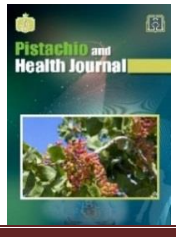

The Effects of *Pistacia Vera* Seed Oil on Anxiety and Depressive-Like Behaviors in Rats with Polycystic Ovary Syndrome

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Abstract

Introduction: Polycystic ovary syndrome (PCOS) is associated with many complications. Neurobehavioral deficits are reported in women with PCOS. Patients with PCOS show a high risk for affective disorders, which impair the quality of life. Finding a safe herbal medicine for these complications is valuable. *Pistacia vera* plays a beneficial role in central nervous disorders. The current study is designed to evaluate the effects of *Pistacia vera* oil on depression and anxiety-like behaviors in female rats with letrozole-induced PCOS.

Methods: Letrozole (1 mg/kg) was used orally in order to induce PCOS. Pistachio oil (1 and 4 ml/kg) was administered with letrozole. After 21 days, elevated plus-maze test, forced swimming test (FST), and open field tests were performed.

Results: The data showed that the PCOS condition led to immobility time enhancement in the FST ($P<0.001$). PCOS animals significantly exhibited anxiety-like behaviors ($P<0.05$). Treatment with pistachio oil with doses of 1 and 4 ml/kg completely blocked the deleterious effects of PCOS on behavioral parameters.

Conclusion: *Pistacia vera* oil could alleviate depression and anxiety in rats with PCOS.

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1. Introduction

Polycystic ovary syndrome (PCOS)[1] is a common endocrine disorder in females in the childbearing age [1]. Multiple characteristics could be detected in women with PCOS, including irregular menstrual cycle, weight gain, acne, hirsutism, increased risk of atherosclerotic cardiovascular disease, hyperandrogenemia, and insulin resistance [2, 3]. This hormone imbalance may lead to psychiatric complications, such as eating disorders, anxiety, and depression [4, 5]. Evidence has reported a high prevalence of depression in women with PCOS and showed an association between PCOS markers and depression [6]. Data has also indicated that patients with PCOS have a high risk of affective disorders, which can impair their quality of life [7]. Women with PCOS experience elevated anxiety [8]. Finding appropriate strategies for affective disorder treatment related to PCOS is of great importance. *Pistacia vera*, a member of the Anacardiaceae family, is an important medicinal plant in Iran, which possesses considerable pharmacological functions [9, 10]. The protective role of *Pistacia* species has been demonstrated in cardiovascular diseases, metabolic syndrome, diabetes, gastrointestinal, respiratory, and liver diseases [11]. Moreover, data indicated that *Pistacia* species could exert neuroprotective properties on the central nervous system[2] disorders, such as seizure [12, 13]. Studies showed that *Pistacia vera* could have anti-anxiety and hypnotic effects in mice [14]. The nutrient profile of *Pistacia vera* includes a variety of antioxidants and anti-inflammatory ingredients that make this nut a valuable target against PCOS [9, 15, 16]. As the effects of *Pistacia vera* have not been investigated against PCOS-related CNS disorders so far, in the current study, it was aimed to evaluate the possible protective role of *Pistacia vera* seed oil on anxiety and depressive-like behaviors in PCOS-induced rats.

2. Materials and Methods

2.1. Plant Material Collection and Oil Extraction

Pistachio with genetic code M30 and from species Akbari was purchased from herbal stores of Rafsanjan, Iran, July 2017. Cold press technique was performed for oil extraction. The oil obtained via this standard method has appropriate quality for experimental studies. The oil was stored at 4°C in amber glass bottles throughout the study [9, 17].

2.2. Animals

Female Wistar rats weighing 150-200 g were used in this study. Animals were kept in four/cages under standard laboratory conditions: 12-hour light/dark cycle, the average temperature of 22± 2°C, and humidity of 55± 2%. Animals had access to food and water. Each animal was used only once during the study. Experiments were performed at the same time of the day. Animals were cared for and treated according to the institutional guideline for the care and use of laboratory

[1] Polycystic Ovary Syndrome (PCOS)
[2] Central Nervous System (CNS)

animals (NIH[3] publications no. 8023, revised 1978) with the approval of Rafsanjan university research and medical ethics committees.

2.3. Polycystic Ovary Syndrome Induction

Polycystic ovary syndrome was induced through a standard model with an aromatase inhibitor, letrozole. This class of drugs inhibits the aromatization step in estrogen biosynthesis. Letrozole was administered orally for 21 days (once daily) at the dose of 1 mg/kg. Letrozole was purchased from Aburaihan Pharmaceutical Co, Iran.

2.4. Experimental Design

Female rats were randomly assigned into four groups (N=7 each group):

1: Control group: healthy normal animals without polycystic ovary syndrome (PCOS)

2: PCOS group: rats with polycystic ovary syndrome.

3: PCOS+PO 1 mg/kg: letrozole-induced PCOS rats treated with pistachio oil with a dose of 1 ml/kg orally for 21 days concurrent with letrozole [18].

4: PCOS+PO 4 mg/kg: letrozole-induced PCOS rats treated with pistachio oil with a dose of 4 ml/kg orally for 21 days concurrent with letrozole [18].

On 22th day of the experiment, after the termination of letrozole and pistachio oil administration, animals were subjected to neurobehavioral pharmacological tests (EPM [4], FST[5], and OFT[6])

2.5. Elevated Plus-Maze Test

The elevated plus-maze (EPM) is a standard model for evaluating the anxiety-like behaviors in rats. The apparatus was made of wood; it consisted of two open and two closed horizontal perpendicular arms with a size of 50 × 10 cm placed 50 cm above the floor and lightened by a 60 W light bulb placed above the center. The 40 cm high walls enclosed the closed arms. The junction of four arms formed a central square platform (10 × 10 cm). Animals were placed in the square platform with their heads facing the open arm for 5 min (free exploration). Animals behavior was recorded concerning the percent of time spent in the open arms [OAT%[7] (the ratio of time spent in the open arms to the total time spent in any arm × 100)], percent of entries into the open arms [OAE%[8] (the ratio of entries into open arms to the total entries × 100)]. The number of total arm entries was considered as a measure of spontaneous locomotor activity. An increase in OAT% and/or OAE% was interpreted as an anxiolytic-like response [19].

2.6. Open Field Test

An open field test (OFT) was used to assess locomotor activity and anxiety-related behavior in animals. Rats were placed separately in a white box (50 × 50 × 50 cm) for 5 minutes, and the following behavioral indices were scored: total distance moved (cm), velocity (cm/s), and total duration in inner zone (s). The activity of animals was monitored and analyzed by Ethovision software. After the 5-minute duration, each animal was removed from the open field, and

[3] National Institutes of Health (NIH)
[4] Elevated Plus-Maze (EPM)
[5] Forced Swimming Test (FST)
[6] Open Field Test (OFT)
[7] Open Arm Time (OAT)
[8] Open Arm Entries (OAE)

the chamber was cleaned with diluted ethanol (10%) and dried [20].

2.7. Forced Swimming Test

FST is a valid method for the assessment of depression and a useful tool for the evaluation of antidepressants' efficacy. Rats were placed in a cylindrical tank (50 cm height × 25 cm diameter) filled with tap water (up to 35 cm of height) having temperature 23-25°C. They were allowed to swim freely for 6 minutes. Immobility was defined as a static state in the water, and this time is recorded as an important index. High immobility time represents depression, while lower immobility time infers an anti-depressive effect [21, 22].

2.8. Statistical Analysis

Data analysis was performed using GraphPad Prism data analysis program version 6 (GraphPad Software San Diego, CA, USA). One-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons was used. The results were expressed as mean ± standard error of the mean (SEM), and P-value less than 0.05 was considered statistically significant.

3. Results

3.1. Effect of Pistachio Oil on Depressive-Like Behaviors in Letrozole-Induced PCOS Rats in the FST Test

Data showed that animals treated with letrozole for PCOS induction developed depression, and immobility time was significantly higher than that of the control group (P<0.001, Figure 1). Interestingly, pistachio oil with both 1 ml/kg and 4 ml/kg could prevent mood disorder in PCOS animals.

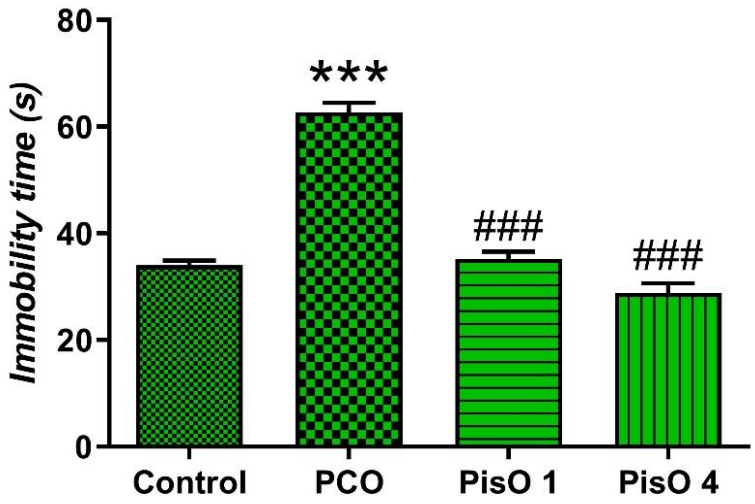

Fig. 1: The anti-depressive effects of pistachio oil on animals with polycystic ovary syndrome

Data expressed as mean±SEM N=7. ***, P<0.001 compared with control. ###, P<0.001 compared with the PCOS group. PCO: polycystic ovary syndrome group. PisO1: PCOS rats treated with pistachio oil 1 ml/kg. PisO4: PCOS rats treated with pistachio oil 4 ml/kg.

3.2. Effect of Pistachio Oil on Locomotor Activity and Anxiety-Like Behaviors in Letrozole-Induced PCOS Rats in the OFT Test

As depicted in figure 2 for anxiety-related behavior, the PCOS group spent less time in the inner zone compared to the control group (P<0.05, A). Figure 2 also represented that for locomotor activity, the traveled distance, as well as velocity of movements, were not significantly different among the experimental groups (B and C).

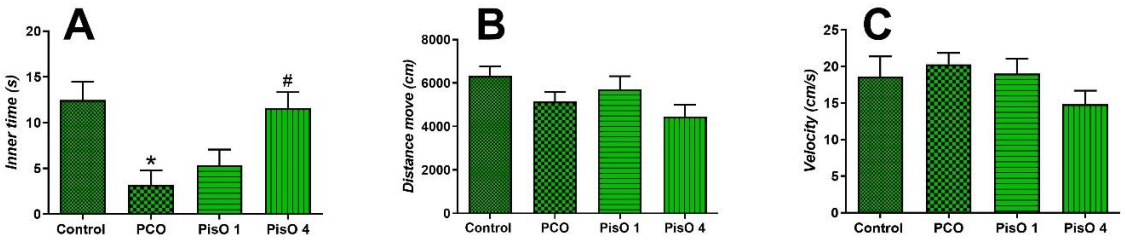

Fig. 2: The effects of pistachio oil on locomotor activity and anxiety under polycystic ovary syndrome condition. Data expressed as mean±SEM N=7.*, P<0.05 compared with control. #<0.05 compared with the PCOS group. PCO: polycystic ovary syndrome group. PisO1: PCOS rats treated with pistachio oil1 ml/kg. PisO4: PCOS rats treated with pistachio oil 4 ml/kg.

3.3. Effect of Pistachio Oil Anxiety-Like Behaviors in Letrozole-Induced PCOS Rats in the EPM Test

As depicted in figure 3, there was a significant difference in OAT% and OAE% parameters in comparison with healthy animals (without PCOS) (P<0.05 for both). Treatment with pistachio oil significantly enhanced factors as mentioned earlier (P<0.05 for OAT% for both doses of oil, P<0.01 and P<0.001 for OAE% in 1 ml/kg and 4 ml/kg of oil, respectively). There was no difference with respect to total arms entries.

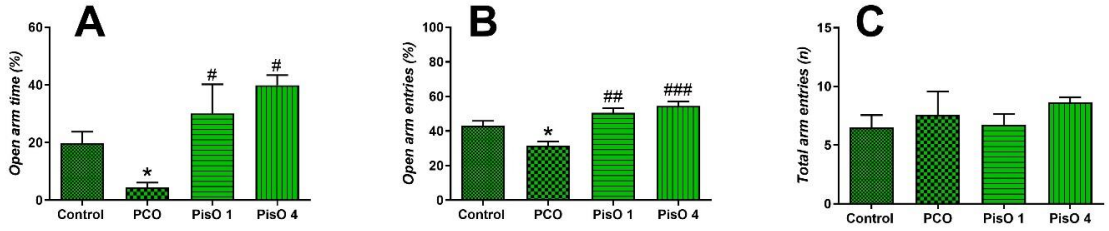

Fig. 3: The effects of pistachio oil on anxiety under polycystic ovary syndrome condition

Data expressed as mean±SEM N=7. *, P<0.05 compared with control. #<0.05 and ##, P<0.01 compared with the PCOS group. PCO: polycystic ovary syndrome group. PisO1: PCOS rats treated with pistachio oil 1 ml/kg. PisO4: PCOS rats treated with pistachio oil 4 ml/kg.

4. Discussion

The data showed that applying PCOS to female rats led to an elevation in immobility time in the FST as a standard screening test for behavioral despair evaluation in rodents. Moreover, PCOS animals reflected an anxiety state compared to the healthy group. Experimental groups exhibited no impairments in locomotor activity. Pistachio oil administration could reverse the deteriorating effects of PCOS on behavioral parameters.

Polycystic ovary syndrome (PCOS) with 5-10% prevalence is a common cause of infertility and has complex pathophysiology [23]. PCOS could be induced in animals through different methods, such as administration of estradiol valerate and dehydroepiandrosterone [24, 25].

Another standard model is letrozole-induced polycystic ovaries [26]. In the present research, letrozole was used in order to induce PCOS. There are reports of PCOS treatment with herbal remedies. Reddy et al. investigated the effects of curcumin in Wistar rats with PCOS [27]. Kort et al. suggested that cinnamon supplementation is effective in women with PCOS [28]. Further, the possible beneficial role of Vitex agnus-castus and soy isoflavones has been investigated [29, 30]. In the present work, the protection of *Pistacia vera* against PCOS behavioral complications is investigated.

Recently, it has been shown that besides metabolic complications, PCOS is associated with an increased risk of depression [31]. Himelein and Thatcher showed that women with PCOS display higher depression and greater body dissatisfaction [32]. Kerchner et al. indicated that there is a higher risk of mood disorders and depression in women with PCOS [33]. Our observation showed that animals with PCOS encountered with depressive disorder in comparison with the non-PCOS group, which is in line with previous studies.

Studies indicated that PCOS might be related to anxiety disorder. Dokras et al. underscored a need for screening PCOS women for mood or anxiety disorders and adequately treat patients who are diagnosed with those conditions [5]. In another investigation, Dokras et al. suggested an increased risk of anxiety symptoms PCOS patients that clarified the follow-up evaluation and treatment necessity as chronic conditions. Moreover, other potential contributors to anxiety, including obesity, infertility, or hirsutism, should be considered [34]. Parallel to the previous study, our data revealed that PCOS condition in animals might be accompanied by anxiety-like behaviors in open field and elevated plus-maze tests.

It is well documented that *Pistacia* species, belonging to Anacardiaceae, contain several active substances that are the main cause of different pharmacological functions, such as phytosterol, flavonoids, alpha- or beta-pinene, alpha-tocopherol and campesterol, as well as stigmasterol and β-sitosterol [9, 35, 36]. These active constituents play anti-inflammatory and antioxidant roles; they are the possible mechanism of action for pistachio beneficial health effects [37].

Different studies demonstrated the neuroprotective properties of *Pistacia* species. Fatehi et al. showed that the hydroalcoholic extract of *Pistacia vera* inhibits the development of seizure following the chronic pentylenetetrazole-induced model of epilepsy in animals [12]. Golchin et al. reported that rat supplementation with pistachio attenuates motor and cognition impairments induced by vincristine or cisplatin [38]. Ziaee and Hosseinzadeh revealed that hydroalcoholic extract of *Pistacia vera* gum exerts muscle relaxant, hypnotic, and anti-anxiety activities. Thus, this herbal medicine may be beneficial in the alleviation of insomnia, anxiety, and muscle contraction disorders [14]. Our observation showed that pistachio oil could be protective in PCOS-induced neurobehavioral deficits.

5. Conclusions

To conclude, the results of this study showed that the administration of *Pistacia vera* oil in female rats with PCOS could alleviate depression and anxiety. Further investigations are necessary to clarify the *Pistacia vera* potential protection against PCOS other complications and probable action mode.

Conflict of interest

The authors declare no conflicts of interest.

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