulate cellular functions, and OS can impair the intracellular milieu resulting in diseased cells or endangered cell survival. In a study (Yang *et al.,* 2014), mice exposed to an oxidative stress inducer and found that inducer could induce DNA damage in endometrial cells and finally embryo loss. Recently, oxidative stress has been reported to have an important role in the normal functioning of the female reproductive system and in the pathogenesis of female infertility (Agarwal and Allamaneni, 2004).

The effect of t-BHP and quercetin administration on plasma estrogen concentration is presented in Figure 3. There were no significant differences for estrogen concentration among treatments. Numerically, injection of t-BHP decreased and quercetin increased the concentration of estrogen as compared with the control group. It was reported (Falany and Falany, 1996; Qian *et al.,* 1998) that quercetin 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 (Falany and Falany, 1996; 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, including quercetin, 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; Walle *et al.,* 1995), which can sulfonate high concentrations of estrogen hormones (Hernandez *et al.,* 1992).

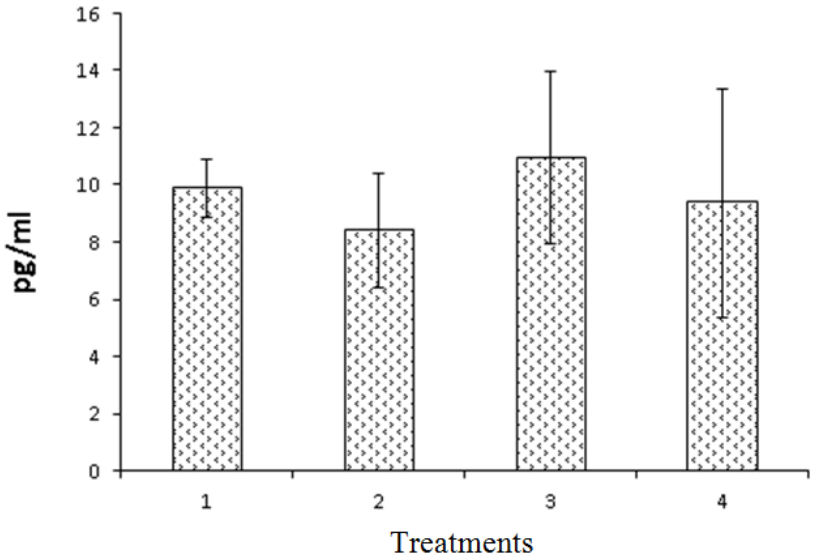

Figure 3: Effect of different treatments (1, control; 2, oxidative stress; 3, quercetin alone; 4, oxidative stress and quercetin administration) on plasma estrogen concentration

The effect of t-BHP and quercetin administration on plasma progesterone concentration is presented in Figure 4. There were no significant differences among treatments for plasma concentration of progesterone. Numerically, t-BHP decreased and quercetin increased progesterone concentration compared to control group. Injection of quercetin to rats exposed to oxidative stress could not improve the concentration of progesterone compared to group exposed to oxidative stress.

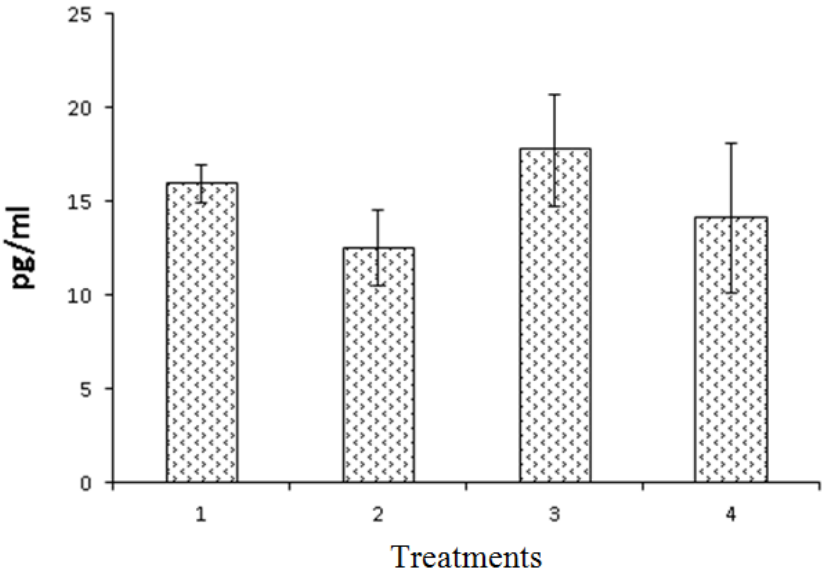

Figure 4: Effect of different treatments (1, control; 2, oxidative stress; 3, quercetin alone; 4, oxidative stress and quercetin administration) on plasma progesterone concentration

Quercetin as an antioxidant agent may be able to protective pituitary gland against oxidative stress. In our study, quercetin administration had effect on gonadotropin hormones, but it had no effect on steroidal hormones. In a study **(**Modaresiand Poor-Naji, 2012**)**, antioxidant administration could not improve plasma levels of LH and FSH as reduced by oxidative stress.

Findings of the present research help us to conclude that the quercetin in dosages used in this study, has improving effects on plasma gonadotropin concentration especially LH in female Wistar rats exposed to oxidative stress. These effects maybe maintain the pregnancy in environmental stress condition.

ACKNOWLEDGMENT
The authors are grateful to the Islamic Azad University for research funding support. We also thank all staffs in the animal house, for the assistance in the care and feeding of the rats used in this research.

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