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Effects of *Cornus mas* Extract (Anthocyanin) and Treadmill Exercise on Hormonal and Histological Effects in the Rat Model of Polycystic Ovary Syndrome

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Abstract

Objectives: This study aimed to assess the protective impact of *Cornus mas* extract and treadmill exercise on hormonal and metabolic parameters in adult rats with polycystic ovary syndrome (PCOS).

Materials and Methods: Forty female Wistar rats were divided into control and experimental groups. The experimental group was further divided into 5 subgroups, namely PCOS control group (G1), PCOS group treated with *C. mas* extract (100 mg/kg/orally/daily) (G2), PCOS group treated with scheduled treadmill exercise (G3), PCOS group treated with both extract and exercise (G4), and PCOS group treated with *C. mas* extract and treadmill exercise (G5). PCOS was induced in G1 by a single injection of estradiol valerate (16 mg/kg, IM). After a 3-week treatment period, all rats were anesthetized and their blood samples were collected for testing the levels of glucose, insulin, aromatase, and hormones (LH, FSH, testosterone, and estrogen). Then their ovaries were removed for histopathological examination.

Results: Serum levels of FBS, insulin, luteinizing hormone [LH], follicle-stimulating hormone [FSH], and testosterone were significantly increased in PCOS compared to the control group ($P<0.05$); however, these levels were significantly decreased in the treated groups compared to PCOS ($P<0.05$). Serum estrogen levels and aromatase activity were significantly decreased in PCOS compared to the control ($P<0.05$); however, they were significantly increased in the treated groups compared to PCOS ($P<0.05$). PCOS led to a reduction in the number of follicles, which was prevented by *C. mas* extract.

Conclusions: In sum, *C. mas* extract and treadmill exercise had a positive impact on LH, FSH, testosterone, estradiol, aromatase, FBS, and insulin levels compared to the PCOS group.

Keywords: Cornus mas, PCOS, Hormones, Ovary, Rat

Introduction

Polycystic ovary syndrome (PCOS) is a prevalent reproductive endocrine disorder affecting 5% to 21% of the women in their reproductive years. Its occurrence varies globally, ranging from 2.2% to 26% in different countries, with higher rates observed in the USA, Germany, and Italy (1). PCOS is characterized by features such as polycystic ovaries, elevated luteinizing hormone (LH), hyperandrogenism, chronic anovulation, oligo-amenorrhea, obesity, and infertility. Furthermore, PCOS is recognized as a metabolic syndrome, posing the risks of insulin resistance, dyslipidemia, impaired glucose tolerance, hypertension, and cardiovascular disease (2).

The complex pathogenesis of PCOS involves hormonal imbalances, including decreased progesterone and increased testosterone, estrogen, and LH. Hyperinsulinemia is strongly correlated with hyperandrogenism, and insulin levels show a positive correlation with adrenal steroid excretion in PCOS patients. Hyperinsulinemia in PCOS is associated with elevated levels of total cholesterol, low-density lipoprotein (LDL), triglycerides, very-low-density lipoprotein (VLDL), and reduced high-density lipoprotein (HDL) concentration. Beyond insulin resistance, the metabolism of lipids in females with PCOS may be influenced by the ovarian or adrenal secretion of sex steroid hormones, which have multifaceted effects on lipid metabolism (2-5).

PCOS is, in addition to the metabolic changes, marked by characteristic alterations in reproductive hormones, including an elevated LH/follicle-stimulating hormone (FSH) ratio and increased circulating androgens, thereby contributing to traditional PCOS symptoms like hirsutism, acne, and acanthosis nigricans (6,7).

Herbal medicine, known for its pharmacologically active constituents affecting the female endocrinology, is commonly used for treating PCOS, diabetes, and cardiovascular disease (8). *Cornus mas* L. (cornelian cherry), a medicinal plant with therapeutic effects

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Key Messages

- PCOS leads to ovarian dysfunction and hormone imbalance in female.
- Treadmill exercise can improve the hormone profile in female rats with PCOS.
- *Cornus mas* extract can improve the ovarian tissue damage related to PCOS.

attributed to compounds like anthocyanins, flavonoids, iridoids, phenolic acids, and tannins, is of particular interest. Experimental studies have suggested that anthocyanins, abundant in cornelian cherry, may improve conditions such as obesity, insulin resistance, inflammation, and dyslipidemia, potentially mitigating cardiovascular risks (9-11).

Given that PCOS induces hormonal and metabolic damage to organs like the ovaries, this study aimed to assess the protective effects of *C. mas* extract and treadmill exercise on ovarian tissue damage and factors related to PCOS.

Materials and Methods

In this study, 40 female Wistar rats aged eight weeks and weighing between 200-250 g were included. The rats were housed in the animal facility under standard conditions, including a temperature of 25°C and a 12-hour light/12-hour dark cycle. The rats were provided with unrestricted access to food and water during the experimental period. The sample size was calculated based on the following formula, and eight rats were considered for each group:

$$n = 1 + 2C\left(\frac{s}{d}\right)^2 = 1 + 2 * 7.85\left(\frac{0.212}{0.556}\right)^2 \approx 4$$

$$\acute{n}=n\sqrt{g-1}=4\sqrt{5-1}=8$$

Experimental Groups and Study Design

The rats were randomly assigned to 5 groups, each consisting of eight rats:

- G1: Control group (Control): Healthy rats without any interventions.
- G2: PCOS control group (PCO): PCOS induced by a single intramuscular injection (IM) of estradiol valerate (16 mg/kg) dissolved in 0.2 mL sesame oil.
- G3: PCOS group treated with *C. mas* extract (*Cornus*): Rats with PCOS treated with hydroalcoholic extract of *C. mas* (100 mg/kg/orally/daily) for 21 days (12).
- G4: PCOS group with treadmill exercise (exercise): PCOS rats subjected to scheduled treadmill exercise for 21 days.
- G5: PCOS group treated with *C. mas* extract and treadmill exercise (*Cornus* + Exercise): PCOS rats treated with hydroalcoholic extract of *C. mas* and treadmill exercise for 21 days.

The estrus cycle of all rats was monitored through daily examination of vaginal smears. After the 21-day treatment period, all rats were anesthetized with ketamine and xylazine, and their blood samples were collected from the heart. The obtained sera were separated by centrifuge and stored at -70 °C. Subsequent tests included measuring glucose, insulin, aromatase activity, and hormone levels (LH, FSH, testosterone, and estrogen) (1, 13).

Preparation of Hydroalcoholic Extract of Cornus mas

To obtain the *C. mas* extract, half a kilogram of the plant was sourced from East Azerbaijan, Iran. The extraction process involved dissolving the plant in 2 L of 96% alcohol and distilled water. This solution was then placed on a shaker (Thermo Fisher) at room temperature for 48 hours. Subsequently, the solution underwent filtration and centrifugation for 5 minutes at 3000 rpm. The resulting solution was transferred to an open-top container, and the solvent was allowed to evaporate. To achieve the desired concentration, the extract was dissolved in normal saline (1,14).

Treadmill Exercise Protocol

After completing the initial 72-hour "Treadmill Exercise Protocol (TEM)," the modified TEM was initiated, consisting of 8 weeks of daily one-hour sessions conducted 5 days a week, maintaining a 0º slope. The focus in the first week was placed on adaption to moderate-intensity exercise, incorporating a gradual increase in both time and speed. Starting from the second week, each session consisted of three periods:

1. "Warm-up" lasting 5 minutes at 30% of Smax1;
2. "Moderate intensity exercise" covering 50 minutes at 60% of Smax1; and
3. Recovery" ranging from 5 minutes to 30% of Smax1.

During these sessions, a low-intensity electric stimulus (1.5 mA - 2.0 mA) positioned at the back of each lane was employed to stimulate the animals (15).

Assays of Serum Glucose Level

The fasting blood sugar (FBS) levels were assessed at the beginning of the study using a portable glucometer and utilizing samples obtained from the tip of the rat's tail vein. After the study period, serum glucose levels were determined using commercial kits from Sigma, Germany. The results were expressed in units of mg/dL.

Assay of Serum Insulin Level

The quantification of serum insulin levels was carried out by adopting an enzyme-linked immunosorbent assay (ELISA) method and utilizing a commercial Rat Insulin kit from Mercodia, Germany. The insulin concentration in the serum was expressed in units of mIU/mL.

Measurement the Serum Levels of Hormones

The serum hormone levels were assessed using ELISA kits for testosterone and estrogen from Sigma, Germany. The absorbance for testosterone and estrogen was measured at 405 nm. Furthermore, the serum levels of LH, FSH, and

aromatase were determined using an ELISA kit sourced from Zelbio, Italy.

Histological Study

To perform histological and histometrical investigations, the tissue sections were obtained from each ovarian sample (n=7), extending from the cortex to the medulla in a spiral and clockwise orientation. On each slide, the enumeration included the count of primary, per antral, antral, cystic follicles, and yellow bodies (16).

Statistical Analysis

The statistical analysis was conducted using SPSS 19 (IBM, USA). The data were expressed as the mean ± standard error (SE). One-way analysis of variance (ANOVA) was employed, followed by the Tukey post hoc test for comparing the values. Statistical significance levels were set at $P<0.05$.

Results

Serum Level of FBS

The serum FBS level was significantly increased in the PCOS group compared to the control group ($P<0.05$). In the therapeutic groups receiving 100 mg/kg of *C. mas* extract and performing treadmill exercise, however, the serum glucose level was notably lower than that of the PCOS group ($P<0.05$) (Figure 1).

Serum Level of Insulin

The serum insulin level was significantly increased in the PCOS group compared to the control group ($P<0.05$), as illustrated in Figure 2. Conversely, there was a significant decrease in the treated groups, including those administered with *C. mas* extract, treadmill exercise, and the combined treatment (G5 with both extract and exercise), when compared to the PCOS group ($P<0.05$).

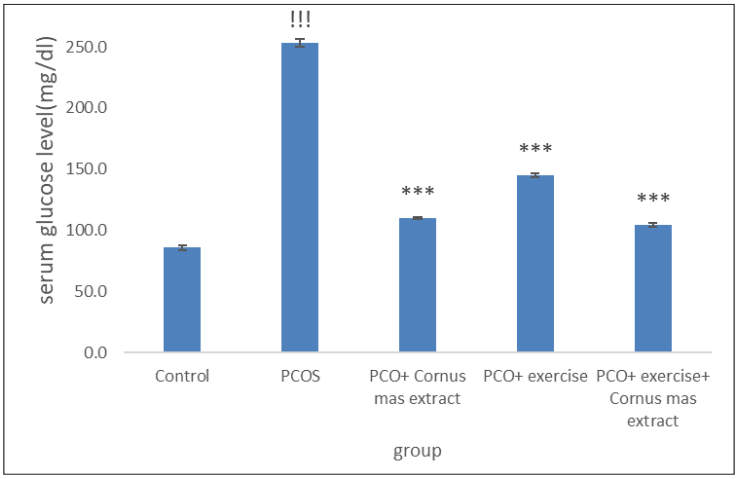

Figure 1. The Serum Level of Glucose in the Study Groups. Control: healthy control group; PCOS: PCOS group that received normal saline by oral gavage; PCO+ Cornus mas extract: PCOS group treated with hydroalcoholic extract 100 mg/kg of *Cornus mas extract*; PCO+ exercise: PCOS group treated with treadmill exercise; PCO+ exercise+ Cornus mas extract: PCOS group treated with treadmill exercise and extract. All data are displayed as mean ± SE. The symbol **!!!** shows significant difference with control group, and the symbol of *** indicates the significant difference with PCOS group ($P<0.0001$).

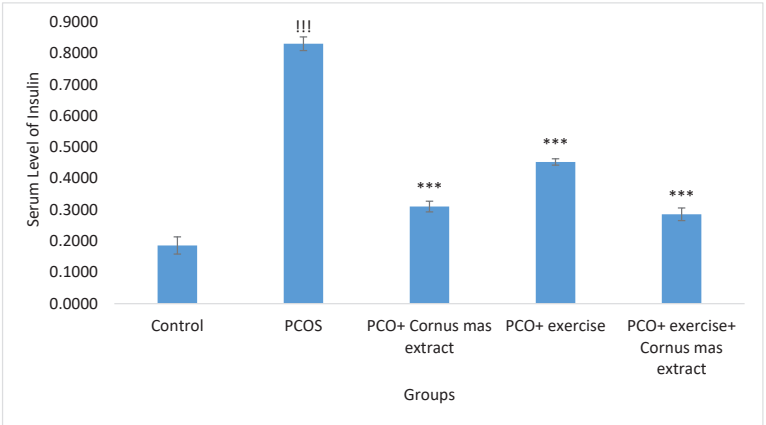

Figure 2. The Serum Level of Insulin in the Study Groups. Control: healthy control group; PCOS: PCOS group that received normal saline by oral gavage; PCO+ Cornus mas extract: PCOS group treated with hydroalcoholic extract 100 mg/kg of *Cornus mas extract*; PCO+ exercise: PCOS group treated with treadmill exercise; PCO+ exercise+ Cornus mas extract: PCOS group treated with treadmill exercise and extract. All data are displayed as mean ± SE. The symbol **!!!** shows significant difference with control group, and the symbol of *** indicates the significant difference with PCOS group ($P<0.0001$).

The Serum Levels of Testosterone, Estrogen, LH, FSH

The serum levels of testosterone, estrogen, LH, and FSH are summarized in Table 1. Notably, the serum testosterone level was significantly elevated in the PCOS group compared to the control group ($P<0.05$). In all therapeutic groups receiving *C. mas* extract, treadmill exercise, and the combined treatment (G5 with both extract and exercise), however, the serum testosterone levels were significantly decreased in comparison to the PCOS group ($P<0.05$).

Regarding serum estrogen levels, a significant difference was observed between the PCOS and control groups ($P<0.05$). However, the serum estrogen levels were significantly increased in the therapeutic groups compared to the PCOS group ($P<0.05$).

PCOS induced a notable increase in serum levels of LH and FSH in the PCOS group compared to the control group ($P<0.05$). However, the serum levels of gonadotropin hormones (LH and FSH) in the groups treated with extract and exercise were significantly lower than those in the PCOS group ($P<0.05$).

The Number of Follicles

Table 2 presents the counts of pre-antral follicles, antral follicles, cystic follicles, and yellow bodies, while Figure 3 depicts the light microscope micrograph of ovaries.

The number of primary follicles exhibited a significant decline in the PCOS group compared to the control group ($P<0.05$). However, a significant difference was observed between the PCOS group and both therapeutic groups ($P<0.05$).

Pre-antral follicles were significantly decreased in the PCOS group compared to the control group ($P<0.05$). In contrast, the number of pre-antral follicles was significantly increased in both therapeutic groups compared to the PCOS group ($P<0.05$).

The count of antral follicles significantly decreased in the PCOS group compared to the control group ($P<0.05$). Conversely, the number of antral follicles in the groups treated with extract and exercise was significantly higher than that in the PCOS group ($P<0.05$).

The count of cystic follicles was significantly increased in the PCOS group compared to the control group ($P<0.05$). Treatment with *C. mas* extract significantly decreased the number of cystic follicles compared to the PCOS group ($P<0.05$).

The evaluation of yellow bodies revealed a significant decrease in the PCOS group compared to the control group ($P<0.05$). There was a significant increase in the number of yellow bodies in all treated groups compared to the PCOS group ($P<0.05$).

Discussion

In the present study, the therapeutic effects of *C. mas* extract and treadmill exercise on a rat model of PCOS were closely investigated. The multifaceted nature of PCOS, characterized by hormonal imbalances and ovarian morphological alterations, prompted an exploration into potential interventions. The comprehensive analysis encompassed various parameters, including serum glucose, insulin, sex hormones, and ovarian histology. The findings shed light on promising avenues for mitigating

Table 1. The Serum Levels of Hormone Profile

Groups	LH (ng/mL)	FSH (ng/mL)	Testosterone (ng/mL)	Estrogen (pg/mL)
Control	1.85± 0.17	1.95±0.15	0.45±0.014	51.0±2.15
PCOS	4.25±0.15[a]	3.32± 0.45[a]	3.15±0.17[a]	22.15 ±1.20[a]
PCO + *Cornus mas* extract	2.35±0.12[b]	2.15±0.25[b]	1.22±0.025[b]	37.35±2.18[b]
PCO + exercise	2.05±0.70[b]	2.35±0.15[b]	1.17±0.033[b]	30.15±2.17[b]
PCO + exercise + *Cornus mas* extract	2.05±0.70[b]	2.28±0.20[b]	1.15±0.053[b]	41.25±2.20[b]

Control: healthy control group; PCOS: PCOS group that received normal saline by oral gavage; PCO + Cornus mas extract: PCOS group treated with hydroalcoholic extract 100 mg/kg of *Cornus mas extract*; PCO+ exercise: PCOS group treated with treadmill exercise; PCO+ exercise+ Cornus mas extract: PCOS group treated with treadmill exercise and extract. All data are displayed as mean ± SE.
The asterisk [a] shows significant difference with control group, and the symbol of [b] means the significant difference with PCOS group ($P < 0.05$).

Table 2. The Number of Primary, Per-antral, Antral, Cystic Follicles and Yellow Body in Different Groups

Group	Primary Follicles	Pre-antral Follicles	Antral Follicles	Cystic Follicles	Yellow Body
Control	50.4±1.50	30±1.35	17.6±1.25	0	9.8±0.53
PCOS	26.6±1.45[a]	15.4±1.20[a]	3.5±1.03[a]	6.4±0.64[*]	1.6±0.53[a]
PCO + *Cornus mas* extract	40.4±2.14[b]	25±2.25[b]	10.4±1.30[b]	2.4±0.44[b]	5.5±0.23[b]
PCO + exercise	36.8±1.73[b]	22±1.24[b]	9.6±1.15[b]	3.2±0.63[b]	4.4±0.24[b]
PCO + exercise + *Cornus mas* extract	42.8±1.63[b]	26±1.50[b]	12.6±1.20[b]	1.5±0.53[b]	6.5±0.52[b]

Control: healthy control group; PCOS: PCOS group that received normal saline by oral gavage; PCO + Cornus mas extract: PCOS group treated with hydroalcoholic extract 100 mg/kg of *Cornus mas extract*; PCO+ exercise: PCOS group treated with treadmill exercise; PCO+ exercise+ Cornus mas extract: PCOS group treated with treadmill exercise and extract. All data are displayed as mean ± SE.
The asterisk [a] shows significant difference with control group, and the symbol of [b] means the significant difference with PCOS group ($P < 0.05$).

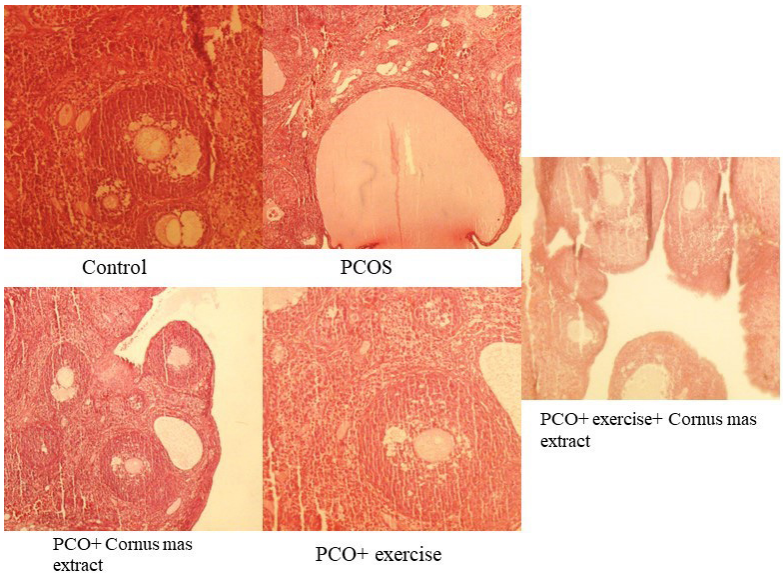

Figure 3. The Histological Findings. Control: healthy control group; PCOS: PCOS group that received normal saline by oral gavage; PCO + *Cornus mas* extract: PCOS group treated with hydroalcoholic extract 100 mg/kg of *Cornus mas* extract; PCO+ exercise: PCOS group treated with treadmill exercise; PCO+ exercise+ *Cornus mas* extract: PCOS group treated with treadmill exercise and extract. H&E staining (X100).

the impact of PCOS.

One of the key observations in this study was the significant increase in FBS levels in the PCOS group, indicative of an altered glucose metabolism. PCOS is frequently associated with insulin resistance, leading to the elevated FBS levels (17,18). Intriguingly, the therapeutic groups receiving *C. mas* extract and performing treadmill exercise demonstrated a notable reduction in serum glucose levels, which was suggestive of the fact that both interventions may have contributed to the improved glucose homeostasis in the context of PCOS (15,19). In this context, Abtahi et al showed that the PCOS induced in rats led to an increase in FBS and metabolic syndrome, and treatment with Antioxidant component may have reduced the FBS (1).

In concordance with the altered glucose metabolism, Moghetti and Tosi investigated the realm of insulin dynamics. The PCOS group exhibited a significant increase in serum insulin levels, underscoring insulin resistance, a common feature of PCOS (20). However, the therapeutic groups, particularly those treated with *C. mas* extract and treadmill exercise, displayed a substantial decrease in insulin levels. This implied that these interventions may have exerted beneficial effects on insulin sensitivity, potentially addressing one of the core pathophysiological aspects of PCOS (21,22). In study by Bannigida et al, it was found that PCOS in women led to insulin resistance and metabolic sundrome (23). Naidu et al also reported that treating PCOS with antioxidant may have reduced the insulin level in serum (24).

The perturbation in sex hormone levels, including testosterone and estrogen, is a hallmark of PCOS. Verrotti et al found a significant elevation in serum testosterone levels in the PCOS group, aligning with the hyperandrogenism characteristic of PCOS (25). Notably, the therapeutic groups exhibited a marked reduction in testosterone levels, indicating the potential of *C. mas* extract and treadmill exercise for modulating androgen levels. Conversely, serum estrogen levels, which revealed a significant change between the PCOS and control groups, were elevated in the therapeutic groups. This shift in estrogen levels may have had implications for restoring hormonal balance in PCOS (26). Khodaeifar et al demonstrated that PCOS resulted in imbalance in sex hormone, and that a treatment with antioxidant may have balanced the level of sex hormone (13). Rashidy-Pour et al also reported that the treadmill exercise may have balanced the sex hormone (27).

Gonadotropins, specifically LH and FSH, play a pivotal role in ovarian function. LH and FSH levels were significantly increased in the PCOS group, indicative of the disrupted gonadotropin dynamics (1,13). Interestingly, LH and FSH levels were significantly decreased in the therapeutic groups, suggesting a potential regulatory effect of *C. mas* extract and treadmill exercise on gonadotropin secretion. This modulation may have contributed to normalizing the altered reproductive hormone milieu associated with PCOS (28). Since granulosa cells in PCOS are damaged and undergo apoptosis, the receptor for gonadotropin hormones is lost and the level of these hormones in the blood serum increases (29).

The histological examination of the ovarian tissue provided valuable insights into the impact of PCOS and the therapeutic interventions. The decline in the number of primary follicles in the PCOS group highlighted the adverse effects on ovarian follicular development (30,31). However, the therapeutic groups exhibited a notable preservation of primary follicles, indicating a protective

effect conferred by *C. mas* extract and treadmill exercise. Additionally, the increase in the number of pre-antral follicles in the therapeutic groups suggested a potential stimulatory effect on early follicular development. Sharma et al argued that PCOS resulted in an increase in the cystic follicle, a reduction in antral and graafian follicles, and a reduction in granulosa cell (31). Rajaei et al determined that antioxidant therapy may have protected the ovary against tissue damage (32). Oxidative stress and the activity of free radicals can lead to damage to ovarian tissue and a reduction in the growth of follicles (14,32). A treatment with antioxidant compounds improves the ovarian tissue and follicle growth by preventing the activity of free radicals, thereby increasing the activity of the antioxidant enzymes and reducing the oxidative stress (8,13). Since *C. mas* extract has antioxidant and anti-inflammatory compounds, on the other hand, it can prevent the ovarian damage (19).

An intriguing finding was a significant decrease in antral follicles recorded for the PCOS group, indicative of the impaired follicular maturation. The therapeutic groups, particularly those treated with *C. mas* extract and exercise, exhibited a significant increase in the number of antral follicles, which was suggestive of the potential of these interventions to promote follicular maturation, a crucial aspect for normal ovarian function (33).

The substantial presence of cystic follicles in the PCOS group underscored the characteristic ovarian cysts associated with the syndrome (30). However, the therapeutic use of *C. mas* extract resulted in a significant decrease in cystic follicles, indicating a potential therapeutic benefit in mitigating cyst formation. Moreover, the evaluation of yellow bodies revealed a significant decrease in the PCOS group, suggesting the impaired luteinization. Notably, all therapeutic groups exhibited a significant increase in yellow bodies, implying a positive impact on luteinization processes. Rahayu et al showed that the exercise may have improved the follicle development and protected the ovarian tissue against oxidative stress (34).

Conclusions

It was concluded that the *C. mas* extract and treadmill exercise produced promising therapeutic effects in a rat model of PCOS. The multifaceted approach, encompassing metabolic, hormonal, and histological parameters, provided a comprehensive understanding of the potential benefits of these interventions. The observed improvements in glucose metabolism, insulin sensitivity, sex hormone levels, and ovarian histology suggested that *C. mas* extract and treadmill exercise may have served as valuable adjuncts in managing the complexities of PCOS. However, it was recommended that further studies and clinical trials should be conducted in order to elucidate the underlying mechanisms and validate the translational potential of these interventions in the context of PCOS.

Authors' Contribution

Conceptualization: Ramesh Baradaran Bagheri, Negin Chavoshinezhad, Bita Barghi, Maryam Soleymaniinallou, Majid Shokoohi.
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Methodology: Majid Shokoohi, Peyman Najafnezhad, Amir Afshin Khaki.
Project administration: Majid Shokoohi.
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Software: Amir Afshin Khaki, Farnaz Dabiri.
Supervision: Amir Afshin Khaki.
Validation: Amir Afshin Khaki, Majid Shokoohi.
Visualization: Amir Afshin Khaki.
Writing–original draft: Ramesh Baradaran Bagheri, Negin Chavoshinezhad, Bita Barghi, Maryam Soleymaniinallou, Majid Shokoohi.
Writing–review & editing: Ramesh Baradaran Bagheri, Negin Chavoshinezhad, Bita Barghi, Maryam Soleymaniinallou, Majid Shokoohi, Amir Afshin Khaki.

Conflict of Interests

Authors declare that they have no conflict of interests.

Ethical Issues

All experiments were performed in accordance with the protocols specified in the guidelines of the Tabriz University of Medical Science. The study received ethical approval under the code IR.TBZMED.VCR.REC.1397. 22.

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References

1. Abtahi-Eivari SH, Moghimian M, Soltani M, et al. The effect of Galega officinalis on hormonal and metabolic profile in a rat model of polycystic ovary syndrome. Int J Womens Health Reprod Sci. 2018;6(3):276-282. doi:10.15296/ijwhr.2018.46
2. Spinedi E, Cardinali DP. The polycystic ovary syndrome and the metabolic syndrome: a possible chronobiotic-cytoprotective adjuvant therapy. Int J Endocrinol. 2018;2018:1349868. doi:10.1155/2018/1349868
3. Ouladsahebmadarek E, Khaki A, Khanahmadi S, Ahmadi Ashtiani H, Paknejad P, Ayubi MR. Hormonal and metabolic effects of polyunsaturated fatty acid (omega-3) on polycystic ovary syndrome induced rats under diet. Iran J Basic Med Sci. 2014;17(2):123-127.
4. Kupreeva M, Diane A, Lehner R, et al. Effect of metformin and flutamide on insulin, lipogenic and androgen-estrogen signaling, and cardiometabolic risk in a PCOS-prone metabolic syndrome rodent model. Am J Physiol Endocrinol Metab. 2019;316(1):E16-E33. doi:10.1152/ajpendo.00018.2018
5. Hosseinpanah F, Barzin M, Erfani H, Serahati S, Ramezani Tehrani F, Azizi F. Lipid accumulation product and insulin resistance in Iranian PCOS prevalence study. Clin Endocrinol (Oxf). 2014;81(1):52-57. doi:10.1111/cen.12287
6. Krishnan A, Muthusami S. Hormonal alterations in PCOS and its influence on bone metabolism. J Endocrinol. 2017;232(2):R99-R113. doi:10.1530/joe-16-0405
7. Gargus ES, Bae Y, Chen J, et al. An ovarian steroid metabolomic pathway analysis in basal and polycystic ovary syndrome (PCOS)-like gonadotropin conditions reveals a hyperandrogenic phenotype measured by mass spectrometry. Biomedicines. 2022;10(7):1646.

doi:10.3390/biomedicines10071646
8. Khodaeifar F, Fazljou SM, Khaki A, et al. 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. Int J Womens Health Reprod Sci. 2019;7(1):92-8. doi:10.15296/ijwhr.2019.15
9. Szczepaniak OM, Kobus-Cisowska J, Kusek W, Przeor M. Functional properties of Cornelian cherry (*Cornus mas* L.): a comprehensive review. Eur Food Res Technol. 2019;245(10):2071-2087. doi:10.1007/s00217-019-03313-0
10. Baldea I, Florea A, Olteanu D, et al. Effects of silver and gold nanoparticles phytosynthesized with *Cornus mas* extract on oral dysplastic human cells. Nanomedicine (Lond). 2020;15(1):55-75. doi:10.2217/nnm-2019-0290
11. Tiptiri-Kourpeti A, Fitsiou E, Spyridopoulou K, et al. Evaluation of antioxidant and antiproliferative properties of *Cornus mas* L. fruit juice. Antioxidants. 2019;8(9):377. doi:10.3390/antiox8090377
12. Ahmadi N, Khaki AA, Khodarahmi P, Baharara J, Zakerbostanabadi S. Effect of anthocyanin on oxidative stress and testis structure in streptozotocin-induced diabetic rats. Crescent J Med Biol Sci. 2023;10(2):56-60. doi:10.34172/cjmb.2023.09
13. Khodaeifar F, Fazljou SM, Khaki A, et al. The effect of hydroalchoholic extract of cinnamon zeylanicum on oxidative damages and biochemical change in adult rats with polycystic ovary syndrome. Crescent J Med Biol Sci. 2019;6(4):511-516.
14. Soltani M, Moghimian M, Abtahi H, Shokoohi M. The protective effect of Matricaria chamomilla extract on histological damage and oxidative stress induced by torsion/detorsion in adult rat ovary. Int J Womens Health Reprod Sci. 2017;5(3):187-192. doi:10.15296/ijwhr.2017.34
15. Hamidi H, Tofighi A, Toluei Azar J, Khaki AA, Razi M. Effect of crocin and treadmill exercise on spermatogenesis and testis structure in streptozotocin-induced diabetic rats: an experimental study. Crescent J Med Biol Sci. 2023;10(2):94-108. doi:10.34172/cjmb.2023.15
16. Tafrishi Nejad SR, Khaki A, Abbasalizadeh S, Shokoohi M, Ainehchi N. Protective effect of hydroalcoholic extract of orange peel on PCNA and FSH-R gene expression in histological damage and oxidative stress due to ovarian torsion in adult rats. Int J Womens Health Reprod Sci. 2021;9(3):205-211. doi:10.15296/ijwhr.2021.38
17. Hasan SS, Sheikh Sahib K. Detection levels of FBS, HbA1c and lipid profile in patients with polycystic ovary syndrome in Diyala province. Biochem Cell Arch. 2022;22(1):183-188.
18. Bennal A, Kerure S. Effect of PCOS on glucose metabolism. Natl J Physiol Pharm Pharmacol. 2013;3(2):167-170. doi:10.5455/njppp.2013.3.220420132
19. Soltani R, Gorji A, Asgary S, Sarrafzadegan N, Siavash M. Evaluation of the effects of *Cornus mas* L. fruit extract on glycemic control and insulin level in type 2 diabetic adult patients: a randomized double-blind placebo-controlled clinical trial. Evid Based Complement Alternat Med. 2015;2015:740954. doi:10.1155/2015/740954
20. Moghetti P, Tosi F. Insulin resistance and PCOS: chicken or egg? J Endocrinol Invest. 2021;44(2):233-244. doi:10.1007/s40618-020-01351-0
21. Marques CM, Motta VF, Torres TS, Aguila MB, Mandarim-de-Lacerda CA. Beneficial effects of exercise training (treadmill) on insulin resistance and nonalcoholic fatty liver disease in high-fat fed C57BL/6 mice. Braz J Med Biol Res. 2010;43(5):467-475. doi:10.1590/s0100-879x2010007500030
22. Fatima Q, Amin S, Kawa IA, et al. Evaluation of antioxidant defense markers in relation to hormonal and insulin parameters in women with polycystic ovary syndrome (PCOS): a case-control study. Diabetes Metab Syndr. 2019;13(3):1957-1961. doi:10.1016/j.dsx.2019.04.032
23. Bannigida DM, Nayak BS, Vijayaraghavan R. Insulin resistance and oxidative marker in women with PCOS. Arch Physiol Biochem. 2020;126(2):183-186. doi:10.1080/13813455.2018.1499120
24. Naidu JN, Swapna GN, Kumar AN, Krishnamma M, Anitha M. Importance of elevated insulin resistance, dyslipidemia and status of antioxidant vitamins in polycystic ovary disease. Free Radic Antioxid. 2013;3(1):17-19. doi:10.1016/j.fra.2013.03.001
25. Verrotti A, D'Egidio C, Mohn A, Coppola G, Parisi P, Chiarelli F. Antiepileptic drugs, sex hormones, and PCOS. Epilepsia. 2011;52(2):199-211. doi:10.1111/j.1528-1167.2010.02897.x
26. Masjedi F, Keshtgar S, Agah F, Karbalaei N. Association between sex steroids and oxidative status with vitamin D levels in follicular fluid of non-obese PCOS and healthy women. J Reprod Infertil. 2019;20(3):132-142.
27. Rashidy-Pour A, Derafshpour L, Vafaei AA, et al. Effects of treadmill exercise and sex hormones on learning, memory and hippocampal brain-derived neurotrophic factor levels in transient congenital hypothyroid rats. Behav Pharmacol. 2020;31(7):641-651. doi:10.1097/fbp.0000000000000572
28. Rimaz S, Rahideh ST, Abolghasemi J, et al. The effect of *Cornus mas* fruit extract on vasomotor symptoms and sex hormones in postmenopausal women: a randomized, double-blind, clinical trial. Menopause. 2023;30(2):201-207. doi:10.1097/gme.0000000000002117
29. Krishna D, Dhoble S, Praneesh G, Rathore S, Upadhaya A, Rao K. Gonadotropin-releasing hormone agonist trigger is a better alternative than human chorionic gonadotropin in PCOS undergoing IVF cycles for an OHSS free clinic: a randomized control trial. J Hum Reprod Sci. 2016;9(3):164-172. doi:10.4103/0974-1208.192056
30. Li D, You Y, Bi FF, et al. Autophagy is activated in the ovarian tissue of polycystic ovary syndrome. Reproduction. 2018;155(1):85-92. doi:10.1530/rep-17-0499
31. Sharma M, Khapre M, Saxena V, Kaushal P. Polycystic ovary syndrome among Indian adolescent girls - A systematic review and metanalysis. Nepal J Epidemiol. 2021;11(3):1063-1075. doi:10.3126/nje.v11i3.38460
32. Rajaei S, Alihemmati A, Abedelahi A. Antioxidant effect of genistein on ovarian tissue morphology, oxidant and antioxidant activity in rats with induced polycystic ovary syndrome. Int J Reprod Biomed. 2019;17(1):11-22. doi:10.18502/ijrm.v17i1.3816
33. Karam M, Najjar H, El Sabban M, Hamade A, Najjar F. Regenerative medicine for polycystic ovary syndrome: stem cell-based therapies and brown adipose tissue activation. Stem Cell Rev Rep. 2023;19(4):853-865. doi:10.1007/s12015-023-10505-5
34. Rahayu FK, Dwiningsih SR, Sa'adi A, Herawati L. Effects of different intensities of exercise on folliculogenesis in mice: which is better? Clin Exp Reprod Med. 2021;48(1):43-49. doi:10.5653/cerm.2020.03937