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Investigating the Role of Hydroalcoholic Extract of *Apium graveolens* and *Cinnamon zeylanicum* on Metabolically Change and Ovarian Oxidative Injury in a Rat Model of Polycystic Ovary Syndrome

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Abstract

Objectives: The present study aimed to compare the role of the hydroalcoholic extract of *Apium graveolens* and *Cinnamon zeylanicum* on metabolically change and ovarian oxidative injury in a rat model of polycystic ovary syndrome (PCOS).

Materials and Methods: In this experimental research, 64 female Wistar rats with breeding were used including the following groups (G): (I) G1: healthy control; (II) G2: PCOS which received no therapy; (III) G3: PCOS + *A. graveolens*; (IV) G4: *A. graveolens*; (V) G5: PCOS + *C. zeylanicum*; (VI) G6: no PCOS + *C. zeylanicum*; (VII) G7: PCOS + *C. zeylanicum* and *A. graveolens*; and (VIII) G8: *C. zeylanicum* and A. *graveolens*. The PCOS was induced by a single dose of the intramuscularly injected estradiol valerate (16 mg/kg). After 14 days, the animals were anesthetized, then their plasma samples were used to check the blood sugar (BS), insulin, and lipid profile. The ovaries of the rats were removed and fixed for histopathological assessment. In addition, the oxidative stress marker in ovarian tissue was evaluated.

Results: The levels of BS, insulin, and lipid profile in plasma significantly enhanced in G2 ($P < 0.05$) while decreasing significantly in the therapy groups, ($P < 0.05$). Moreover, a significant decline was observed in the serum level of high-density lipoprotein (HDL) in G2 ($P < 0.05$) while it enhanced significantly in the therapeutic animals ($P < 0.05$). Furthermore, a negative change was found in the PCOS group on the ovarian tissue. Besides the oxidative stress enhanced in this tissue while in the treated groups this change was improved.

Conclusions: Generally, it was revealed that the extract of *A. graveolens* and *C. zeylanicum* had a useful impact on regulating the serum levels of fast blood sugar (FBS), insulin, lipid profile, and oxidative stress markers in the palliation of the PCOS complications.

Keywords: *Apium graveolens, Cinnamon zeylanicum*, PCOS, Lipid profile

Introduction

Polycystic ovary syndrome (PCOS) is known as a publicized derangement of the endocrine system in women who can be fertilized (1, 2). Other complications of this syndrome in long-term consist of type 2 diabetes, insulin resistance syndrome, hypertension, and cardiovascular diseases. In addition, the risk of hyperplasia and cancer of the endometrium is higher in women with PCOS who do not receive any treatment (3). In the PCOS patients and hyperandrogenism, there is an extensive attachment between the hyperinsulinemia and hyperandrogenism as the level of insulin shows an intense correlation with the exclusion of the steroid from the adrenal (4). Besides, in these patients, hyperinsulinemia is accompanied by elevated levels of low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), triglyceride, total cholesterol (5), and a decline in the serum level of high-density lipoprotein (HDL) (6). In addition to resistance to insulin, the lipid metabolism of the females with PCOS is probably transformed by ovarian or adrenal secretion of the sex steroid hormones. The effect of sex steroids on fat metabolism that involved the actions of estrogens and androgens is complicated in this scenario (7, 8).

Using medicinal plants to treat female sexual disorders in Iran has a broad background and studies have shown that the use of these plants has a positive effect on treating metabolic diseases including diabetes and PCOS (9, 10).

Apium graveolens includes a lot of antioxidant

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compounds such as vitamin C, which reduce the edema of the body cells, and also eliminate and act against rheumatism and cancerous diseases (11). The *A. graveolens* extract has the potential to interdict the activity of free radicals and stopper of lipid peroxidation. Accordingly, this plant has antioxidant and anti-diabetes confidants. Additionally, previous studies showed that the extract of this herb is anti-inflammatory and analgesic and can decrease cholesterol and triglyceride (12,13).

The skin of *Cinnamon zeylanicum* includes 0.5%-2.5% essential oil. Besides, it comprises more than fifty various combinations of which 65%-80% is aldehyde cinematic. The major components of cinnamon are aldehyde (65%-80%) and eugenol (10%) which have high antioxidant activities and antibacterial properties (14).

As a result, given that the effects of these herbs have not been studied on PCOS, the present research sought to test the impact of *C. zeylanicum* and *A. graveolens* extracts on the ovarian oxidative damage and metabolically change in rats altered due to the PCOS.

Materials and Methods

In this research, 64 female Wistar rats weighing 200-250 g were used. All the animals were purchased from Razi Institute of Mashhad and were intently kept in the animal laboratory under standard conditions (Temperature of 22°C & 12/12 hours of the light-dark cycle) and had access to food and water.

Study Design and Groups

All the rats were divided into 8 groups as follows:

1. Healthy control group: daily oral intake of physiological serum
2. PCOS group: received no treatment
3. PCOS group that received therapy using the *A. graveolens* (200 mg/kg) extract for 14 days, orally (PAG)
4. Sham group: including 8 rats that were orally treated with the *A. graveolens* (200 mg/kg) extract for 14 days, without PCOS induction (AG)
5. PCOS group that received therapy with *C. zeylanicum* (200 mg/kg) extract for 14 days, orally. (PCA)
6. Sham group: including 8 rats that were treated with the C. *zeylanicum* (200 mg/kg) extract for14 days without PCOS induction (CA)
7. PCOS group that received therapy with the *C. zeylanicum* (200 mg/kg) and *A. graveolens* (200 mg/kg) extracts for 14 days (PCO+ C +A)
8. No PCOS + *C. zeylanicum* and *A. graveolens* (C+A).

The PCOS was induced by estradiol valerate with a single dose (16 mg/kg) which was intramuscularly injected (Sigma, Germany) and solved in 200 lambdas of sesame oil (7). The smear of the vagina was daily examined and confirmed the estrus cycle in PCOS rats. After the therapy, all the animals were anesthetized using ketamine and xylazine (5/1 mg/kg) (15). Then, the blood samples were derived from the inferior vena cava and the serum was separated by centrifuge at 3000 g. The taken serum was kept at -70°C for further assessment.

Preparation of *Apium graveolens* and *Cinnamon zeylanicum* Hydroalcoholic Extracts

To provide the *A. graveolens* and *C. zeylanicum* extracts, 0.5 kg of each plant was first powdered, then solved in one liter of ethanol 50% and kept on a shaker (Thermo Fisher) at the room temperature for 48 hours. Next, the dilution was filtered and centrifuged at 3000 rpm for 10 minutes. Afterward, the solution was infused into a dish to vaporize the solvent. And finally, to attain a suitable concentration, the obtained extract was solved in sterile physiological serum.

Assessing Plasma Glucose, HDL, LDL, and Cholesterol Levels

At the end of the study, serum glucose, HDL, LDL, triglyceride (TG), and cholesterol levels were defined by mercantile kits (Parsazmun, Iran). The quantity was expressed as mg/dL.

Assay of Serum Insulin Level

Insulin levels in plasma were measured by a method based on enzyme-linked immunosorbent assay (ELISA) using the kit of Rat Insulin (Mercodia).

Assessing Oxidative Stress Markers in the Ovarian Tissue

To evaluate the markers of oxidative stress in the tissue, the ovarian tissues were homogenized. Malondialdehyde (MDA) level was assessed to investigate the lipid peroxidation in the ovary as well. In order to provide a solution of TBA-TCA-HCL, 375 mg of thiobarbituric acid (TBA) was solved in 2000 µL of HCL (hydrochloric acid). Next, it was added to 100 mL of the trichloroacetic acid (TCA) 15%. To complete the resolution of the sediment, water bath with a temperature of 50°C was used. Then, the ovarian tissue was weighed and immediately homogenized with potassium chloride 5.1% to create 10% homogenized admixture. Subsequently, 1000 lambdas of homogenized tissue mixture was dissolved in two ccs of TBA-TCA-HCl solution, afterward, it was put in the boiling water for 45 minutes to heat in order to obtain a pink-orange solution. After cooling the solution, it was centrifuged at 1000 rpm for 10 minutes. The absorption was read by a spectrophotometer. The superoxide dismutase (SOD) and glutathione peroxidase (GPX) levels were recognized in the ovarian tissue by an ELISA reader device (Antus) conforming the protocols of the kits (Randox, & Ransod, UK) (16).

Histological Assessment

After euthanasia, the ovary tissues were accumulated

from each rat. Then, the tissues were fixed in the 10% neutral-buffered formalin followed by embedding in paraffin. They were then divided into 5 µm sectioned, deparaffinized, stained by hematoxylin & eosin staining (H&E), and dehydrated in ethanol 95%, 90%, and 70%. Afterward, they were cleared by the xylene. The sections were observed by a microscope (Olympus).

Statistical Analysis

All the data were analyzed by the SPSS software, version 19 (USA). Moreover, the data were presented as mean ± SEM (standard error of the mean) and were compared using one-way ANOVA and Tukey post hoc test. The level of $P < 0.05$ was considered significant.

Results

Plasma FBS and Insulin Level

Based on Figure 1, the plasma level of FBS in the PCOS group was significantly higher than that of the control ($P < 0.001$). Besides, the plasma glucose level in the treated groups that received *A. graveolens* and *C. zeylanicum* extracts was lower than that of the PCO group ($P < 0.05$). Figure 2 shows that the plasma level of insulin was statistically higher in the PCOS group compared to that of the control group ($P < 0.05$). Alternatively, a significant decline was found the insulin level of plasma in groups that were treated with *A. graveolens* and *C. zeylanicum* extracts as compared to that of the PCOS group ($P < 0.05$).

Lipid Profile

Plasma Cholesterol Level

The cholesterol level in plasma significantly enhanced in the PCOS group when compared with that of the control ($P < 0.05$). However, the plasma cholesterol level declined in all those groups that were treated with *A. graveolens* and *C. zeylanicum* extracts as compared with that of the control group ($P < 0.05$) (Table 1)

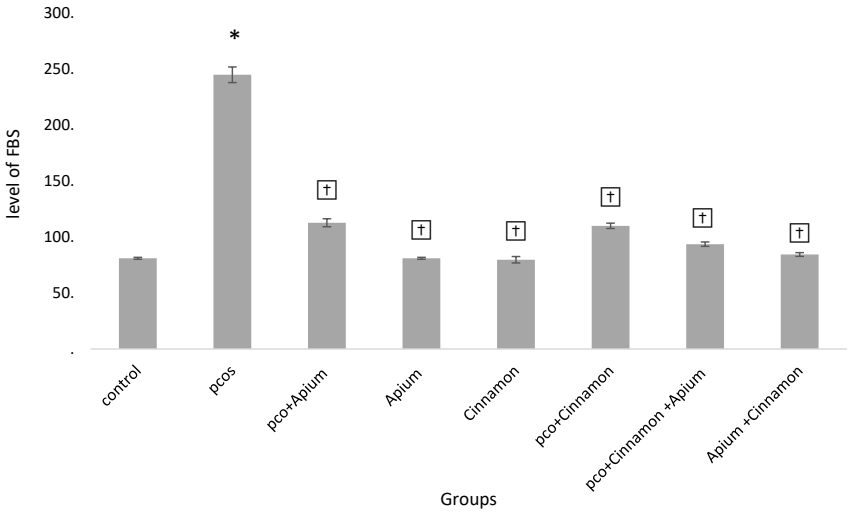

Figure 1. Serum Level of FBS. The asterisk * shows significant difference with control group and the symbol of † means the significant difference with PCO group.

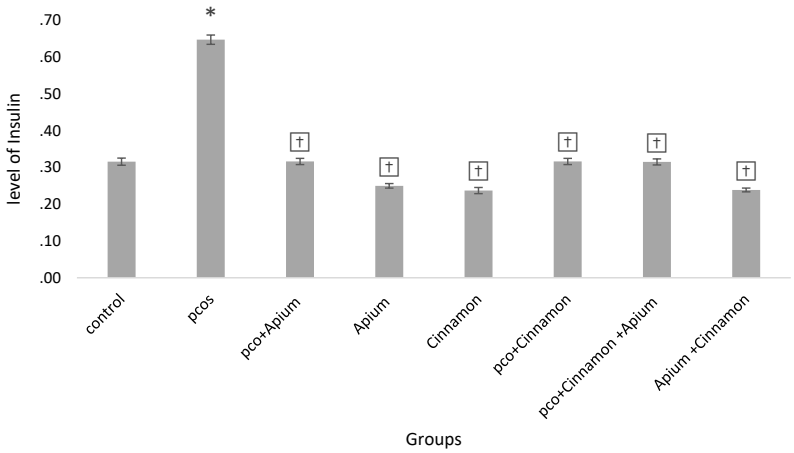

Figure 2. Serum Level of Insulin. The asterisk * shows significant difference with control group and the symbol of † means the significant difference with PCO group.

Table 1. Comparing the Serum Level of Cholesterol, HDL, LDL, and TG in the Study Groups

Group	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	TG (mg/dL)
Control	68.050±2.32	45.694±1.59	11.144±1.51	35.215±1.58
PCOS	80.989±1.04[b]	28.922±0.53[b]	23.228±3.68	60.372±4.58[b]
PGA	70.5726±3.37[a]	40.213±1.15[a]	15.912±2.03[a]	40.780±4.13[a]
AG	69.526±3.37[a]	42.213±1.15[a]	10.052±2.03[a]	36.789±4.13[a]
PCZ	72.726±3.37[a]	39.213±1.15[a]	16.952±2.03[a]	38.895±4.13[a]
CZ	67.050±2.32	43.894±1.59	10.174±1.51	35.565±1.58
PCO+C+ A	66.576±3.37[a]	42.413±1.15[a]	14.152±2.03[a]	42.785±4.13[a]
C + A	67.150±2.32	46.894±1.59	11.474±1.51	37.505±1.58

Note. Control: Control group; PCOS: PCO (polycystic ovary syndrome) group that received normal saline by oral gavage; PGA: PCOS group that were treated with hydroalcoholic extract of *Apium* (A.) *graveolens* (200 mg/kg); AG: Healthy group which received the hydroalcoholic extract of *A. graveolens* (200 mg/kg); PCA: PCOS group that received therapy with *C. zeylanicum* extract (200 mg/kg/orally); CA: Healthy group which received the hydroalcoholic extract of *C. zeylanicum* (200 mg/kg); PCO+C+A: PCOS group that received therapy with *C. zeylanicum* (200 mg/kg) and *A. graveolens* (200 mg/kg) extracts; C+A: No PCOS + *C. zeylanicum* and *A. graveolens.*

[a] A substantial difference with the control group; [b] The notable difference with PCOS group.

Serum Level of Low-Density Lipoprotein

The LDL plasma level also increased in the PCOS group compared to that of the control group ($P > 0.05$). In addition, a significant decline was observed in the plasma LDL level among the therapy groups that received *A. graveolens* and *C. zeylanicum* extracts compared to that of the PCOS group ($P < 0.05$) (Table 1).

Plasma Level of High-Density Lipoprotein

The level of HDL in plasma significantly decreased in the PCOS group when compared with that of the control group ($P < 0.05$). However, in the therapy groups, it enhanced significantly compared to that of the PCOS group ($P < 0.05$) (Table 1)

Plasma Level of Triglycerides

The TG level in plasma a significant increase was detected in the PCOS group compared with that of the control ($P < 0.05$). In addition, there was a significant decline in the serum level of TG in the groups treated with *A. graveolens* and *C. zeylanicum* extracts compared to TG serum level of the PCOS group ($P < 0.05$) (Table 2)

Table 2. The Markers of Oxidative Stress in Ovarian Tissue

Groups	MDA ± SD	SOD ± SD	GPx ± SD
Control	80 ± 6.5	1.63 ± 0.21	33 ± 3.21
PCOS	140 ± 7.35[b]	0.60 ± 0.11[b]	14.35 ± 2.32[b]
PGA	100 ± 5.7[a]	0.96 ± 0.18[a]	26.75 ± 3.6[a]
AG	82 5.25±[a]	1.57 ± 0.24[a]	33.65 ± 3.25[a]
PCZ	105 ± 10.2[a]	1.06 ± 0.15[a]	27.56 ± 4.2[a]
CZ	79 ± 3.5	1.65 ± 0.25[a]	34.65 ± 2.25[a]
PCO+C+ A	95 ± 8.4	1.18 ± 0.15[a]	27.25± 4.05[a]
C + A	75 ± 3.2	1.70 ± 0.25[a]	35.05 ± 3.15[a]

Note. Control: Control group; PCOS: PCO group that received normal saline by oral gavage; PGA: PCOS group treated with hydroalcoholic extract of *Apium graveolens* (200 mg/kg); AG: Healthy group which received the hydroalcoholic extract of *A. graveolens* (200 mg/kg); PCA: PCOS group that received therapy with C. *zeylanicum* extract (200 mg/kg/orally); CA: Healthy group received the hydroalcoholic extract of *C. zeylanicum* (200 mg/kg); PCO+C+A: The PCOS group that received therapy with *C. zeylanicum* (200mg/kg) and *A. graveolens* (200 mg/kg) extracts; C+A: No PCOS + *C. zeylanicum* and *A. graveolens*.

[a] A substantial difference with the control group; [b] The notable difference with PCOS group.

Oxidative Stress Markers in Ovarian Tissue

A significant level of MDA was observed in group 2 as compared to the control group ($P < 0.05$). However, in therapy groups that received *A. graveolens* and *C. zeylanicum* extracts, separately and together, the malondialdehyde level in ovarian tissue declined significantly ($P < 0.05$). The SOD and GPX levels in ovarian tissue showed a significant decrease in group 2, ($P < 0.05$). However, in therapeutic groups, it increased significantly compared to that of the group 2 ($P < 0.05$).

Histological Changes

The histological assessment showed a normal histology of the ovarian tissue in the control group. Besides, the number of follicles was normal in this group. However, in the PCOS group, PCOS caused damage to the ovarian tissue and the number of normal follicles decreased in this group as well. In addition, the atretic follicles were observed in the ovarian tissue of the rats with PCOS. Moreover, treatment with *A. graveolens* and *C. zeylanicum* extracts was found to decrease the number of atretic follicles while enhancing the normal follicles (Figure 3).

Discussion

The results indicated that the PCOS led to an increase in the plasma level of the FBS and insulin. In this context, recent research has shown that the PCOS can induce the metabolic syndrome. Besides, it mostly depends on insulin resistance attended by compensatory hyperinsulinemia, resulting in an enhanced risk for the expansion of type 2 diabetes mellitus (17,18). Moreover, the results also showed that the *A. graveolens* and *C. zeylanicum* extracts

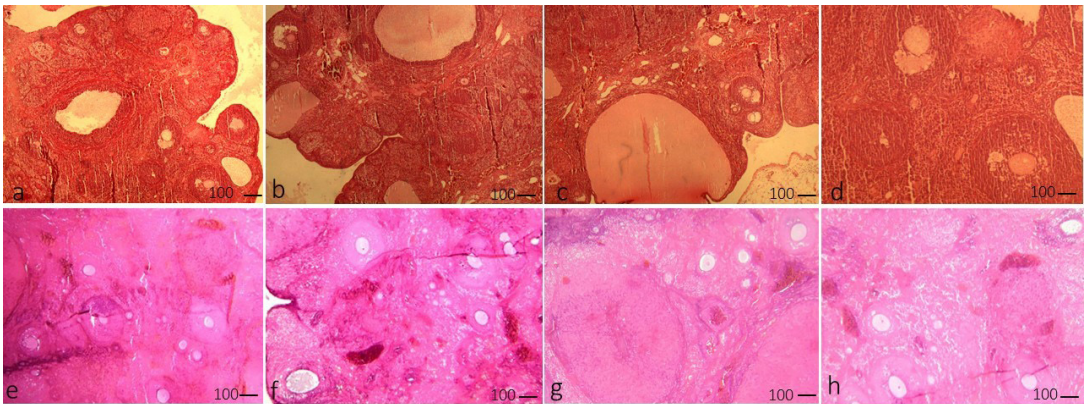

Figure. 3. The Histological Findings in Studied Groups. a: control group; b: PCO group that intake normal saline by oral gavage; c: PCOS group treated with hydroalcoholic extract of *Apium graveolens* (200 mg/kg); d: healthy group intake the hydroalcoholic extract of *Apium graveolens* (200 mg/kg); e: PCOS group that received therapy with extract of *Cinnamon Zeylanicum* (200 mg/kg/orally); f: healthy group intake the hydroalcoholicextract of *Cinnamon zeylanicum* (200 mg/kg), g: PCOS group that received therapy with extract of *Cinnamon Zeylanicum* (200 mg/kg) and *Apium graveolens* (200 mg/kg); h: No PCOS + *Cinnamon zeylanicum* and *Apium graveolens*.

significantly affected the plasma levels of fast blood sugar (FBS) and insulin. The antioxidant properties of this plant that prevented the oxygen species activity and reduced the oxidative stress may be a justification for this result. Gutierrez et al indicated that administering *A. graveolens* extract could decrease the level of FBS in plasma of the diabetic rat (19). The decline in insulin level by *A. graveolens* extract may stem from the presence of compounds such as luteolin flavonoids and other compounds such as apoysyl glucoside 7-O., Apigenin 7-O apysyl glucoside, and Cryosurial 7-O apysyl glucoside (20). Notably, the reduction in body capacity of antioxidant has been shown to have a connection with the insulin resistance (21). Furthermore, the *C. zeylanicum* extract could decrease the plasma level of FBS and insulin. Besides, it reduced the insulin resistance which is feasible due to the presence of an antioxidant combination such as limonene and linalool, aldehyde transcine, tannin, coumarin, and resin. It was indicated that these compounds can decline the insulin resistance (14,22,23). Considering the beneficial effects of both herbal medicines on the plasma levels of FBS and insulin in the groups that received both extracts together, the level of insulin and FBS decreased significantly.

Assessment of lipid profile in this research revealed that administration of *A. graveolens* and *C. zeylanicum* extracts had considerably positive effects on regulation of plasma lipids profile level such as a reduction in serum level of LDL, TG, and cholesterol and an increase in the level of HDL. Tuzcu et al also found that the C. *zeylanicum* extract could inhibit the hyperlipidemia and increase the level of HDL (24). Furthermore, Kooti et al claimed that the *A. graveolens* extract could decrease the serum lipid and regulate the lipid profile (25). Given that the extract used in the current study had antioxidant properties and could reduce the oxidative stress, administration of both extracts declined the plasma level of cholesterol, LDL, and TG and enhanced the level of HDL.

In this research, it was observed that the PCOS caused damage in ovarian tissue such as the production of cystic follicles and atretic body in the ovary and also a decline in the number of normal follicles. Moreover, assessing oxidative stress marker in the ovarian tissue showed that the PCOS led to the increment in lipid peroxidation and also a reduction in SOD and GPX level in the ovarian tissue. These phenomena are possible originated from hyperandrogenism that led to the production of cystic follicles and stepped down in the normal follicles (26). Several studies demonstrated that enhancing the reactive oxygen species (ROS) level in the tissue resulted in oxidative stress and tissue damage (16,27-31). Mannera et al, for example, showed that the PCOS enhanced the atretic follicles while reducing the healthy antral follicles (32). Apparently, treatment with *A. graveolens* and *C. zeylanicum* extracts could protect the ovarian tissue from the oxidative damage by their antioxidant property. Besides, the ability of these extract in the prevention of hyperinsulinemia and hyperandrogenism may be a justification for these results.

Conclusions

Based on the results it was revealed that *A. graveolens* and *C. zeylanicum* extracts significantly influenced the regulation of serum levels of FBS, insulin, TG, LDL, and cholesterol in the palliation of the PCOS complications. Moreover, administering this extract separately and together could protect the ovarian tissue damages induced by the PCOS.

Ethical Issues

The present study was approved by the Ethics Committee of Tabriz University of Medical Sciences (TBZMED) under the ethical code of IR.TBZMED.VCR.REC.1397.22.

Conflict of Interests

There is no conflict of interests.

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