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Effect of Thymus Vulgaris Ethanol Extract, on Serum Total Antioxidant in Experimental Induced Poly Cystic Ovarian Syndrome (PCOS) Rats

Moslem Jafarisani[1], Masoomeh Masoomikarimi[2], Seyedeh Sareh Kazemi[3], Sareh Mirzaeidelaviz[4], Zahra Naderi[5], Reza Ahmadi[6*]

[1] Dept. of Clinical of Biochemistry, School of Medicine, Shahroud University of Medical Science, Shahroud, Iran.
[2] Dept. of Immunology, Torbat Heydariyeh University of medical science, Torbat Heydariyeh, Iran.
[3] Dept. of Biochemistry, Damghen Branch of Islamic Azad University, Damghan, Iran.
[4] School of Specialized Veterinary Science, Islamic Azad University, Science and Research Branch, Tehran, Iran.
[5] Firouzgar Hospital, Iran University of medical science, Tehran, Iran.
[6] Clinical Biochemistry Center, Shahrekord University of Medial Science, Shahrekord, Iran.

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Abstract

Background:*Poly cystic ovary syndrome is one of the most common female endocrine disorders. One of the side effects of PCOS is oxidative stress.. Here we investigated antioxidants effects of Thymus vulgaris ethanoli extract on experimental PCOS induced rats by estradiol-valerat (PPA).*

Methods*: Wistar female rat (n=70) were divided into 7 groups including C1: an equal volume of (0.9% NaCl) as placebo; C2: extract (0.6cc/rat/orally/daily); C3: induced PCO by single injection of estradiol-valerate (4mg/rat/IM), and T1: PCOS induced rats+an equal volume of (0.9% NaCl) as placebo, T2: PCOS induced rats + extract(0.2cc/rat/orally/daily), T3: PCOS induced rats+extract (0.4cc/rat/orally/daily), T4:PCOS induced rats+extract (0.4cc/rat/orally/daily)test groups, were received extract supplement, for 60 consequence days. Animals were kept in standard conditions. In last day of study the blood samples of rats in whole groups were obtained and prepared to biochemical analysis.*

Results*: Total antioxidant capacity level, superoxide dismutase and catalase activity were significantly increased in PCOS treated groups (P<0.03), these parameters in PCOS groups that did not receive extract significantly decreased (P<0.05) in comparison to control. Level of MDA in PCOS groups were significantly increased as compared to control and extract treated groups (P<0.01).*

Conclusions*: Our results showed that administration of Thymus vulgaris ethanol extract significantly improved tissue antioxidants level in PCOS induced rats.*

Keywords: Poly cystic ovary syndrome, Thymus vulgaris extract, Antioxidant, Superoxidedismutase, Catalase, MDA.
***Corresponding to:** R Ahmadi**, Email:**a119b118@gmail.com
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Introduction

Polycystic ovarian syndrome (PCOS) is one of the most common endocrine disorders in reproductive-aged women, with prevalence ranging from 5% to 21%.[1,2] It is characterized by elevated luteinizing hormone, chronic anovulation with oligoamenorrhea, enlarged cystic ovaries, obesity, hyperandrogenism, and infertility. Insulin resistance is also a common feature of the syndrome, which may lead to diabetes mellitus type II.[3,4] The etiology of PCOS remains unclear and is possibly multifactorial. Women with PCOS are at greater risk of cardiovascular disease.

Furthermore, oxidative stress (OS) may play a distinct role in the pathophysiology of PCOS.[5-8] Reactive oxygen species (ROS) are formed in the human body as a result of normal cellular function and metabolism. Natural defense mechanisms in the form of antioxidants are deployed to modulate ROS. Antioxidants are natural substances that suppress or delay OS, by scavenging biologically important reactive oxygen species (ferryl; O2−; OH−; H2O2; HOCl; alkoxyl, and peroxyl).[1,9,10] Oxidative stress can be generated when there is an imbalance between antioxidant defenses and oxidant molecules (reactive oxygen and nitrogen species). The role of OS in infertility in both males and females has been demonstrated.

ROS production increases in response to PCOS.[6,11] Thus, the antioxidant effect is critical to counteract the infertility caused by oxidative stress.[1] Nowadays, the use of antioxidants to manage PCOS is widely established. Several characteristics of PCOS, including abdominal adiposity, insulin resistance, obesity, and androgen excess, have the potential to enhance oxidative stress status in these patients.[1]

Phytomedicines have been used for therapeutic purposes for centuries.[12] Thymus vulgaris L., commonly known as thyme, a species of the mint family, Lamiaceae, is native to Iran (shirazes, garden, daenensis), and to southern Europe, from the western Mediterranean to southern Italy. Growing from 15 to 30 cm tall and by 40 cm wide, it is a bushy, woody-based evergreen subshrub with small, highly aromatic, gray-green leaves and clusters of purple or pink flowers in early summer.[13] Thyme is constituted by a number of flavonoids: thymol (45.21%), p-cymene (12.90%), γ-terpinene (8.07%), carvacrol (5.15%), linalool (3.43%), and (E)-caryophyllene (2.45%), which have an antioxidant effect.[14]

Numerous studies indicate that thymus petal ethanol extract possesses antidepressant, anti-inflammatory, antinociceptive, antihypertensive, anti-cancer, and antitumor properties. Recently, the antioxidant and antimicrobial properties of thyme have been empirically investigated. The beneficial antioxidant activity of thyme has been attributed largely to phenolic compounds.[14-19]

The aim of this study is to assess the effect of thymus vulgaris ethanol extract antioxidants on the total ovary antioxidant level in rats with experimentally induced PCOS.

Materials and Methods

In this experimental study, 7-week old adult Wistar albino female rats (n=70) weighing 250±10 g were obtained from the Animal Facility of Pasture Institute, Iran. The animals were housed and kept in controlled temperature conditions (25°C) and 12 h/12 h light/dark cycle with constant humidity (40–70%) for 1 week prior to use in experimental procedures. All rats were treated in accordance with the principles of Laboratory Animal Care [NIH]. All rats were fed a standard diet and water. The Wistar female rats were allocated to one of the two major groups. The control group (n = 30) was divided into subgroups: C1: an equal volume of (0.9% NaCl) as placebo; C2: extract (0.6 cc/rat/orally/daily); C3: induced PCO by single injection of estradiol-valerate (4 mg/rat/IM), and T1: PCOS-induced rats + an equal volume of (0.9% NaCl) as placebo, T2: PCOs induced rats+extract (0.2 cc/rat/orally/daily), T3: PCOS-induced rats+extract (0.4 cc/rat/orally/daily), T4: PCOS-induced rats+extract (0.4 cc/rat/orally/daily). All groups were treated for 60 continuous days. On the 60th day of the study, 5cc blood samples were drawn from rats in all groups and prepared for biochemical analysis. Animals were kept in standard conditions.

The Thymus vulgaris petals were harvested at autumn from Gonabad, KhorasanRazavi Provence, and air dried on laboratory tables at room temperature (25±2°C) away from fluorescent light for three days. They were later ground using a crusher. In total, 100 g of each of the ground substances were infused separately in double distilled water and 50% ethanol, for 72 h with periodic stirring. Each prepared extract was filtered repeatedly using a sterile muslin cloth, cotton wool, and filter paper. The prepared ethanol extract was freeze dried and stored at −80°C.

For daily using of thymus ethanol extract, a 0.3 mg/ml solution was prepared and stoked. The provided supplement was administered by the gavage method to the treatment groups.

TAC was measured in serum, using TAC Colorimetric Assay Kit (K274-100, Biovision, USA). In this method, Cu2+ ion is converted to Cu+ by both small molecule and protein. The protein mask prevents Cu2+ reduction by protein, enabling the analysis of only the small molecule antioxidants. The reduced Cu+ ion is chelated with a colorimetric probe giving a broad absorbance peak around 570 nm, proportional to the TAC (mmol/L).

The activity of SOD was measured according to the method of Beyer and Fridovich (1987), using the SOD assay kit (cat no: 7500-100-K, Funakoshi, Japan)

GPX was assayed using GPX assay kit (K762-100, Biovision, USA). In BioVision's GPX activity assay, GPX reduces cumenehydroperoxide while oxidizing GSH to GSSG. The generated GSSG is reduced to GSH with consumption of NADPH by GR. The decrease in NADPH (easily measured at 340 nm) is proportional to GPX activity with a detection sensitivity of approximately 0.5 mU/ml of GPX in samples.

Serum CAT activity was measured using the CAT Activity Colorimetric/Fluorometric Assay Kit (k773-100. Biovision, USA). In the assay, CAT first reacts with H_2O_2 to produce water and oxygen. Unconverted H_2O_2 reacts with OxiRed™ probe to produce a product, which can be measured at 570 nm (Colorimetric method).

Tissue MDA levels were determined by the thiobarbituric acid method and expressed as nmol MDA formed/mL. Plasma MDA concentrations were determined using a spectrophotometer. A calibration curve was prepared by using 1,1′,3,3′-tetramethoxypropane as standard.

Statistical analysis was performed using the ANOVA and t-test to comparison data in the control groups with the experimental groups using SPSS19 software. The results are expressed as mean±SD (standard deviation) and considered significant at the level of P<0.05.

Results

In PCOS rats, CAT activity indicated a significant decrease compared to normal controls (P=0.03). Treatment with thymus vulgaris ethanol extract for 60 continuous days significantly increased CAT activity in experimental groups, compared with control groups, in a dose-dependent manner (P=0.001; Figure 1).

Treatment with 0.4, 0,6 cc/rat/orally/daily of thyme ethanol extract for 60 consecutive days significantly increased Superoxide Dismutase (SOD) activity in experimental groups, compared with the control 3 and treatment 1 groups (P=0.001). Furthermore, PCOS rats showed a significant decrease in SOD activity compared to controls (P=0.04, Figure 2).

As illustrated in Figure 3, administration of thyme ethanol extract for 60 consecutive days significantly increased GPX activity in experimental groups, compared with the C3 group (P=0.001) in a dose-dependent manner (Figure 3). GPX activity decreased significantly in PCOS rats compared to control and treatment groups (P=0.03).

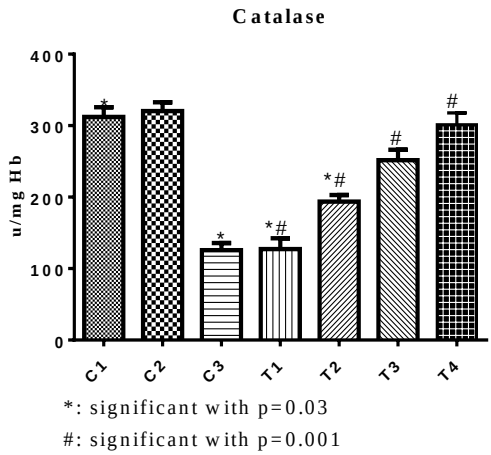

Figure 1. Catalase activity in serum of PCOs induced rats

As a result of oxidative stress, MDA concentration in PCOS rats was significantly elevated (P=0.001). Administration of thyme ethanol extract for 60 consecutive days significantly

decreased MDA concentration in experimental groups compared to control groups (P=0.003, Figure 4).

As illustrated in Figure 4, administration of thyme ethanol extract for 60 consecutive days significantly increased TAC concentration in experimental groups compared to control groups (C1: P=0.005, C3: P=0.003). In PCOS rats, however, TAC decreased significantly (P=0.001, Figure 4).

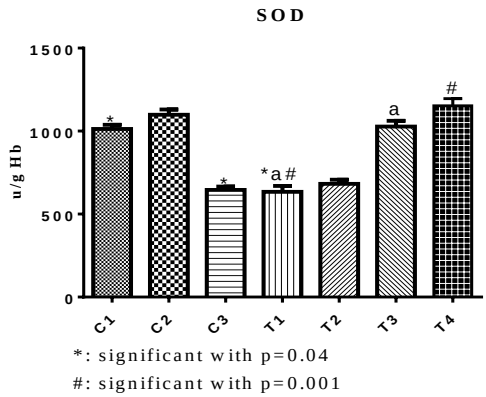

Figure 2. SOD activity in serum of PCOs induced rats

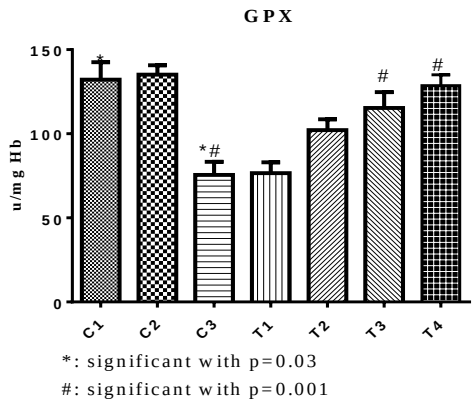

Figure 3. GPX activity in serum of PCOs induced rats

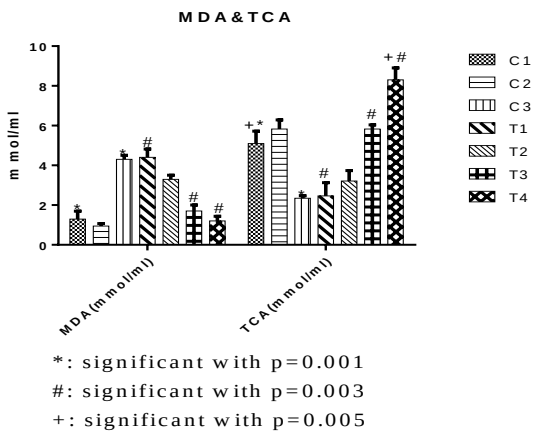

Figure 4. MDA and TCA concentration in serum of PCOs induced rats

Discussion

Oxidative stress is the result of an imbalance between production of free radicals that contain unpaired electrons, which increase chemical reactivity, and antioxidant defenses buffering oxidative damage.[6,11]It was recently reported that MDA levels as an indicator of lipid peroxidation and SOD activity increased, and erythrocyte reduced glutathione (GSH) levels decreased in women with PCOS.[5,20] These observations suggest increased oxidant stress and decreased antioxidant levels in PCOS.[6]

In the female reproductive tissue, elevated ROS levels are pursued by active steroidogenesis and metabolism.[21-23]This may cause oocyte and DNA damage. In addition, ROS may play a role in the irregular growth of ovarian mesenchyme. In a pathological condition such as PCOS, excessive oxidative stress might contribute to ovarian mesenchyme hyperplasia. During the previous decade, several studies have reported that there is an increase in coronary heart disease risk factors in women with PCOS.[24] Some studies point to a relationship between hyperinsulinemia, which is a consequence of insulin resistance, and increased risk of cardiovascular disease in PCOS.[25] ROS causes lipid peroxidation, potentially damaging DNA and/or altering cell signaling and cellular function. OS can damage the DNA of the ovarian epithelium or cause cell apoptosis. However, oxidative status in the cell modulates follicular growth, corpus luteum formation, endometrial differentiation, and embryonic growth. Oxidative stress might also cause preeclampsia, miscarriage, endometriosis, infertility, mole hidateform, and free radical-induced birth defects; hence, evaluating and protecting against OS is critical in reproductive science.[26,27] It is necessary to reduce ROS using antioxidant agents.

Enzymatic antioxidant defense systems include GPX, SOD, and CAT. Non-enzymatic antioxidants include ascorbic acid (Vitamin C), α-tocopherol (Vitamin E), carotenoids, flavonoids, glutathione (GSH), and other antioxidants. In a cell, there is typically a balance between the activities and levels of these antioxidants that is vital for the health of the organism. SOD generated in cumulus oophorus cells is closely related to oocyte maturation and GPX and CAT are critical for reproductive functions; GSH as a redox system is usually found in large amounts in the oocytes.[1] Despite oxidative stress having many physiological roles, increased production of these agents might increase the risk of ovarian pathology, which might be intensified under conditions of reduced antioxidant status.[1]

Plants are always suggested as rich sources of antioxidant compounds, including a wide variety of active phytochemicals, such as flavonoids, terpenoids, sulfides, coumarins, polyphenolics, lignans, carotenoids, saponins, curcumins, plant sterols, and phthalides.[28,29]

A wealth of research on the medicinal characteristics of thyme indicates that thyme has a potent antioxidant activity, attributed to the presence of thymol as major compound in its extract.[14,30-35]

In the current study, crude extract was used. This contains all components, as well as thymol, p-cymene, γ-terpinene, carvacrol, linalool, and (E)-caryophyllene. The results indicate a significant difference in oxidative stress in PCOS rats

compared to control and thyme treated rats. According to previous studies, MDA concentration in PCOS rats is significantly elevated as a result of oxidative stress. In this group, SOD, GPX, TAC, and CAT showed significant reductions in activity or concentration, which may cause oxidative damage.[7,24,36-38]

Our results showed that thyme extract in non-PCOS rats was unable to increase antioxidant defense systems. However, PCOS rats treated with thyme ethanol extract had significantly decreased concentrations of MDA owing to the antioxidant effect of thyme ethanol extract. This was confirmed by elevated CAT, SOD, and GPX activity, and increased TAC concentration.

The results indicate that, after treating with thyme ethanol extract in the rats with induced PCOS, antioxidant levels (CAT, SOD, GPX, and TAC) increased in this group, whilst these antioxidants were low in the rats that did not receive thyme. MDA as an oxidative species was also decreased in the PCOS (treatment) group. Based on the findings of this and previous studies, PUFAS is important as an antioxidant to improve and modulate PCOS outcome.

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Conflict to Interest

The authors declare that they have no conflict of interest.

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