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Effect of Trans-Anethole on Gene Expression of Steroidogenic Enzymes in the Ovary of Polycystic Ovary Syndrome Model Rate

Maryam Asadian [1, 2], Hashem Yaghoubi [1✉], Fariba Mahmoudi [2], Khadijeh Haghighat Gollo [2]

[1] Department of Biology, Ardabil Branch, Islamic Azad University, Ardabil, Iran
[2] Faculty of Sciences, University of Mohaghegh Ardabili, Ardabil, Iran

Abstract

Background: The process of steroidogenesis is crucial to the normal function of the ovaries. In individuals with polycystic ovary syndrome (PCOS), the activity of related enzymes in this process is disrupted. In the present study, the effect of trans-anethole was investigated on gene expression of steroidogenesis enzymes in PCOS model rats. **Materials and Methods:** In this experimental study, thirty female rats were divided into six groups (n=5 per group). Fifteen PCOS rats in three groups received intraperitoneal injections of distilled water, 50, and 80 mg/kg of trans-anethole, respectively. Also, 15 intact rats in three groups received intraperitoneal injections of distilled water, 50, and 80 mg/kg trans-anethole. The expression of steroidogenesis genes was determined using real-time reverse transcription polymerase chain reaction. **Results:** The mRNA level of *Cyp19* significantly increased in intact rats receiving 80 mg/kg trans-anethole compared to the control group. The *Cyp19* level in PCOS groups was significantly reduced compared to the control group. The mRNA level of *Cyp19* in PCOS groups that resived 50 or 80 mg/kg trans-anethole increased compared to PCOS rats, but this increase was not statistically significant. The mRNA level of *Cyp17* did not significantly change in intact and PCOS rats that received trans-anethole compared to the control group. **Conclusion:** Trans-anethole may improve PCOS complications due to its involvement in regulating steroidogenesis.

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Keywords: Polycystic Ovary Syndrome; Trans-anethole; *Cyp17*; Aromatase; Steroidogenesis

Introduction

Reproductive hormones, including estrogen, progesterone, and testosterone are essential for the reproductive cycle because they play a vital role in regulating several physiological processes, such as the menstrual cycle and fertility [1]. Also, these hormones are essential for the growth and function of various tissues [1]. The synthesis of steroid hormones is mediated by the function of various enzymes through several consecutive pathways [2]. The process of transforming cholesterol into steroid hormones is defined as steroidogenesis. Cholesterol converts to pregnenolone by

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Email:info@gmj.ir

✉ **Correspondence to:**
Hashem Yaghoubi, Department of Biology, Faculty of Sciences. Islamic Azad University, Ardabil, Iran
Telephone Number: +989125798735
Email Address: Yaghoubi_h@iauardabil.ac.ir

the function of the side chain cleavage enzyme (CYP11A) enzyme. Then, in the theca cells of the ovary, the 3 beta-hydroxysteroid dehydrogenase (3β-HSD) and 17-alpha hydroxylase/17, 20 lyase (*Cyp17*) enzymes convert pregnenolone to progesterone and 17- hydroxyprogesterone, respectively. The 17- hydroxyprogesterone, in turn, converts to androstenedione by the *Cyp17* enzyme. Then, testosterone is produced by 17 beta-hydroxysteroid dehydrogenase (17β-HSD) enzyme, which in turn converts to 17-beta-estradiol by the function of aromatase (*Cyp19*) in the granulosa cells of the ovary [3-5].

Polycystic Ovary Syndrome (PCOS) is a chronic endocrine disorder that impacts 15-20% of women of reproductive age [6]. The condition is defined by hyperandrogenism, chronic anovulation, and excessive androgen synthesis by the ovarian [7]. Bakhshalizadeh *et al*. showed that higher production of androgens and disruption of the steroidogenesis process is a crucial component of aberrant folliculogenesis and failure in dominant follicle selection in hyperandrogenic PCOS patients [8].

Trans-anethole (1-methoxy-4-[1-propenyl] benzene), an aromatic organic substance, is a colorless, minorly volatile liquid with a characteristic smell. In nature, trans-anethole is found in large amounts in plants such as *Foeniculum vulgare, Pimpinella anisum L.,* and *Illicium verum* [9]. Also, trans-anethole has various pharmacological properties, including antioxidant, anti-diabetic, cardioprotective, anti-inflammatory, anti-cancer, and estrogenic activities [10]. Also, the inhibitory effects of trans-anethole on reproductive parameters were shown [11]. We aimed to investigate the effects of intraperitoneal injection of trans-anethole on gene expression of *Cyp17* and aromatase (*Cyp19*) in the estradiol valerate-induced PCOS rat model.

Materials and Methods

Chemicals and Animals

In this experimental study, trans-anethole was purchased from Sigma Aldrich (USA). Thirty Wistar rats weighing 180-220 g were supplied by the Iran University of Medical Sciences. Animals were maintained under standard laboratory conditions (12-hours light/dark cycle at 25±2 °C, 50-60% humidity) with enough food and water. Also, the ethics committee of Ardabil University of Medical Sciences approved the protocol of study (code: IR.ARUMS.REC.1399.055).

Induction of PCOS

The vaginal smear was performed for two consecutive weeks to determine the stage of estrus (proestrus, estrous, metestrus, and diestrus). For induction of PCOS, 2 mg/rat estradiol valerate was dissolved in 0.2 ml sesame oil and injected intramuscularly into animals in the estrous phase as a single dose. The PCOS status was confirmed 60 days after the estradiol valerate injection by observing persisting cornification epithelium cells with a vaginal smear and examination under a light microscope.

Groups

The intact or PCOS rats were grouped into six groups (n=5 per group) as follows:

Group (I): intact control rats received 0.5 ml of distilled water.

Group (II): intact rats received 50 mg/kg trans-anethole.

Group (III): intact rats received 80 mg/kg trans-anethole.

Group (IV): PCOS rats received 0.5 ml of distilled water.

Group (V): PCOS rats received 50 mg/kg trans-anethole.

Group (VI): PCOS rats received 80 mg/kg trans-anethole.

The animals received saline or trans-anethole for 14 consecutive days at 9:00-9:30. One day after the last injection, rats were anesthetized using ketamine-xylazine, and ovarian samples were removed after a cut in the animal's abdomen surface and immediately stored at temperature-80 °C.

Real-Time Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Total RNA was isolated from the ovarian tissue samples using the TRIzol reagent kit (Qiagen Co, Germany). After RNA extraction, the purity and concentration of RNA were determined using a Nanodrop 2000 Spectrophotometer (Thermo Fisher

Scientific, Waltham, MA, USA) to read light absorption at wavelengths of 260 and 280 nm. According to the kit instructions, the cDNA was synthesized from 1 µg of total RNA (Vivantis Co., Malaysia). Changes in gene expression levels were determined using the Corbett rotor gene 6000 (Qiagen Co, Germany) real-time PCR detection system and SYBR Green I kit (Takara Bio Inc., Japan) in a final volume of 25 µl according to manufacturer instructions. The PCR cycling conditions were as follows: one cycle (2 minutes, 95 °C) and 40 cycles (94 °C for 15 seconds, 60 °C for 30 seconds, and 72 °C for 15 seconds). Nucleotide sequences for sense and antisense primers for *GAPDH, Cyp19,* and *Cyp17* genes were as follows: *Cyp19*: F: 5′-CGTCATGTTGCTTCTCATCG-3′, and R: 5′-TACCGCAGGCTCTCGTTAAT-3′[12]; *Cyp17*: F:5'-ACTGAGGGTATCGTGGATGC-3', and R:5'-TCGAACTTCTCCCTGCACTT -3', and GAPDH: F: 5'-AAGTTCAACGGCACAGTCAAG-3', and R: 5'-CATACTCAGCACCAGCATCAC-3' [12]. The *Cyp19, Cyp 17,* and *GAPDH* amplified products were 149, 160, and 120 base pairs, respectively. The *GAPDH* gene was used to normalize the values obtained for each sample. Equation $2^{-\Delta\Delta CT}$ was used to calculate the relative gene expression levels of the target mRNAs.

Statistical Analysis

The data were analyzed using SPSS software version 16 (SPSS Inc. USA). The one-way ANOVA and Tukey's Post-Hoc tests were performed to compare the significant differences between the groups. The results were presented as a mean±SEM. P-values≤0.05 were considered as statistically significant.

Results

The mRNA level of *Cyp19* significantly increased in intact rats that received 80 mg/kg trans-anethole in comparison to the control group (Figure-1, P<0.05). The mRNA level of *Cyp19* in the PCOS group was significantly reduced compared to the control group (Figure-1, P<0.05). The mRNA level of *Cyp19* in PCOS groups that received 50 and 80 mg/kg trans-anethole increased compared to PCOS rats, but this increase was not statistically significant (Figure-1).

The mRNA level of *Cyp17* was not significantly changed in intact rats that

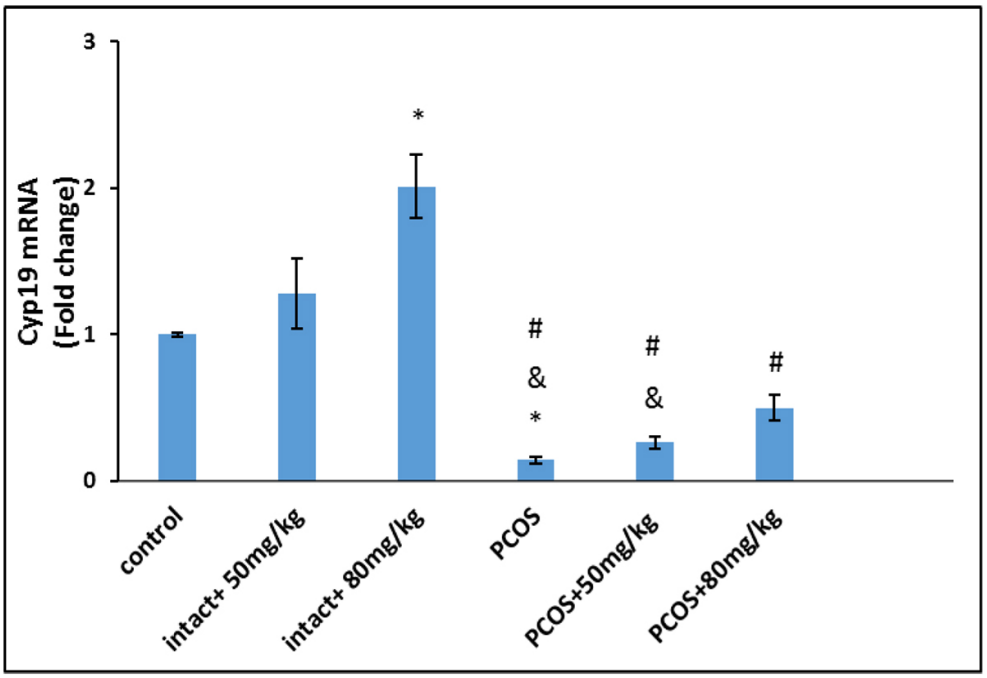

Figure 1. Effects of trans-anethole on relative *Cyp19* gene expression in the ovaries of intact and PCOS rats. The results are presented as mean±standard error of the mean (SEM), and significance was defined by P≤0.05. * vs. control, & vs. intact 50 mg/kg, # vs. intact 80 mg/kg

received 50 and 80 mg/kg trans-anethole compared to the control group (Figure-2). Also, the mRNA level of *Cyp17* in the PCOS group was not significantly changed compared to the control group (Figure-2). Also, significant change was not observed in the mRNA level of *Cyp17* in PCOS groups that received 50 and 80 mg/kg trans-anethole compared to the PCOS group (Figure-2).

Discussion

The present study showed that in rat's ovaries, the mean relative gene expression of *Cyp19* was significantly reduced compared to the control group. The present results are consistent with previous reports, which demonstrated a decrease in *Cyp19* gene expression in the ovary [13]. Intraperitoneal injection of trans-anethole to intact rats caused an increase in *Cyp19* mRNA level in comparison with the control group. However, trans-anethole did not exert a significant stimulatory effect on *Cyp19* in PCOS rats. The effects of trans-anethole on adrenal carcinoma cells were demonstrated previously and showed that trans-anethole increases the expression of the *Cyp19* gene in these cells [14]. Another study showed that trans-anethole was not significantly increase *Cyp19* gene expression [15].

In female reproduction, sex steroid hormones play an important role in women's fertility health. Also, various factors, such as peripheral hormones, neurotransmitters, different signaling pathways, and oxidative stress could play a role in controlling the enzymes of the estrogen synthesis pathway. Previous studies established the role of oxidative stress in metabolic and endocrine diseases [16]. The *Cyp19* enzyme activity is impaired by increasing the production of reactive oxygen species (ROS) and antioxidant degradation in most postmenopausal women or those with PCOS. Evidence revealed that trans-anethole has a protective role in cells against an increase in ROS. [17-18]. It could be concluded that trans-anethole has a protective effect on the enzyme *Cyp19* against oxidative stress. In patients with PCOS, the activity of *Cyp19* production decreases [19]. This reduction may be due to oxidative stress in these patients. Trans-anethole, by reducing the destructive effects of ROS, regulates the function of the *Cyp19* enzyme in the steroidogenesis pathway, and it may ultimately improve ovarian function [19].

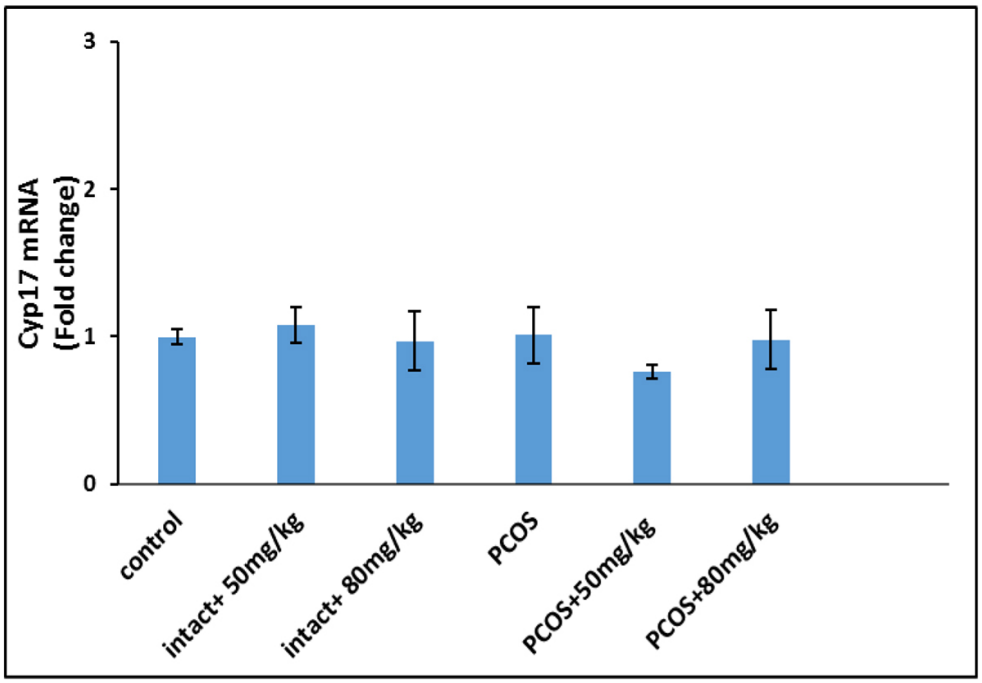

Figure 2. Effects of trans-anethole on relative *Cyp17* gene expression in the ovaries of intact and PCOS rats. The results are presented as mean±standard error of the mean (SEM)

PCOS is an endocrine disorder, and various pathophysiological factors play a role in its development [19]. The present data showed that in PCOS rats, the relative gene expression of *Cyp17* could not significantly change compared to the control group. However, Al-Omar *et al*. and Jahromi *et al*. showed increased gene expression of *Cyp17* in PCOS model rats [20, 21]. Also, the present results demonstrated that intraperitoneal injection of trans-anethole in Wistar female rats does not change *Cyp17* gene expression in the ovary. Whereas in vitro studies on antral follicles showed that trans-anethole increases the activity of the *Cyp17* enzyme [14, 15].

Disruption of any enzyme involved in the steroidogenesis pathway leads to ovarian dysfunction and the development of cysts in the ovaries, and infertility in women [22]. A close relationship was reported between interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) on ovarian function, ovulation, and fertility in PCOS patients so that the serum levels of IL-6 and TNF-α increase in individuals with PCOS [23, 24]. Trans-anethole interference with critical signaling pathways, including protein kinase A (PKA) and inflammatory pathways such as IL-6 and TNF-α, has been reported in vitro conditions [25, 26]. The stimulation of steroidogenic cells leads to the activation of PKA, which activates the function of genes involved in steroidogenesis [27]. Other evidence suggests that inflammatory factors increase the levels of the *Cyp17* enzyme via stimulating androgen production in theca cells [28]. In addition, some researchers reported that trans-anethole alters steroidogenesis by activating the PKA signaling pathway [14].

Also, trans-anethole may improve ovarian function in PCOS rats by altering the gene expression of steroidogenesis enzymes partly via reducing inflammatory factors.

Conclusion

Disrupting steroidogenesis enzymes could lead to ovarian dysfunction, PCOS, and infertility. The present study showed decreased *Cyp19* mRNA levels in trans-anethole-received rats. However, intraperitoneal injection of trans-anethole showed no effect on the *Cyp17* mRNA levels. Trans-anethole may be useful for improving PCOS complications due to its involvement in regulating steroidogenesis.

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Conflict of Interest

There was no conflict of interest.

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