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ORIGINAL ARTICLE

Safranal's therapeutic effects in rat models of polycystic ovary syndrome

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Abstract

Polycystic ovary syndrome is one of the leading causes of female infertility in reproductive age. In this work, the protective effects of safranal against letrozole-induced polycystic ovary syndrome in rats were examined. For this purpose, 32 Wistar albino female rats were split into four groups. Each group received the following treatments for 21 days: Group 1 received carboxymethylcellulose (1%, 2 ml/kg); Group 2, letrozole (1 mg/kg), Group 3, safranal (200 mg/kg); and Group 4 letrozole and safranal via oral gavage. We identified estrus cycles in the rats and analyzed various parameters in their serum and ovarian tissues, as well as histopathologic findings. The parameters studied included C-reactive protein, glucose, total cholesterol, triacylglyceride, high-density lipoprotein, low-density lipoprotein, follicle-stimulating hormone, estradiol, and luteinizing hormone levels in serum. Additionally, the study measured malondialdehyde, glutathione, glutathione peroxidase, and catalase levels in ovarian tissue. We also examined tumor necrosis factor-alpha, interleukin-6, and interleukin-1beta parameters in serum and ovarian tissues, as well as nuclear factor erythroid 2-related factor-2 and heme oxygenase-1 protein levels. In the letrozole group, the estrus cycle was disrupted, and all parameters, except for glutathione and glutathione peroxidase, showed impairments compared to the control group. The findings showed that glucose, triacylglyceride, catalase, and heme oxygenase-1 levels slightly improved after safranal treatment, however, other parameters showed statistically significant improvements. Furthermore, safranal treatment reduced the development of cystic follicles while preserving tissue architecture, as revealed by histopathologic findings. Based on the results obtained, it may be argued that safranal's antioxidant and anti-inflammatory properties, along with its ability to regulate sex hormone levels and manage dyslipidemia, make it a promising solution for patients with polycystic ovary syndrome.

Keywords Hyperandrogenism · Oxidative stress · Inflammation · Phytochemical · Herbal medicine

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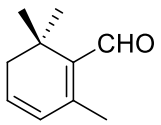
Introduction

Polycystic ovarian syndrome (PCOS) is a complex disorder that affects women during their reproductive years and presents with a wide range of symptoms. Pathological conditions such as infertility, insulin resistance, oligomenorrhea, hirsutism, hyperandrogenism, acne, amenorrhea, and obesity may develop in women with PCOS. Polycystic ovarian syndrome, a leading cause of female infertility, accounts for approximately 40% of cases (Demirel et al. 2016; Nautiyal et al. 2022). It has been suggested that PCOS may raise the risk of endometrial cancer, particularly in obese women, by producing excessive luteinizing hormone (LH) secretion and long-term anovulation (Hardiman et al. 2003). The elevated luteinizing hormone (LH)/follicle-stimulating hormone (FSH) ratio leads to an inability of ovarian

granulosa cells to convert androgens into estrogen, resulting in decreased estrogen levels and anovulation (Badawy and Elnashar 2011). Despite the diverse biochemical and clinical symptoms of PCOS are changes in ovarian morphology are consistently observed. Hyperandrogenemia is considered the most significant indicator of polycystic ovarian morphology, primarily driven by theca cells within the ovaries. In the context of PCOS, disruptions in follicular development occur during the antral stages, preventing the maturation of follicles for ovulation. This abnormality in follicular maturation is associated with an underlying endocrine disorder. Whether it is triggered by an intrinsic issue with the folliculogenesis process remains unknown (Webber et al. 2003). Obesity is one of the factors contributing to disruptions in the follicular cycle. Specifically, cytokines and adipokines secreted by adipose tissue contribute to insulin resistance and chronic inflammation, both characteristic features of PCOS (Cortón et al. 2007). Given the relationship between obesity, diabetes, and PCOS, it can be said that these conditions can mutually exacerbate each other. Another element in the development of PCOS that has been reported is oxidative stress, as it serves as the root cause of various disorders (Rani et al. 2022).

Apart from surgery, various treatments are employed for PCOS, including aromatase inhibitors, insulin-sensitizing agents, tamoxifen, glucocorticoids, or gonadotropin (Badawy and Elnashar 2011). Clomiphene citrate is one of the primary medications administered for the treatment of infertility in women diagnosed with PCOS. However, drug resistance may develop in some patients, and the efficacy of this medication for inducing ovulation is approximately 35% (Wang et al. 2017). Another commonly used medication is metformin, which operates by reducing hepatic glucose production, lipid biogenesis, and gluconeogenesis while enhancing fatty acid oxidation rates. These actions lead to decreased levels of glucose and insulin in the bloodstream (Johnson 2014).

Given the limited efficacy and potential side effects associated with the medications used to treat PCOS, there is crucial need to develop drugs that are more effective and have fewer adverse effects (Abasian et al. 2018). Dietary phytochemicals, known for their diverse bioactivities, are recognized for their potential in the treatment of certain disorders (Türk et al. 2022). One such phytochemical is safranal (**1**), a primary component of saffron, which has a long history of traditional use in various cultures for the treatment of specific ailments. The medicinal properties of saffron are attributed to its apocarotenoids, with safranal being one of them (Cerdá-Bernad et al. 2022). Safranal is known to possess antiapoptotic, antioxidant, antidiabetic, antihyperlipidemic, and antidepressant effects, which may be beneficial in the treatment of PCOS (Cerdá-Bernad et al. 2022). Letrozole, on the other hand, is a non-steroidal aromatase inhibitor that inhibits the conversion of testosterone to estradiol, resulting in decreased estrogen levels. Letrozole induces polycystic ovarian changes similar to those observed in women with PCOS, including a thickened theca interna cell layer, increased ovarian weight and size, and anovulation (Xu et al. 2020). The objective of the current study was to evaluate the effectiveness of safranal co-treatment in rats with experimentally induced polycystic ovarian syndrome using letrozole. The study's findings provide biochemical and histological evidence of safranal's protective effects.



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Material and Methods

Animals

The study included thirty-two Wistar albino female rats. Initially, fifty rats were part of the experiment to identify those with regular estrus cycles for inclusion in the study. After an 11-day observation period, a sufficient number of rats with regular estrus cycles were identified. Out of the forty-four rats exhibiting regular estrus, thirty-two were selected for the study. The estrus cycles of the animals were monitored for 11 days, beginning at the start of the experiment, and differences among the experimental groups were observed. To determine the length of the estrous cycle, the order of the cycle, and the identification of its stages in rats, changes in vaginal cells were examined. Vaginal cytological testing techniques were applied and assessed following the method described by Cora et al. (2015). Vaginal cell samples were collected using the vaginal lavage method during the 11-day period, typically between 11 am and 1 pm, to assess the length, order, and stages of the estrous cycle. Daily vaginal smear collections were conducted, and animals displaying two consecutive regular 4-day cycles, as determined through Giemsa staining and microscopic analysis, were included in the experiment. Throughout the study, the rats had access to tap water and a standard commercial pellet feed at all times. The experimental environment was set up in accordance with the guidelines outlined in the article by Kuzu et al. (2020).

Chemicals and Kits

FSH, estradiol (E2), LH, progesterone, testosterone, tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and interleukin-1 beta (IL-1β) ELISA kits were purchased from Bioassay Technology Laboratory, China. Commercial preparation Femara (Letrozole, 2.5 mg; lot: TJJ81; Novartis) and safranal (HPLC purity ≥90%; lot: STBJ2200; Sigma Aldrich) were purchase. Other chemicals and solutions used in the study were purchased in analytical grade from Merck KGaA, Darmstadt, Germany.

Experimental Design

A rat model of polycystic ovary syndrome was established following the method outlined by Kafali et al. (2004). The safranal dosage for treatment was determined based on existing literature (Karafakioğlu et al. 2017). The study was designed to span 21 days, and the experimental groups were organized as follows:

Group 1 (Control group): using the oral gavage method, 2 ml/kg of 1% carboxymethylcellulose solution was given once a day during the experiment.

Group 2 (Letrozole): Letrozole, dissolved in 1% carboxymethylcellulose solution was administered once daily at a dose of 1 mg/kg via oral gavage throughout the experiment.

Group 3 (Safranal): safranal at a dose of 200 mg/kg was administered orally once daily in 1 ml of physiological saline throughout the course of the experiment.

Group 4 (letrozole + safranal, L+S): This group received combined treatments, with applications from Groups 2 and 3.

During the experiment, estrus cycles were identified microscopically during the experiment by staining vaginal smears with Giemsa and collecting daily samples.

Vaginal Cell Analysis

To collect the cells from the vaginal canal, 0.2 ml of isotonic NaCl was drawn into a pipette. The pipette tip was gently inserted 5–10 mm into the vaginal opening and the saline was flushed into the vagina and back out, repeating this process 2-3 times. The second and third flushing were skipped if the initial lavage fluid sample was cloudy. After lavage, a drop of the remaining fluid from the pipette was distributed over the slide as a thin layer and allowed to air-dry. Wright's Giemsa staining technique was used to fix the dried preparations with methyl alcohol and stain them. With 10x and 40x objectives, the preparations were examined under the microscope.

Serum Analysis

Under ketamine (60 mg/kg, *i.m.*) + xylazine (10 mg/kg, *i.m.*) anesthesia, blood samples were collected from the tail vein to non-anticoagulant blood tubes 24 h after the last treatment in all groups. Serum samples were obtained by centrifuging blood samples at 2200 × *g* for 15 min. Some of the samples were reserved for ELISA testing and assessment of inflammatory parameters. An autoanalyzer was also used to determine the levels of C-reactive protein (CRP), glucose, total cholesterol, triacylglyceride (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL).

Tissue Samples Preparation

After the experiment, the rats were anesthetized, and anesthesia-induced decapitation was performed. Subsequently, their ovarian and uterine tissues were extracted. Prior to the analysis of oxidative stress and inflammatory markers, the tissue samples were rinsed in a 0.9% NaCl solution and appropriately stored. With a 1/10 ratio of 1.15% KCl, the samples of ovarian tissues were homogenized. After some were set aside for malondialdehyde (MDA) analysis, the remaining portion of the homogenate was centrifuged at 3670 × *g* for 1 h (4 °C), and the supernatant was collected. Samples of uterine and ovarian tissue were preserved in 10% formaldehyde for histological analysis.

Oxidative Stress Parameters

Malondialdehyde, reduced glutathione (GSH), catalase (CAT), and glutathione peroxidase (GPx) enzyme activities were measured manually using spectrophotometric techniques to assess the extent of oxidative damage and antioxidant activity in ovarian tissue samples. Malondialdehyde levels were assessed following the method developed by Ohkawa et al. (1979), while GSH levels were determined using the procedure developed by Beutler et al. (1963). CAT activity was measured according to Aebi's method (Aebi 1984), and GPx activity was measured following Beutler's approach (Beutler 1975). Protein concentrations in the samples were determined using the technique described by Lowry et al. (1951).

ELISA Tests

The hormone levels of FSH, E2, LH, and testosterone in freshly prepared serum samples were assessed using ELISA kits. To evaluate the degree of inflammation, TNF-α, IL-6, and IL-1β protein levels in serum and ovarian

tissues were measured using the ELISA method, following the protocols provided by the manufacturer's kit contents.

Western Blot Analysis

The protein levels of heme oxygenase-1 (HO-1) and nuclear factor erythroid 2-related factor 2 (Nrf-2) in ovarian tissue were measured using the Western blot technique, following the procedure reported by Cellat et al. (2022).

Histopathological Examination

Tissue samples from the uterus and ovaries were fixed in a 10% buffered formalin solution for histopathological analysis. After being embedded in paraffin following a series of treatments with alcohol and xylol, standard procedures were used to prepare five µm thick slices. The sections, following deparaffinization in xylol and a series of alcohol solutions (100, 96, 80, and 70%) were examined under a light microscope (Olympus CX31) and photographed using an Olympus DP12 camera after staining with hematoxylin-eosin (H&E).

Statistical Evaluation

Statistical evaluations were conducted using SPSS Statistics 23 software. The histopathological results were assessed semi-quantitatively. The normality of the parameters was determined using the Shapiro–Wilk normality test. Differences between the groups were detected using one-way analysis of variance (ANOVA). Post hoc Duncan tests were applied for pairwise comparisons. All values were reported as mean ± standard error (±S.E.M.), and significance was considered at $p < 0.05$.

Results

Vaginal Cell Samples

Out of the initial fifty rats in the study, six did not exhibit regular estrus, while forty-four did. The duration of normal estrus cycles in rats was determined to be 4.5 days. After the treatment, irregular estrus cycles were observed in 7 rats in the letrozole group, while regular cycles were observed in 8 rats from the control group, 8 rats from the safranal group, and 6 rats from the L+S group (Fig. 1). It was noted that rats with normal estrus cycles had cycles lasting 4.5 days in the safranal group and 5 days in the L+S group.

Serum Parameter Results

C-reactive protein, glucose, total cholesterol, TG, HDL, and LDL values were measured in serum samples. The findings revealed that glucose and TG levels increased in the letrozole group as compared to the control group. There was a slight decrease when the L+S group was compared to the letrozole group, although this was not statistically significant ($p > 0.01$). Additionally, CRP level increased in the letrozole group compared to the control group, while the HDL level fell. These values were statistically close to the control group levels in the L+S group ($p < 0.01$). The total cholesterol and LDL levels in the letrozole group increased significantly when compared to the control group, while they decreased the L+S group when compared to the letrozole group ($p < 0.01$, for all, Table 1).

Oxidative Stress Results

Malondialdehyde, GSH levels, and GPx and CAT enzyme activities were evaluated in ovarian tissue to assess the oxidative stress resulting from the study's treatment. The safranal group exhibited the lowest MDA value, while the letrozole group showed a significant increase compared to the other groups In the treatment group, the MDA value approached that of the control group ($p < 0.01$). Regarding GSH levels and GPx activities, no significant differences were observed between the groups ($p > 0.05$, for all). However, letrozole significantly reduced CAT activity compared to the control and safranal groups. Although this decrease was partially prevented in the treatment group, it did not reach statistical significance ($p < 0.01$, Table 2).

ELISA Test Results

ELISA kits were used to measure FSH, LH, E2, and testosterone hormone levels in serum samples from each group. FSH level in the letrozole group decreased significantly compared to the other groups, while the LH level increased significantly ($p < 0.001$, for all). The treatment group showed significant improvements in LH and FSH hormone levels when compared to the letrozole group. E2 level decreased in the letrozole group compared to the control group but increased in the L+S group compared to the letrozole group ($p < 0.001$). The letrozole group had higher testosterone levels than the control group, while the L+S group had lower levels compared to the letrozole group ($p < 0.001$). Progesterone levels were lower in the letrozole group than in the control group, but they were higher in the L+S group compared to the letrozole group ($p < 0.01$, Table 3).

Additionally, proinflammatory cytokines TNF-α, IL-6 and IL-β were measured in both serum and ovarian tissue samples. In terms of serum values, TNF-α, IL-6 and IL-β levels were higher in the letrozole group compared to the control group, but all values were improved in the L+S group ($p < 0.001$, for all, Table 4). Similarly, all cytokine

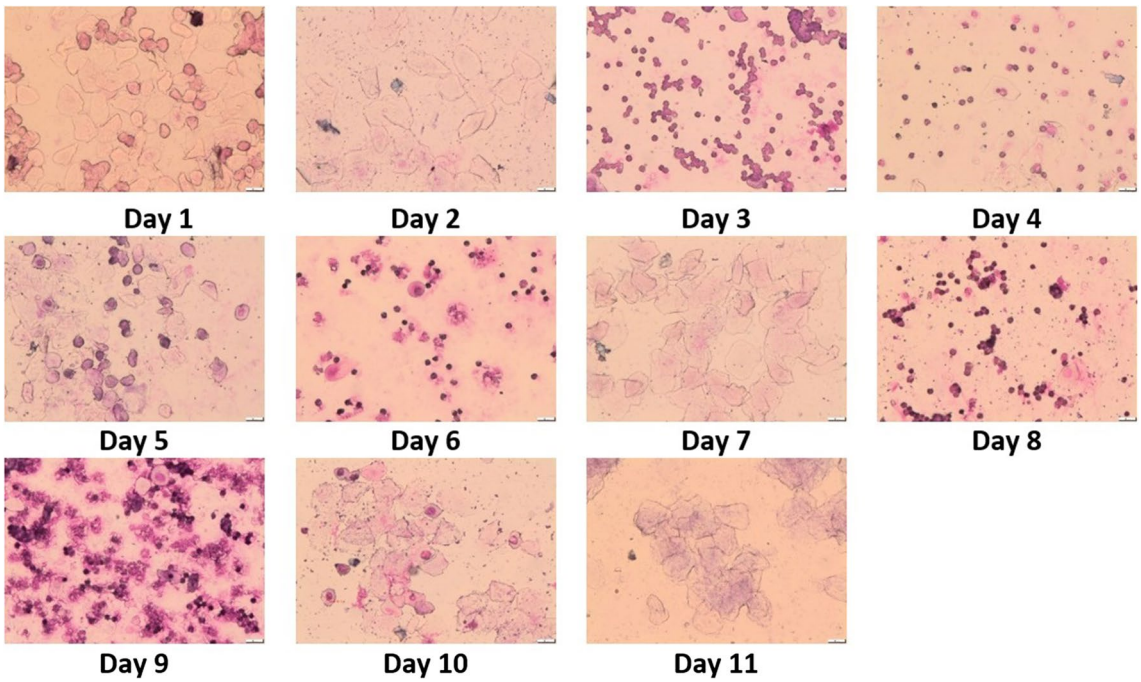

Fig. 1 Vaginal cytological observation of the rat in the L+S group with regular estrus

Table 1 Serum biochemical parameters

Parameter /Group	MDA	GSH	GPx	CAT
Control	8.873±0.18^{b}	7.563±0.89	24.744±2.11	43.174±2.21^{b}
Letrozole	10.920±0.37^{c}	7.500±0.50	21.398±1.14	35.162±0.46^{a}
Safranal	7.230±0.35^{a}	7.197±0.90	26.701±1.55	46.582±1.54^{b}
L+S	8.466±0.22^{b}	6.707±0.55	22.291±0.90	40.095±1.72^{ab}

Values represent mean ± SEM for each group. Different superscript letters (a, b, c) within the same column show statistically significant differences between the groups ($p < 0.01$). MDA: malondialdehyde; GSH: glutathione; GPx: glutathione peroxidase; CAT: catalase.

Table 2 Ovarian tissue oxidative stress parameter measurement results

Parameter/Group	CRP	Glucose	Total Cholesterol	TG	HDL	LDL
Control	5.866±0.52^{ab}	95.833±2.98^{a}	35.000±1.91^{a}	79.666±2.21^{a}	30.000±1.39^{a}	13.833±0.90^{a}
Letrozole	12.166±0.81^{c}	143.333±4.37^{b}	68.833±2.52^{c}	109.666±3.10^{b}	16.333±1.14^{b}	28.500±1.64^{c}
Safranal	3.950±0.33^{a}	91.833±3.05^{a}	38.500±2.68^{a}	77.166±3.40^{a}	30.833±1.30^{a}	14.666±1.14^{a}
L+S	6.650±0.63^{b}	131.666±3.77^{b}	49.666±2.38^{b}	100.00±2.97^{b}	26.000±1.67^{a}	22.333±1.20^{b}

Values represent mean ± SEM for each group. Different superscript letters (a, b, c) within the same column show statistically significant differences between the groups ($p < 0.01$). CRP: C-reactive protein; TG: triacylglyceride; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

values in ovarian tissue were significantly higher in the letrozole group than in the other groups, similar to the values seen in serum levels. There was no statistically significant difference between the L+S group and the control group ($p < 0.001$, for all, Table 4).

Western Blot Analysis

Protein levels of Nrf-2 and HO-1 in ovarian tissue were assessed using the Western blot method. The letrozole group exhibited significantly lower levels of Nrf-2 and HO-1

Table 3 Serum hormonal parameters

Parameter/ Group	FSH	LH	E2	Testosterone	Progesterone
Control	7,477±0,35	2,044±0,04	71,264±2,08	7,114±0,68	46,151±3,17
Letrozole	4,412±0,25*	2,830±0,08*	48,616±3,68*	13,209±1,02*	1,840±2,31*
Safranal	7,663±0,12	2,071±0,07	70,089±2,27	7,451±0,61	50,343±3,01
L+S	6,813±0,43	2,170±0,08	70,874±2,09	8,567±0,65	41,036±2,74
p-Value	0,000	0,000	0,000	0,000	0,002

Values represent mean ± SEM for each group. *Shows statistical difference when compared to other groups. FSH: follicle-stimulating hormone; LH: luteinizing hormone; E2: estradiol.

compared to the control group. When comparing the L+S and letrozole groups, increased Nrf-2 levels were observed (Fig. 2A), but HO-1 levels did not show a statistically significant difference (Fig. 2B).

Histopathological Results

Histopathological analysis of ovarian tissues in the control group revealed normal cortex and medulla. Healthy primordial, primary, secondary, and tertiary follicles, granulosa, theca layer, uniform round cells, and corpus luteum with eosinophilic cytoplasm were observed in the ovarian sections at various developmental stages (Fig. 3A). Rats treated with letrozole had large cystic follicles with insufficient granulosa cells or multiple large cysts lacking a granulosa cell layer. Degenerative changes in granulosa cells and necrotic cells were observed in follicles with reduced cell layer and cyst-shaped follicles (Fig. 3B). With the exception of one rat, the rats in the letrozole group had significantly more cystic follicles than those in the control group, and there was also a noticeable reduction in the granulosa cell layer. Treatment with L+S decreased the number of cystic follicles in the ovaries of the treated rats (Fig. 3C), and mitotic figures were observed in the granulosa cells of the letrozole-treated animals. Additionally, it was found that healthy follicles at various developmental stages were similar to the control and safranal groups in the rats treated with L+S when their ovaries were compared to those of the polycystic ovary group. Normal follicles at all developmental stages were comparable to the control group in rats given Safranal, while vacuolated granulosa cells were significantly more prominent (Fig. 3D). Only one rat's corpus luteum had granulosa cells that had undergone necrotic changes. The uterine tissues of the control group showed hyperemia, mononuclear cell infiltrations with few neutrophils, and lymphocytes (Fig. 4A). Both the letrozole and L+S groups exhibited thickening of the endometrium. The epithelial cells in the letrozole, L+S, and safranal groups were identical in size and cuboidal compared to the control group, despite the fact that hyperplasia in the lamina epithelialis was observed in the uterus of the rats treated with letrozole. In both the L+S and safranal groups, endometrial gland hyperplasia was observed. The letrozole group's propria exhibited hyperemia, mononuclear cell infiltration, notably lymphocytes, and hemosiderin pigmentation (Fig. 4B). Lymphocyte-containing mononuclear cell infiltrations and an increase in the number of glands in the propria were noted in the L+S group (Fig. 4C). Hyperemia, an increase in the number of endometrial glands, particularly neutrophil granulocytes and a small number of mononuclear cell infiltrations were observed in the safranal group (Fig. 4D).

Table 4 Serum and ovarian inflammation parameters

	Parameter/ Group	TNF-α	IL-6	IL-1β
Serum	Control	79.589±2.38	3.615±0.14	320.733±7.61
	Letrozole	105.503±2.17*	5.791±0.18*	440.233±7.94*
	Safranal	78.967±4.02	3.682±0.09	305.630±6.14
	L+S	83.736±1.77	4.107±0.10	324.801±7.99
Ovarian	Control	103.893±2.00	5.080±0.06	393.783±13.68
	Letrozole	135.602±1.49*	7.452±0.22*	538.410±23.19*
	Safranal	98.674±3.01	5.001±0.09	398.660±6.12
	L+S	104.247±1.40	5.172±0.09	411.443±5.72
	p-Value	0.000	0.000	0.000

Values represent mean ± SEM for each group. *Shows statistical difference when compared to other groups. TNF-α: tumor necrosis factor-alpha; IL-6: interleukin-6; IL-1β: interleukin-1beta.

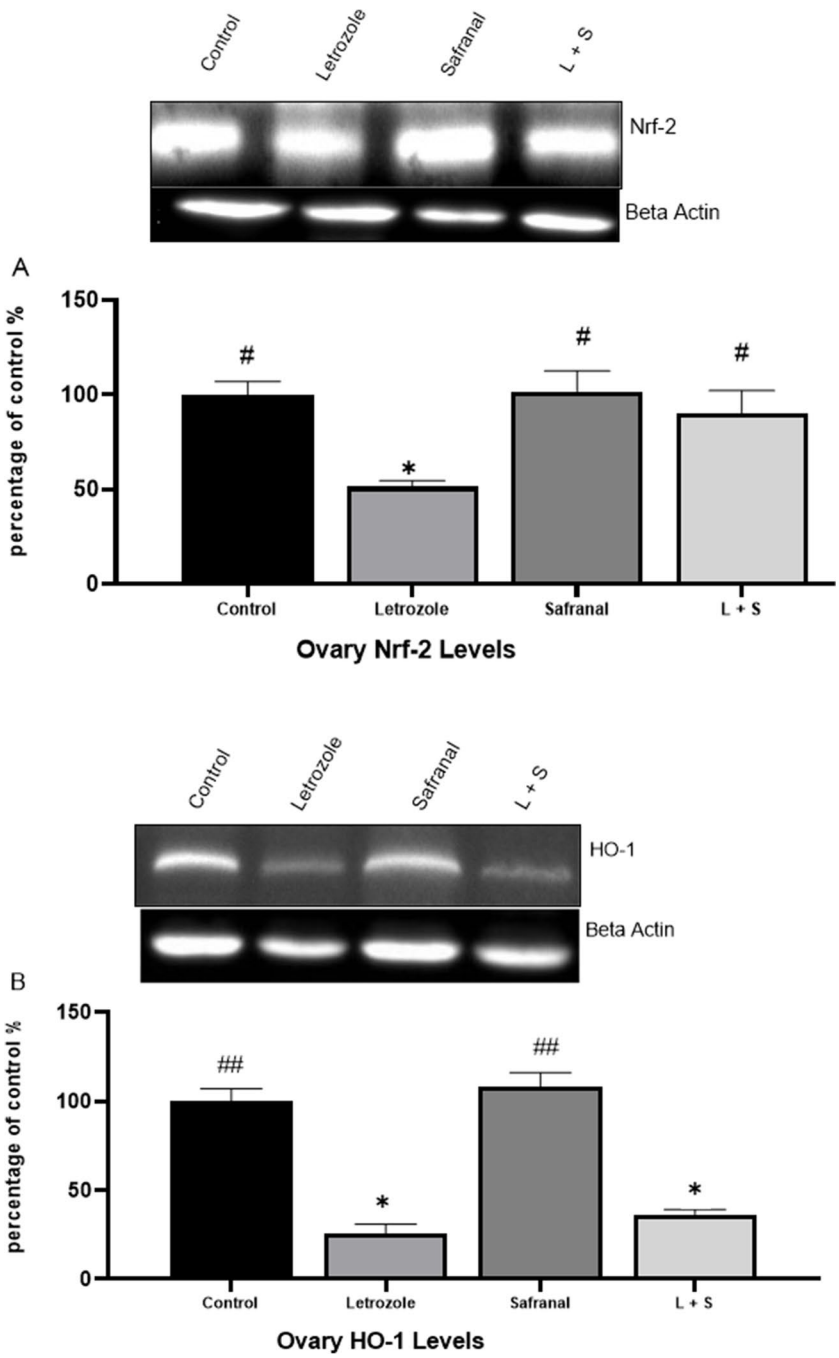

Fig. 2 Effect of safranal (**1**) on western blot analysis of Nrf-2 and HO-1 in the ovary tissue of control and experimental rats. Protein expression is normalized to β-actin. Data are shown as percent of control. A. Representative western blot picture of Nrf-2 and Beta Actin in ovary. Mean Nrf-2 expression levels (%) B. Representative western blot picture of HO-1 and Beta Actin in ovary. Mean HO-1 expression levels (%). Data are expressed as mean ± SEM. # *$p < 0.05$, **$p < 0,01$ versus control group. Nrf-2: nuclear factor erythroid 2-related factor-2; HO-1: heme oxygenase-1

Discussion

Chronic inflammation, oxidative stress, and metabolic disorders are all linked to PCOS, which can have an impact on a patient's quality of life and long-term health. The available therapies do not sufficiently address PCOS. The induction of a PCOS model in rats using letrozole is a widely employed technique in literature. Anovulation results from hormonal changes that hinder or halt follicle the maturation. Since animals exhibit many characteristics resembling human PCOS, such as hyperandrogenism, abnormal follicles, hyperglycemia, and oxidative stress, the letrozole-induced PCOS model is a preferred approach (Ibrahim et al. 2022). In our study, letrozole was administered successfully for 21days to induce PCOS in rats, and the findings have been evaluated in the context light of existing research.

Female sex hormones, particularly estrogen and progesterone, play a pivotal role in regulating the menstrual cycle. Patients with PCOS often experience irregular menstruation due to hormonal irregularities (Patel et al. 2022). Studies have indicated that rats with a PCOS model develop cystic follicles and experience disruptions in their estrous cycle

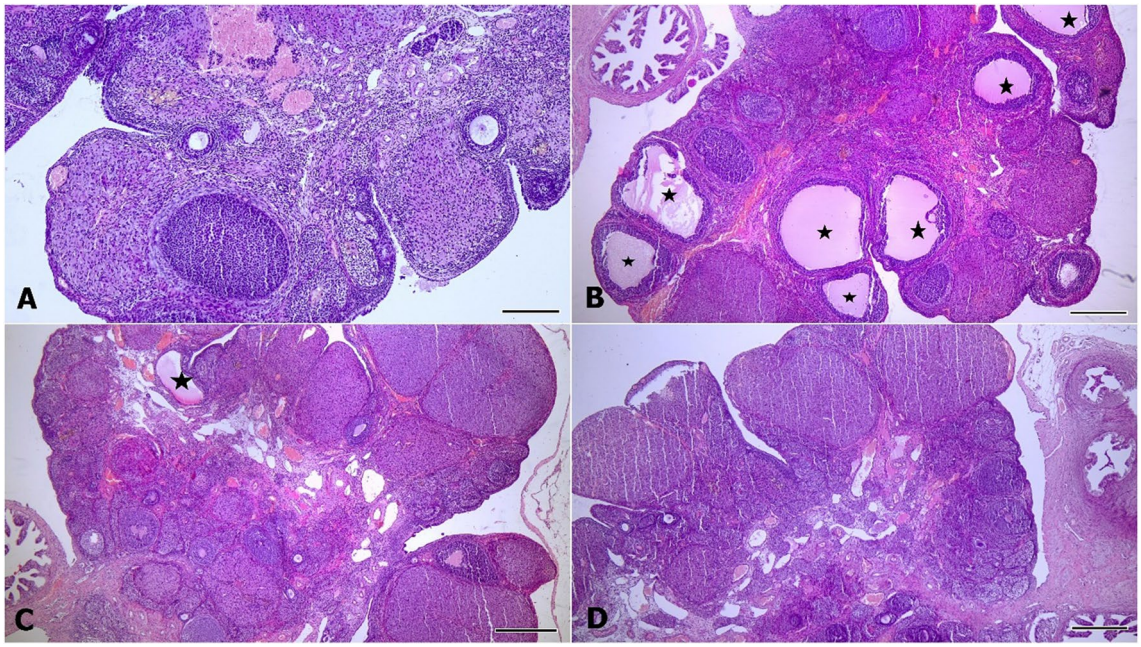

Fig. 3 Histopathology of the ovary, A. Control group; Normal follicles and hyperemia, B. Letrozole group; Cystic follicles (stars) and hyperemia, C. L+S group; Cystic follicle (star) and hyperemia in only three rats, D. Safranal (**1**) group; Hyperemia, H&E. Bars: 200 µm (A), 500 µm (B-D)

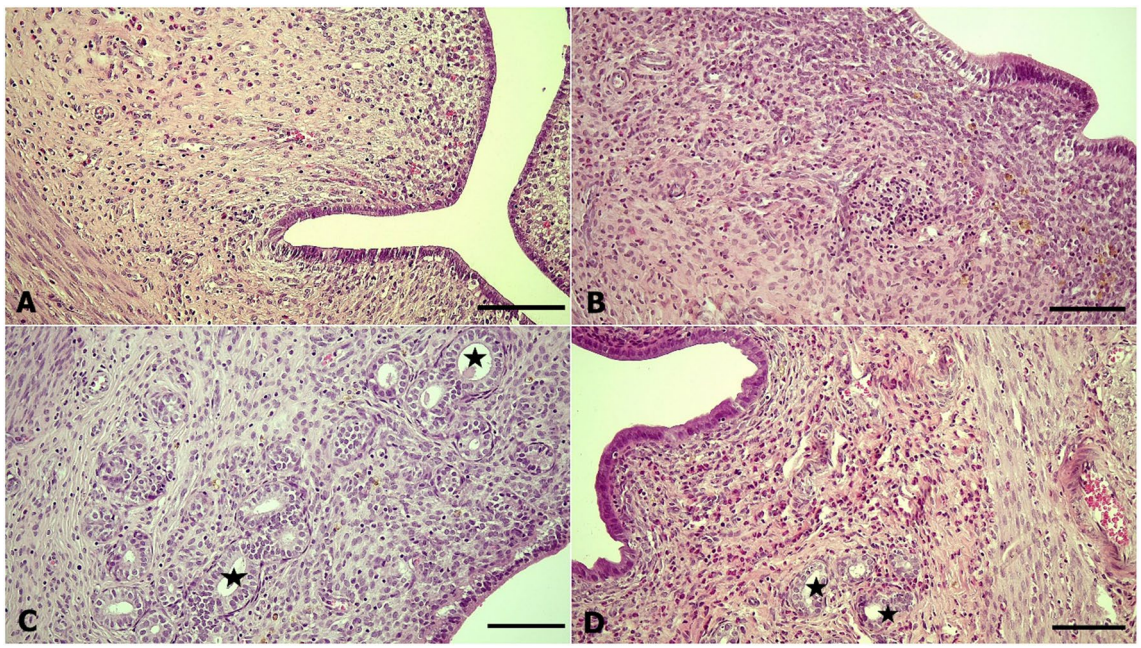

Fig. 4 Histopathology of uterine tissues. A. Control group; Hyperemia and cell infiltrations, B. Letrozole group; hyperplasia in epithelial cells and mononuclear cell infiltration, C. L+S group; Mononuclear cell infiltration and hyperplasia of endometrial glands (stars), D. Safranal group; Hyperemia, neutrophil granulocyte infiltration and glandular hyperplasia (stars), HE. Bars: 100 µm

(Feng et al. 2022). Our study revealed that 87.5% of rats in the letrozole group had disrupted estrous cycles, whereas this percentage decreased to 25% in rats treated with safranal. The improvement in the estrous cycle suggests the potential elimination of anovulation and the restoration of normal ovarian functions (Soumya et al. 2016). Maintaining

proper follicular form and function depends on the interaction between granulosa and theca cells. Hyperplasia of theca interna, however, is the primary contributor to ovarian dysfunction in PCOS. Rats with PCOS have been observed to develop several ovarian cysts larger than other follicles, a thin granulosa cell layer, and hyperplastic theca cell layers within cystic follicles (Ali et al. 2021). Our histological analysis indicated that safranal treatment successfully reversed PCOS-induced changes in uterine and ovarian tissues in PCOS-affected rats. Consequently, the use of safranal in treating PCOS-affected rats shows promise for restoring normal ovarian function.

PCOS has been associated with various forms of dyslipidemia, including elevated levels of total cholesterol, triacylglycerides, and LDL, as well as reduced levels of HDL (Pasquali et al. 2011). Our study's findings demonstrated that the letrozole group exhibited increases in cholesterol, triacylglycerides, LDL, and glucose levels compared to the control group. Conversely, the level of HDL decreased. Treatment with safranal for PCOS in rats led to an approach of HDL levels to those of the control group, along with a significant improvement in total cholesterol and LDL levels compared to the letrozole group. Although triacylglyceride and glucose levels decreased in response to safranal treatment, the changes were not statistically significant. Sabir et al. (2022) reported that safranal treatment had a therapeutic effect on dyslipidemia in rats with non-alcoholic fatty liver disease. According to some reports, the saffron's impact may be attributed to its influence on metabolic processes and enzymes involved in cholesterol synthesis and metabolism.

Free radicals play a crucial physiological regulatory role, but their excessive production may promote ovarian disease by changing the redox equilibrium of ovarian tissue. Thus, it is claimed that oxidative stress contributes to the pathophysiology of PCOS (Elmosalamy et al. 2022). To identify the therapeutic effects of safranal in PCOS-affected rats, oxidative stress parameters were analyzed. While the MDA level of the Letrozole group increased significantly compared to the control group, safranal treatment significantly decreased the MDA level. CAT activity decreased in the letrozole group but showed only a slight improvement in the L+S group. GSH levels and GPx activity, on the other hand, were statistically similar between groups. P Imbalances in the antioxidant-prooxidant equilibrium have been described to have pathological effects on oocyte maturation, ovulation, fertilization, implantation, and embryo development (Haslan et al. 2021). As a result of its antioxidant effects, safranal treatment can be considered to have a therapeutic impact on PCOS.

The main clinical sign of PCOS is hyperandrogenism, which occurs as a result of steroid production dysregulation. It has been shown that LH hypersecretion and hyperinsulinemia boost androgen production synergistically, and rats given letrozole had low estrogen and high testosterone levels (Patel and Shah 2018). FSH deficiency inhibits the formation of tiny ovarian follicles, leading to polycystic ovaries and anovulation (Tena et al. 2011). Progesterone blood levels are also typically low in women with PCOS, which is attributed to anovulation and a reduced number of corpora lutea (Khosrowpour et al. 2022). In our study, we observed a significant decrease in FSH, progesterone, and E2 levels, accompanied by elevated LH and testosterone levels in rats with PCOS. This contrasts with previous research findings where FSH levels decreased, while LH and testosterone levels increased significantly (Zhou et al. 2022). Notably, safranal treatment in our current study effectively prevented the decline in FSH levels. The imbalance in LH/FSH ratios has been associated with the proliferation of ovarian theca cells, leading to increased steroidogenesis and subsequent hyperandrogenism (Helal et al. 2021). Additionally, Begum et al. (2022) reported higher testosterone levels in rats with letrozole-induced PCOS compared to the control group, along with lower progesterone and E2 levels. Fukui et al. (2011) found that the scent of saffron extract reduced testosterone levels in the follicular phase while increasing E2 levels in the follicular and luteal phases, suggesting that saffron may have a regulatory effect on sex hormones. Changes in hormone levels can lead to structural changes in the ovaries of mice. According to the letrozole-induced PCOS model, the number of cystic follicles increased while the corpus luteum decreased (Begum et al. 2022). Other reported ovarian changes in PCOS include ovarian hypertrophy, hyperplasia in the ovarian stroma, an increase in fibrous subcapsular follicle cysts, thickening of the capsule, and early luteinization of theca cells (Zeng et al. 2022). In light of its potential to regulate sex hormones, safranal, a primary component of saffron, holds promise for the treatment of PCOS.

Proinflammatory cytokines are believed to play a significant role in the pathogenesis of PCOS development, as PCOS is considered a chronic inflammatory disorder (El-Saka et al. 2021). Reports indicate that patients with PCOS exhibit elevated levels of circulating CRP, indicating sporadic low-grade chronic inflammation. Notably, this increase in CRP levels is not necessarily linked to obesity (Mohammadi et al. 2017). Our findings revealed that ovarian and serum TNF-α, IL-6, and IL-1β levels in PCOS rats were significantly higher than in the control group. Additionally, serum CRP levels were elevated compared to the control group. All of these parameters showed improvement when comparing the L+S group to the letrozole group. Previous research has also reported increased serum TNF-α, IL-6, IL-1β, and ovarian IL-1β levels in rats with PCOS compared to controls (Ibrahim et al. 2022). Given the elevated levels of inflammatory cytokines, particularly interleukins and CRP, in PCOS patients, polycystic ovaries are considered a chronic inflammatory condition (Sadoughi et al. 2017). Safranal treatment had an anti-inflammatory effect against CCl_4-induced inflammation by reducing TNF-α, IL-6, and

IL-1β levels in Alayunt et al.'s study (2019). It has been demonstrated that the reduction in CRP associated with metformin administration, a common treatment for PCOS, is correlated with the alleviation of metabolic symptoms in PCOS patients (Mohammadi et al. 2017). Given its anti-inflammatory effects, safranal may be considered as a potential alternative for the treatment of PCOS, aligning with the outcomes of our study.

The essential molecule Nrf-2 plays a pivotal role in controlling the antioxidant response to maintain cell function in various tissues, including female reproductive organs (Li et al. 2022). In our study, letrozole significantly reduced the levels of the proteins Nrf-2 and HO-1, while safranal treatment exhibited a therapeutic effect, particularly concerning Nrf-2 levels. Consistent with these findings, Ibrahim et al. reported significantly lower levels of the proteins Nrf-2 and HO-1 in rats with PCOS compared to the control group (Ibrahim et al. 2022). Astaxanthin, a powerful antioxidant, was shown in another study to boost Nrf-2 expression in PCOS patients. Since Nrf-2 can be a strong survival factor for follicle preservation, it has been suggested that activation of the Nrf-2 signaling pathway may be important in reducing oxidative stress in patients with PCOS (Gharaei et al. 2022).

Accurate diagnosis and treatment of PCOS are crucial to effectively manage the condition and mitigate associated metabolic disorders and health risks (Chaturvedi et al. 2023). Our study indicates that safranal treatment regulates the estrus cycle, reduces oxidative stress, balances sex hormone levels, ameliorates dyslipidemia, decreases inflammation, and maintains ovarian tissue architecture in the letrozole-induced PCOS model. Based on these findings, it can be inferred that safranal treatment holds promise as a therapeutic approach for PCOS patients.

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Data availability All the data will be available on request.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethics approval This study was carried out with the approval of Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee, numbered 2020/06-05.

Informed consent Not applicable

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