Original Article

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Effect of Aqueous Extract of Artemisia absinthium L. on Sex Hormones, Inflammatory Cytokines and Oxidative Stress Indices of Ovarian Tissue in Polycystic Ovary Syndrome Rat Model

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ABSTRACT

BACKGROUND AND OBJECTIVE: Hormonal disorders along with oxidative stress and inflammation in ovarian tissue lead to anovulation in polycystic ovary syndrome (PCOS) patients. Considering antioxidant effect of Artemisia absinthium, purpose of this study is to determine the effect of aqueous extract of Artemisia absinthium on serum levels of sex hormones, inflammatory cytokines and oxidative stress indices of ovarian tissue in polycystic ovary syndrome rat model.

METHODS: In this study, 32 female Wistar rats were divided into 4 equal groups: groups of control, PCOS control and two groups with PCOS which are under treatment through aqueous extract of Artemisia absinthium (100 and 200 mg/kg). Polycystic ovarian syndrome was induced by single intramuscular injection of estradiol valerate (4mg/kg). Aqueous extract of Artemisia absinthium was intraperitoneal injection to PCOS treated groups, for 24 days. At the end of treatment period, serum levels of LH, FSH, Estradiol, Testosterone, TNF-α, IL-1β and IL-6 and levels of SOD, CAT, GPX enzymes and level of MDA in ovarian tissue were measured through ELISA method.

FINDINGS: Compared to PCOS control group (LH: 14.30±2.52, FSH: 2.35±0.28, Estradiol: 17.61±2.44, Testosterone: 10.29±1.56, TNF-α: 178.65±4.35, IL-1β: 121.52±5.17, IL-6: 162.28±5.83, SOD: 20.51±1.84, CAT: 64.42±3.70, GPX: 35.15±2.88, MDA: 87.32±3.40), MDA tissue level (55.46±4.73), serum levels of LH (8.26±1.36), Estradiol (7.76±1.55), Testosterone (6.40±1.04) and TNF-α (115.35±5.83), IL-1β (70.25±5.74) and IL-6 (89.15±4.52) cytokines in group under treatment with 200 mg/kg aqueous extract of Artemisia absinthium significantly decreased (p=0.008) and serum level of FSH (7.52±1.21) and levels of SOD (71.58±5.19), CAT (128.30±5.11) and GPX (88.21±5.51) antioxidant enzymes of ovarian tissue significantly increased (p=0.005).

CONCLUSION: Aqueous extract of Artemisia absinthium by decreasing the serum levels of inflammatory cytokines and increasing the activity of the ovary tissue antioxidant enzymes has a favorable effect on the improvement of hormonal parameters in rats with polycystic ovary syndrome.

KEY WORDS: *Polycystic Ovarian Syndrome, Artemisia absinthium, Oxidative stress, Cytokines, Rats*.

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Introduction

Polycystic Ovary Syndrome (PCOS) is a common disease in women of reproductive age (1). Research has shown that this disease disrupts the pituitary-gonadal axis and disrupts ovarian function (2). Its main characteristic is to reduce puberty of follicles, reduce ovulation and increase the level of androgens in the circulation, which ultimately causes infertility (3). Studies have shown that cytokines such as Tumor Necrosis Factor-α (TNF-α), Interleukin-1 beta (IL-1β) and Interleukin-6 (IL-6) indicates systemic and localized inflammation in the body.

Also there is evidence that shows a direct and close relationship between systemic and local inflammation with the polycystic ovary (4). Research has shown that the imbalance between the production of free radicals and the antioxidant defense system can damage cellular components such as proteins, lipids, nucleic acids, and decrease the activity of intracellular antioxidants enzymes (5).

Oxidative stress is also effective in increasing the production of androgens, disrupting the developmental stages of ovarian follicles and ovarian tissue damage in patients with polycystic ovary. On the other hand, abnormal increase in lipid peroxidation causes damage to cell membranes and cellular organelles (6). Several therapeutic methods have been proposed to treat this syndrome. At present, the most important treatment is the use of drugs such as clomiphene citrate, metformin and gonadotropins (7).

Due to the side effects of these drugs, it is important to identify and provide alternative and effective drugs. Due to the fact that plants are considered as important sources of antioxidants, studies in this field are increasing. Artemisia absinthium belongs to Magnoliophyta, Magnoliopsida, Asterales and Asteraceae (8).

Recent research has shown that the compounds in the Artemisia plant have antioxidant properties and inhibitory effects of free radicals (9). Also, high levels of phenolic compounds and flavonoids present in this plant have a significant antioxidant effect. One of the antioxidant effects of this plant is the protection of cells against oxidative damage.

In addition, research has shown that the methanolic extract of Artemisia causes a balance in the levels of superoxide dismutase and glutathione peroxidase and, by increasing the activity of antioxidant enzymes and inhibition of oxygen reactive species, reduces systemic inflammation (9, 10). It has also been shown that Artemisia plant compounds have anti-inflammatory activity and can reduce the levels of pre-inflammatory cytokines such as IL-6, TNF-α and IL-1-β (11).

Considering that till now no report on the effect of Artemisia plant on the complications of polycystic ovaries has been presented, the aim of this study was to determine the effect of aqueous extract of Artemisia on serum levels of gonadotropins, estradiol, testosterone, inflammatory cytokines and oxidative stress indices of ovarian tissue in rats with polycystic ovarian syndrome..

Methods

In this experimental study, 32 adult female Wistar rats weighing 170±8 and 95±5 days old from the center of propagation and maintenance of experimental animals of Payame Noor University of Mashhad were purchased. Animals were kept at ambient temperature of 24±3 °C, relative humidity of 35±4% and 12 hours darkness period.

This research was conducted at the Animal Research Laboratory of Payame Noor University of Mashhad after approval by the Ethics Committee of Neyshabour University of Medical Sciences with code 47.1395IR.NUMS.REC. Artemisia blueberry extract was prepared using a Soxhlet device (Electrothermal, UK) (12). Before the start of the study, LD50 and ED50 of the aqueous extract of Artemisia leaf were examined and the concentration of 1000 mg / kg for LD50 and range of 50-300 mg/kg for ED50 was determined. Rats were randomly divided into 4 groups of 8.

Group 1: Control rats received 0.5 ml of saline solution for 24 days and administered intraperitoneally as a solvent.

Group 2: Rats in control group with polycystic ovary received 0.5 ml saline solution for 24 days and intraperitoneally as solvent.

Group 3: Rats of the treated polycystic ovaries group received 0.5 ml of aqueous extract of Artemisia at a concentration of 100 mg/kg for 24 days and intraperitoneally.

Group 4: Rats of the treated polycystic ovaries group received 0.5 ml of aqueous extract of Artemisia at a concentration of 200 mg/kg for 24 days and intraperitoneally.

To conduct this study, mice were first selected from 2 to 3 regular estrous cycleduring 12-14 days of vaginal smear. Rats that were in the estrous cycle were

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selected for later stages of the study. The vaginal smear in the estrus stage has more horn cells than the epithelial cells and does not have leukocyte (13). Polycystic ovaries were induced by an intramuscular injection of estradiol Valerate (Pharmacy of Abu Reihan, Iran) at a dose of 4 mg/kg.

The time required to induce polycystic ovary was about 60 days after the injection of estradiol Valerate. Vaginal smear and inducing polycystic ovary tissue studies were confirmed (13). At the end of the treatment period, the rats were anesthetized by diethyl ether. Then, the skin of the chest, sternum and ribs was cut and bypassing the biceps and ribs from the left ventricle of the heart, blood samples were taken by a 2 milliliter syringe (13).

Serum levels of LH, FSH, estradiol, testosterone, tumor necrosis factor-α, interleukin-1 beta, and interleukin-6 were measured by ELISA, ICA 2100 (Stat Fax, USA), and Finetest kits, (Finetest, China). The ovaries were removed from the body of rats. After washing with saline solution-Tris buffer (Sigma-Aldrich, Germany), it was homogenized at 5,000 rpm for 5 minutes with Homogenizer T25 digital ULTRA-TURRAX (IKA, Germany).

The homogenized solution was centrifuged by a refrigerated centrifuge machine of the Z366 model (Hermle, Germany). In order to prevent the degradation of enzymes and proteins, all steps were carried out at 4°C (refrigerated centrifuges) and treated with 0.5 mM Phenylmethylsulfonyl fluoride (Sigma-Aldrich, Germany) as protease inhibitor (14). After centrifugation, a clear overlay solution was deposited from the underside, separated and used for measuring. The level of antioxidant enzymes of superoxide dismutase, catalase, glutathione peroxidase, and MDA in ovarian tissue were measured by ELISA and Fine Test kits. The obtained data was analyzed by SPSS-20 software using kolmogorov smirnov test, one-way ANOVA and Tukey's post hoc test. P<0.05 was considered significant.

Results

Based on the results, serum levels of LH, estradiol and testosterone in the control group with polycystic ovary syndromesignificantly increased and the serum FSH level decreased significantly (p<0.05). Intraperitoneal administration of aqueous extract Artemisia at 200 mg/kg to rats with polycystic ovary, in comparison to control group with polycystic ovary, caused a significant decrease in serum levels of LH hormones, Estradiol and testosterone and also a significant increase in serum FSH levels (p<0.05) (table 1).

Serum levels of tumor necrosis factor alpha, interleukin-1 beta, and interleukin-6 in the control group with polycystic ovaries were significantly increased (p<0.05). Compared to the control group with polycystic ovary, serum level of tumor necrosis factor alpha, interleukin-1 beta, and interleukin-6 in the polycystic ovary group treated with 200 mg / kg aqueous extract of Artemisia were significantly reduced (p<0.05) (table 2). The activity of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase in the ovary tissue in the control group with the polycystic ovary significantly decreased and the level of malondialdehyde significantly increased (p<0.05).

Compared with the control group with polycystic ovary, the activity of antioxidant enzymes of superoxide dismutase, catalase, glutathione peroxidase in ovarian tissue in a group treated with a 200 mg/kg aqueous extract of Artemisia significantly were increased and malondialdehyde significantly decreased (p<0.05)(table 3).

Table1. Comparison of serum levels of LH, FSH, estradiol and testosterone hormones by studied groups

Variable / Group	LH(mIU/ml) Mean±SD	FSH(mIU/ml) Mean±SD	Estradiol(ng/ml) Mean±SD	Testosterone (ng/ml) Mean±SD
Control	6.35±0.43 [a]	10.08±1.47 [a]	5.15±0.84 [a]	4.64±0.47 [a]
control PCOS	14.3±2.52 [b]	2.35±0.28 [b]	17.61±2.44 [b]	10.29±1.56 [b]
PCOS treated with 100 mg/kg Artemisia extract	12.88±1.85 [b]	3.18±0.53 [b]	15.83±2.74 [b]	9.87±2014 [b]
PCOS treated with 200 mg/kg Artemisia extract	8.26±1.36 [a]	7.52±1.21 [a]	7.76±1.55 [a]	6.4±1.04 [a]
Significance level (one-way analysis of variance)	0.014	0.008	0.011	0.019

The non-identical letters in one column show a significant difference and the same letters indicate no significant difference (p<0.05)

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Table2. Comparison of mean serum level of inflammatory cytokines by group

Variable / Group	TNF-α(pg/ml) Mean±SD	IL-1β(pg/ml) Mean±SD	IL-6(pg/ml) Mean±SD
Control	85.4±5.23 [a]	48.12±3.23 [a]	71.42±6.08 [a]
PCOS control	178.65±4.35 [b]	121.52±5.17 [b]	162.28±5.83 [b]
PCOS treated with 100 mg/kg Artemisia extract	169.14±6.52 [b]	104.07±6.62 [b]	157.42±8.7 [b]
PCOS treated with 200 mg/kg Artemisia extract	115.35±5.83 [a]	70.25±5.74 [a]	89.15±4.52 [a]
Significance level (one-way analysis of variance)	0.016	0.007	0.011

The non-identical letters in one column show a significant difference and the same letters indicate no significant difference ($p<0.05$)

Table3. The average activity of antioxidant enzymes and MDA of ovarian tissue by group

Variable / Group	SOD(pg/ml) Mean±SD	CAT(mIU/ml) Mean±SD	GPX(pg/ml) Mean±SD	MDA(ng/ml) Mean±SD
Control	80.14±3.71 [a]	145.26±7.56 [a]	107.14±8.46 [a]	40.28±5.11 [a]
PCOS control	20.51±1.84 [b]	64.42±3.7 [b]	35.15±2.88 [b]	87.32±3.4 [b]
PCOS treated with 100 mg/kg Artemisia extract	26.7±2.14 [b]	75.47±5.06 [b]	42.75±4.25 [b]	81.5±6.02 [b]
PCOS treated with 200 mg/kg Artemisia extract	71.58±5.19 [a]	128.3±5.11 [a]	88.21±5.51 [a]	55.46±4.73 [a]
Group/Parameter	0.001	0.006	0.002	0.021

The non-identical letters in one column show a significant difference and the same letters indicate no significant difference ($p<0.05$)

Discussion

In this study, the polycystic ovary in rats was induced at a dose of 4 mg / kg for about 60 days after a single intra-muscular injection of estradiol valerea. Alizadeh et al. used the same method to induce the phenotype of polycystic ovary syndrome using hormonal induction by intramuscular injection of estradiol valerate (15). In this study, serum levels of LH, estradiol and testosterone in the control group with PCOS significantly increased and the serum level of FSH decreased significantly. Polycystic ovary syndrome has been shown to increase the serum levels of testosterone and beta-estradiol as a result of endocrine disorders, and this causes ovulation and infertility disorders (16).

Nabiuni and colleagues reported in their study that in rats with PCOS, serologic changes are as reduced FSH, progesterone and as increased LH, estradiol, and testosterone (17). Another study showed that in rats with polycyclic ovarian syndrome, changes in sex hormones were significantly as increased LH, estradiol and testosterone (15). Based on the results of this study, the serum levels of tumor necrosis factor-α, interleukin-1 β, and interleukin-6 in control rats with polycystic ovary increased significantly compared to the control group.

In line with the results, a study was conducted to evaluate the cytokines of the tumor necrosis factor-α, interleukin-1α, and interleukin-1 beta in the serum of patients with polycystic ovary, and revealed that serum levels of IL-1α and β in patients with polycystic ovary increased, but the tumor necrosis factor-α did not change significantly. Polycysticization of the ovaries may also be a chronic inflammatory disease, since inflammatory cytokines, especially interleukins, are greatly increased in these patients (18). In a study, Yaghmaei et al. investigated the rate of inflammation in patients with polycystic ovaries and revealed that the serum levels of inflammatory cytokines, serum level of C-reactive protein, and red blood cell sedimentation rate as diagnostic indices for inflammation in women with polycystic ovaries increases (19). In this study, the activity of antioxidant enzymes in ovarian tissue in rats with polycystic ovary decreased and lipid peroxidation increased. It has been reported that the increase in the stress-oxidative stress in patients with polycystic ovary syndrome is directly related to hypertension, diabetes, and reduced ovulation (20).

Fenkci et al., according to the results of this study, have shown that in the ovarian tissue of the polycystic ovarian syndrome, lipid peroxidation is increased and the activity of antioxidant enzymes decreases (21). The results of this study showed that intraperitoneal administration of aqueous extract of Artemisia at concentration of 200 mg/kg for 24 days to rats with polycystic ovaries improved hormonal disorders, decreased serum levels of inflammatory cytokines,

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increased Antioxidant enzyme activity and decreases lipid peroxidation of ovarian tissue.

Wan et al. reported that the compounds in the Artemisia plant have an effect on internal antioxidant synthesis systems that can be effective in the production and protection of intracellular antioxidants, and ultimately lead to the removal of free radicals. It has also been shown that polyphenolic and flavonoid compounds of Artemisia extract can protect cells against oxidative damage by decreasing lipid peroxidation and increasing the activity of antioxidant enzymes such as glutathione reductase, glutathione peroxidase and catalase (22).

A research was conducted to determine the protective capacity of Artemisia plant as a strong antioxidant against free radical damage and it was found that the extract of Artemisia inhibited the lipid peroxidation and hemolysis of red blood cells significantly. It can also reduce the activity of hydroxyl radicals, nitric oxide and hydrogen peroxide and increase the activity of intracellular antioxidant enzymes (23). In another study, Aggarwal et al. reported that extract of Artemisia can reduce systemic inflammation in diabetic people by reducing the synthesis of pre-inflammatory cytokines (interleukin-6). It also increases the secretion of insulin from pancreatic beta cells by reducing inflammation in the pancreatic tissue (24). Research has shown that Artemisia extract due to the presence of phytosterols has an inhibitory effect on the activity of 5-alpha-reductase enzyme. Reducing this enzyme decreases the plasma concentration of the dihydrotestosterone hormone (25). In addition, phytosterols reduce the sensitivity of tissues to androgens and reduce the activity of androgens, including testosterone, by inhibition of aromatase and 5-alpha-reductase enzymes. It has also been shown that phytosterols reduce the synthesis of testosterone by reducing cholesterol (25). According to the results obtained, it can be concluded that aqueous extract of Artemisia plant with increased activity of ovary antioxidant enzymes and reduction of serum level of inflammatory cytokines can lead to significant changes in serum level of pituitary-axis hormones in rats with Polycystic ovarian syndrome. Also, due to the approximate return of serum levels of LH, estradiol, and testosterone to the natural threshold, it is possible that the process of ovulation and normal growth of the follicles can be restored, which can be confirmed by an increase in the serum level of FSH. Therefore, the compounds present in Artemisia as a natural product can be used to reduce the symptoms of polycystic ovary syndrome.

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