Study of the effect of exposure to Phytoestrogens (Genistein) and estradiol (17β-estradiol) in the level of gene expression (CYP19A1) and quantification of aromatase in female rats

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**ABSTRACT**

The current study was carried out to investigate the role of Phytoestrogens (genistein) in different concentrations and estrogens manufactured in tablets coated with or without progesterone at the level of gene expression of CYP19A1 and quantitative estimation of aromatase compared to control group. The results of the present study showed a significant increase in (P <0.05) in the level of quantitative estimation of serum aromatase enzyme and for all treatments compared to the control group (124.12). The highest level of the enzyme (322.00) in the group treated with genistein in combination with estradiol, while, the lowest rise in the level of aromatase enzyme (164.37) in the group treated with genistein combined with progesterone. There was also a significant decrease in the concentration of ribosomal RNA and all the treatments compared to the control group (1068.95), the Lower of RNA concentration was (373.70) in the group treated with genistein at a concentration of 50 mg | kg. While Less low concentration of RNA (1068.95) was observed in the groups treated with concurrent genistein with estradiol. The results of the present study indicate that there was a significant increase (P <0.05) in the level of gene expression of the aromatase enzyme in ovarian tissue and for all the treatments compared to control group (305.51). The highest level of gene expression of the enzyme (7.189) was observed in the groups treated with concurrent genistein with estradiol, While the lowest level of gene expression of aromatase (2.204) was observed in the groups treated with genistein combined with progesterone.

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INTRODUCTION

Phytoestrogens are Non-steroidal biphenol compounds possess chemical structures similar to estrogen that have the ability to bind to the active receptors of estrogen in mammals’ tissues (Eden 2012; Zorrilla and Yatsenko 2013). Soybean, red clover, flaxseed, cauliflower and cabbage are among the most important foods with estrogen (Hajirahimkhan, Dietz et al., 2013). Phytoestrogens genistein (5,7,40-trihydroxyisoflavone) is found mainly in soybeans and other legumes (Preedy 2013; Baber 2010). The biological activities of phytoestrogens in mammals are closely related to their similarity (17β-Estradiol) and its ability to bind to the estrogen receptor (ER) (Collins, McLachlan et al., 1997, Leclercq, De Cremoux et al., 2011).

The similarities between estrogen (17β-Estradiol) and plant estrogen at the molecular level can reduce the latter effect of estrogen or metabolise its effect, as it can bind to the estrogen receptor to increase its effect or prevent its association with its inhibitory effect (Yildiz 2005 and Hashem and Soltan 2016). Although the phytoestrogens are not
exactly identical to normal female estrogen, they mimic their biochemical properties, amazingly.

Phytoestrogen contribute to a lower risk of cancer by stopping or inhibiting the work of enzymes that cause malignancy, which contribute to the proliferation and growth of cells, or reduce their genetic expression as aromatase (Sirotkin 2014, Yanagihara, Zhang et al., 2014), which converts the androgens to estrogen in the endocrine tissue and increases the chance of its association with its receptors, particularly alpha receptors that mediate cell proliferation (Rietjens, Sotoca et al., 2013). There is also convincing evidence that a diet rich in soy protein, as genistein, has an effect on the hormonal status and regulation of menstrual cycle for premenopausal women (Cassidy, Bingham et al., 1994). At the molecular level, knowing the role of genes involved in the biosynthesis of steroid biosynthesis or participation in the hypothalamic-pituitary-ovarian axis is one of the goals of molecular genetic studies at present as it plays a key role in causing some forms of infertility and hormone-dependent cancers. Among these genes is the aromatase gene, also called CYP19A1, Which is one of the most important genes related to fertility in women and also plays an active role in stimulating the conversion of androgens carrying 19 carbon atoms C19 androgens, which include Testosterone Testosterone and Androstenedione Androstenedione to estrogens, which carry 18 carbon atoms C18 estrogens, including Estradiol and Estrone Estrone .This is the most important step in biosynthesis of steroid hormones in women, making this gene a prominent feature in genetic studies (Sebastian and Bulun 2001 and Altmäe, Haller et al. 2009).

This study was designed to know the genetic expression of CYP19A1 by measuring the mRNA expression using Rial time PCR and quantification of the aromatase enzyme Associated with the use of Phytoestrogens (genistein) compared to control group and Industrial estrogens group with or without progesterone.

MATERIAL AND METHODS

Experimental animals

Forty-eight female Norwegian NorvegicusRattus rats were between 12 and 10 weeks of age between 250 and 200 grams. Obtained from the Animal House of the Faculty of Veterinary Medicine at the University of Qadisiyah. The rats were placed in plastic cages with six rats per cage. Each cage had a metal cap with a water bottle and a food place. The cedar flooring was replaced weekly to maintain the cleanliness of the rats. The locally produced flour was used from wheat flour, corn and animal protein. The mice were subjected to laboratory conditions from a light cycle that was divided into 12 light hours and 12 hours of darkness and temperature throughout the experiment.

Design of study

In the present study, 48 animals from the female, white rats have randomly divided into six groups, and each group had eight rats and were treated as follows:

1. The control group (T1) treated with 1 ml of normal saline (NS) solution at 0.9% concentration for 1 month by oral dosage
2. The second group (T2) treated with Estradiol valerate (2 mg/kg), which was manufactured in the form of tablets coated with normal soluble saline for one month by oral dosage.
3. The third group (T3) treated with a mixture of estrogen and progesterone, which was manufactured in tablets containing 2 mg/kg Estradiol valerate and (0.5mg / kg) Progesterone and soluble with normal saline for one month by oral dosage.
4. The fourth group (T4) treated with phytoestrogens (50 mg/kg) dissolved in the normal saline for one month.
5. The fifth group (T5) treated with phytoestrogens (100 mg/kg) dissolved in the saline solution for one month by oral dosage.
6. The sixth group (T6) treated with a solution of Estradiol valerate tablets 2 mg/kg for one month and orally treated with phytoestrogens 100 mg/kg dissolved in normal saline.

The animals were dissected after anaesthetising them with a mixture of ketamine and xylocaine, and the blood was withdrawn directly from the heart by means of a 5-ml plastic syringe. It was placed in a plastic tubing and was sterile in order to prevent the blood from reaching it for more than 30 minutes at room temperature until blood clotting and then separating the serum using a centrifugal device (5000) cycles / min for 10 minutes to ensure the availability of serum-free of red blood cell effects, to measure levels of aromatase enzyme.

Using the Enzyme-linked Immunosorbent Assay (ELISA) immunosorbent assay technique, using the ELISA device and an aromatase kit designed by the German company Cusabo-uman. The tissue samples were also collected to extract the acid DNA (DNA), and to examine the interaction of the polymerization chain in real time quantitatively (reverse transcription) to measure the quantitative levels of transcribed DNA (mRNA transcript levels) to indicate the amount of gene expression (GAPDH) gene was used as a standard regulator to calculate gene expression. As well as
the determination of the concentration of ribosomal DNA ng/μlRNA and the measure of RNA purity by reading the absorbance at (260/280 nm) using the Nanodrop spectrophotometer and according to the method attached to the kit processed by the Korean company Pioneer.

**Primers**

These prefixes are designed in this study using NCBI GenBank Data and using Primer3 plus. This prefix is supplied by Korean Pioneer as shown in Table (2).

**Statistical analysis**

The results were statistically analysed using the Statistical Package for Social Sciences (SOVS) by the One-Way Analysis (ANOVA) test to identify the differences between the parameters studied for the different groups. The differences were significant between the averages at P00.05 and the lowest difference Moral (LSD) Least Significant Difference Test.

**RESULTS**

**Level of aromatase enzyme**

The results of the current study shown in Table (3) at (P < 0.05) showed a significant increase in the level of quantitative estimation of serum aromatase enzyme for all treatments compared to control group (124.12). The highest level of the enzyme (322.00), was observed in the (T5) groups While the lowest level of aromatase (164.37) was observed in the (T2) groups.

The treatment with estradiol significantly increased the quantitative estimation of aromatase with an increased rate of 189.87 compared with control group (124.12) and T2 group 164.37 While representing a significant decrease compared with T3 (212.87) and T4 group (258.75) and T5 (322.00).

The treatment with estrogen associated with progesterone showed a significant increase in the level of the enzyme (164.37) compared to control group (124.12), while it remained to represent a significant decrease compared to the group treated with estradiol (189.87), T3 group (212.87) and T4 group (258.75) and T5 group (322.00).

Treatment with Genistein at a concentration of 50 mg/kg significantly increased the level of the aromatase enzyme (212.87) compared with control group (124.12) and T1 group (189.87) and T3 group (164.37) while it remained to represent a significant decrease compared to the T4 group (258.75) and T5 (322.00).

The treatment of the Genistein at a concentration 100 mg/kg showed a significant increase in the level of the aromatase concentration (258.75) compared with control group (124.12) and T1 group (189.87) and T2 group (164.37) and T3 group (212.87), but still showed a significant decrease compared with T5 group (322.00).

The results of the treatment with the concurrent genistein with estradiol were significantly higher in the quantitative estimation of the aromatase enzyme (322.00) compared to control group (124.12), T1 group (189.87), T2 group (164.37), T3 group (212.87) and T4 group (258.75).

**Molecular Analysis**

**Changes in concentration and purity of RNA**

The results of the current study shown in Table (3) showed a significant decrease in the concentration of ribosomal RNA for all the treatments compared to the control group (1068.95). The highest significant decrease of RNA concentration was (373.70) in the T3 group While The lowest nonsignificant decrease in RNA concentration was (1066.90) in the T5 group.

The concentration of ribosomal RNA was significantly decreased in the treatment group with estradiol (801.434) compared to the control group (1068.98) and T5 group (1063.90). While representing significantly increased compared to T2 (605.80), T3 (373.70) and T4 group (770.73).

The treatment with estrogen associated with progesterone resulted in a significant decrease in the concentration of rRNA, (605.80) compared with the control group (1068.98), the estradiol group (801.433), T5 (1063.90), and the T4 group (770.73), While significantly increasing the concentration of rRNA compared to T3 group (373.7).

The treatment with genistein at concentrations of 50 mg/kg was more significant in reducing the concentration of rRNA (373.7) compared with the control group (1068.98), T1 (801.433), T2 (605.80), T4 (770.73) and T5 group (1063.90). While the treatment with the genistein at the concentration 100 mg/kg was a significant effect on the reduction of the rRNA concentration (770.73) compared with the control group (1068.98), T1 (801.433), T5 group (1063.90), While representing significantly increasing compared to T2 (605.80) and T3 group (373.7).

The treatment with genistein concurrent with estradiol showed no significant decrease in rRNA concentration (1066.90) compared to the control group (1068.98).
The results of the study regarding DNA purity showed a significant decrease in all treatments compared to the control group.

**The level of gene expression of the aromatic enzyme CYP19A1**

The results of the current study in Table (3) showed a significant increase in the level of gene expression of the aromatase enzyme in the ovarian tissue for all the treatments compared to the control group (1.034). The highest level of gene expression of the enzyme (7.189) was observed in the estradiol treatment group, while the lowest level of gene expression of aromatase (2.204) was observed in the group, treatment with estradiol associated with progesterone.

The treatment with estradiol significantly increased the level of gene expression of aromatase (3.719) compared to control group (1.034), T2 group (2.204) While it represents a significant decrease compared to T3(4.639), (5.133) and T5 group (7.189).

The treatment with estrogen associated with progesterone showed a significant increase in the level of the gene expression of aromatase (2.204) compared with control group (1.034), while it remained a significant decrease compared to the group treated with estradiol (3.719), T3 |group (4.639), as well as T4 (5.133) and T5 group (7.189).

Treatment with genistein at concentrations 50 mg/kg showed significantly increased of gene expression of aromatase (4.639) compared with control group (1.034), T1 (3.719), T2 (2.204), While it was representing a significant decrease compared to T4(5.133) and T5 group (7.189).

Treatment with genistein at concentrations 100 mg/kg showed significantly increased of gene expression of aromatase (5.133) compared with control group (1.034), T1 (3.719), T2 (2.204), and
T3 (4.639) group, but still showed a significant decrease compared to T5 group (7.189).

The treatment with genistein concurrent with estradiol showed a more significant increase of aromatase gene expression, (7.189) compared with the control group and all treatment groups.

**DISCUSSION**

The process of gene expression, the regulation of aromatase synthesis and its effect is a complex process that is influenced by many factors, such as sex (Labrie et al., 1997; Wenjie, 2012); menstrual cycle (Sano et al., 1981Wenjie, 2012) (Marsh et al., 2011), as well as the effect of the enzyme on genetic variables (Ahsan et al., 2005). Unlike most cytochrome enzymes, the gene expression of aromatase in healthy liver tissue is low (Carruba 2009).

In contrast, the expression of aromatizophthalate is very high in the ovaries of women in Premenopausal age where estrogen synthesis to perform its functions in target tissues (Simpson 2003 and Bulun, and Simpson, 2008). In post-menopause and men, the Peritoneal synthesis estrogens by the effectiveness of aromatase are the primary route of estrogen production in women after ovarian function declines during menopause or after a pathological change or surgical intervention that reduces or removes ovarian function (Pritts 2010).

Depending on the results of the current study, the genetic expression of aromatic has increased significantly in comparison to the control group and all treatments. The ovary is a non-conventional target tissue of estrogen, in addition to that expression of estrogen receptor type alpha is very low or cannot be measured in contrast, beta-estrogen receptor expression is high in ovarian tissue (Saji, Jensen et al., 2000)

The Affinity of genistein to beta-type estrogen receptors is approximately 20-30 times greater than of alpha-estrogen receptors compared with estradiol (Morito, Hirose et al., 2001). The similarities between estrogen (17βEstadiol) and phytoestrogen genistein at the molecular level allow the latter to reduce the effect of estrogen or counteract its effect when associated with estrogen receptors and prevent its effect (Yildiz 2005; Hashem and Soltan 2016). At the same time, the level of estrogen in the blood increases and the increase in the concentration of estrogen stimulates the production of sex hormone-binding globulin (SHBG). This leads to faster removal of sex hormones, including estrogens, Thus, target cells need estrogen more than phytoestrogen provides, In addition, the hypothalamus-ovarian-ovarian axis leads to negative feedback with high estrogen levels to control the secretion of hormones released

In addition, the hypothalamus-ovarian-ovarian axis leads to negative feedback with high estrogen levels to control the secretion of Gonadotrophin Releasing hormones, thus inhibiting secretion of pituitary hormones, therefore, the lack of the level of hormone FSH inhibits the formation of folliculogenesis in the vesicular stage and delayed maturity and thus a low level of ovarian estrogen. Therefore, the body resorts to the production of peripheral estrogen through aromatase of peripheral tissue outside the Extra gonadal tissues. Therefore, the level of gene expression and aromatase concentration has increased significantly.

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plant flavonoids in the catecholamine system."
