Original Article

# The Protective Effects of Trans-Anethole against Polycystic Ovary Syndrome Induced Histopathological and Metabolic Changes in Rat

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## Abstract

**Background:** Aim of the study was to evaluate the protective effects of trans-anethole, against polycystic ovary syndrome (PCOS) induced histopathological and biochemical changes in female Wister rats.

**Materials and Methods:** In this experimental study, forty-eight animals were randomly assigned into 6 groups: control; PCOS; PCOS+trans-anethole (20, 40, 80 mg/kg); and PCOS+metformin (300 mg/kg). Testosterone (1 mg/kg/day) was injected intraperitoneally for 35 days to induce PCOS. After PCOS induction, animals were treated by trans-anethole and metformin (30 days oral gavage). Finally, serum oxidative stress and insulin levels as well as histological changes in ovaries, kidneys and liver were evaluated.

**Results:** In PCOS group, the serum level of malondialdehyde (MDA) was 1.391 ± 0.18 mmol/L and significantly increased (P=0.000) compared to the control group with the MDA level of 0.35 ± 0.08. Meanwhile the activity of superoxide dismutase (SOD) and catalase (CAT), and total thiol levels were significantly decreased (P=0.000 for all groups), compared to the control group. In the trans-anethole (80 mg/kg) treated group, insulin (P=0.000) and MDA (P=0.000) levels were significantly decreased while total thiol (P=0.001) and activity of SOD (P=0.000) and CAT (P=0.007) were significantly increased compared to the PCOS group. In the metformin treated group the insulin level (P=0.03) decreased compared to the PCOS group. Histological evaluation showed multiple cysts in the ovarian tissue, an increase in inflammatory cells in the liver, and a loss of order in the structure of the tubules and glomeruli of the kidney in the PCOS group. Tissue damage was reduced in the trans-anethole treated group.

**Conclusion:** Tarns-anethole at a dose of 80 mg/kg improved metabolic status, oxidative stress, liver and kidney damage as well as the cystic mass of ovarian tissue. To understand the exact protective effects of trans-anethole in PCOS, more experimental or clinical studies are suggested.

***Keywords:*** Histopathology, Metformin, Oxidative Stress, Polycystic Ovarian Syndrome, Trans-Anethole

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## Introduction

Polycystic ovary syndrome (PCOS) is considered as an endocrine-metabolic disease, which highly increases the risk of infertility and metabolic disorders including obesity, insulin resistance, and type2 diabetes (1). Hyperandrogenism signs or symptoms such as alopecia, acne, and hirsutism are common in most women with PCOS. A major feature of PCOS is the increase of androgen production and insulin levels that result in suppressing the production of sex hormone binding globulin (SHBG) and its release from the liver. As a result, the level of free testosterone would increase in the PCOS patients (2). Moreover, PCOS is associated with elevation of free radicals and decrease in activities of antioxidant enzymes and serum levels of antioxidants. However, the role of oxidative stress markers in the pathogenesis of PCOS needs to be completely determined. It was suggested that oxidative stress might alter ovarian steroid synthesis, which leads to increased androgen levels, disturbance of follicular development, and infertility in PCOS patients (3).

Induction of insulin resistance and hyperglycemia in PCOS have also been identified as factors in promoting oxidative stress (4). Insulin resistance might result in ovarian cysts by increasing tumor necrosis factor-α production (5). Lipid peroxidation and reactive oxygen species (ROS) formation have been shown to cause

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oxidative stress damage due to the conversion of the glutathione reduced form (GSH) to the Glutathione disulfide (GSSG) oxidized form (6). Factors contributing to oxidative stress, including chronic inflammation and infection, obesity, and insulin resistance have been shown to be related to an excess of oxidative stress markers in women with PCOS (2). Different types of anti-PCOS drugs are prescribed for reducing these complications, but these drugs have no definitive therapeutic effects, and in most PCOS patients these drugs have undesirable side effects (7). Nowadays, the tendency to use herbal medicines and natural products for the treatment of disease has been increased. *Foeniculum Vulgare (F. Vulgare)* is a functional food plant widely used to treat hormonal disorders and regulation of menstrual cycles for its estrogenic properties (8). *F. Vulgare* contains protein, calcium, phosphorus, iron, potassium, vitamins A and C, and has antimicrobial and antioxidant properties (9). Trans-anethole is one of the most important active ingredients of *F. Vulgare*, with many pharmacological properties (10).

Trans-anethole has antioxidant, anti-inflammatory and estrogenic properties (11). In a previous study, 35 days testosterone injection in rats increased the serum levels of testosterone, dehydroepiandrosterone and lipids which are markers of PCOS induction (12). However, to the best of our knowledge the effects of trans-anethole against metabolic and histological changes of PCOS induced by testosterone had not been evaluated. Moreover, metformin is one of the most common medications prescribed to PCOS patients which can modulate oxidative stress and decreases serum androgen levels in PCOS patients (13). Therefore, in the present study, our aim was to examine the effects of trans-anethole and metformin on the histological changes in the ovaries, liver and kidneys as well as serum biochemical markers of testosterone-induced PCOS rats.

## Materials and Methods

Drugs and materials were obtained as follows: Testosterone enanthate (IM) (Caspian, Iran), trans-anethole (Sigma, China), metformin (Sigma, India), and olive oil (Farabi, Iran). Enzyme immunoassay kits were used for measurement of insulin (Cayman Chemical, USA) by ELIZA (14) and materials for measurement of oxidative stress marker were obtained from Merck (Germany). All chemicals and reagents for histological assessments were purchased from Farzan Azma (Iran).

### Animals

In this experimental study, forty-eight female Wister rats (8 weeks, 180-210 g) were obtained from the animal care facility of the Faculty of Medicine, Mashhad University of Medical Sciences, Iran. During the experiment, animals were kept under standard conditions (22 ± 2°C with 12 hour light-dark cycles) with free access to water and food. The study protocol was performed in accordance with ethical policies and principles approved by the Committee on Animal Research of Mashhad University of Medical Sciences (IR.MUMS.MEDICAL.REC.1399.075).

### Experimental design

Animals were randomly divided into six groups (n=8) as: control; PCOS non-treated; PCOS treated group with metformin (300 mg/kg) which is represented as metformin; PCOS treated groups with 3 doses of trans-anethole (20, 40, 80 mg/kg) (12, 15); which are represented as trans20, trans40 and trans80. To induce the PCOS model, dissolved testosterone in olive oil (1 mg/kg/day) was intraperitoneally injected for 35 days (12). After PCOS induction, treatments were given orally by gavage for the next 30 days. At the end of the study, for measurement of serum biochemical and oxidative stress parameters, rats were fasted overnight and blood samples were collected, serum was isolated and kept at -20°C. The serum levels of insulin, MDA, total thiol content, and activities of CAT, and SOD were assayed. In addition, for histological examination, the liver, ovary, and kidney tissues were dissected and isolated.

### Measurement of superoxide dismutase activity

SOD activity was measured by the Madesh and Balasurbamanian colorimetric methods, using 96-well microtiter plates (16). Briefly, the appropriate amount of serum, MTT [3-(4, 5-dimethylthiazol-2-yl) 2, 5-diphenyltetrazolium bromide] and pyrogallol were poured into the wells and incubated for 5 minutes at room temperature and in a dark environment. After adding the dimethyl sulfoxide inhibitor, its absorption was read at 570 nm, and the results were reported in u/ml.

### Measurement of catalase activity

The CAT activity was assayed according to the Abei colorimetric method. The decomposition of hydrogen peroxide (30 mM) micromoles per milligram of protein sample was considered as one unit of CAT activity. The reduction was measured using a spectrophotometer at 240 nm (17).

### Measurement of malondialdehyde

MDA Measurement was performed to determine serum lipid peroxidation. The method was done as previously described (18).

### Measurement of total thiol content

Total thiol content (mM) was assayed using the method of Ellman (19).

### Histology

For histology, after removing the organs, (kidneys, ovaries, liver) they were placed in 10% formalin solution. After 2-3 hours, the tissues were divided in half and placed in formalin for another 48-72 hours, then the tissues were washed with running water and distilled water and numbered. The samples were dehydrated using alcohol with ascending degrees of 70, 80, 90, 95, and 100%. The time of placing the samples in each of these concentrations

of alcohol was two hours. After dewatering, the samples were clarified with xylene, then molding was done using paraffin by TBS 88 machine; 5-micron sections were prepared by a Lietz 1512 microtome machine. Next, the slides were stained with hematoxylin-eosin (H&E). The prepared slides were checked to examine the tissue damage by considering necrosis, inflammation, number of cysts, size of the ovarian follicles, cell swelling, loss of tissue integrity, increased glomerular space in the kidney, and dilation of the veins in the liver. Then the samples were photographed using a Olympus BX51 microscope (20).

### Statistical analysis

Experimental data were analyzed by SPSS 22 software (SPSS, Inc., Chicago, IL, USA). The method of Kolmogorov and Smirnov was used for evaluation of data distribution, which was not normal. Therefore, a non-parametric Kruskal Wallis test was used for data analysis and was expressed as mean ± SEM. The significant level was considered as $P<0.05$.

## Results

### Effects on serum insulin level

The serum insulin levels of the PCOS animals were higher than those of the control animals, but the difference was not significant. One month treatment of animals with trans-anethole (80 mg/kg) or metformin significantly reduced the insulin levels compared to the PCOS animals (P=0.000 and P=0.03 respectively). The insulin levels in all treated groups were nearly the same as the control group (Table 1).

### Effects on oxidative stress parameters

#### Total thiol

Serum total thiol levels were significantly decreased in the PCOS and trans-anethole 40 groups compared to the control group (P=0.000 and P=0.015, respectively). PCOS animals treated group with trans-anethole 80 showed a significant increase in total thiol levels compared to the PCOS group (P=0.001). There was no significant difference between total thiol levels in PCOS animals treated with trans-anethole (20, 40 and 80 mg/kg) and metformin (Fig.1A).

### Malondialdehyde

Serum MDA levels were significantly increased in the PCOS group compared to the control group (P=0.000). MDA level in the trans-anethole 40 and 80 groups were significantly decreased in comparison to the PCOS group (P=0.023 and P=0.000, respectively). There was no significant difference between the MDA levels in all treated groups (Fig.1B).

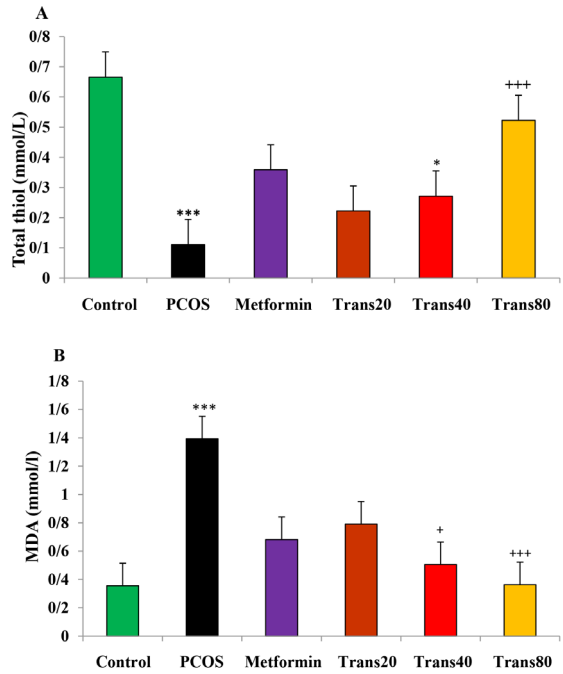


**Fig.1:** Effect of trans-anethole and metformin on serum levels of total thiol and malondialdehyde (MDA). **A.** Total thiol, and **B.** MDA levels in control, polycystic ovary syndrome (PCOS), PCOS+metformin (metformin) and PCOS+trans-anatole (trans20, 40, and 80) groups. Values are expressed as mean ± SEM. $^{*}$; $P<0.05$, $^{***}$; $P<0.001$, as compared to control group, $^{+}$; $P<0.05$, and $^{+++}$; $P<0.001$, compared to PCOS group (n=8).

### Activity of superoxide dismutase

SOD activity was significantly decreased in the PCOS, trans-anethole 20 and 40 groups compared to the control (P=0.000, P=0.003, and P=0.04, respectively). The SOD activity in PCOS animals receiving trans-anethole 80 was significantly increased in comparison to the PCOS group (P=0.000). In addition, SOD activity was significantly higher in the trans-anethole 80 treated group than in the trans-anethole 20 (P=0.017, Fig.2A).

### Activity of catalase

CAT activity was significantly decreased in the PCOS group compared to the control (P=0.000). In the group receiving trans-anethole 80 CAT activity was significantly increased in comparison to the PCOS group (P=0.007, Fig.2B).

**Table 1:** Serum insulin levels

| Groups | Control | PCOS | Metformin | Trans20 | Trans40 | Trans80 |
|---|---|---|---|---|---|---|
| Insulin (µU/mL) | 6.33 ± 1.50 | 24.67 ± 4.20 | 6.98 ± 2.6$^{+}$ | 10.35 ± 3.09 | 8.27 ± 2.09 | 2.96 ± 1.46$^{+++}$ |

**Values are expressed as mean ± SEM. Kruskal Wallis test was used for data analysis. $^{+}$; $P<0.05$, $^{+++}$; $P<0.001$, compared to PCOS group (n=8), and PCOS; Polycystic ovary syndrome, trans20, 40, 80, PCOS+trans-anethole (20, 40, 80 mg/kg).**

## Histological results

### Ovarian tissue examination

The results showed that in the control group, the morphology of the ovaries, follicle numbers, and the follicle cell layers of theca and granulosa cells were normal (Fig.3A). In the PCOS group the number of immature follicles increased, granulosa cell destruction (atresia) and cystic follicles were seen (Fig.3B). In PCOS treated group with trans-anethole 80 mg/kg, the number of follicles and cystic follicles compared to the PCOS group was decreased and had the greatest effect in comparison between the 3 treated groups (Fig.3C-F). In groups receiving trans-anethole 20 and 40 mg/kg, and metformin, the number of follicles was decreased, but irregularities in the theca and granulosa cells were seen (Fig. 3C, D, F).

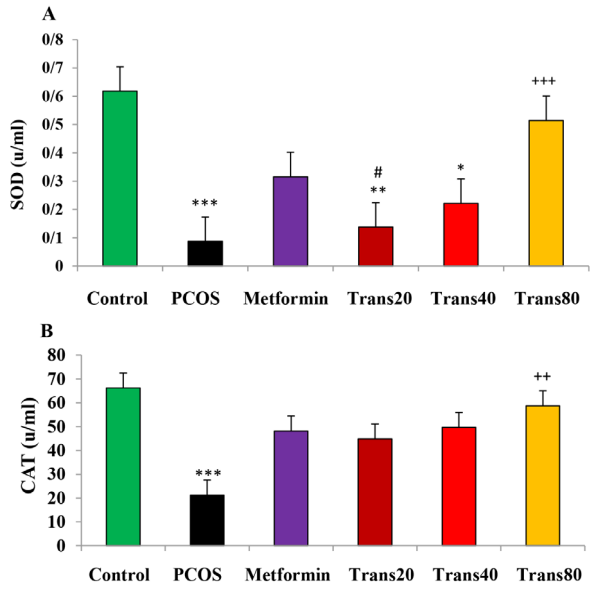


**Fig.2:** Effect of trans-anethole and metformin on serum activity of superoxide dismutase (SOD) and catalase (CAT). **A.** SOD and **B.** CAT activity in control, polycystic ovary syndrome (PCOS), PCOS+metformin (metformin) and PCOS+trans-anethole (trans20, 40, and 80) groups. Values are expressed as mean ± SEM. $^{*}$; P<0.05, $^{**}$; P<0.01, $^{***}$; P<0.001, compared to the control group, $^{++}$; P<0.01, $^{+++}$; P<0.001 as compared to PCOS group, and #; P<0.05, compared to trans-anethole 80 (n=8).

### Liver tissue examination

Histological evaluation of the liver showed that in the control group, veins and sinusoids were healthy with a regular structure (Fig.4A). In the PCOS group, tissue structure was irregular, veins were dilated, inflammatory cells infiltration, coalesced vacuoles, and lipid droplets were present, which indicate progress towards fatty liver (Fig.4B). In the PCOS treated with doses of 20 and 40 mg/kg trans-anethole inflammatory cells and dilated sinusoids were still visible (Fig.4C, D). In the PCOS group which received trans-anethole 80 mg/kg, inflammatory cells infiltration and the distance between the sinusoids were decreased, and in general, the structure of the liver became more regular and very close to the control group (Fig.4E). In the PCOS group treated with metformin, the sinusoids and veins became more regular, but inflammation and irregular structure were still present (Fig.4F).

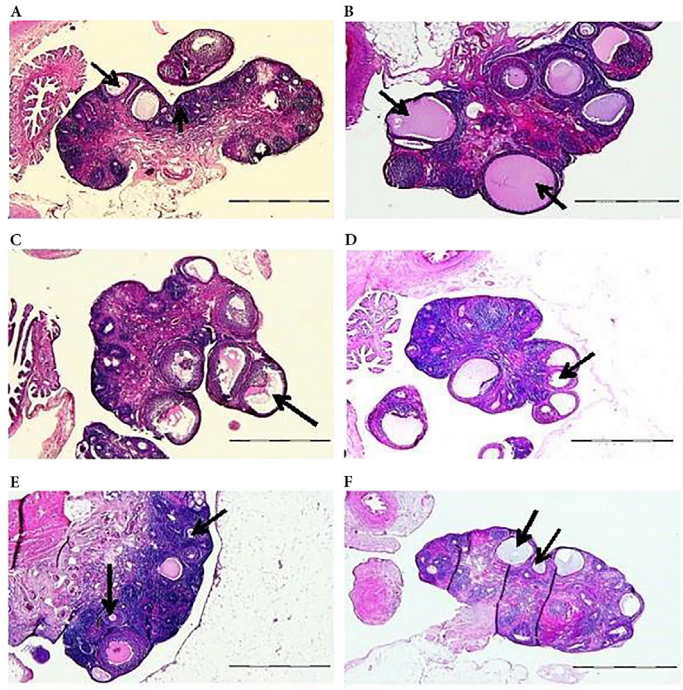


**Fig.3:** Photomicrograph of ovarian tissue section with a scale of 1000 µm and H&E staining. **A.** Light microscopic examination of a healthy control group showed the regular structure in parenteral and antral follicles. **B.** In PCOS group, increased number of follicles, cystic follicles, and irregular ovarian structures were observed. **C.** PCOS treated group with trans-anethole 20 mg/kg, **D.** PCOS treated group with trans-anethole 40 mg/kg. **E.** PCOS treated group with trans-anethole 80 mg/kg, demonstrates a decrease in the number of follicles, and **F.** PCOS treated group with metformin 300 mg/kg. Black arrow; Cystic follicles.

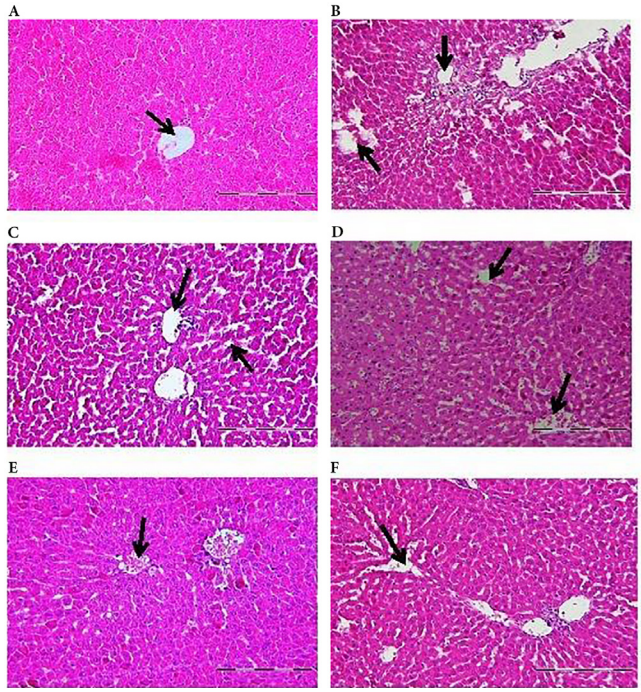


**Fig.4:** Photomicrograph of liver tissue section with the scale of 200 µm and H&E staining. **A.** Light microscopic evaluation of the control group, veins (arrowed) and sinusoids with a regular structure. **B.** In the PCOS group, areas of inflammatory cells infiltration, coalesced vacuoles, dilated vein (arrowed) and irregular structure were seen, **C.** PCOS treated group with trans-anethole 20 mg/kg, **D.** PCOS treated group with trans-anethole 40 mg/kg; **E.** In PCOS treated group with trans-anethole 80 mg/kg, less inflammatory-cell infiltration, coalesced vacuoles, dilated vein (arrowed) and more regular structures were seen, and **F.** PCOS treated group with metformin 300 mg/kg.

**Kidney tissue examination**

Histological examination of the kidneys showed that in the control group the tubules, glomeruli, and brush border were normal, the number of cells were normal and the distal and proximal tubes were separable (Fig.5A). In the PCOS group, glomeruli were damaged, the Bowman spaces were increased, inflammatory cells were seen, and the distal and proximal tubules were indistinguishable (Fig.5B). In all treated groups, the Bowman spaces were reduced, but inflammatory cells and tubular destruction were still observed. Among the treatment groups, the highest improvement was observed with a dose of 80 mg/kg trans-anethole, which reduced the damage (Fig.5C-F).

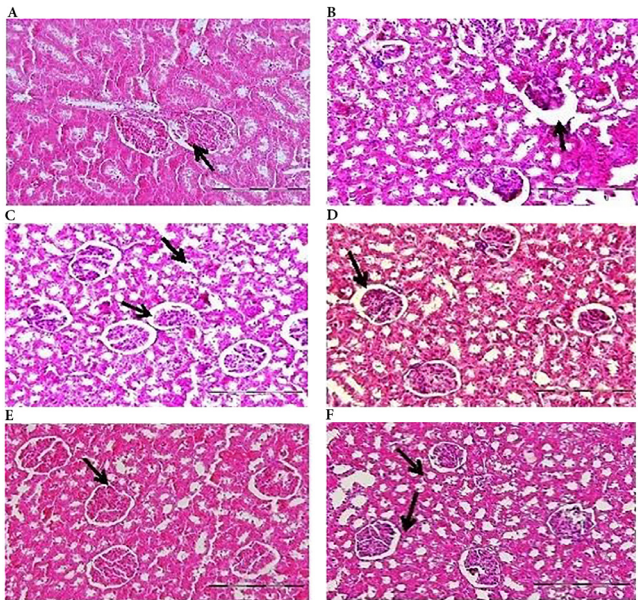


**Fig.5:** Photomicrograph of kidney tissue section with a scale of 200 µm and H&E staining. **A.** In light microscopic analysis of healthy controls, the number of cells were normal, and generally regular structure was seen. **B.** In the polycystic ovary syndrome (PCOS) group, tubules and glomeruli were damaged and irregular kidney structure was seen. **C.** PCOS treated group with trans-anethole 20 mg/kg, **D.** PCOS treated group with trans-anethole 40 mg/kg. **E.** PCOS treated group with trans-anethole 80 mg/kg which decreased injury rate, and **F.** PCOS treated group with metformin 300 mg/kg. Black arrow; Damaged glomeruli and irregular kidney structure.

## Discussion

Elevated testosterone and high insulin levels due to increased insulin resistance, hirsutism, ovarian cysts, irregular menstruation, and lack of ovulation are hallmarks of PCOS in patients (21). In this study, PCOS induction in animals resulted in a significant increase in insulin levels, and the polycystic feature of the ovaries as well as pathological changes in the liver and kidneys which demonstrated a metabolic disturbance similar to non-alcoholic fatty hepatic disease. A significant increase in insulin and testosterone levels in PCOS animals suggested an association between increased androgens and hyperinsulinemia. Insulin resistance plays a pivotal role in anovulation and metabolic disorders in PCOS disease. Insulin promotes PCOS metabolic distribution via mitogen-activated protein kinase and phosphoinositide 3-kinases (PI3K) signaling pathways. Insulin induces inhibition of PI3K in the follicular cells of polycystic ovaries and reduces 17α-hydroxylase, proposing that insulin might enhance steroid synthesis through the PI3K pathway, which enhances hyperandrogenism (22).

In addition, imbalanced serum oxidative stress markers in the PCOS group might support the hypothesis that there is an early association between insulin resistance and impaired oxidative metabolism. It has been indicated that oxidative stress induced by ROS overproduction might have a role in the development of hyperandrogenism and insulin resistance in PCOS patients (23). Elevation of the MDA level, and reduction of thiol content and activity of antioxidant enzymes (SOD and CAT) in PCOS rats had been reported previously and might explain some features of tissue damage and metabolic disturbance of PCOS patients. Abdominal adiposity and hyperlipidemia in PCOS patients might contribute to the development of local and systemic oxidative stress. In PCOS patients and animal models blood lipids and weight usually increase, which could induce a vicious cycle contributing to oxidative stress and insulin resistance and play an important role in PCOS pathogenesis. However, there is no effective treatment for these complications (24).

In recent decades the therapeutic effects of medicinal plants, among them *F. vulgare* has been shown in PCOS patients. *F. Vulgare* oil has antioxidant capacities and the effectiveness of the plant in many gynecological diseases including premenstrual syndrome, heavy menstrual bleeding, menopause, vaginal atrophy, amenorrhea, hirsutism, infertility, and PCOS have been demonstrated. The main therapeutic constituent of *F. Vulgare* is trans-anethole. Moreover, the therapeutic effects of *F. Vulgare* on the genitals and mammary glands have been attributed to the estrogenic properties of trans-anethole (25). In this study, treating PCOS rats with trans-anethole and metformin, returned the elevated serum insulin levels to normal; these results are in line with the study of Salehi et al. (26), who found that treatment with trans-anethole significantly reduced plasma insulin in PCOS rats. The metabolic effect of trans-anethole against insulin resistance might be related to its antihyperlipidemic, hepatoprotective, estrogenic and antioxidant properties.

In this study MDA decreased significantly after treatment with trans-anethole and metformin which indicates that both trans-anethole and metformin can suppress the oxidative stress induced in PCOS. In addition, SOD activity was significantly increased in animals

receiving trans-anethole 80mg/kg, which indicates that trans-anethole improves oxidative stress in PCOS rats in a dose dependent manner. All doses of trans-anethole resulted in a significant increase in CAT activity while the serum level of thiol was only increased in the group receiving trans-anethole 80 mg/kg. The antioxidant and protective effects of trans-anethole in PCOS patients have been attributed to estrogen and phytoestrogen compounds (26). Metformin might prevent oxidative stress induced damage in diabetic patients and have a potent antioxidant activity (27). As can be seen in this study, in general, trans-anethole increased antioxidant factors and decreased oxidative factors. The effects of trans-anethole on oxidative stress might be related to the IL-6 signaling pathway. The modulatory activity of trans-anethole on the IL-6 inflammatory pathways which is an important factor in the pathological changes and ovulation processes of PCOS patients might decrease androgens and improve ovulation processes (28). In the present study, the number of cystic follicles with destroyed granulosa cell layers was increased in the ovaries of PCOS rats, while trans-anethole, especially at a dose of 80 mg/kg, reduced the number of cystic follicles and improved those histological features. Our findings are in agreement with a study conducted by Yavangi et al. (28).

Ovarian histological changes in the PCOS group support other current and previous study results such as high serum testosterone and insulin levels, accumulation of glycogen and lipids in hepatocytes, and irregularly shaped and dilated veins in the liver, which are markers of progression to fatty liver. However, trans-anethole especially at a dose of 80 mg/kg decreased the number of inflammatory cells very close to the control group. In general, rats treated with trans-anethole showed significant restorative changes in the tissue structure of the liver. Based on these findings it may be suggested that trans-anethole which is a component of the essential oils of F. Vulgare, has protective effects on the liver (29). Insulin resistance, abdominal obesity and hyperlipidemia are the most typical endocrinopathies in both nonalcoholic fatty liver disease and PCOS patients.

In PCOS patients, hyperandrogenism resulted in hyperinsulinemia and insulin resistance, which in turn might induce steatohepatitis, abdominal adiposity and dyslipidemia (30). Moreover, metabolic disturbance, chronic low-grade inflammation, and cardiovascular events (hypertension) in PCOS patients have shown to be associated with renal injury markers such as decreased glomerular filtration rate and microalbuminuria and increase in kidney dysfunction (31). A definitive relationship between PCOS and kidney injury have been demonstrated previously. In PCOS patients, clinical analyses have showed that the urine albumin to creatinine ratio and urinary protein excretion levels is higher and is correlated to serum testosterone, which are reflective of injury in the kidney tubules (32). In kidney tissue, mesangial glomerular cells, primary tubules, and cortical collecting ducts contain androgen receptors, so the kidneys can be affected by androgens (33). Therefore, hyperandrogenism and its related metabolic consequences have been proposed as a main factor in inducing immature cystic follicles, oligoanovulation, and pathological injury in the kidney and liver (2, 33). In the present study, in the PCOS group, glomeruli were damaged and inflammatory cells were seen due to high serum testosterone levels. Trans-anethole had a positive effect on glomeruli and tubules and reduced the inflammatory features in the kidney of treated groups. These findings indicate the protective effect of trans-anethole against the destructive activity of high testosterone on kidney tissue. In line with our finding, a study by Sadrefozalayi et al. showed that *F. Vulgare* improves kidney structure and kidney function in PCOS female rats (25).

In PCOS patient metformin was showed to prevent metabolic and endocrine disturbances which are the contributors to liver and kidney injury by decreasing serum androgen levels, oxidative stress and low grade inflammation (13) and also having insulin-sensitizing and hypolipidemic effects (34). In the present study, although treatment of PCOS rats with metformin decreased the insulin level and the number of cystic follicles in the ovary, and improved kidney and liver histopathology, there was not significant improvement in oxidative stress markers. However, a between groups comparison indicated that, both pathological changes in the ovaries, liver and kidneys, and the levels of serum insulin and oxidative stress markers were significantly improved in the trans-anethole 80 group. These findings showed the higher effectiveness of trans-anethole 80 in comparison to metformin. In this study and the previous one, trans-anethole showed ameliorating effects against PCOS induced hormonal and metabolic disturbance by reducing insulin resistance, hyperlipidemia, and excess androgen and ROS production (12). Therefore, it might be safer than hormone replacement therapy such as estrogen-progestin contraceptives, which is prescribed in combination with metformin, and spironolactone for PCOS patients (35). Although the safety of trans-anethole at a dose of 80 mg/kg was confirmed in this and previous studies (12, 15), more studies are needed to determine the potency of the estrogenic effects of this compound.

Novel insights: Tarns-anethole (especially at a dose of 80 mg/kg) improved metabolic status, oxidative stress, liver and kidney damage as well as the cystic mass of ovarian tissue.

Established facts: Elevated testosterone and high insulin levels due to increased insulin resistance,

hirsutism, ovarian cysts, irregular menstruation, and lack of ovulation are present in PCOS patients.

## Conclusion

These results indicated that trans-anethole especially in higher doses (80 mg/kg) has a therapeutic effect on PCOS induced histological changes and metabolic complications. More clinical studies are necessary to uncover the beneficial effects of trans-anethole in PCOS patients, and further experimental findings are needed to reveal the mechanism of its hepato and renal protective activity.

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## Authors' Contributions

F.M.N.; Participated in study design, data collection, evaluation, drafting, and statistical analysis. M-A-R.H.; Contributed to conception of study design, manuscript drafting and was responsible for overall supervision. Z.G.; Contributed extensively in interpretation of the data and the conclusion, and revising the manuscript. F.S.; Drafted the manuscript and contributed to statistical analysis. Z.S.N.; Participated in study design, data collection and evaluation, and drafting. All authors read and approved the final manuscript.

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