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THE EFFECT OF QUERCETIN ON PLASMA GONADOTROPIN AND STEROID HORMONES IN RATS EXPOSED TO BISPHENOL A

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ABSTRACT

Bisphenol A is an estrogenic compound that is pervasive in the soil, water and food. The presence of bisphenol A in urine of 95% of human is controversial because of the endocrine disruption. Quercetin, a naturally flavonoid compound commonly detected in most fruits and vegetables, has been reported to possess antioxidant and estrogenic properties. There is no report concerning administration of bisphenol A and quercetin, in combination, on gonadotropical and steroidal hormones, therefore this study was carried out. Twenty newly weaned rats were randomly divided into four groups: control, quercetin alone (30 mg/kg BW, two times per week), bisphenol alone (50 mg/kg BW, two times per week) and combination of bisphenol and quercetinthat were administered intraperitoneally for4 weeks.There was no significant difference among treatments for plasma concentration of gonadotropins, nor for bisphenol or quercetin administration alone, neither their combination. Administration of bisphenol and quercetin, in combination, had no effect on LH, but it decreased numerically plasma FSH concentration as compared to control group.Bisphenol administration decreased plasma concentration of estrogen ($P<0.008$) and progestrone ($P<0.006$). The main effect of administration of quercetin showed no significant effect on estrogen and progestrone concentration. Quercetin administration alone increased progestrone concentration as compared to control group. Combination of bisphenol and quercetin numerically decreased estrogen, but significantly decreased progestrone concentration. Findings of the present research help us to conclude that the bisphenol in dose

used in this study, has adverse effects on plasma gonadotropin and steroid concentration especially LH and progestrone female Wistar rats exposed to bisphenol. Administration of quercetin could not ameliorate the effects of bisphenol on reproductive hormones.

Key words:Gonadotropin, Steroid hormones, Bisphenol, Quercetin, Rat

INTRODUCTION

Bisphenol is the major estrogenic compound that leaches into nearby water and food supplies [1] with a high production volume chemical and an endocrine disruptor. It has been detected in 95% of human urine samples, which indicates that environmental exposure is widespread [2]. Sheep fetuses and adult sheep and rats prenatally exposed to a human-relevant exposure dose of bisphenol showed increased reactive nitrogen species[3] and it also increased reactive oxygen species [4]. Reactive nitrogen species act together with reactive oxygen species to damage cells, causing nitrosative and oxidative stress.

This type of stressis one of the factors that cause infertility or recurrent miscarriages, endometriosis, polycystic ovarian syndrome and other disorders related to pregnancy [5]. The hypothalamic-pituitary-adrenal axis is a major component of the stress response [6] 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.Nitrosative and oxidative stress occurs as a result of an imbalance between pro-oxidants and antioxidants [7]. Addition of antioxidant supplement to diet is a key way to increase the antioxidant capacity of the animal body. Quercetin is a potent antioxidantand estrogenic compound belonging to a group of plant-derived non-steroidal compounds known as phytoestrogens [8]. Few reports are published concerning the combinational effect of bisphenol and phytoestrogens on cancer and reproductive system. The results are contradictory. Katchy*et al*. [9] reported that co-exposure to bisphenol and soy-based phytoestrogens results in additive estrogenic effects, and may contribute to estrogen-linked diseases. Hwan *et al*. [10] reported that genistein has an inhibitory effect on the growth of estrogen-dependent cancers promoted by bisphenol. In the literature, there was limited information concerning the effect of bisphenol administration together with quercetin on gonadotropin and steroids hormones in female animals. Based on their estrogenic effects, we hypothesized that administration of bisphenol and quercetin together and alone reduce gonadotropic and in

consequence steroidal hormones secretion in female rats.

Therefore, the present study was carried out to investigate the effects of bisphenol and quercetin administration on the plasma concentration of gonadotropic and steroidal hormones in female rats.

MATERIAL 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.BisphenolA was purchased from Sigma Chemical Company (CAS Registry No: 80-05-7).

Animals and experimental design

Twenty newly weaned female Wistar albino rats (50-55 g body weight) were purchased from the RaziInstitute (Karaj, Iran). The animals were housed in polycarbonate 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. After one week of acclimatizationto the animal house, rats were randomly divided into fourexperimental groups (5 rats in each) as follows: The first groupserved as control group and was injectedHPMC solution intraperitoneally. Rats of the second group received bisphenol A 50 mg/kg BWtwo times per week for 4 weeks.The third group received with quercetin at a dose of 30 mg/kg body weighttwotimes per week for 4 weeks. The fourth groupreceivedbisphenol and quercetin.Quercetintreatment started one week before bisphenol and continued throughoutthe duration of the experiment. The doses of bisphenol andquercetin were calculated according to the animal's body weight beforeeach injection.

Blood sampling

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 thencentrifuged and the plasma was collected and kept at -20 °C for the determination ofluteinizing 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 platewas washed four times. After addition of the substrate solution, the concentration of progesterone was inversely proportional to the optical density measured.

Statistical Analysis

Collected data were analyzed using completely randomized design using ANOVA procedure of SAS (SAS Institute, Cary, NC). To evaluate the differences between the control and treatments, significant means were analyzed using Duncan's multiple range tests. In all cases, $P \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

The main purpose of this study was to evaluate the effect of bisphenol and quercetin administration, alone and together, on plasma concentration of reproductive hormones. In the literature, therewas no informationabout the effects of administration of bisphenol and quercetin, in combination, on reproductive hormones concentration in animals and we conducted this research with hypothesis that these substances could negatively affect the secretion of reproductive hormones.

The main effects and interaction of bisphenol and quercetin administration on plasma concentration of LH,FSH, estrogen and progestronearepresented in Table 1. There wasno significant differenceamong treatmentsneither forplasma concentration of gonadotropins, nor for bisphenol or quercetin administration alone, neither their combination.Numerically, bisphenol administration decreased FSH and LH

concentration and quercetin increased LH, but it decreased FSH. Administration of bisphenol and quercetin, in combination, had no effect on LH, but it decreased numerically plasma FSH concentration as compared to control group.

In contrast to our finding, a study [11] reported that female rats fed 100 or 200 mg/kgbisphenol had significantly higher levels of serum estradiol and lowerFSH compared to control group. It has been reported that steroidogenesis is a major target for bisphenol [12]. In line with our finding some researchers [13,14,15] reported that bisphenol may interfere with steroid hormone synthesis pathways and the release of the more potent endogenous steroid hormones into circulations.

Table 1: The plasma concentration of FSH, LH, Estrogen and progesterone hormones in rats exposed to bisphenol or received quercetin

Items	LH ng/ml	FSH ng/ml	Estrogen pg/ml	Progesterone µg/ml
Main effects				
Bisphenol				
Yes	**59.3**	**369**	**15.7^{b}**	**13.7^{b}**
No	**61.4**	**402**	**20.4^{a}**	**17.0^{a}**
Quercetin				
Yes	**61.7**	**382**	**18.0**	**15.9**
No	**59.0**	**389**	**18.1**	**14.8**
Interaction effects				
Control	**63.6**	**414**	**20.8^{a}**	**15.6^{ab}**
Bisphenol Yes, QuercetinNo	**54.4**	**364**	**15.4^{c}**	**14.0^{b}**
Bisphenol No, Quercetin Yes	**59.2**	**390**	**20.1^{ab}**	**18.5^{a}**
Bisphenol Yes, Quercetin Yes	**64.2**	**375**	**16.0^{bc}**	**13.4^{b}**
P Value				
Bisphenol	**0.721**	**0.384**	**0.008**	**0.006**
Quercetin	**0.646**	**0.844**	**0.971**	**0.249**
Bisphenol * Quercetin	**0.241**	**0.633**	**0.640**	**0.095**

^{a,b} Means within a column with different superscript are significantly differ (P < 0.05)

The change in serum sex hormone levels may cause subsequent reproductive dysfunction by interfering with the feedback regulatory mechanisms of the hypothalamic-pituitary-adrenal axis. Our data supported this notion as the altered plasma level of estrogen was detected in the bisphenol exposed rats.

Bisphenol administration decreased plasma concentration of estrogen (P<0.008) and progestrone (P<0.006). The main effect of administration of quercetin showed no significant effect on estrogen and progestrone concentration. Quercetin administration alone increased progestrone concentration as compared to control group. Combination of bisphenol and quercetin numerically decreased estrogen, but significantly decreased progestrone concentration. The highest progestrone concentration was for rats

received quercetin and the lowest one was for those received bisphenol alone.

In contrast to our finding, some studies [16,17]reported that quercetin increased mean estradiol concentration by inhibiting estrogen sulfotransferase. Quercetin in our study increased progestrone concentration and a study [16] indicated that progesterone stimulate expression of estrogen sulfotransferase. However, quercetin had no effect on estrogen concentration. A recent study indicated that dietary flavonoids, including quercetin, may inhibit estrogen sulfatase, suggesting a protective effect of these dietary polyphenols [18]. Previous studies have demonstrated that flavonoids can be potent inhibitors of a human sulfotransferase [20], which can sulfonate high concentrations of estrogen hormones [21].

Quercetin as an antioxidant agent could not able to protective pituitary gland against oxidative stress induced by bisphenol. In our study, quercetin administration had no effect on gonadotropin and steroidal hormones. In line with ourfinding, a study[22] found thatantioxidant 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 bisphenol in doseused in this study, has adverse effects on plasma gonadotropinand steroid concentrationsespecially LH and progestrone female Wistar rats exposed to bisphenol. Administration of quercetin could not ameliorate the effects of bisphenol on reproductive hormones.

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