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Effect of Maca root (*Lepidium meyenii*) on some biochemical and antioxidant parameters in rats with experimental polycystic ovary syndrome

Efecto de la raíz de maca (*Lepidium meyenii*) sobre algunos parámetros bioquímicos y antioxidantes en ratas con síndrome de ovario poliquístico experimental

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ABSTRACT

In this study, the potential therapeutic effect of Maca root (*Lepidium Meyenii*) on oxidative damage and histopathological changes in ovaries induced by experimental polycystic ovary syndrome (PCOS) in rats was evaluated. The study involved thirty–five female Sprague Dawley rats, each aged 2.5 months. These rats were allocated into five distinct groups. The first group did not receive any treatment or intervention. Carboxymethyl cellulose (CMC) (1%) was applied to 2nd group. Maca root was applied to the 3rd group at a dose of 2 g·kg^{-1}·d^{-1} body weight by gavage for 7 days (d). 1 mg·kg^{-1}·d^{-1} letrozole (PCOS agent) dissolved in 1% CMC was applied to the 4th group by gavage for 21 d. Letrozole and maca root were applied together to the 5th group. Maca root application was started on the 15th d of letrozole application and was applied for 7 d, while letrozole was applied for 21 d starting 14 d before maca root application and both applications were terminated on the 22nd d. In the PCOS group, malondialdehyde (MDA) levels were observed to be elevated compared to the control group, whereas reduced glutathione (GSH) levels, along with the activities of catalase (CAT), glutathione peroxidase (GSH–Px), glutathione–S–transferase (GST), and superoxide dismutase (SOD), were found to be reduced. In the PCOS+maca root group, differences were determined compared to the PCOS applied group, and the all parameter values were found to be close to the control group values (except GSH–Px and Follicle–Stimulating Hormone (FSH)). The number of atretic follicles were significantly decreased in the PCOS group and PCOS+maca group compared to the control group ($P \leq 0.01$). It was noted that the number of cystic follicles increased statistically significantly in the PCOS groups compared to the other groups ($P \leq 0.001$). In the development of PCOS–related ovarian toxicity and oxidative stress, PCOS may contribute to a discrepancy between oxidants and antioxidants, while Maca root may help alleviate the severe side effects caused by PCOS.

Key words: PCOS; *Lepidium Meyenii*; malondialdehyde; testosterone; follicle

RESUMEN

En este estudio se evaluó el posible efecto terapéutico de la raíz de maca (*Lepidium meyenii*) sobre el daño oxidativo y los cambios histopatológicos en los ovarios inducidos por el síndrome de ovario poliquístico experimental (SOP) en ratas. El estudio involucró a treinta y cinco ratas Sprague Dawley hembras, cada una de 2,5 meses de edad. Estas ratas se dividieron en cinco grupos distintos. El primer grupo no recibió ningún tratamiento ni intervención. Se aplicó carboximetilcelulosa (CMC) (1 %) al segundo grupo. Se aplicó raíz de maca al tercer grupo en una dosis de 2 g·kg^{-1}·día^{-1} de peso corporal por sonda durante 7 días (d). Se aplicó 1 mg·kg^{-1}·d^{-1} de letrozol (agente para SOP) disuelto en 1% de CMC al cuarto grupo por sonda durante 21 d. Se aplicaron letrozol y raíz de maca juntos al quinto grupo. La aplicación de raíz de maca se inició el día 15 de la aplicación de letrozol y se aplicó durante 7 d, mientras que el letrozol se aplicó durante 21 d comenzando 14 d antes de la aplicación de raíz de maca y ambas aplicaciones finalizaron el d 22. En el grupo con SOP, se observó que los niveles de malondialdehído (MDA) estaban elevados en comparación con el grupo de control, mientras que los niveles de glutatión reducido (GSH), junto con las actividades de catalasa (CAT), glutatión peroxidasa (GSH–Px), glutatión–S–transferasa (GST) y superóxido dismutasa (SOD), estaban reducidos. En el grupo de SOP + raíz de maca, se determinaron diferencias en comparación con el grupo de SOP aplicado, y se encontró que todos los valores de los parámetros estaban cerca de los valores del grupo de control (excepto GSH–Px y Hormona folículo estimulante (FSH)). El número de folículos atrésicos disminuyó significativamente en el grupo de SOP y el grupo de SOP + maca en comparación con el grupo de control ($P \leq 0.01$). Se observó que el número de folículos quísticos aumentó estadísticamente de manera significativa en los grupos de SOP en comparación con los otros grupos ($P \leq 0.001$). En el desarrollo de la toxicidad ovárica relacionada con el SOP y el estrés oxidativo, el SOP puede contribuir a una discrepancia entre oxidantes y antioxidantes, mientras que la raíz de maca puede ayudar a aliviar los efectos secundarios graves causados por el SOP.

Palabras clave: SOP, *Lepidium Meyenii*, malondialdehído, testosterona, folículo

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INTRODUCTION

The most prevalent endocrine condition affecting female human is polycystic ovarian syndrome (PCOS), impacting around 8 to 13% of individuals during their reproductive life. Despite its high prevalence, PCOS remains a complex condition that presents challenges in diagnosis and management due to the variability of symptoms based on age and the need for individualized treatment approaches [1, 2]. Polycystic ovarian morphology, biochemical or/and clinical hyperandrogenism, and prolonged anovulation are the hallmarks of the syndrome. Diagnosis is made based on the ''Rotterdam criteria'' [3], requiring at least two of these three features while excluding other potential causes [4].

The pathogenesis of PCOS is multifaceted, involving genetic, environmental, and intergenerational factors. These lead to ovarian and adrenal hyperandrogenism by interfering with the hypothalamic–pituitary–ovarian axis. Insulin resistance is another hallmark of PCOS, exacerbated by adipose tissue accumulation linked to hyperandrogenism, lipotoxicity, and oxidative stress [2]. PCOS patients frequently have diminished Follicle–Stimulating Hormone (FSH) output, high Luteinizing hormone (LH), and persistently elevated Gonadotropin–releasing hormone (GnRH) secretion. These hormonal imbalances drive increased androgen production and ovulatory dysfunction. Furthermore, insulin resistance is prevalent in most individuals with PCOS, contributing to higher androgen levels and reduced synthesis of sex hormone binding globulin, thereby aggravating the condition [5].

Up to 80% of women may experience physical, mental and emotional symptoms associated with hormonal imbalances. Signs and symptoms that may indicate hormonal imbalances include irregular menstrual cycles, heavy menstrual periods, premenstrual syndrome, infertility, hot flashes, weight gain, and hair decaying. Functional medicine practitioners can help identify the possible causes of hormonal dysregulation and recommend an integrated plan that can support hormonal balance. Maca has gained popularity due to its potential to support hormonal health, especially in relation to the symptoms associated with the menopausal transition. Numerous studies have examined maca's possible health benefits, including its effects on menopausal symptoms, sexual dysfunction, and semen quality. Species indigenous to North America, Europe, China's Yunnan province, and the Andes have received special attention [5, 6].

Reactive oxygen species (ROS) are believed to contribute to a wide array of health issues [6]. Excessive ROS production can compromise cellular structures, leading to membrane damage, compromised permeability and integrity, protein malfunction, DNA damage, and eventually cellular apoptosis [7]. However, ROS are naturally generated as part of the mitochondrial electron transport process. Under normal conditions, a well–functioning antioxidant defense system can effectively mitigate the risks posed by ROS [8]. Consequently, enhancing comprehension of oxidative damage regulation and optimizing antioxidants are critical for developing pharmacological and nutraceutical strategies aimed at preventing and treating conditions linked to oxidative imbalance [9].

For all these reasons, in the study, therapeutic effect of maca root on oxidative damage and histopathological changes in ovaries induced by PCOS in rats was evaluated.

MATERIALS AND METHODS

Animals and experimental procedure

In the investigation, female Sprague–Dawley rats *(Rattus norvegicus*) weighing 250–300 g and 2.5 months of age were employed. The animals were sourced from the Laboratory Animals Breeding Unit at Firat University. The study was granted ethical approval by the Local Ethics Committee for Animal Experiments at Firat University (Protocol No: 2022/21–06). The rats were housed in climate–controlled environments, with a temperature set at 25 ± 2°C and humidity levels maintained between 60–65%, following a 12–hour light/dark cycle to ensure consistent environmental conditions.

Experimental protocol

The amounts of maca root and letrozole (Letrozole is an aromatase inhibitor that is not steroidal in nature) utilized in the study were established based on prior research [10, 11]. The rats were separated into five groups, with no treatment administered to the first group. The second group was planned as a solvent. Carboxymethyl cellulose (CMC) (1%), the solvent of letrozole, was applied to this group. CMC is a good solvent for letrozole, a PCOS agent. Therefore, letrozole was prepared by dissolving it in 1% CMC. Maca root (aniqherbs®) was administered to group 3 at a dosage of 2 $g \cdot kg^{-1} \cdot day^{-1}$ (d) body weight for a duration of 7 d via gavage. Maca root was prepared through dissolution in steril tap water. 1 $mg \cdot kg^{-1}$ letrozole (PCOS agent, Cas No: 112809–51–5, PHR1540, Merck,) dissolved in 1% CMC was applied to group 4 by gavage for 21 d. Letrozole and maca root were applied to group 5 at the doses and times specified above. Letrozole applied to this group was prepared by dissolving it in CMC in the same way as in the second group. Maca root application was started on the 15th d of letrozole application and was applied for 7 d, while letrozole was applied for 21 d starting 14 d before maca root application and both applications were terminated on the 22nd d (FIG. 1.). During the study period, rats were monitored for sexual cycles by vaginal smear method.

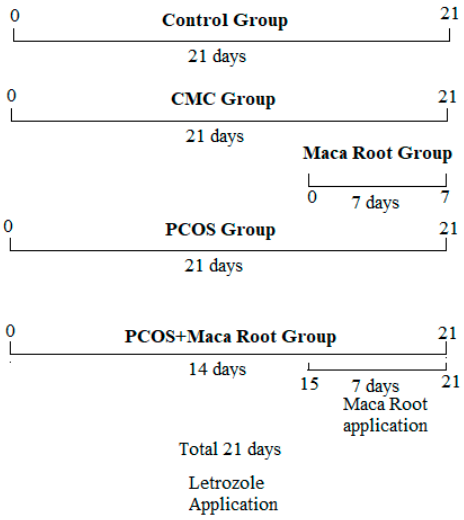

FIGURE 1. Experimental groups and application periods

Biochemical analyses

At the conclusion of the experiment, rats from both the control and experimental groups were euthanized by decapitation, and both ovaries tissue samples were promptly collected. Right ovaries were used for biochemical analyses and left ovaries were used for histopathological analyses. Before analysis, the tissues were rinsed with physiological saline (0.9% NaCl) and homogenized in distilled water at a 1:10 (W/V) using a homogenizer (CAT R50D, Germany). The homogenates were subjected to centrifugation (NUVE NF800R, Turkey) at 4°C for 15 min at 3000 g to evaluate malondialdehyde (MDA), reduced glutathione (GSH), catalase (CAT), glutathione–S–transferase (GST), and superoxide dismutase (SOD), and for 55 min at 13000 g to determine glutathione peroxidase (GSH–Px) activity. Serums were examined for FSH, LH, testosterone, glucose, insulin, and cholesterol levels using an Advia 1800 Chemistry Analyzer (Siemens Healthineers–Advia 1800 Chemistry Analyzer, Erlangen–Bavaria, Germany).

Biochemical assays were carried out using spectrophotometry (Thermo Scientific, Genesys 10S UV–VIS Spectrophotometer, USA). MDA levels were measured following the procedure outlined by Placer *et al*. [12], which involves the reaction between thiobarbituric acid (TBA) and MDA. The GSH levels were determined using Ellman's technique [13], which relies on the consistent yellow hue formed sulfhydryl groups. The CAT activity was assessed utilizing Aebi's technique, which measures the breakdown of hydrogen peroxide (H_2O_2) [14]. The activity of GSH–Px was assessed utilizing Beutler's technique, which observes the transformation of GSH to its oxidized version (GSSG) in a solution of H_2O_2 [15]. The GST activities were quantified using the methodology of Habig *et al*. [16], which involves the interaction between 1–chloro 2,4–dinitrobenzene (CDNB) and GSH. SOD activity was evaluated based on the decrease of nitroblue tetrazolium, as described by Sun *et al*. [17]. Protein levels were measured using the technique introduced by Lowry *et al*. [18].

Histopathological examination

The left ovaries taken from the rats were fixed in 10% formalin solution for 72 hours (h). The tissues were dehydrated by keeping them in ascending ethanol series (70%, 90%, 96% and 100%, respectively) for 24 h. Then, they were kept in 100% ethanol for 30 min twice and cleared in xylene series for 30 min three times. Following this, the tissues were kept in soft and then in hard liquid paraffin in an oven (JSR, Model JSOF–050, Korea) at 56°C for 45 min each and encased in paraffin blocks. Sequential sections with a thickness of 5 μm were obtained from these blocks using a microtome (Roundfin Rd–475, Chine). 10 sections were taken from each ovarium at 10–section intervals and stained with the hematoxylin–eosin (HE) method and inspected using a light microscope (Olympus CX21, Model CX21FS1, Olympus Corporation, Japan) [19].

Statistical analyses

This study employed SPSS software, version 22.0, for statistical analysis to assess the importance of disparities among distinct groups. The normality of the raw data for all measured parameters was checked using the "Shapiro–Wilk test", which verified that the data adhered to a normal distribution. For comparing differences between groups, a one–way analysis of variance (ANOVA) was performed. If substantial disparities were identified, post hoc comparisons were conducted utilizing the Tukey test to identify specific group differences.

RESULTS AND DISCUSSIONS

Biochemical results

TABLE I displays the concentrations of GSH and MDA in ovarian tissue, along with the functions of antioxidant enzymes, including CAT, GST, GSH–Px, and SOD, for both the control and experimental groups. TABLE II provides the measurements of FSH, LH, testosterone, cholesterol, insulin, and glucose.

The results showed that MDA levels in the ovarian tissue were markedly elevated in the PCOS group compared to the control group (P<0.001). However, treatment in the PCOS+maca group appeared to restore MDA levels closer to normal. Additionally, in comparison to the control group, the PCOS group exhibited significantly lower GSH levels and reduced activities of antioxidant enzymes, including CAT, GST, GSH–Px, and SOD. There were no differences observed among the control, maca root, and CMC groups across all parameters. GSH levels and the activities of CAT, GST, GSH–Px, and SOD were significantly higher in the PCOS+maca group compared to the PCOS group, with the increase reaching

TABLE I
Effect of maca root application in rats with PCOS on MDA and GSH levels and CAT, GSH–Px, GST, and SOD activities in ovaries tissues

	Control	Maca Root	CMC	PCOS	PCOS+Maca	*P*
MDA (nmol·g tissue^{-1})	0.86 ± 0.05^{ab}	0.66 ± 0.04^{a}	0.86 ± 0.05^{ab}	1.21 ± 0.06^{c}	0.92 ± 0.07^{b}	≤0.001
GSH (μmol·mL^{-1})	2.04 ± 0.06^{ab}	0.08 ± 0.05^{a}	2.13 ± 0.05^{a}	1.46 ± 0.06^{c}	1.87 ± 0.02^{b}	<0.001
CAT (k·g prot.$^{-1}$)	19.89 ± 0.79^{a}	19.03 ± 1.15^{a}	19.34 ± 1.04^{a}	11.76 ± 1.08^{b}	17.30 ± 0.69^{a}	<0.001
GSH–Px (U·mg prot.$^{-1}$)	0.15 ± 0.003^{a}	0.16 ± 0.002^{a}	0.15 ± 0.003^{a}	0.10 ± 0.003^{c}	0.13 ± 0.003^{b}	<0.001
GST (U·mg prot.$^{-1}$)	26.97 ± 0.36^{ab}	27.37 ± 0.36^{a}	27.07 ± 0.31^{a}	21.46 ± 0.62^{c}	25.39 ± 0.32^{c}	<0.001
SOD (U·mg prot.$^{-1}$)	3.15 ± 0.02^{a}	3.14 ± 0.01^{a}	3.14 ± 0.06^{a}	2.85 ± 0.02^{b}	3.09 ± 0.07^{a}	<0.001

Means with distinct letters ($^{a, b}$, and ^{c}) within rows differ considerably (P<0.05). MDA: malondialdehyde, GSH: reduced glutathione, CAT: catalase, GSH–Px: glutathione peroxidase, GST: glutathione–S–transferase, SOD: superoxide dismutase, CMC: Carboxymethyl cellulose, PCOS: Polycystic Ovary Syndrome

TABLE II
Effect of Maca root application in rats with PCOS on LH, FSH, testosterone, glucose, insulin and cholesterol levels in serum

	Control	Maca Root	CMC	PCOS	PCOS+Maca	*P*
LH (mIU·mL^{-1})	2.49 ± 0.04^{ab}	2.38 ± 0.06^{a}	2.39 ± 0.06^{a}	3.89 ± 0.18^{c}	2.88 ± 0.08^{b}	≤0.001
FSH (mIU·mL^{-1})	5.25 ± 0.03^{a}	5.42 ± 0.08^{a}	5.48 ± 0.12^{a}	2.36 ± 0.06^{c}	4.51 ± 0.30^{b}	<0.001
Testosterone (ng·mL^{-1})	2.48 ± 0.07^{a}	2.44 ± 0.04^{a}	2.56 ± 0.04^{a}	4.96 ± 0.09^{b}	2.72 ± 0.11^{a}	<0.001
Glucose (mg·dL^{-1})	75.57 ± 1.29^{a}	74.28 ± 1.02^{a}	75.28 ± 1.23^{a}	124.14 ± 5.82^{b}	83.0 ± 3.12^{a}	<0.001
Insulin (ng·mL^{-1})	2.13 ± 0.05^{a}	2.09 ± 0.07^{a}	2.28 ± 0.04^{a}	4.42 ± 0.31^{b}	2.66 ± 0.19^{a}	<0.001
Cholesterol (mg·dL^{-1})	77.86 ± 1.42^{a}	75.71 ± 1.15^{a}	77.43 ± 1.32^{a}	106.57 ± 5.03^{b}	81.14 ± 1.83^{a}	<0.001

Means with distinct letters ($^{a, b}$, and ^{c}) within rows differ considerably (P<0.05). MDA: malondialdehyde, GSH: reduced glutathione, CAT: catalase, GSH–Px: glutathione peroxidase, GST: glutathione–S–transferase, SOD: superoxide dismutase, CMC: Carboxymethyl cellulose, PCOS: Polycystic Ovary Syndrome

statistical significance. It was found that, except for GSH–Px, the values of all other antioxidant parameters closely resembled those of the control group, with no statistically significant differences observed. GSH–Px activity was found to be different from the PCOS group in the PCOS+maca group but it was observed that it did not reach the control group averages statistically.

When the changes in LH, FSH, testosterone, glucose, insulin and cholesterol levels, which are important laboratory findings in PCOS, were evaluated, In the PCOS group, LH, FSH, testosterone, glucose, insulin and cholesterol levels were significantly higher compared to the control group, whereas FSH levels were lower. When compared to the PCOS group, all values were observed to be significantly higher in the PCOS+maca group. It was found that the values of all other parameters except FSH approached the control group values when compared with the control group and there was no statistical difference with the control group. FSH levels were observed to be different from the PCOS group in the PCOS+maca group but it was observed that it did not reach the control group averages statistically.

Histopathological results

The follicles in the ovaries of the rats in the control and experimental groups were evaluated morphologically and the follicles in various stages of folliculogenesis were counted as primary, secondary, tertiary (Graff), atretic, cystic follicles and corpus luteum were summarized in TABLE III. It was noted that the ovaries in the control, Maca and CMC groups had a normal appearance (FIG. 2. A, D, E). All follicle numbers were similar in these groups. No significant statistically change was identified in the primary, secondary and tertiary follicle numbers in all groups (P≥0.05). The number of atretic follicles were significantly lower in both the PCOS and PCOS+maca groups compared to the control group (P≤0.01). It was noted that the number of cystic follicles increased statistically significantly within the PCOS applied group, compared to the other groups (P≤0.001). The same significant difference was found in the PCOS group and the group treated with PCOS+maca. It was observed that the granulosa layer in cystic follicles were considerably thinned, and there were abundant follicular fluid and granulosa cells and macrophages spilled into the antrum (FIG. 2. B, C). It was determined that the number of corpus luteum (CL) within the PCOS applied group was significantly reduced compared to the other groups. It was noted that the CL number in the PCOS+maca group was similar compared to the control, maca and CMC groups.

Oxidative stress can be assessed from various perspectives, but one of the most notable areas of interest is its connection to patients with PCOS. A multitude of studies have suggest that oxidative stress levels are significantly elevated in individuals with PCOS, often linked to several associated disorders [20, 21, 22, 23]. These conditions may arise independently, as part of different diseases, or in combination, complicating both treatment and diagnosis. The development of PCOS, in the scope of oxidative stress, may involve disturbances in cellular tissues and organelles, as well as biochemical and molecular processes. In the study, it was intended to explore the possible ramifications of maca root

TABLE III
Average follicle numbers at various stages of folliculogenesis in the control and experimental groups

	Control	Maca Root	CMC	PCOS	PCOS+Maca	SH	*P*
Primary Follicle	5.25 ± 0.25	5.00 ± 0.46	5.13 ± 0.44	4.88 ± 0.67	4.88 ± 0.67	0.22	NS
Secondary Follicle	3.75 ± 0.25	3.50 ± 0.38	3.63 ± 0.32	2.75 ± 0.73	3.38 ± 0.26	0.19	NS
Tertiary Follicle	0.75 ± 0.25	0.63 ± 0.26	0.75 ± 0.16	0.38 ± 0.18	0.50 ± 0.19	0.09	NS
Atretic Follicle	4.38 ± 0.50 ^{a}	3.25 ± 0.68 ^{ab}	3.25 ± 0.31 ^{ab}	2.13 ± 0.44 ^{b}	2.63 ± 0.36 ^{b}	0.24	0.01
Cystic Follicle	0.00 ± 0.00 ^{c}	0.00 ± 0.00 ^{c}	1.00 ± 0.38 ^{c}	5.63 ± 1.07 ^{b}	9.13 ± 1.03 ^{a}	0.65	0.001
Corpus Luteum	8.00 ± 0.57 ^{a}	7.75 ± 0.77 ^{a}	7.88 ± 0.66 ^{a}	4.38 ± 0.71 ^{b}	7.38 ± 0.56 ^{a}	0.37	0.01

$^{a, b, c}$: Different letters in the same row are statistically significant (P<0.001). NS: The difference is not statistically significant. CMC: Carboxymethyl cellulose, PCOS: Polycystic Ovary Syndrome

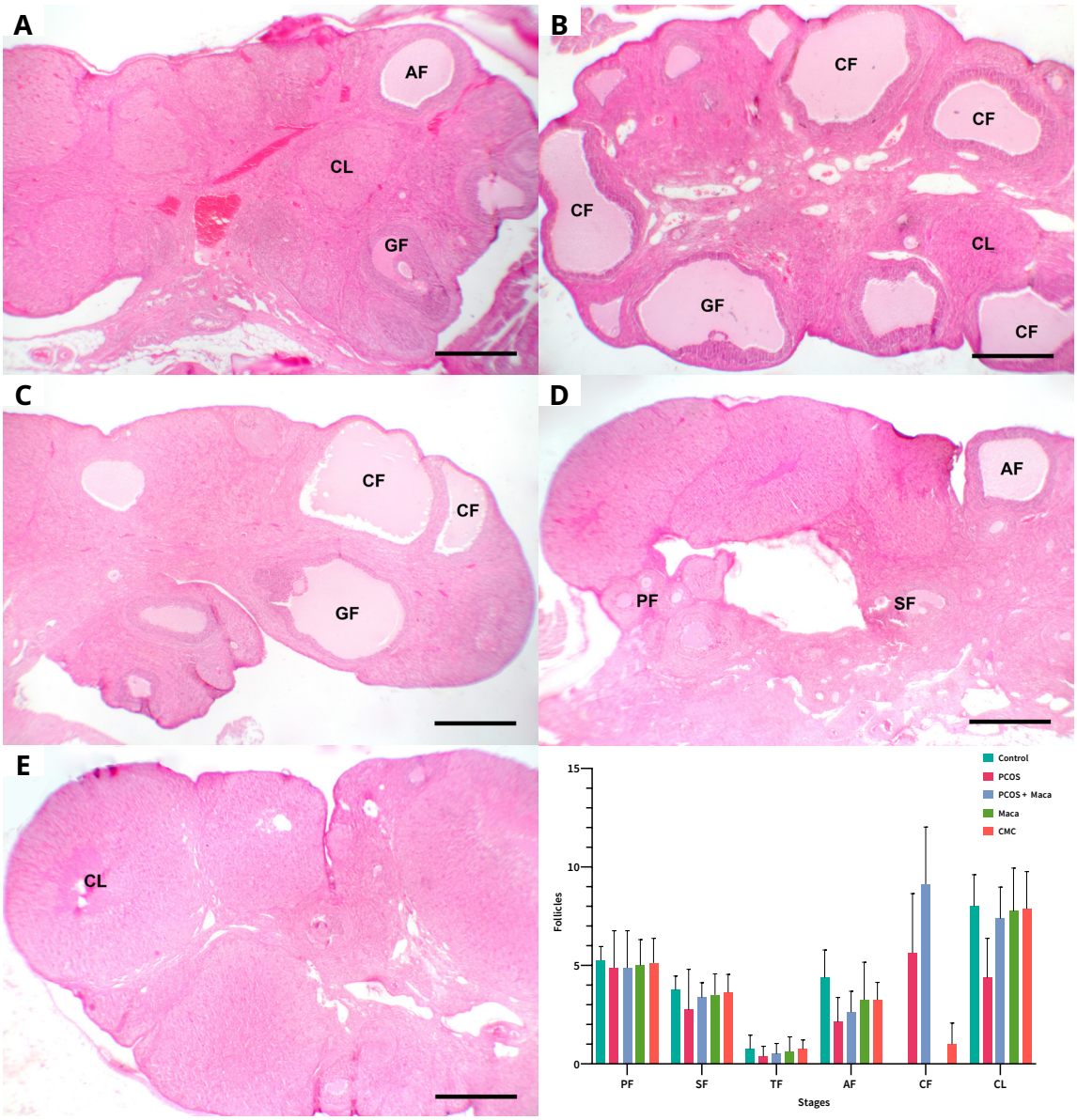

FIGURE 2. A. Microscopic appearance of the ovarium in the control group, B. Numerous cystic follicles (CF) in the PCOS group, C. Cystic follicle (CF) formations in the PCOS+Maca group, D. Normal histological appearance of the ovarium in the Maca group, E. Histological appearance of the ovarium in the CMC group, Bar=500 µm, HE 20×. CL: Corpus luteum, GF: Graff Follicle (TF: Tertiary Follicle), AF: Atretic Follicle, SF: Secondary Follicle, PF: Primary Follicle

on oxidative stress induced by experimental PCOS and its impact on ovarian histopathological changes in rats by analyzing various biochemical and histopathological parameters.

Abnormal oxidative status is linked to various diseases, including PCOS. Oxidative damage plays a role in the pathogenesis of PCOS by exacerbating insulin resistance, promoting excess androgen synthesis, and inducing chronic inflammation. Principal circulating indicators of oxidative stress comprise MDA, asymmetric dimethylarginine (ADMA), SOD, GSH, and paraoxonase 1 (PON1). Women with PCOS generally display modified amounts of these oxidative stress indicators. The disparity in total serum antioxidant levels in PCOS results in heightened cellular damage and diminished protective mechanisms [24].

PCOS is associated with several metabolic and hormonal abnormalities, with oxidative stress significantly contributing to its pathogenesis. Oxidative stress denotes a disparity between ROS and the organism's capacity to counteract them via antioxidants. This imbalance can lead to cellular damage, inflammation, and contribute to the development of insulin resistance, increased androgen levels (such as testosterone), and chronic inflammation–all of which are hallmark features of PCOS [25].

The accumulation of free radicals leads to excessive MDA production. Its concentration serves as an indicator of antioxidants levels and oxidative damage in cancer patients. Notably, MDA levels are elevated in both PCOS and obesity [26]. Ghowsi *et al.* [27] investigated the impact of resveratrol on oxidative damage in the

serum and liver of rats with PCOS and found that serum MDA levels were elevated compared to the control group, while MDA levels were decreased when resveratrol was administered. Excessive androgen levels may cause oxidative stress independently of obesity and many other diseases. Hyperandrogenism has been suggested to induce the premature activation of mononuclear cells in people with PCOS, leading to an increase in ROS production independent of other conditions [28]. However, the exact mechanisms behind ROS generation in PCOS remain unclear. Certain research indicate that NADPH oxidase may contribute to reactive oxygen species production in diverse cell types [28]. In the current investigation, the rise in testosterone and LH levels and the decrease in FSH levels support the increase in lipid peroxidation due to ROS increase. Also these changes may occur due to changes in redox balance. The findings of the current investigation regarding MDA levels in rats with PCOS are in agreement with a study in which some investigators reported that MDA levels were increased in PCOS applied grup compared with controls in famales who was not obese with PCOS [29]. This study results indicate the presence of oxidative damage in rats with testosterone–induced polycystic ovarian syndrome. Research indicates that insulin resistance may play a role in the onset of PCOS and intensify oxidative damage [30], and the findings in this study, particularly concerning insulin levels and oxidative damage biomarkers in the PCOS group, support this notion.

Pandey *et al*. [31] studied the impact of some parameters PCOS in rats. They observed a reduce in the antioxidant.enzymes activities (SOD and CAT) on the 7th d, followed by an increase on the 15th and 21st d. Besides this found an increase to lipid peroxidation and testosterone levels. Although the CAT and SOD data on the 7th d are consistent with this study findings, there is a difference in the data on the 21st d. The researchers proposed that the rise in oxidative damage and inflammatory biomarkers occurs comparatively early in the development of PCOS. In the present study and many other studies, the changes were found to be evident on the 21st day [11, 32, 33]. Hassan and Al–Husseini [34] determined the some oxidative stress indicators level in PCOS patients and found that CAT enzyme activities decreased significantly and MDA levels increased in PCOS patients. Huang and Zhang [35] showed that luteolin alleviated polycystic ovarium syndrome in rats by addressing oxidative stress and insulin resistance, and determined that the levels of antioxidants, whose activities were determined in this study, decreased. Uçkan *et al.* [36] found that GST activity, which is an antioxidant enzyme, decreased in the PCOS patient group and thought that this decrease was due to its use in the fight against ROS.

PCOS is associated with oxidative damage, potentially initiated by hydroxyl (OH·) radicals. GSH and GST play vital roles in protecting cell and tissues from the deleterious consequences of PCOS. GSH interacts directly or serves as a cofactor/coenzyme, utilizing its – SH group in a manner similar to how ROS interact. Since biological membranes are particularly susceptible to peroxidation, GSH provides essential protective benefits [36]. The current investigation found a decrease in GST activity, likely reflecting its increased utilization in counteracting ROS generated during PCOS progression. Moreover, CAT activity was diminished in PCOS group, indicative of ovarium toxicity. Reduced CAT activity facilitates the formation of OH· from H_2O_2, exacerbating oxidative stress. This reduction may result from disrupted antioxidant defenses, potentially as an adaptive response to elevated oxidative stress levels in ovarian tissue. The antioxidant enzyme GSH–Px, an early defense against ROS, also showed decreased activity in PCOS–affected groups, accompanied by a decline in its substrate, GSH. The primary reason for the reduced activity of GSH–Px is the inhibition of enzymatic function due to ROS. Moreover, the binding of ROS to proteins can lead to structural alterations and oxidation, further reducing enzymatic activity in the context of oxidative stress induced by PCOS [37].

Hormonal imbalances in women with PCOS lead to various pathological symptoms. Several hormones, including insulin, ghrelin, growth hormone (GH), gonadotropin–releasing hormone (GnRH), LEAP–2, LH, FSH, estrogens and testosterone levels are determined to be irregular in these females These hormonal irregularities are linked to metabolic issues such as insulin resistance, diabetes, obesity, irregular menstrual cycles and infertility in PCOS patients [38]. Numerous studies [30, 31, 32, 33, 35] have demonstrated alterations in hormone levels and biomarkers such as FSH, insulin, LH, glucose, cholesterol, and testosterone. In general, due to endocrinopathy in individuals with PCOS, increases in the levels of LH hormone, which initiates ovulation and provides growth of the corpus luteum, decreases in FSH levels, which provide maturation of ovarian follicles, increases in testosterone levels, which is an androgen, and increases in insulin, glucose, and cholesterol levels, again as a direct or indirect result of endocrinopathy, have been determined. Similar results were found in the current study, and it was stated that these changes occur as a result of hyperandrogenism, polycystic ovaries, chronic anovulation, and metabolic disorders. In studies emphasizing the changes in the histopathological structure of the ovarian tissue in rats with PCOS, it was found that the quantity of atretic and cystic follicles increased [32, 38], and in another study, A small reduction in the average number of primordial and primary follicles, as well as corpus luteum, was observed in the PCOS–induced group compared to the control group [32]. In the current investigation, despite the elevated quantity of cystic follicles seen in the PCOS and PCOS+maca groups, the fact that the number of corpus luteum was higher in the PCOS+maca group compared to the PCOS group and approached the control group values indicates that the necessary environment for healthy sexual development has been re–established.

Traditionally and scientifically it has been proven that the maca plant can increase fertility in humans and animals [39], as evidence indicates that the maca plant can enhance libido and boost sperm count in male and reduce sexual dysfunction in postmenopausal female in animals [40] and possibly increase female fertility [41, 42]. The precise mechanism of action remains unknown, further studies will shed light on this.

Ali Amin *et al.* [43] examined the effect of maca root extract on the improvement of sperm status in obese diabetic male rats and found that CAT activities, which decreased after maca administration, increased in proportion to the dose and decreased the increased lipid peroxidation (MDA) levels. Researchers also emphasized that it provided improvement in sex hormone (Testosterone, FSH, LH), glucose and cholesterol levels, which it was tried to emphasize in this study. The researchers thought that the maca plant achieved this by preserving the equilibrium between oxidant and antioxidant levels. When maca or antioxidant compounds were used at various doses in rats and mice, certain effects were observed in response to stress factors [44, 45]. Qui *et al.* [46] suggest that maca enhances the activity of some antioxidant enzymes, regardless of the active

ingredients makaid and makaene, and that the antioxidant effects are not linked to these bioactive compounds. The current study revealed that increased lipid peroxidation levels decreased, decreased antioxidant parameters increased and ovarium histopathology improved after maca root administration. Although the quantities may vary, maca contains several significant antioxidant compounds, including phenols, glucosinolates, alkylamides, and polysaccharides. These compounds have been shown in scientific studies to have various metabolic functions and antioxidant effects.

Maca has been shown that maca consumption in mice and rats can improve female fertility with an increase in progesterone levels [47]. In another study, Ruiz–Lina *et al.* [48] emphasized that pregnancy rate, larger number of offspring and postnatal survival rate can be increased in the maca–treated group. Balanced effects on FSH, estradiol and progesterone were recorded. Meissner *et al.* [49] showed that maca powder application reduced levels of progesterone and estradiol. in the 4th week. Other researchers have shown that it can cause an increase in uterine separation in mice [48]. The present study found that maca root improved oxidative stress–related parameters and biomarkers, including LH, FSH, testosterone, insulin, glucose, and cholesterol, in rats with PCOS.

CONCLUSION

This study results indicate that maca root is a potent flavonoid antioxidant capable of addressing metabolic and endocrine comorbidities associated with PCOS. Maca root demonstrated positive effects on the lipid profile, hormonal levels, and glucose metabolism. Additionally, it exhibited strong antioxidant properties, helping to repair ovarian cysts, promote healthy follicles, and restore the structure of the follicles. The restoration of ovarian function and the anti–androgenic effects of maca root may provide a beneficial treatment option for PCOS. It may also be a preliminary study for studies on the use of maca for PCOS, one of the most common reproductive problems in humans.

Author's contribution

Çelikdemir N., Mamur M. N., Üstün İ., Yilmaz S., Kaya E. were participated in the study design, animal procedures, and laboratory experiments (biochemical), and evaluation of results. Çeribaşı A.O. was contributed to the laboratory experiments (histopathological), histopathological evaluation of ovarian tissue, and evaluation of results. All authors analyzed the data, thoroughly reviewed the manuscript for significant intellectual content, and approved the final version.

Data availability

All data that support the conclusions of this article are provided within the article.

Conflict of interests

The authors report no financial conflicts of interest.

Ethics approval and consent to participate

The experiments received approval from the Firat University Animal Studies Local Ethics Committee (Protocol No: 2022/21-06).

Disclosure statement

The authors confirm that there are no conflicting interests. Additionally, this article is based on the master's thesis of the first author, Nisanur Çelikdemir.

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