



**ORIGINAL ARTICLE**

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## **The Effect of Thymoquinone on Plasma Gonadotropin and Steroid Hormones of Rats Exposed to Oxidative Stress**

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### **ABSTRACT**

*An imbalance between free radicals and antioxidants is a reason for the start and spread of damage in the reproductive processes. Oxidative stress is one of the factors that cause infertility or recurrent miscarriages, endometriosis, polycystic ovarian syndrome and other disorders related to pregnancy. Thymoquinone (TQ) that is the active component of black seed can reduce the negative effects of oxidative stress on cell structure and function, because of its antioxidant properties. In this study, twenty newly weaned rats were randomly divided into four groups: control, TQ (15 mg/kg), tert butyl hydroperoxide (t-BHP) at 0.2 mmol/kg and combination of t-BHP and TQ 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 progesterone ( $p < 0.05$ ), but had no significant effect on FSH, LH and estrogen ( $P > 0.05$ ). Thymoquinone administration increased progesterone ( $P < 0.05$ ), but had positively affected other hormones numerically. Therefore, oxidative stress induced by t-BHP reduced the level of progesterone and thus could result in fetus loss, but TQ administration can increase the level of progesterone in animals exposed to oxidative stress and finally maybe help to better efficiency of female reproductive system.*

**Keywords:** Oxidative stress; tert butyl hydroperoxide; Thymoquinone; female reproductive system.

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### **INTRODUCTION**

Oxidative stress occurs as a result of an imbalance between pro-oxidants and antioxidants [3]. This imbalance is due to increased levels of reactive oxygen species, nitrogen species or decreased antioxidant defense system occurs [7,8,14]. 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 [16].

Thymoquinone (TQ) as the main constituent of *Nigella sativa* essential oil is a scavenger that makes cleanup superoxide, hydroxyl radical and single oxygen molecule [5]. *Nigella sativa* is a herbal plant. It is known as black cumin. *Nigella sativa* oil and TQ inhibit oxidative stress induced by gentamicin and cyclosporine (that suppress the immune system), and overproduction of nitric oxide in the rat kidney [17]. The anti-oxidative effect of TQ is related to the redox properties of the Quinone structure of TQ molecule [15]. Badary *et al.* [5] reported that the ability of TQ to cross morpho-physiological barriers and its easy access to subcellular compartments could facilitate the radical scavenging effect.

We hypothesized that TQ is capable to prevent the adverse effects of oxidative stress on gonadotropic and steroidal hormones in female rats. Adverse effects of oxidative stress on sperm quality and testicular tissue is well documented, but its adverse effects on ovary, uterus and female sex hormones have not

been fully clarified. Therefore, the present study was carried out to investigate; 1: the effects of oxidative stress induced by tert butyl hydroperoxide (t-BHP) on the concentration of gonadotropic and steroidal hormones in female rats, and 2: the effects of thymoquinone, alone or together with oxidative stress, on the concentration of gonadotropic and steroidal hormones.

## MATERIAL AND METHODS

### *Chemicals*

Tert butyl hydroperoxide (2-Methylpropane-2-peroxol) and thymoquinone (2-isopropyl-5-methyl-1, 4-benzoquinone) were purchased from Sigma-Aldrich Chemical Co.

### *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 rats were maintained under controlled conditions of a 12 h light-dark cycle, room temperature of 22-25 °C, relative humidity of 40-50%. Rats were allowed free access to standard rat chow diet and water. 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 olive oil. Rats of the second group were intoxicated with 0.2 mmol/kg body weight t-BHP [11] two times per week for 4 weeks. The third group was treated with TQ at a dose of 15 mg/kg body weight [1] two times per week for 4 weeks. The fourth group was treated with both t-BHP and TQ. The t-BHP was dissolved in sterilized distilled water and TQ was dissolved in sterilized olive oil and both were injected intraperitoneally. TQ treatment started 1 week before t-BHP injection and continued throughout the duration of the experiment. The doses of t-BHP and TQ were calculated according to the animal's body weight before each injection. All rats were handled in accordance with the standard guide for the use and care of laboratory animals.

### *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 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  $P < 0.05$ .

## RESULTS

### *Effects on FSH concentration*

The effects of t-BHP and thymoquinone injection on FSH, LH, progesterone and estrogen concentrations are shown in Table 1. There were no differences between main effects of t-BHP and thymoquinone administration. Also, there were no differences ( $P > 0.05$ ) among treatments for interaction. Numerically the lowest concentration was for those exposed to oxidative stress and the highest was for rats received thymoquinone. Administration of thymoquinone could not improve the concentration of FSH in rats exposed to oxidative stress.

There were no significant differences among treatments for the main and interaction effects. Numerically, in the interaction effects the highest concentration of LH was found for rats received t-BHP and thymoquinone and the lowest was for those exposed to oxidative stress alone.

There were significant differences among treatments for the main and interaction effects. In the main effect, injection of t-BHP decreased ( $P < 0.05$ ) progesterone concentration and thymoquinone had no effect. In the interaction effects, injection of t-BHP decreased progesterone concentration ( $P < 0.05$ ) compared to control group, but its effect was not significant ( $P > 0.05$ ). Injection of thymoquinone increased ( $P < 0.05$ ) progesterone concentration. Injection of thymoquinone to rats exposed to oxidative stress could not improve the concentration of progesterone compared to group received thymoquinone alone.

There were no significant differences for estrogen concentration among treatments for the main and interaction effects. In the interaction effect, injection of t-BHP and thymoquinone numerically decreased concentration of estrogen as compared with the control group.

## DISCUSSION

There are some studies concerning the effect of thymoquinone on hormones concentration in rats [4,10], but in the literature review, there was no report on the effects of thymoquinone on gonadotropic and steroidal hormones. Consistent to our finding, in the study by Gökçeet *al.* [9], oxidative stress induced by t-BHP reduced sex hormones level that this reaction was significant only about progesterone. It was reported [13] 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 [18], 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 [2,6].

In this study, administration of TQ increased the level of progesterone, consistent with the study [12] which hydro-alcoholic extract of *Nigella sativa* enhanced the level of progesterone in mice. TQ as an antioxidant agent may be able to protective reproductive system against oxidative stress. In our study, TQ administration had no effect on others hormones. In a study [13], 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 thymoquinone in dosages used in this study, has improving effects on plasma progesterone concentration in female wistar rats exposed to oxidative stress. These effects would maintain the pregnancy in environmental stress condition.

Table 1. Level of FSH, LH, Estrogen and progesterone hormones (ng/ml) in plasma of rats exposed to oxidative stress (induced by t-BHP) or received thymoquinone (TQ).

Items	LH	FSH	Estrogen	Progesterone
<b>Main effects</b>				
<i>Oxidative stress (t-BHP injection)</i>				
Yes	58	379	17.1	12.59 <sup>b</sup>
No	56	433	18.45	15.38 <sup>a</sup>
<i>Thymoquinone injection</i>				
Yes	58.67	394	17.08	14.38
No	55.33	419	18.47	13.59
<b>Interaction effects</b>				
control	57.33	446	19.37	14.39 <sup>ab</sup>
t-BHP yes, TQ no	53.33	392	17.57	12.79 <sup>b</sup>
t-BHP no, TQ yes	54.67	421	17.53	16.37 <sup>a</sup>
t-BHP yes, TQ yes	62.67	366	16.63	12.39 <sup>b</sup>
<i>MSE</i>	9.828	62.172	2.348	1.626
<b>P Value</b>				
t-BHP	0.734	0.17	0.348	0.018
TQ	0.573	0.503	0.337	0.426
t-BHP * TQ	0.675	0.472	0.57	0.061

<sup>a,b</sup> Means within a column with different superscript are significantly differ ( $P < 0.05$ ).

MSE: standard errors of mean

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