



The Effect of Hydroalcoholic Extract of *Cinnamom zeylanicum* on Oxidative Damages and Biochemical Change in Adult Rats With Polycystic Ovary Syndrome

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Abstract

Objectives: Polycystic ovary syndrome (PCOS) is known as one of the most usual hormone disorder in women of childbearing age. Accordingly, the aim of this research was to investigate the effect of hydroalcoholic extract of *Cinnamom zeylanicum* (CZ) on oxidative damages and biochemical change in adult rats with PCOS.

Materials and Methods: In our experimental research, 32 Wistar female rats were used (n=8 per group) including control group (G1), PCOS group without any therapy (G2), rats with PCOS that received a daily intake of hydroalcoholic extract (the 200 mg/kg/orally) of CZ for 2 weeks (G3), and the group with no PCOS while with a daily intake of the extract of CZ (200 mg/kg) for 2 weeks (G4). PCOS was induced by estradiol valerate with a single injection dose (16 mg/kg) intramuscularly. After 14 days, all animals were anesthetized with ketamine and xylazine, followed by obtaining the blood sample and using their plasma for checking the blood glucose, insulin, follicle-stimulating hormone (FSH), luteinizing hormone (LH), estrogen, and testosterone. Finally, the ovaries of all animals were removed and fixed for assessing the histological damage.

Results: As regards the plasma levels of blood glucose, insulin, LH, FSH, and testosterone, a significant change was observed between G2 and G1 groups while G3 and G4 groups demonstrated a significant decline in terms of such parameters as compared to G2 group. In addition, the level of estrogen in the plasma decreased significantly in G2 as compared to G1 while in G2 and G1, it was significantly higher when compared to the PCOS group. Eventually, the number of follicles reduced in PCOS group while it indicated an increase in the groups which were treated with CZ extract.

Conclusions: The results of the present research showed that the hydroalcoholic extract of CZ has a beneficial effect on regulating the plasma levels of testosterone, estradiol, LH, FSH, FBS, and insulin in the palliation of PCOS complications.

Keywords: *Cinnamom zeylanicum*, Oxidative damages, PCOS, Hormones, Rat

Introduction

Polycystic ovary syndrome (PCOS) is considered as one of the most common hormone disorders in women of childbearing age, which includes 5%-10% of the disorders in this age group. The symptoms of PCOS include acne (due to excessive androgen production), menstrual dysfunction, ovulation, and infertility (1). In addition, the long-term complications of this syndrome encompass type 2 diabetes, hypertension, and cardiovascular disorder. Further, the risk of hyperplasia and cancer in the endometrium is higher in patients with non-treated PCOS (2). Previous research shows that in PCOS, a number of endocrine disorders including the defects in the functioning of hypothalamic-pituitary axis (i.e., ovarian and adrenal functions) exacerbate and enhance the other

functions (3). In fact, this syndrome is associated with abnormal gonadotropin secretions, as well as increased secretion of ovarian androgens and insulin resistance. The level of luteinizing hormone (LH) increases, specifically in PCOS patients, which is due to more secretion of this hormone. The ovaries preferentially enhance the production of androgens when the concentration of LH increases compared to that of follicle-stimulating hormone (FSH). The insulin levels and insulin-like growth factors also represent an increase in women who enhance the synthesis of androgens in the cells thus enhancing the function of LH (3,4). Furthermore, PCOS can induce the dysregulation in the steroidogenesis of ovary, namely, folliculogenesis including polycystic and irregular ovaries or no ovulation (5,6), along with the enhanced index

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of oxidative stress (7,8). Although there is no especial approved drug for the therapy of PCOS, the common signs and symptoms of PCOS are often treatable (9,10).

Herbal medicine has long been used for treating sexual disorders in females since it includes several components with a pharmacological activity exhibiting promising impacts in the treatment of PCOS and diabetic patients (11,12).

Cinnamon zeylanicum (CZ) is dried in tubular shapes with the outer and interior surfaces of cyan and brown, respectively (13). The skin of CZ includes 0.5-2.5% essential oil, including more than fifty variant compounds of which 65-80% is aldehyde cinematos. The other components are cinnamic acid, phenolic syntax (e.g., eugenol, fledron, and safrole), terpene components (e.g., limonene and linalool), aldehyde transcine, tannin, coumarin, resin, phenylpropane compounds (e.g., aldehyde hydroxy cinnamate and mannitol). Moreover, the main components of CZ encompass aldehyde (65%-80%) and eugenol (10%), which have the highest antibacterial activity related to cedar aldehyde. CZ has a high antioxidant activity, as well as having antibacterial properties (14).

Considering that this plant has a lot of antioxidant properties and is used in traditional medicine, the current study used CZ to evaluate the effects of the extract of this herb on PCOS which was induced in rats.

Materials and Methods

Thirty-two animals (female Wistar rats weighing 200±20 g) were utilized in the present study. All the rats were purchased from the Razi Institute, then intently kept in the animal laboratory of the Faculty of Medicine of Tabriz University of Medical Sciences at a standard situation (The temperature of 25°C and 12 h light/12 h dark), and had free access to food and water.

Study Design and Experimental Groups

All rats were randomly separated into 1 healthy group and 3 experimental groups as follows.

1. Healthy group (control), that were prescribed the normal saline (orally and daily);
2. Polycystic ovary syndrome (PCOS) group (PCO), in which PCOS was induced by a single dose of estradiol valerate (16 mg/kg) intramuscularly (Sigma, USA) that was solved in 0.2 mL sesame oil (15, 16);
3. PCOS group + prescribing *Cinnamon zeylanicum* (CZ) extract orally (200 mg/kg) for 14 days (PCA);
4. Sham group (CA) + prescribing the extract of CZ orally (200 mg/kg) without PCOS induction for 14 days.

The estrus cycle was confirmed by the vaginal smear in all rats with PCOS. According to Roushangar and Rad (17), all the studied animals were anesthetized by ketamine and xylazine (5/1 mg/kg) after the remedy period, followed by deriving the blood samples from the left ventricle of the

rat heart and centrifuging the samples at 3000 g for plasma separation. The serum sample was kept at -80°C until the subsequent assessment.

Preparation of the Hydroalcoholic Extract of *Cinnamon zeylanicum*

About 0.5 kg of this herbal medicine was purchased to obtain the CZ extract. First, the plant was powdered and then solved in ethanol 50% and kept at the room with 25°C temperature on a shaker (Thermo Fisher) for 48 hours in order to provide the extract. Next, the produced solution was filtered and centrifuged at 3000 rpm for 5 minutes, followed by infusing the eventuated solution into an open-top dish and vaporizing the solvent. Finally, the resulted extract was solved in normal saline to attain a suitable concentration.

Measurement of the Serum Levels of Testosterone, Estrogen, Follicle-stimulating Hormone, and Luteinizing Hormone

The hormone levels in plasma were quantified by ELISA Kits (Demeditec Diagnostics, Germany) for estrogen and testosterone with an analytical sensitivity of 0.08 ng/mL. Correspondingly, the plasma levels of FSH and LH were examined with a Cusabio Kit (China) specified for the rats with a sensitivity of 0.15 mLU/mL using the ELISA method.

Measurement of Oxidative Stress Marker

The level of malondialdehyde (MDA) in the plasma was assessed by locating 0.20 mL of serum into a microtube which included 3.0 mL of glacial acetic acid then, 1% thiobarbituric acid in 2% NaOH was added to the microtube. Next, the tube was placed in the boiling water for 15 minutes. After cooling the microtube, the absorbance of the pink-colored product was read at 532 nm. Additionally, the calibration curve was produced by MDA tetrabutylammonium salt obtained from Sigma, the USA (18,19). The plasma levels of superoxide dismutase (SOD) and glutathione peroxidase (GPX) were measured by an ELISA reader device (Antus) according to the protocols of the kits (Randox and Ransod, the UK, with a sensitivity of <S1 standard value).

Histological Study

After tissue sampling, the ovarian was placed in formalin solution 10% for fixation. Then, the samples were dehydrated by placing in ethanol and embedded in paraffin. Likewise, the cut of the tissue samples was created with five microns thick and then deparaffinized and stained with haemotoxylin and eosin. To accomplish histopathological studies, the tissue sections of each ovarian sample were auscultated from the ovary cortex to the medulla in a spirally and clockwise direction, followed by enumerating the number of primary, per-antral, antral, cystic follicle, and yellow body in each slide. The above-

mentioned parameters were compared between the study groups (19-21).

Statistical Analysis

All the obtained data were analyzed by SPSS software, version 19 (USA), presented as mean ± SEM, and finally, compared by one-way ANOVA and Tukey post hoc test. $P < 0.05$ was considered as statistically significant.

Results

The Serum Levels of Testosterone, Estrogen, Luteinizing Hormone, and Follicle-Stimulating Hormone

The plasma level of testosterone was significantly higher in polycystic ovary (PCO) group when compared to the control group ($P < 0.05$). On the other hand, the testosterone levels in the plasma significantly reduced in the treated groups (PCA & CA) as compared to polycystic ovary syndrome (PCOS) group ($P < 0.05$). However, a significant difference was observed between the PCOS and control groups in terms of plasma estrogen levels ($P < 0.05$). In other words, the plasma level of estrogen significantly enhanced in the treated groups with the extract of CZ as compared to PCO ($P < 0.05$). Similarly, the ratio of LH/FSH levels in PCOS group was significantly higher compared to the control group ($P < 0.05$). Conversely, the levels of gonadotropin hormones in the plasma demonstrated a significant reduction in groups that were treated with CZ extract relative to PCOS group ($P < 0.05$), the details of which are provided in Table 1.

Plasma Level of Superoxide Dismutase, Glutathione Peroxidase and Malondialdehyde

The plasma levels of GPX and SOD significantly decreased in PCOS group compared to control ($P \leq 0.05$) and the therapy groups that were treated with the extract of CZ Aromaticum significantly enhanced the level of SOD and GPX when compared with PCOS. In addition, the plasma MDA level was higher in the PCOS group when compared to the control group ($P < 0.05$). On the other hand, in PCA and CA groups, the level of MDA was significantly lower than the PCO group ($P < 0.05$). Figures 1-3 summarize the data related to this section.

The Results of Follicles Counting

Table 2 demonstrates the count of primary, pre-antral,

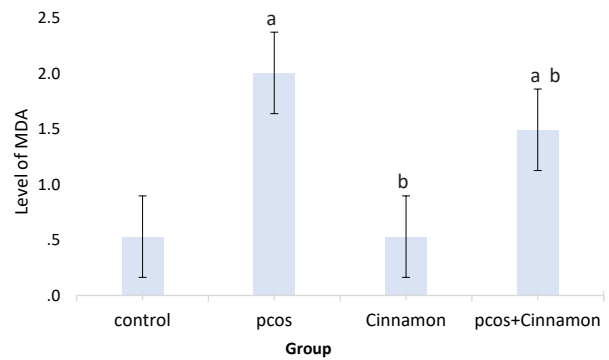


Figure 1. Serum Level of Malondialdehyde.

Note. Control: Control group; PCOS: PCOS group that received normal saline; PCA: PCOS group that was given the hydroalcoholic extract of *Cinnamon zeylanicum* (200 mg/kg), CA: Healthy group which took the hydroalcoholic extract of *Cinnamon zeylanicum* (200 mg/kg); PCOS: Polycystic ovary syndrome. The symbol a indicates a substantial difference with the control group and symbol b displays the notable difference with PCOS group ($P < 0.05$).

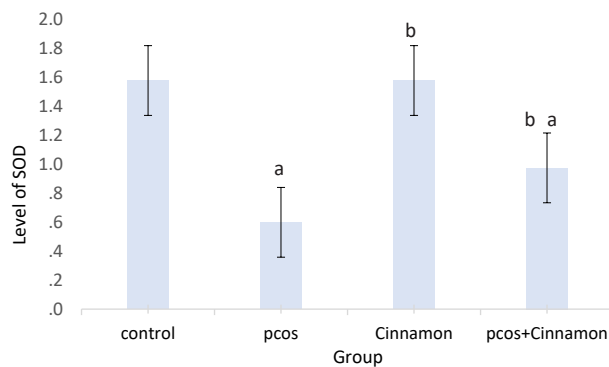


Figure 2. Serum Level of Superoxide Dismutase.

Note. Control: Control group; PCOS: PCOS group that received normal saline; PCA: PCOS group that was given the hydroalcoholic extract of *Cinnamon zeylanicum* (200 mg/kg), CA: Healthy group which took the hydroalcoholic extract of *Cinnamon zeylanicum* (200 mg/kg); PCOS: Polycystic ovary syndrome. The symbol a indicates a substantial difference with the control group and symbol b displays the notable difference with PCOS group ($P < 0.05$).

antral, and cystic follicles, along with the number of yellow bodies, and the micrograph of ovaries is illustrated in Figure 4. The primary follicles count significantly reduced in group 2 compared to group 1 ($P < 0.05$). Moreover, a notable diversity was found between PCO and treated groups ($P < 0.05$). The pre-antral follicles count in the PCOS group represented a significant decrease as

Table 1. The Plasma Testosterone, Estrogen, LH, and FSH Levels in All Groups

Groups	LH (ng/mL)	FSH (ng/mL)	Testosterone (ng/mL)	Estrogen (pg/mL)
Control	2.01±0.131	2.52±0.149	0.53±0.050	50.71±3.45
PCOS	3.27±0.159 ^a	2.58±0.369	2.99±0.078 ^a	35.00±2.72 ^a
PCA	2.39±0.052 ^b	2.77±0.113	1.59±0.064 ^b	44.50±1.26 ^b
CA	2.07±0.049 ^b	2.51±0.168	0.52±0.030 ^b	50.14±3.23 ^b

Note. Control: Control group; PCOS: PCOS group that received normal saline; PCA: PCOS group which received the hydroalcoholic extract of *Cinnamon zeylanicum* (200 mg/kg); CA: Healthy group that took the hydroalcoholic extract of *Cinnamon zeylanicum* (200 mg/kg); LH: luteinizing hormone; FSH: follicle-stimulating hormone; PCOS: polycystic ovary syndrome. All data are exhibited as mean ± SE.

The symbol a Shows a substantial difference with the control group and symbol and symbol b means the notable difference with PCOS group ($P < 0.05$).

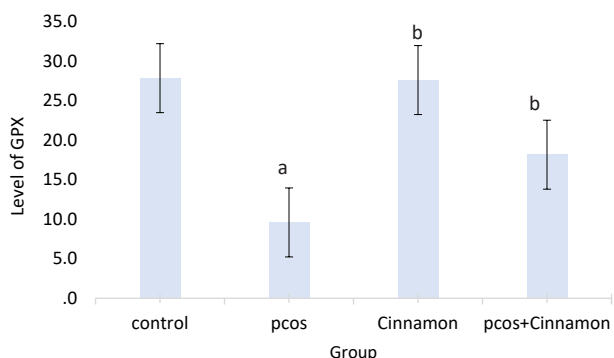


Figure 3. Serum Level of Glutathione Peroxidase.

Note. Control: Control group; PCOS: PCOS group that received normal saline; PCA: PCOS group that was given the hydroalcoholic extract of *Cinnamon zeylanicum* (200 mg/kg), CA: Healthy group which took the hydroalcoholic extract of *Cinnamon zeylanicum* (200 mg/kg); PCOS: Polycystic ovary syndrome. The symbol a indicates a substantial difference with the control group and symbol b displays the notable difference with PCOS group ($P < 0.05$).

compared to control group ($P < 0.05$) while this count was significantly higher in both therapy groups compared to PCOS group ($P < 0.05$). However, PCOS group showed a substantial reduction in counting the antral follicles when compared with the control group ($P < 0.05$). On the other hand, the count of antral follicles significantly increased in groups that received 200 mg/kg CZ extract relative to the PCOS group, ($P < 0.05$). However, the number of cystic follicles significantly enhanced again in the PCOS group compared to the control group ($P < 0.05$) while the count of cystic follicles significantly decreased by treatment with CZ extract as compared to PCO groups that received no treatment ($P < 0.05$). In addition, evaluating the count of yellow bodies represented a significant decline in the PCO group relative to the control group ($P < 0.05$). Eventually, a significant enhancement in the count of yellow bodies was observed in PCA and CA groups as compared with the group which was affected by PCOS ($P < 0.05$).

Discussion

Some researchers reported that the PCOS patients have a high level of LH/ FSH, and testosterone while estrogen levels are lower in these patients (16,22). According to these studies, the aromatase level in plasma reduced

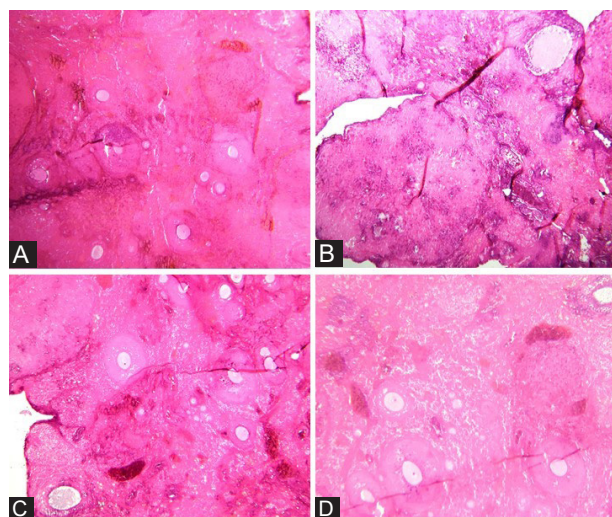


Figure 4. The Histological Findings in the Studied Groups.

Note. 1: Control group; 2: PCO group that was given the normal saline by oral gavage; 3: CA: Healthy group which received the hydroalcoholic extract of *Cinnamon zeylanicum* (200 mg/kg); 4: PCA (PCOS group) that was treated with the hydroalcoholic extract of *Cinnamon zeylanicum* (200 mg/kg); PCOS: Polycystic ovary syndrome.

in PCOS patients (16,22), which can be a reason for a reduction in the estrogen level in PCOS patients.

The results of our research showed that PCOS induced a significant enhancement in the plasma levels of testosterone and the ratio of LH/FSH while leading to a significant reduction in the plasma levels of estrogen in PCOS group as relative to the control group. Nonetheless, therapy with CZ extract can increase the plasma estrogen levels while it decreases the serum levels of gonadotropins and testosterone. In PCOS groups, several studies indicated that plants with antioxidant property can decrease the serum levels of gonadotropins and insulin (23-25). On the one hand, the findings of another study revealed that the decline of plasma aromatase level in PCOS group is probably related to a decrease in plasma estrogen level (26).

Cytochrome P450c-17 α is considered as one of the enzymes whose activity is enhanced in some diseases such as PCOS. Further, it is the basic enzyme in the production of androgens which is straightly incited by insulin in PCOS. According to this subject, CZ can lead

Table 2. The Count Offollicles and Yellow Body in Studied Groups

Groups	Primary Follicles	Pre-antral Follicles	Antral Follicles	Cystic Follicles	Yellow Body
Control	18.4±1.14	27.8±1.92	16.2±1.30	0	9.4±1.83
PCO	7.4±1.14 ^a	7.2±1.30 ^a	3.8±1.14 ^a	6.8±0.54 ^a	1.8±0.89 ^a
PCA	14.4±2.07 ^b	18.4±1.14 ^b	12.4±1.94 ^b	1.4±0.54 ^b	7.6±0.70 ^b
CA	19.2±2.38 ^b	27.8±2.48 ^b	13.6±2.07 ^b	0 [†]	6.6±1.89 ^b

Note. Control: Control group; PCOS: PCOS group that received normal saline; PCA: PCOS group which received the hydroalcoholic extract of *Cinnamon zeylanicum* (200 mg/kg); CA: Healthy group that took the hydroalcoholic extract of *Cinnamon zeylanicum* (200 mg/kg); LH: luteinizing hormone; FSH: follicle-stimulating hormone; PCOS: polycystic ovary syndrome. All data are exhibited as mean ± SE.

The symbol a Shows a substantial difference with the control group and symbol and symbol b means the notable difference with PCOS group ($P < 0.05$).

to a decrease in plasma insulin levels and the regulation of the level of gonadotropins hormone in plasma (23,24). By acting on the liver, insulin can prevent the secretion of sex hormone-binding protein globulin, therefore, it increases the level of blood androgens, namely, sex hormones (27). Furthermore, metformin can increase the spontaneous ovulation in women with PCOS because it can step down the insulin levels and lead to a reduction in androgen levels (28).

Other complications of PCOS include enhancement in oxidative stress and intracellular reactive oxygen species, which are known as the main pathways preoccupied in PCOS pathogenesis (7,29,30). The results of our research showed that the plasma MDA level significantly enhanced while the SOD and GPX levels in the serum significantly reduced in rats with PCOS. Therefore, the positive effect of antioxidant, associated with the regulatory effect on endocrine characteristics, might prepare new management of PCOS (7, 29). In this study, treatment with the hydroalcoholic extract of CZ could increase the level of SOD and GPX in the plasma and a decline in the level of MDA was probably due to high antioxidant capacity and antioxidant compounds available in this plant.

Moreover, the results further revealed that the count of follicles, first, demonstrated a significant reduction in PCOS group while a significant enhancement was later detected in the count of cystic follicles in this group. These phenomena are probably attributed to hyperandrogenism that results in the production of cystic follicles and steps down in the count of the other normal follicles (31). Based on the findings of previous research, the count of atretic follicles enhanced in PCOS whereas the count of healthy antral follicles represented a decline (32). Additionally, Badawy et al. found that the number of healthy follicles decreased in rats with PCOS (33). The results of the present study showed that therapy with CZ extract could increase the number of normal follicles while reducing the count of cystic follicles that are derived from the effects of CZ extract on a decline in plasma insulin level and insulin resistance. This possibly pertains to the presence of antioxidant compounds such as limonene and linalool, aldehyde transcin, tannin, coumarin, resin, as well as phenylpropane compounds such as aldehyde hydroxy cinnamate and mannitol in CZ. It was also indicated that these compounds can reduce insulin resistance and prevent the hyperandrogenism (14,23,24). However, the impact of CZ on preventing the manufacturing of cystic follicles in the groups treated with CZ extract (PCA & CA) may be due to the anti-hyper androgenic properties of this plant.

Conclusions

Overall, our results revealed that CZ extract can regulate the level of gonadotropin and steroid hormones, decrease the oxidative stress, and finally, enhance the activity of

the antioxidant enzyme. Eventually, the hydroalcoholic extract of CZ could prevent cystic follicle production while increasing the number of normal follicles.

Conflict of Interests

Authors have no conflict of interests.

Ethical Issues

All experimental proceeding was accomplished upon obtaining permission from the Ethics Committee of Tabriz University of Medical Sciences under the ethical code of IR.TBZMED.VCR.REC.1397.22.

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