

Galangin Mitigates Letrozole-Induced Polycystic Ovary Syndrome in Rats by Restoring PI3K/pAKT/PTEN Signaling

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Abstract

Purpose:

Polycystic ovary syndrome (PCOS) is a complex endocrine and metabolic disorder characterized by hyperandrogenism, ovulatory dysfunction, and the formation of ovarian cysts. Key contributors to its pathophysiology include oxidative stress, inflammation, and altered intracellular signaling, especially within the PI3K/pAKT/PTEN pathway. Galangin, a dietary flavonoid derived from *Alpinia galanga*, exhibits antioxidant, anti-inflammatory, and estrogen- modulatory properties. This study investigated the protective effects of galangin in a PCOS rat model induced by letrozole and explored its underlying molecular mechanisms.

Methods:

Thirty-six adult female Wistar rats were divided into six groups: control, galangin (8 mg/kg), letrozole (1 mg/kg), letrozole + galangin (4 or 8 mg/kg), and letrozole + metformin (20 mg/kg). All treatments were administered orally for 21 days. Serum hormones, oxidative stress biomarkers, inflammatory mediators, and key proteins in the PI3K/pAKT/PTEN pathway were assessed, along with histopathological and immunohistochemical analyses.

Results:

Letrozole administration induced characteristic PCOS-like features, including cystic follicle formation, hormonal imbalances, oxidative stress, inflammation, and suppression of PI3K/pAKT signaling, accompanied by an increase in PTEN levels. Galangin pretreatment improved ovarian morphology, restored hormonal balance, reduced oxidative and inflammatory responses, and reactivated PI3K/pAKT signaling while downregulating PTEN. These effects were comparable to those observed with metformin.

Conclusion:

Galangin provides multidimensional protection against letrozole-induced ovarian dysfunction by alleviating oxidative stress, inflammation, and dysregulation of the PI3K/pAKT/PTEN pathway. These findings support the potential of galangin as a safe, multitarget natural adjunct for managing PCOS.

1. Introduction

Polycystic ovary syndrome (PCOS) is a widespread endocrine and metabolic disorder that affects 8–13% of women of reproductive age worldwide (Almhoud et al. 2024). It is a leading cause of infertility and metabolic dysfunction (Wal et al. 2024). PCOS is typically characterized by excessive androgen production, irregular menstrual cycles, and the presence of polycystic ovaries. It is often associated with insulin resistance, obesity, and dyslipidemia (Rajabi et al. 2024). Despite extensive research, the

underlying mechanisms of PCOS remain complex and multifactorial, involving various hormonal, genetic, and environmental factors (Bai et al. 2024).

The pathogenesis of PCOS is influenced by complex interactions among oxidative stress, chronic inflammation, and dysregulated intracellular signaling. An imbalance between reactive oxygen species (ROS) generation and the antioxidant defense system leads to oxidative damage in ovarian tissue (Ihim et al. 2024; Mishra et al. 2024). Research shows that patients and experimental models of PCOS exhibit increased levels of lipid peroxidation products and decreased activity of key antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) (Talat et al. 2022; Mahmud et al. 2022; Lan et al. 2024). This imbalance disrupts follicular development, impairs steroid hormone production, and increases insulin resistance. Additionally, elevated levels of pro-inflammatory mediators, including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF- α), and nuclear factor-kappa B (NF- κ B), exacerbate oxidative stress and interfere with ovarian function (Kar et al. 2024; Bansal et al. 2025).

Several signaling pathways play a role in the development of PCOS, particularly the phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKT) pathway. This pathway is crucial for regulating glucose metabolism, follicular survival, and ovarian cell proliferation (Rabah et al. 2023; Li et al. 2025). The negative modulator of this pathway, phosphatase and tensin homolog (PTEN), functions as a tumor suppressor by inhibiting PI3K/AKT activation (Hsueh et al. 2015; De Felici and Klinger 2021). In conditions marked by oxidative stress and inflammation, PTEN expression is upregulated, which suppresses AKT signaling. This suppression can lead to impaired follicular development, anovulation, and hormonal imbalances (Ghafari et al. 2025). Therefore, the interplay between oxidative stress, inflammatory cytokines, and the dysregulation of the PI3K/AKT/PTEN pathway is a critical triad that underlies the molecular pathology of PCOS.

There are various therapeutic strategies for managing PCOS, including lifestyle modification, pharmacological treatments, and assisted reproductive techniques (Bai et al. 2024). Common pharmacological options include metformin, clomiphene citrate, and combined oral contraceptives, which are used to address metabolic and reproductive disturbances. However, these treatments can be associated with side effects, limited effectiveness, and the recurrence of symptoms upon discontinuation (Melin et al., 2024). As a result, there is an increasing interest in exploring safer, naturally derived compounds that can target multiple pathogenic pathways and holistically restore ovarian function (Cowan et al. 2023).

Galangin (3,5,7-trihydroxyflavone) is a naturally occurring flavonoid primarily found in *Alpinia galanga* and propolis (Wang et al. 2023). It has attracted increasing attention due to its extensive pharmacological properties, which include antioxidant, anti-inflammatory, anti-fibrotic, and metabolic regulatory effects (Aladaileh et al. 2021; Abukhalil et al. 2021). As an antioxidant, galangin helps maintain redox balance by reducing oxidative stress and protecting against fructose-induced renal injury, demonstrating dose-dependent improvements in insulin resistance and oxidative markers in rats (Sivakumar et al. 2010). Its anti-inflammatory effects are well-documented; galangin modulates several

molecular pathways, including p38 MAPK, NF- κ B, NLRP3 inflammasome, and PI3K/AKT, that play crucial roles in inflammatory signaling and oxidative damage (Deng et al. 2022; Thapa et al. 2023). In bone tissue, galangin inhibits RANKL-induced osteoclastogenesis by targeting the MAPK and NF- κ B pathways, further confirming its broad anti-inflammatory capabilities (Li et al. 2021). Additionally, *Alpinia officinarum* extracts, a significant natural source of galangin, have shown antihyperlipidemic activity by regulating lipid metabolism and improving serum lipid profiles in experimental models (Shin et al. 2002).

In reproductive tissues, galangin and its structurally related flavonoid, pinocembrin, have been shown to displace dihydrotestosterone from sex hormone-binding globulin, suggesting a modulatory influence on androgen availability (Hillerns et al. 2005). Other flavonoids, including galangin, have demonstrated estrogenic potential in assays using recombinant yeast and MCF-7/BUS cells, indicating their ability to interact with estrogen receptors (Resende et al. 2013). A recent molecular docking study has also revealed that phytoconstituents from *Phyllanthus niruri*, including galangin analogs, may target MMP-9 and IL-1 β , contributing to anti-inflammatory and anti-angiogenic effects in the treatment of endometriosis (Wulandari et al. 2024).

This study aimed to evaluate the protective effects of galangin against experimentally induced PCOS in female rats and to elucidate the underlying molecular mechanisms involved. The findings suggest that galangin could serve as a potential natural adjunctive therapy for managing PCOS, providing a safer and more effective alternative to conventional treatments.

2. Materials and Methods

2.1 Chemicals and Reagents

Letrozole and metformin were obtained from Merck (Darmstadt, Germany). Galangin ($\geq 98\%$ purity) was purchased from Aktin Chemicals Inc. (Chengdu, China). All other reagents and solvents used in this study were of analytical grade and sourced from reputable commercial suppliers.

2.2 Animals

This study involved thirty-six adult female Wistar rats, aged 2–3 months and weighing between 180 and 200 grams. The animals were housed under controlled laboratory settings ($24 \pm 2^\circ\text{C}$, 12-hour light/dark cycle) with unrestricted access to standard feed and water. All experimental protocols were reviewed and approved by the Research Ethics Committee of the Faculty of Pharmacy at King Abdulaziz University in Jeddah, Saudi Arabia (Approval No. PH-1445-35). Furthermore, all animal procedures complied with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023, revised 1978).

2.3 Induction of Letrozole-Induced Polycystic Ovary Syndrome (PCOS) in Female Rats

Thirty-six female Wistar rats were confirmed to be in the same stage of estrous cycle by vaginal smears before grouping. The animals were then randomly divided into six experimental groups (n = 6). The induction of PCOS model was adapted from previous studies (Namlı Kalem et al. 2023). Treatments were administered once daily by oral gavage for 21 consecutive days as follows:

1. **Control:** Received 1% carboxymethylcellulose (CMC; 1 ml/kg/day, p.o.) and 1% dimethyl sulfoxide (DMSO) in normal saline (10 ml/kg/day, p.o.).
2. **Galangin:** Administered galangin (8 mg/kg/day, p.o.) and 1% DMSO in normal saline (10 ml/kg/day, p.o.).
3. **PCOS:** Administered letrozole (1 mg/kg/day, p.o.) to induce PCOS, alongside with 1% CMC (1 ml/kg/day, p.o.)
4. **PCOS + Galangin (4 mg/kg):** Received galangin (4 mg/kg/day, p.o.) one hour prior to letrozole (1 mg/kg/day, p.o.).
5. **PCOS + Galangin (8 mg/kg):** Received galangin (8 mg/kg/day, p.o.) one hour prior to letrozole (1 mg/kg/day, p.o.).
6. **PCOS + Metformin:** Received metformin (20 mg/kg/day, p.o.) one hour prior to letrozole (1 mg/kg/day, p.o.).

Letrozole was used to inhibit aromatase activity and increase androgen production, effectively mimicking the endocrine and morphological characteristics of PCOS. Galangin and metformin treatments were administered concurrently with letrozole to assess their potential protective effects. The doses selected for these treatments were based on previously published experimental studies that demonstrated optimal efficacy and safety in rodent models (Aloud et al. 2017, 2018).

2.4 Sample Collection and Preparation

At 24 hours after the final treatment, all animals were weighed and anesthetized with ketamine (80 mg/kg, i.p.) and xylazine (8 mg/kg, i.p.). Blood samples were collected from the retro-orbital plexus using capillary tubes, then centrifuged at 3,000 rpm for 15 minutes to separate the serum. The obtained serum was stored at – 80°C until subsequent hormonal and biochemical analyses. Following blood collection, the animals were sacrificed by cervical dislocation. Both ovaries were carefully excised, cleared of adherent fat and connective tissue, and rinsed with physiological saline. The right ovary was fixed in 10% neutral buffered formalin for histopathological and immunohistochemical examination, whereas the left ovary was snap-frozen in liquid nitrogen and stored at – 80°C for biochemical assays.

2.5 Histopathological Investigation of Ovarian Tissue

Ovarian tissues fixed in 10% neutral buffered formalin were processed for routine histological examination. The fixed samples underwent dehydration through a graded series of ethanol solutions (70%, 80%, 95%, and 100%) to remove water and formalin. Once dehydrated, the specimens were cleared in xylene and embedded in paraffin wax to create uniform tissue blocks. The paraffin-embedded ovaries were then sectioned to a thickness of 5 µm using a rotary microtome, and the sections were mounted on clean glass slides. For microscopic evaluation, the paraffin sections were deparaffinized in xylene and

rehydrated through descending grades of ethanol (100%, 95%, 80%, and 70%). They were then stained with hematoxylin and eosin (H&E) to assess general ovarian morphology (Bancroft 2008). The stained slides were examined under a light microscope (Carl Zeiss Axiostar Plus, Oberkochen, Germany) by a histopathologist who was blinded to the experimental groups. The evaluation included an examination of follicular development, the presence of cystic follicles, the integrity of granulosa and theca cells, corpora lutea, and stromal organization. Representative photomicrographs were selected from at least three sections per ovary from three animals per group to ensure consistency and reproducibility of observations.

2.6 Measurement of Serum Hormone Levels

Serum levels of estradiol, LH and anti-Müllerian hormone (AMH) were measured using enzyme-linked immunosorbent assay (ELISA) kits specifically designed for rats. These kits were obtained from MyBioSource (San Diego, CA, USA) and were used according to the manufacturer's instructions. The specific kits utilized were: estradiol (Catalog No. MBS2607338), LH (Catalog No. MBS764675), and AMH (Catalog No. MBS726534). Absorbance readings were taken at a wavelength of 450 nm using a microplate reader, and hormone concentrations were determined from standard calibration curves created with known reference concentrations.

2.7 Assessment of Oxidative Stress Biomarkers

The levels of malondialdehyde (MDA) and GSH, along with the activities of SOD and CAT, were measured in ovarian tissue homogenates using commercial colorimetric assay kits (Bio-Diagnostic, Giza, Egypt). The corresponding product codes were: MD 25 29 for MDA, GR 25 11 for GSH, SD 25 21 for SOD, and CA 25 17 for CAT. All assays were performed according to the manufacturer's protocols. The results for MDA and GSH were expressed as concentrations, whereas SOD and CAT were expressed as enzyme activities, normalized to the protein content of the tissue homogenate.

2.8 Immunohistochemical Analysis

Immunohistochemical (IHC) staining was conducted to assess the ovarian expression of inflammatory markers, including IL-6, TNF- α , and NF- κ B, as well as key proteins involved in the PI3K/AKT/PTEN signaling pathway. Paraffin-embedded ovarian sections (5 μ m thick) were deparaffinized in xylene, rehydrated through a series of graded alcohol concentrations, and subjected to antigen retrieval by boiling in a 10 mM sodium citrate buffer (pH 6.0) for 5 minutes. After cooling, the sections were rinsed with phosphate-buffered saline (PBS) and incubated in 5% bovine serum albumin (BSA) in Tris-buffered saline (TBS) for 1 hour at room temperature to block non-specific binding. The slides were then incubated overnight at 4°C with one of the following primary antibodies: IL-6 (ab9324, Abcam, UK), TNF- α (ab307164, Abcam, UK), NF- κ B p65 (8242, Cell Signaling Technology, USA), PI3K (MA1-74183, Invitrogen, USA), p-AKT (sc-514032, Santa Cruz Biotechnology, USA), and PTEN (ab170941, Abcam, UK). The following day, the sections were washed and incubated with the appropriate secondary antibody for 30 minutes at room temperature. The slides were examined and photographed using an Olympus BX53 light

microscope (Tokyo, Japan). The percentage of immunopositive area for each marker was quantified using ImageJ software (version 6, National Institutes of Health, USA).

2.9 Statistical analysis:

All data are presented as the mean $\pm$ standard deviation (SD). The Shapiro–Wilk test was used to assess the normality of the data prior to analysis. Statistical comparisons among groups were conducted using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison post hoc test to identify differences between groups. Analyses were performed using GraphPad Prism software (version 10; GraphPad Software, San Diego, CA, USA). A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1 Effect of Galangin on Histological Changes in Letrozole-Induced PCOS

Histological analysis, as shown in Fig. 1, revealed normal ovarian follicular architecture in both the control group (I) and the galangin-only group (II). In contrast, the letrozole-treated group (III) exhibited significant pathological changes, including severe vacuolation of the granulosa cell layers, epithelial desquamation, and marked congestion of ovarian blood vessels. A key finding in this group was the presence of numerous cystic follicles, which are follicles that have arrested in their development and failed to ovulate. These cystic follicles are characterized by a thin granulosa cell layer, vacuolated theca interna, and desquamated epithelial lining. The group pretreated with 4 mg/kg of galangin (IV) also displayed notable vacuolation in the granulosa cells. However, the group treated with 8 mg/kg of galangin (V) showed only mild vacuolation, indicating a better preservation of follicular structure. The metformin-treated group (VI) exhibited mostly normal ovarian morphology, although some follicles still showed disruption of the zona pellucida.

Table 1
Semi-quantitative evaluation of histopathological changes in the ovary.

Histopathological Feature	Control	GAL 8 mg/kg	LET 1 mg/kg	LET + GAL 4 mg/kg	LET + GAL 8 mg/kg	LET + MET 20 mg/kg
Healthy Follicles	Present	Present	Decreased	Decreased	Decreased	Present
Corpora Lutea	Present	Present	Decreased	Decreased	Decreased	Present
Cystic Follicles	Absent	Absent	Severe	Severe	Mild	Subtle
Zona Pellucida Disruption	Absent	Absent	Severe	Severe	Mild	Subtle
Fibrosis	Absent	Absent	Severe	Severe	Mild	Absent
Inflammation	Absent	Absent	Present	Present	Mild	Subtle
Angiogenesis in Ovarian Cortex	Absent	Absent	Present	Present	Mild	Subtle

3.2 Effect of Galangin Pretreatment on Serum Estradiol, LH, and AMH Levels in Letrozole-Induced PCOS Rats

Pretreatment with galangin significantly improved the hormonal disturbances induced by letrozole in the PCOS rat model (Fig. 2). Panel A shows that the control group (I) and the galangin-only group (II) exhibited similar estradiol levels, indicating normal ovarian steroidogenesis. In contrast, the letrozole-treated group (III) displayed a significant suppression of estradiol (– 72% compared to control), confirming the expected inhibition of aromatase activity. However, pretreatment with galangin at doses of 4 mg/kg (IV) and 8 mg/kg (V) dose-dependently restored estradiol levels by approximately 84% and 127%, respectively, relative to the letrozole group, indicating partial recovery of follicular function. The metformin-treated group (VI) further increased estradiol levels by 234% compared to the letrozole group, approaching near-normal values.

Panel B illustrates that LH levels were significantly elevated in the letrozole group (III), showing an approximate 279% increase relative to the control group, consistent with the hypergonadotropic characteristics of PCOS. Galangin pretreatment significantly reduced LH concentrations by about 33% (4 mg/kg) and 50% (8 mg/kg) compared to the letrozole group, indicating a dose-dependent recovery of gonadotropin balance. Notably, the metformin-treated group (VI) also exhibited a substantial reduction in LH levels (64% lower than the letrozole group), achieving levels comparable to those observed with the high dose of galangin (8 mg/kg).

Panel C depicts that AMH levels were significantly increased in the letrozole group (+ 77% compared to control), reflecting impaired folliculogenesis. Galangin pretreatment at 4 mg/kg (IV) and 8 mg/kg (V) reduced AMH levels in a dose-dependent manner by 12% and 27%, respectively, compared to the

letrozole group. The metformin-treated group (VI) showed the most pronounced reduction, with a 46% decrease compared to the letrozole group, restoring AMH levels closer to normal.

3.3 Impact of Galangin on Oxidative Stress Markers in Letrozole-Induced PCOS Rats

Pretreatment with galangin significantly reduced the oxidative stress induced by letrozole in PCOS rats (Fig. 3). Panel A illustrates the levels of MDA, a marker of lipid peroxidation. The control group (I) and the galangin-only group (II) showed normal MDA levels. In contrast, letrozole administration (III) resulted in a substantial increase in MDA, approximately 420% higher compared to the control, indicating significant oxidative damage. However, pretreatment with galangin at doses of 4 mg/kg (IV) and 8 mg/kg (V) decreased MDA levels by 29% and 48%, respectively, compared to the letrozole group, suggesting a dose-dependent protective effect. Furthermore, metformin (VI) led to a 62% reduction in MDA compared to letrozole, bringing levels close to normal.

Panel B presents the activity of SOD, a primary enzymatic antioxidant. Letrozole markedly suppressed SOD activity by 84% compared to the control group. In contrast, galangin pretreatment significantly enhanced SOD activity, showing increases of 100% at 4 mg/kg (IV) and 228% at 8 mg/kg (V) relative to the letrozole group. Metformin produced the highest increase in SOD activity, showing a 351% improvement compared to letrozole. These results indicate that galangin enhances superoxide radical scavenging in a dose-dependent manner.

Panel C highlights the activity of CAT, another important antioxidant enzyme. Letrozole administration reduced CAT activity by 78% compared to the control. However, pretreatment with galangin significantly restored CAT activity, with increases of 131% at 4 mg/kg and 210% at 8 mg/kg relative to the letrozole group. Metformin achieved the greatest improvement, with a 275% increase compared to letrozole, consistent with its established antioxidant effectiveness.

Panel D shows levels of reduced GSH, a major non-enzymatic antioxidant. Letrozole exposure resulted in an 81% reduction in GSH compared to the control group, indicating a depletion of antioxidant reserves. Conversely, galangin pretreatment markedly elevated GSH levels by 120% (4 mg/kg) and 250% (8 mg/kg) compared to the letrozole group. Metformin treatment produced a remarkable 356% increase in GSH, nearly restoring levels to normal.

3.4 Galangin Reduces Inflammatory Marker Expression Induced by Letrozole in PCOS Rats

Pretreatment with galangin significantly reduced the ovarian inflammatory response caused by letrozole in PCOS rats (Fig. 4). Panel A presents representative immunohistochemical staining results for IL-6, TNF- α , and NF- κ B expression in ovarian tissues from different experimental groups. The control group (I) and the galangin-only group (II) showed weak or negligible immunoreactivity for all three markers, indicating a normal ovarian inflammatory status. In contrast, the letrozole-treated group (III) exhibited

intense brown immunostaining for IL-6, TNF- α , and NF- κ B, primarily localized within the granulosa and theca cell layers. This reflects a significant upregulation of pro-inflammatory mediators compared to the control. Pretreatment with galangin at doses of 4 mg/kg (IV) and 8 mg/kg (V) significantly reduced this inflammatory response in a dose-dependent manner, with noticeably fewer positively stained cells and weaker staining intensity.

Panel B provides a quantitative analysis of the immunopositive area percentages for IL-6, TNF- α , and NF- κ B. Administration of letrozole resulted in a dramatic increase in the positive staining area for all markers compared to the control and galangin groups. Galangin pretreatment significantly diminished these increases in a dose-dependent manner: IL-6 levels decreased by 82% and 91%, TNF- α by 34% and 81%, and NF- κ B by 68% and 87% for the 4 mg/kg and 8 mg/kg groups, respectively, compared to the letrozole group. Metformin showed the most substantial reduction, achieving a 99% suppression for all markers and restoring expression levels close to normal.

3.5 Galangin Restores PI3K/pAKT/PTEN Signaling Disrupted by Letrozole in PCOS Rats

The pretreatment with galangin effectively modulated the PI3K/pAKT/PTEN signaling pathway that was disrupted by letrozole in rats with PCOS, as illustrated in Fig. 5. In Panel A, representative immunohistochemical staining for PI3K, p-AKT, and PTEN in ovarian sections from various experimental groups is displayed. The control group (I) and the galangin-only group (II) demonstrated moderate expressions of PI3K and p-AKT, along with normal PTEN immunoreactivity, indicating typical follicular signaling activity. In contrast, the group treated with letrozole (III) exhibited a significant reduction in PI3K (– 98%) and p-AKT (– 96%), along with a dramatic overexpression of PTEN (+ 4319%). This reflects a suppression of follicular growth signaling and activation of inhibitory pathways.

The pretreatment with galangin restored the disrupted signaling in a dose-dependent manner. In the 4 mg/kg group (IV), there was a partial recovery, with PI3K and p-AKT expression increasing approximately sixfold (around + 621%) and elevenfold (around + 1162%), respectively. At the same time, PTEN decreased by 55% compared to the letrozole group. The group receiving 8 mg/kg of galangin (V) showed an even stronger recovery, with PI3K and p-AKT increasing approximately nineteenfold (around + 1933%) and sixteenfold (around + 1645%), respectively, while PTEN decreased by 85% relative to the letrozole group. The metformin-treated group (VI) resulted in the most significant effect, with PI3K and p-AKT rising by nearly fiftyfold (approximately + 5088% and + 3992%), and PTEN reduced by 98%, thereby restoring expression patterns to near-normal levels. Panel B quantitatively confirms these results.

4. Discussion

Polycystic ovary syndrome (PCOS) is one of the most prevalent endocrine and metabolic disorders affecting women of reproductive age, characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology (Rajabi et al. 2024; Almhoud et al. 2024). Oxidative stress, chronic inflammation, and insulin resistance are recognized as key contributors to its pathophysiology, leading to

impaired folliculogenesis and infertility (Ihim et al. 2024; Mishra et al. 2024). Current therapeutic approaches, such as metformin or clomiphene citrate, offer limited efficacy and may be associated with side effects, highlighting the need for safe, multitarget natural agents that can restore hormonal and metabolic balance (Lan et al. 2024; Kar et al. 2024).

Letrozole is a potent aromatase inhibitor that prevents the enzymatic conversion of testosterone and androstenedione into estradiol. This leads to decreased estrogen levels and increased androgen levels (Kaltsas et al. 2025). By disrupting estrogen synthesis, letrozole consistently induces a PCOS-like phenotype characterized by elevated LH levels, hyperandrogenism, and follicular arrest with multiple cyst formations. Consistent with earlier reports (Adelakun et al. 2022; Lan et al. 2024; Kar et al. 2024), this study confirmed these endocrine and histopathological abnormalities. Notably, treatment with galangin significantly reversed these alterations, especially at a dosage of 8 mg/kg, suggesting a restoration of steroidogenic balance and folliculogenesis. Although galangin is not a potent estrogen receptor agonist, previous studies indicate that it can interact with estrogen receptors and modulate their signaling indirectly, potentially through effects on aromatase activity or hypothalamic–pituitary regulation (Resende et al. 2013; Safna Hussan et al. 2024). Similar modulatory behavior has been reported for structurally related flavonoids such as quercetin and biochanin A (Lephart 2015).

Oxidative stress is now recognized as a significant contributor to the pathophysiology of PCOS, influencing insulin resistance, inflammation, and oocyte maturation (Ihim et al. 2024; Mishra et al. 2024). In the current study, letrozole administration markedly elevated ovarian MDA levels and reduced the activities of antioxidant enzymes (SOD and CAT) and GSH content, confirming the induction of oxidative stress consistent with earlier reports (Adelakun et al. 2022; Lan et al. 2024). In the present study, galangin significantly reduced the concentration of MDA while increasing the activities of antioxidant enzymes such as SOD and CAT, as well as elevating the level of the tripeptide antioxidant GSH. These results demonstrate galangin's potent antioxidant capacity. The underlying mechanism of this antioxidant activity has been attributed to its hydroxyl groups at positions 3 and 5, which readily donate hydrogen atoms to neutralize reactive species, and to its conjugated C2–C3 double bond, which enables resonance stabilization of the resulting radicals (Spiegel 2024). The findings are consistent with previous reports describing galangin's ability to scavenge free radicals and neutralize ROS in models of diabetes, nephropathy, and cardiotoxicity (Aladaileh et al. 2021; Fang et al. 2023). By mitigating oxidative injury, galangin helps preserve granulosa cell viability and prevents membrane damage resulting from lipid peroxidation, thereby maintaining follicular integrity. This underscores galangin's therapeutic potential as a natural adjunct in the treatment of this condition.

Chronic low-grade inflammation is a key mechanism underlying PCOS. Elevated levels of ovarian IL-6, TNF- α , and NF- κ B can disrupt insulin signaling and steroidogenesis. In the present study, letrozole administration markedly increased the expression of these pro-inflammatory mediators, confirming the establishment of an inflammatory state consistent with previous findings in letrozole-induced PCOS models (Kar et al. 2024). Treatment with galangin effectively attenuated these elevations, supporting its anti-inflammatory potential. Moreover, galangin appears to inhibit the activation of p38 MAPK, NF- κ B,

and the NLRP3 inflammasome, as thoroughly reviewed by (Hassanein et al. 2023) and (Thapa et al. 2023). Similar anti-cytokine effects have been observed with other phenolic compounds, such as sinapic acid, which mitigates ovarian inflammation by suppressing NF- κ B signaling (Lan et al. 2024). By reducing inflammatory signaling, galangin may help restore steroidogenic enzyme activity and improve the quality of the oocyte microenvironment, ultimately promoting follicular maturation.

A significant aspect of this study is the demonstration that galangin restored the PI3K/pAKT/PTEN pathway disrupted by letrozole. In the present model, letrozole administration markedly suppressed PI3K and pAKT expression while upregulating PTEN, consistent with previous studies showing inhibition of the PI3K/pAKT signaling cascade in letrozole-induced PCOS (Moustafa et al. 2024). The PI3K/pAKT pathway is essential for regulating cellular survival, metabolism, and folliculogenesis, while PTEN serves as a negative regulator whose overexpression can lead to follicular arrest (Hsueh et al. 2015; De Felici and Klinger 2021). In models of PCOS, oxidative stress and hyperandrogenism suppress PI3K/pAKT signaling while enhancing PTEN activity, resulting in increased apoptosis of granulosa cells and anovulation (Gao et al. 2021; Namlı Kalem et al. 2023). In this study, galangin treatment reversed these alterations by upregulating PI3K and pAKT expression and downregulating PTEN, suggesting a recovery of survival signaling. A similar PI3K/pAKT-mediated cytoprotective mechanism of galangin has been observed in other tissues, such as dopaminergic neurons, where it attenuated MPTP-induced injury through an autophagy-dependent pathway (Huang et al. 2025). Therefore, galangin appears to act on a key regulatory pathway linking oxidative stress, inflammation, and cell survival, which may underlie its coordinated hormonal and histological benefits.

Collectively, this study demonstrates that galangin exerts significant protective effects against the endocrine, oxidative, and inflammatory disturbances associated with letrozole-induced PCOS in rats. Pretreatment with galangin ameliorated the alterations in estradiol, LH, and AMH levels, indicating partial restoration of ovarian endocrine balance. Additionally, it reduced lipid peroxidation, enhanced antioxidant enzyme activity, and suppressed the ovarian expression of IL-6, TNF- α , and NF- κ B. Furthermore, galangin modulated the PI3K/pAKT/PTEN signaling pathway, suggesting that its beneficial actions extend beyond symptomatic relief to include regulation of molecular mechanisms involved in follicular survival and maturation (Fig. 6).

5. Conclusion

In conclusion, galangin alleviates the hormonal, oxidative, and inflammatory alterations associated with letrozole-induced PCOS in rats, partly through modulation of the PI3K/pAKT/PTEN pathway. These findings highlight galangin's promise as a natural, multitarget compound for PCOS management.

Declarations

Ethics approval and consent to participate

The experimental protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia (Approval No. PH-1445-35).

Competing Interests

The authors state that they have no conflicts of interest to disclose.

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Author Contribution

Lenah S. Binmahfouz: Supervision, Data Curation, Formal Analysis, Writing – Original Draft, Project Administration, Funding Acquisition. Amina M. Bagher: Validation, Investigation, Writing – Review & Editing. Najlaa S. Binmahfouz: Histopathology, Data Curation, Visualization. Rasheed A. Shaik: Methodology, Investigation, Data Curation. Ashraf B. Abdel-Naim: Conceptualization, Supervision, Writing – Review & Editing. Khadijah B. Alkinani: Provision of galangin reagent and technical support related to experimental materials. Sarah Alsaggaf, Salma Karkashan, Ahad Bangita, and Shimaa Shukr: Animal Experimentation, Sample Collection, Laboratory Analysis. Basma G. Eid: Conceptualization, Validation, Supervision.

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Data Availability

All data supporting this study are included within the published article.

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Figures

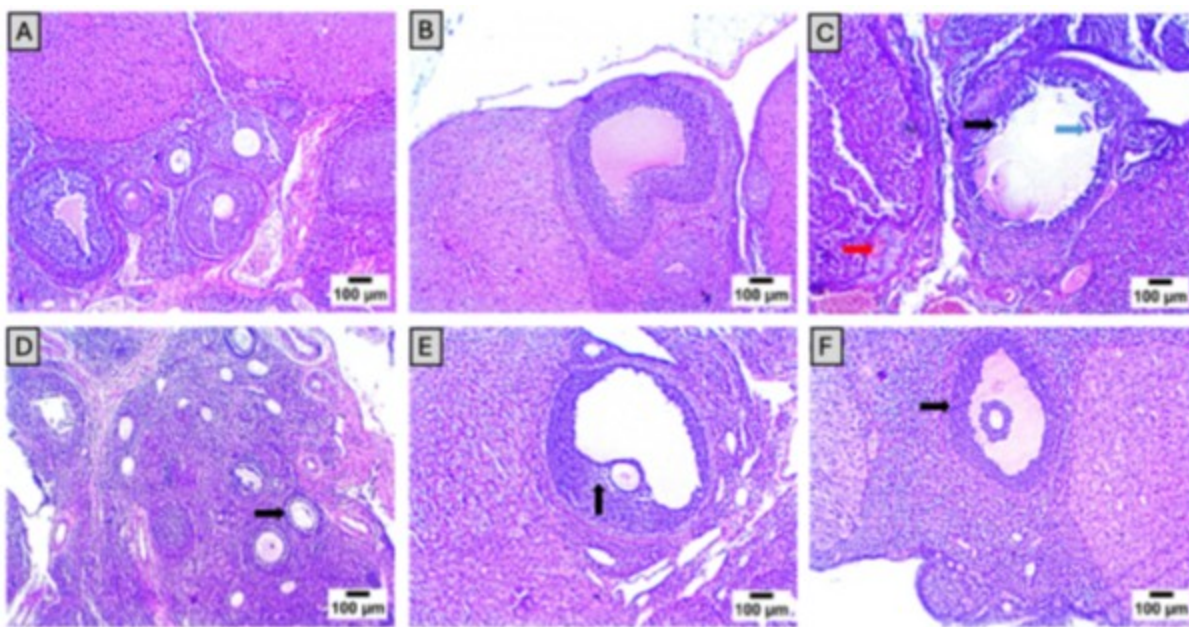


Figure 1

Representative ovarian histological sections stained with hematoxylin and eosin (H&E) from different experimental groups in Letrozole-Induced PCOS rats. (A) Control group showing normal ovarian architecture with healthy growing follicles and corpora lutea. (B) Galangin-only (8 mg/kg) group showing preserved follicular morphology, similar to the control. (C) Letrozole (1 mg/kg) group displaying characteristic cystic follicles (black arrow) with thin granulosa layers, vacuolated theca cells, and desquamated epithelium (blue arrow); also note marked congestion of ovarian blood vessels (red arrow). (D) Letrozole + Galangin (4 mg/kg) group showing persistent cystic follicles (black arrow) and evident vacuolations. (E) Letrozole + Galangin (8 mg/kg) group exhibiting mild vacuolation (black arrow) with improved follicular structure. (F) Letrozole + Metformin (20 mg/kg) group showing overall normalized morphology, although some follicles still exhibit disruption of the zona pellucida (black arrow).

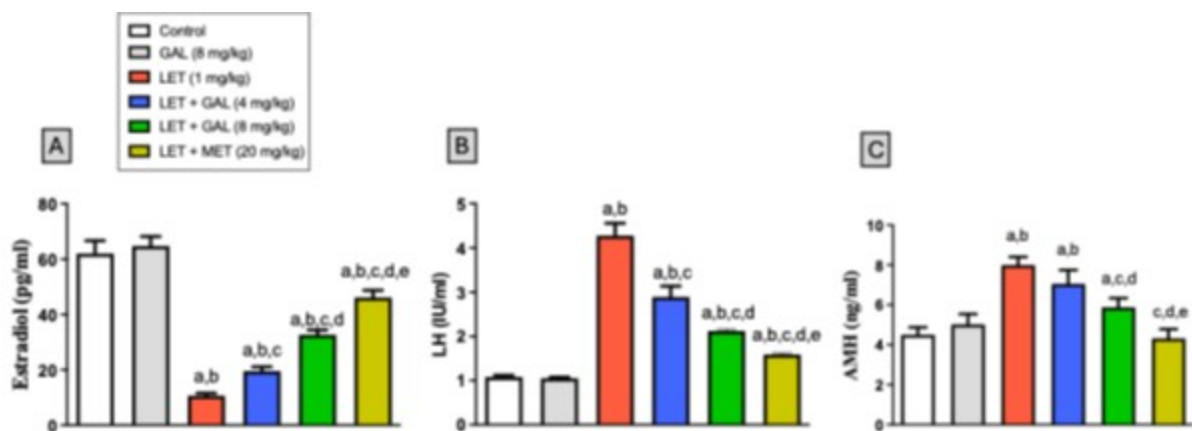


Figure 2

Galangin pretreatment helps restore hormonal balance by normalizing estradiol, luteinizing hormone (LH), and anti-Müllerian hormone (AMH) levels in rats with letrozole-induced PCOS. Serum levels of estradiol (A), LH (B), and AMH (C) were measured in six groups: control (I), galangin-only (II), letrozole (III), letrozole + galangin 4 mg/kg (IV), letrozole + galangin 8 mg/kg (V), and letrozole + metformin (VI). The values are expressed as mean $\pm$ SD (n=6). Statistical significance was determined with the following comparisons: a = vs. control, b = vs. galangin, c = vs. letrozole, d = vs. galangin 4 mg/kg, e = vs. galangin 8 mg/kg; $p < 0.05$.

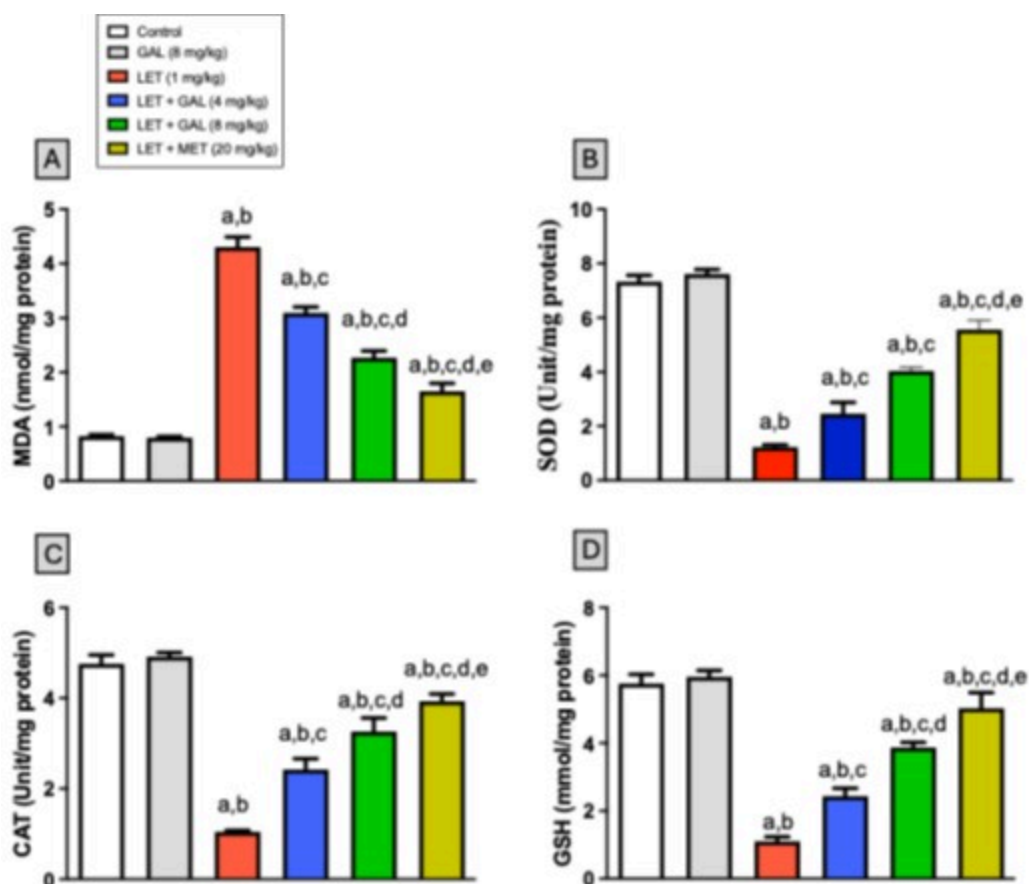


Figure 3

Galangin pretreatment attenuates oxidative stress by reducing lipid peroxidation and restoring antioxidant enzyme activities in letrozole-induced PCOS rats. The levels of malondialdehyde (MDA; **A**), superoxide dismutase (SOD; **B**), catalase (CAT; **C**), and reduced glutathione (GSH; **D**) were measured in the following groups: control (I), galangin-only (II), letrozole (III), letrozole + galangin 4 mg/kg (IV), letrozole + galangin 8 mg/kg (V), and letrozole + metformin 20 mg/kg (VI). Values are expressed as mean $\pm$ SD (n=6). Statistical significance was determined with the following comparisons: a = vs. control, b = vs. galangin, c = vs. letrozole, d = vs. galangin 4 mg/kg, e = vs. galangin 8 mg/kg; $p < 0.05$.

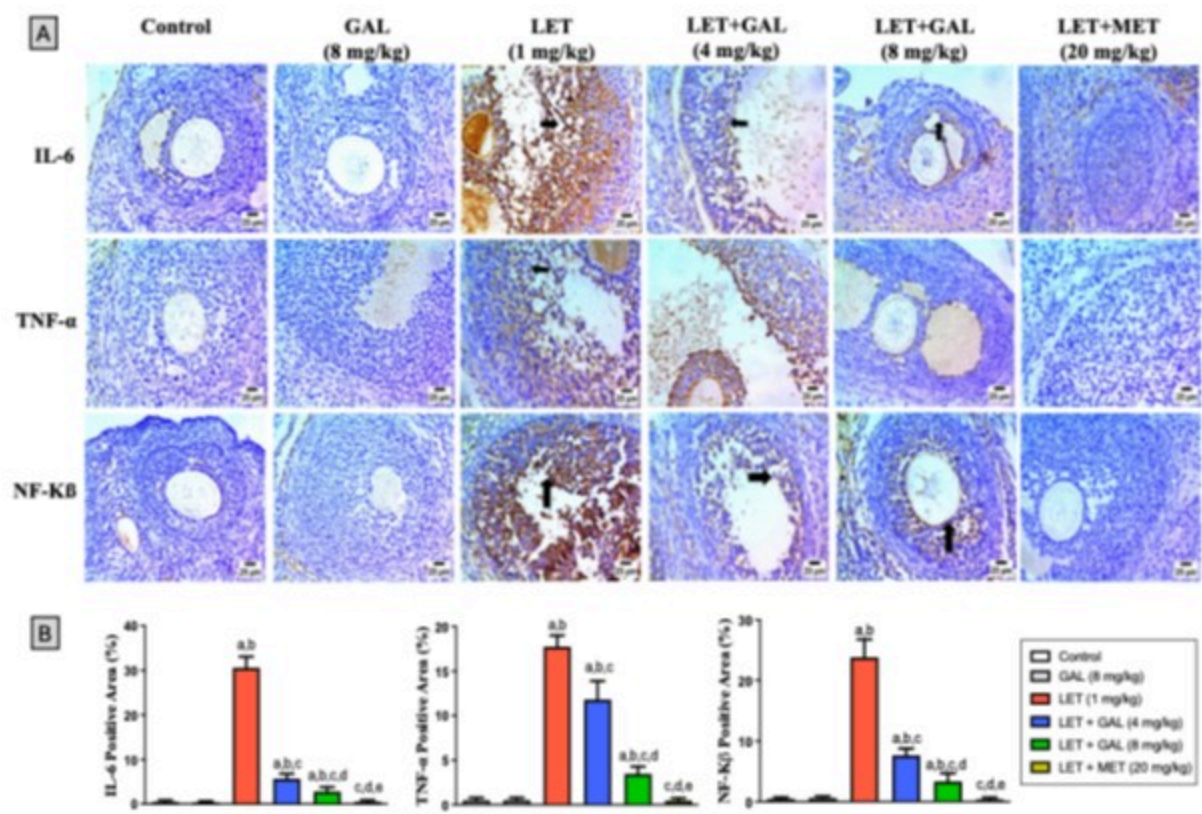


Figure 4

Galangin pretreatment reduces ovarian inflammation by lowering the expression of IL-6, TNF-α, and NF-κB proteins in letrozole-induced PCOS rats. (A)Representative immunohistochemical staining of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and nuclear factor-κB (NF-κB) in ovarian sections from the following groups: control (I), galangin only (II), letrozole (III), letrozole + galangin 4 mg/kg (IV), letrozole + galangin 8 mg/kg (V), and letrozole + metformin 20 mg/kg (VI). (B) Quantitative analysis of the immunopositive areas for IL-6, TNF-α, and NF-κB (as a percentage). Values are expressed as mean $\pm$ SD (n=6). Statistical significance was determined with the following comparisons: a = vs. control, b = vs. galangin, c = vs. letrozole, d = vs. galangin 4 mg/kg, e = vs. galangin 8 mg/kg; $p < 0.05$.

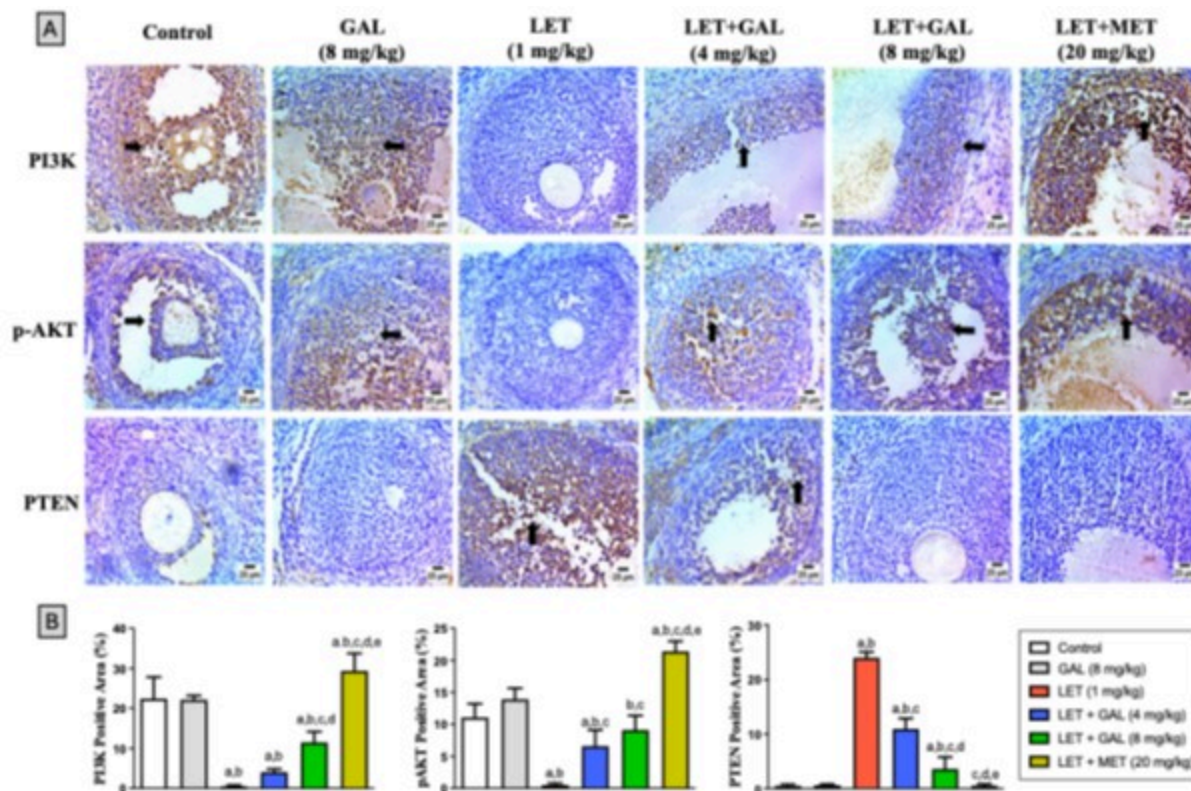


Figure 5

Galangin pretreatment restores the PI3K/pAKT/PTEN signaling pathway in the ovaries of rats with letrozole-induced PCOS. (A) The panel shows representative immunohistochemical staining of phosphatidylinositol-3-kinase (PI3K), phosphorylated AKT (p-AKT), and phosphatase and tensin homolog (PTEN) in ovarian sections from different groups: control (I), galangin-only (II), letrozole (III), letrozole + galangin 4 mg/kg (IV), letrozole + galangin 8 mg/kg (V), and letrozole + metformin 20 mg/kg (VI). (B) The quantitative analysis presents the percentage of immunopositive areas for PI3K, p-AKT, and PTEN. Values are expressed as the mean $\pm$ standard deviation (n=6). Statistical significance is indicated as follows: a = compared to control, b = compared to galangin, c = compared to letrozole, d = compared to galangin 4 mg/kg, e = compared to galangin 8 mg/kg; $p < 0.05$.

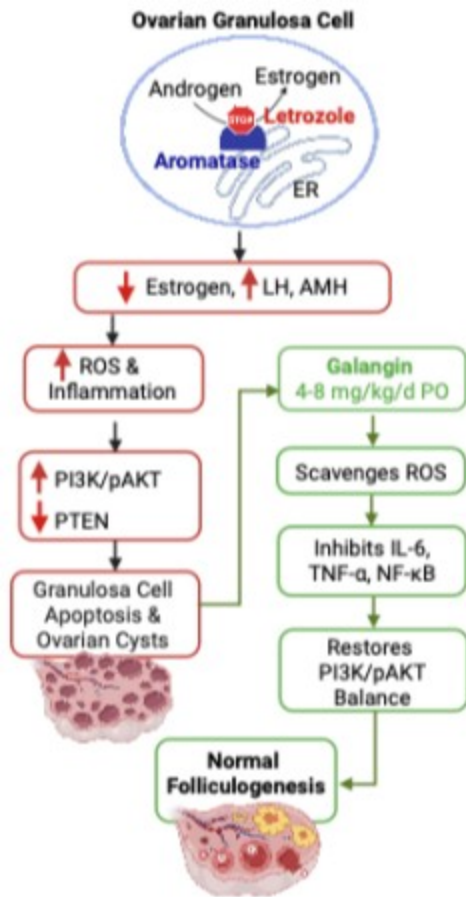


Figure 6

Proposed mechanism of letrozole-induced ovarian dysfunction and the protective actions of galangin in promoting normal folliculogenesis. Letrozole inhibits aromatase activity in ovarian granulosa cells, leading to reduced estrogen synthesis and subsequent oxidative stress, inflammation, and PI3K/pAKT/PTEN signaling imbalance that contribute to granulosa cell apoptosis and cyst formation. Galangin (4–8 mg/kg/day, p.o.) **ameliorates these disturbances** by scavenging reactive oxygen species (ROS), suppressing the expression of IL-6, TNF- α , and NF- κ B, and **modulating the PI3K/pAKT/PTEN pathway**, thereby supporting restoration of normal folliculogenesis.