



The Effectiveness of Ethanol Extract of *Zingiber officinale* var. *rubrum* on the Expression of TNF- α , IRS, and Follicles in Preventing Polycystic Ovary Syndrome (PCOS) in Reproductive Health

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Abstract

Polycystic ovary syndrome (PCOS) involves impaired ovulation and hyperandrogenism, elevating insulin and TNF- α levels, and disrupting insulin receptor substrate (IRS) formation. *Zingiber officinale* var. *rubrum* has been shown to suppress TNF- α , thereby enhancing IRS function and follicular development. This study evaluated whether red ginger extract attenuates TNF- α , upregulates IRS, and improves follicular development as a PCOS prevention strategy. Using a randomised post-test-only control group design, thirty *Rattus norvegicus* were divided into five groups (K-, K+, P1, P2, P3) and administered ethanol extract at 200, 400, or 800 mg/kg BW alongside testosterone propionate (100 mg/kg BW, i.p.) for 21 days. Tissues were assessed via HE staining and immunohistochemistry; LC-MS characterised extract composition. Ethical approval was obtained (No. 2.KEH.59.04.2025, Faculty of Medicine, Airlangga University). Red ginger extract significantly reduced TNF- α , increased IRS expression, and promoted follicular development. One-way ANOVA showed significant differences across all parameters ($p = 0.001$), with P1, P2, and P3 each differing significantly from the positive control.

Keywords

zingiber officinale var.

rubrum;

TNF- α expression;

IRS;

follicle;

PCOS.

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Introduction

Perturbations of downstream insulin signaling cascades and insulin receptor integrity are potential consequences of this process. Such molecular disruptions may establish pathophysiological links to both insulin resistance and PCOS (Zuo et al., 2016). Insulin resistance augments GnRH pulse frequency and LH secretory patterns. Such neuroendocrine disturbances trigger compensatory hyperinsulinemic responses and stimulate excessive ovarian androgen production (Rojas et al., 2014).

Polycystic Ovary Syndrome (PCOS) constitutes a clinical condition marked by anovulation coexisting with elevated androgen levels. Affected individuals frequently exhibit insulin resistance, a metabolic phenotype resembling that observed in type 2 diabetes mellitus, thereby impairing normal follicular development (Broskey et al., 2018; Desreza & Raiyani, 2023; Rojas et al., 2014). Among insulin-resistant patients with Polycystic Ovary Syndrome, excessive insulin secretion is a common finding. This hyperinsulinemic state may diminish the therapeutic effectiveness of ovulation stimulation (Franks & Berga, 2012). The underlying mechanism involves the disproportionately high LH:FSH ratio prevalent among women exhibiting insulin resistance. Such gonadotropin dysregulation impairs the oocyte's developmental competence (Hassani et al., 2019). The inability of ovulation induction agents to suppress elevated serum insulin, LH, and androgen levels necessitates alternative therapeutic strategies. Pharmacological management of hyperinsulinemia in this patient population facilitates augmented folliculogenesis, culminating in restored ovulation and elevated conception rates (Baptiste et al., 2010). Insulin resistance plays an important role in the pathogenesis of PCOS (Tang et al., 2017).

Overall, these findings indicate that insulin resistance is not only a major metabolic disorder in PCOS but also a key factor influencing reproductive outcomes and treatment response. In this context, understanding the magnitude of PCOS as a public health problem becomes important before discussing the experimental and mechanistic models used to study its pathophysiology.

Reproductive-aged women exhibit a Polycystic Ovary Syndrome (PCOS) prevalence of 5-21%. In affected individuals, insulin resistance occurs in 50-75% of cases (Teede et al., 2018), with insulin sensitivity diminished by 35-40% compared to normal physiological standards (Barber et al., 2019). Mareta et al. (2018) study identified obesity in 92.2% of 249 PCOS patients. This high figure is explained by the fact that the study was performed at a private fertility practice, where the majority of patients presented with complaints of infertility and reproductive disorders. This condition caused the study sample to be dominated by patients with more severe manifestations of PCOS, including obesity and metabolic disorders. The researchers also stated that the study data could not represent the general PCOS population as a whole due to the specific characteristics of patients seeking treatment at that practice, with infertility prevalence among these subjects ranging between 61% and 89.6%. Differences in sample inclusion criteria and methods of identifying PCOS and infertility across individual studies also contributed to the variation in reported prevalence. Furthermore, women with PCOS demonstrate an 8.5 times greater likelihood of infertility relative to women without the syndrome (Kusumawati et al., 2025). Ovarian cells induced by testosterone propionate at 100 mg/kg/BW intraperitoneally exhibit elevated testosterone levels, which precipitate cellular inflammation and engender an imbalance between protective and deleterious factors within ovarian cells, thereby constituting a primary pathophysiological mechanism underlying PCOS (Zuo et al., 2016).

Due to hyperinsulinemia, the liver decreases SHBG production, while the ovaries increase androgen production (K. Wang et al., 2023). Hyperglycemia-associated insulin resistance induces oxidative stress, which is further exacerbated by elevated circulating free fatty acids (Pratama & Zhang, 2025). The genesis of oxidative stress reflects an imbalance favoring reactive oxygen species generation over antioxidant protective mechanisms. NF κ B upregulation ensues, mediating TNF- α

formation, AP-1 stimulation, and HIF-1 expression as inflammatory effectors (Garza-Garza & Delgadillo-Guzmán, 2020). Through reciprocal activation by TNF- α , IL-6, and IL-1, NF- κ B perpetuates a positive feedback loop, thereby sustaining chronic inflammatory conditions (Barrero, 2017). The amplification of inflammatory signaling ultimately promotes serine phosphorylation of insulin receptor substrates (IRS), a molecular event implicated in the pathogenesis of hyperandrogenism (Velaga et al., 2017). Researchers have demonstrated that subcutaneous injection of testosterone propionate, when combined with high-fat dietary protocols, successfully generates a PCOS-IR mouse model (Lai et al., 2014; M.-X. Wang et al., 2020). These mice exhibit phenotypic features resembling PCOS pathology and associated endocrine dysregulation; however, the introduction of exogenous androgens may alter the endocrine profile representative of the syndrome (Taylor et al., 2019).

By suppressing the formation of reactive oxygen species, the ethanolic extract derived from *Zingiber officinale* var. *rubrum* enhances the production of superoxide dismutase. Furthermore, it attenuates the levels of specific inflammatory markers, notably TNF- α and IL-1, while concurrently increasing IRS expression. The work conducted by Lakshmi Priya and colleagues established the multifaceted bioactivity of this extract, confirming its antioxidant, antidiabetic, and antimicrobial potential (Gopalakrishnan et al., 2016). The antidiabetic properties of *Zingiber officinale* var. *rubrum*, particularly in insulin-resistant diabetic states, encompass the reduction of TNF- α inflammation, as evidenced in murine investigations. Additionally, Chinedu et al. demonstrated that ethanol extract administration significantly potentiates insulin receptor sensitivity ($p < 0.0001$), thereby substantiating its role in insulin resistance amelioration (De Leo et al., 2016). This study seeks to evaluate the effectiveness of ethanol extract derived from *Zingiber officinale* var. *rubrum* upon TNF- α and IRS expression, as well as follicular morphology, in the prophylaxis of Polycystic Ovary Syndrome (PCOS) within reproductive health parameters.

Materials and Methods

The present research was conducted in April 2025 at the laboratories of the Faculty of Medicine, Airlangga University (Surabaya) and the Faculty of Medicine, Brawijaya University (Malang). Ethical clearance for the study (No. 2.KEH.59.04.2025) was secured from the Faculty of Medicine, Airlangga University, on April 28, 2025. A randomized post-test-only control group design was employed. Thirty *Rattus norvegicus* were allocated into five distinct groups: a negative control (K-), a positive control (K+), and three treatment groups (P1, P2, P3), which were administered graded doses of *Zingiber officinale* var. *rubrum* ethanol extract at 200, 400, and 800 mg/kg/BW, respectively. Subjects in the treatment cohorts received the extract, followed by an injection of testosterone propionate (100 mg/kg/BW) one hour later. This protocol was repeated daily for 21 days. All animals were sacrificed on day 22, and ovarian tissue samples were harvested and stored in citrate buffer for subsequent analysis.

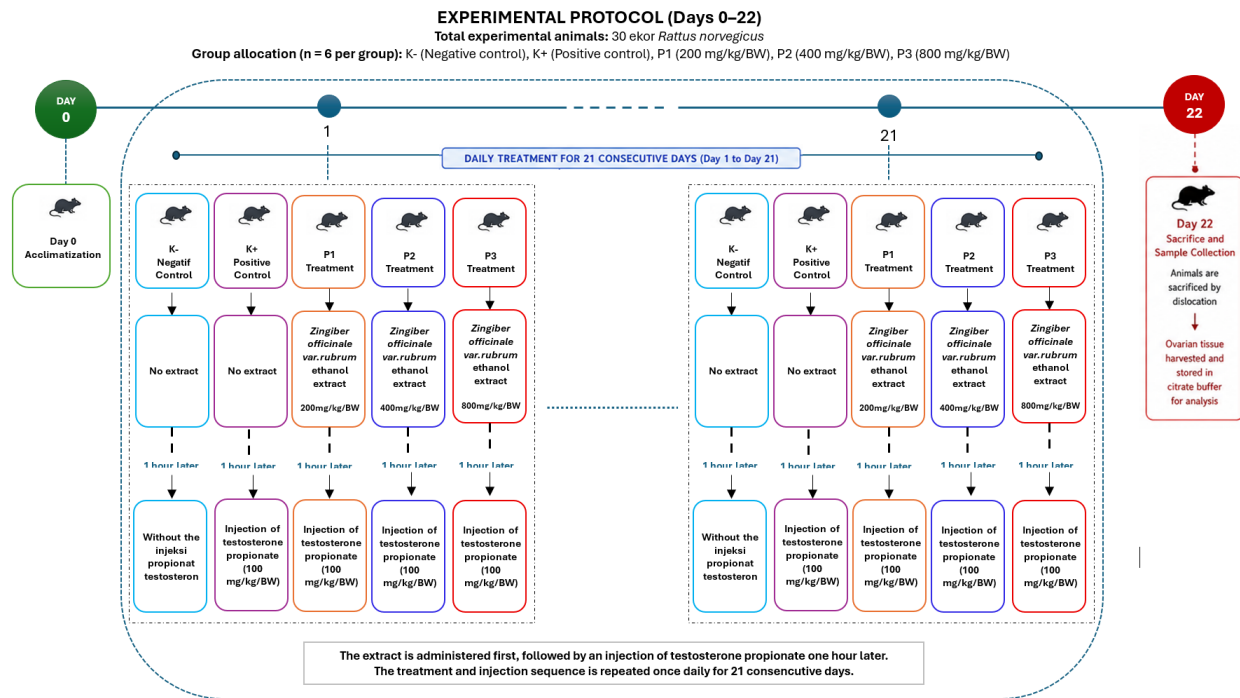


Figure 1. Timeline of the Experimental Protocol

Microtome sectioning, which is essential for subsequent Hematoxylin-Eosin staining, yielded thin, translucent specimens suitable for microscopic analysis (Chlipala et al., 2020). Pathology: Anatomy of the ovary of *Rattus norvegicus*; researchers use *Rattus norvegicus* because it is easy to breed and has anatomical, genetic, and physiological structures similar to those of humans (Muttaqin, 2009). *Rattus norvegicus* ovaries have a structure similar to that of humans, are easy to handle, have more flexible tissue, are inexpensive, and are readily available (Lizneva et al., 2016). Immunohistochemical analysis was used to evaluate TNF- α expression in ovarian tissue. TNF- α is an important cytokine involved in proinflammatory and inflammatory processes (Mareta & Amran, 2018). Ovarian TNF- α quantification was performed on tissue specimens obtained directly from the ovaries of *Rattus norvegicus*. Immunohistochemical (IHC) analysis, performed using a Cx41 microscope (Olympus, Japan), was utilized to assess Insulin Receptor Substrate (IRS) levels, a critical component of the insulin signaling pathway at the receptor level. Follicular enumeration involved direct microscopic or histopathological examination of ovarian tissue derived from PCOS model mice to identify follicular structures and polycystic morphology. Folliculogenesis was evaluated by quantifying developing follicles in ovarian sections under microscopic observation, yielding a comprehensive overview of follicular development across all phases. Observations were conducted at magnifications ranging from 400 $\times$ to 1000 $\times$, with developmental stages systematically enumerated.

Results and Discussions

Results

The investigative protocol encompassed a specialized methodological framework for quantifying TNF- α and IRS expression, alongside follicular enumeration, in *Rattus norvegicus*. Statistical analysis proceeded in a hierarchical sequence: an initial Shapiro–Wilk normality assessment, followed, when appropriate ($p > 0.05$), by ANOVA. Significant omnibus findings ($p < 0.05$) triggered post hoc comparisons, with Tukey's test applied under homogeneous conditions and

Brown–Forsythe/Games–Howell procedures employed when variance homogeneity was violated. All computational analyses were conducted using JASP (version 0.19.2).

Influence of administration of *Zingiber officinale* var. *rubrum* on the parameter TNF- α

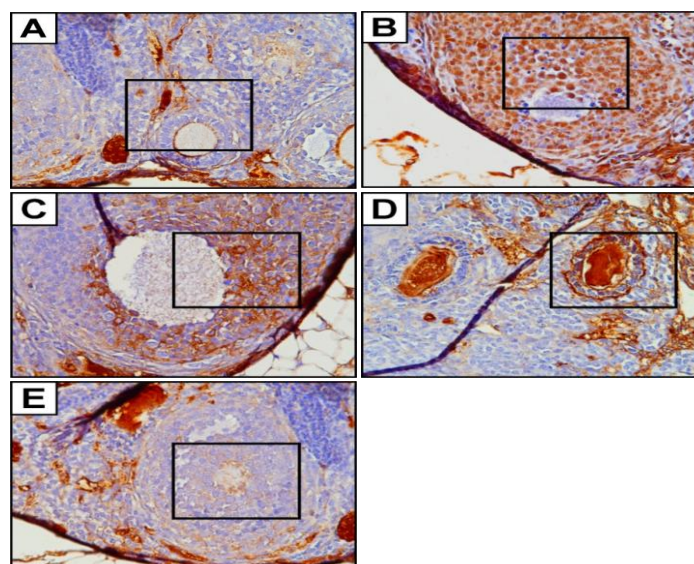
Immunohistochemical analysis revealed increased TNF- α expression in ovarian granulosa cells. Data for each sample were assessed using a modified semi-quantitative Remmele method, in which the Remmele index (Immunoreactive Remmele Score, IRS) is calculated by multiplying the percentage of immunoreactive cells by their color intensity score (Sam, 2019). Data for each sample is the TNF- α average observed in a roomy view. Prior to inferential analysis, all examination data were assessed for normality. Shapiro-Wilk testing demonstrated that TNF- α measurements across all experimental groups conformed to normal distribution ($p > 0.05$). Subsequent analysis included homogeneity testing, followed by an ANOVA to compare TNF- α levels between groups. The ANOVA results show a significant difference between groups ($p < 0.05$), so the test requires further analysis to determine which group differs from the others using Post Hoc Tests. This shows that TNF- α in the control group is negative (K-), significantly different from the control-positive (K+) group and all group treatments. The group positive control (K+) differs significantly from the group negative control (K-) and all group treatments.

Table 1
Results of the test of differences in TNF- α expression between groups ($p < 0.05$)

Group	N	Mean $\pm$ SD	p-value
K-	6	3.00 $\pm$ 1.27	< 0.001*
K+	6	10.5 $\pm$ 1.34	< 0.001*
P1	6	7.50 $\pm$ 2.88	< 0.001*
P2	6	4.33 $\pm$ 2.07	< 0.001*
P3	6	2.50 $\pm$ 0.84	< 0.001*

(*): significant $\alpha=0.05$

Immunohistochemical analysis, as detailed in Table 1, revealed highly significant differences in TNF- α expression. A marked disparity was observed between the negative (K-) and positive (K+) control groups ($p < 0.001$). Furthermore, TNF- α levels in treatment groups P1 and P2 differed significantly from those in group P3, with both comparisons attaining p-values < 0.001 .



Immunohistochemical examination results of TNF- α expression in granulosa cells (A) negative control group, (B) positive control group, (C) treatment group one, (D) treatment group two, (E) treatment group three

Subsequent ANOVA analysis revealed statistically significant intergroup differences in TNF- α expression ($p < 0.05$). Post hoc testing subsequently demonstrated that the negative control group (K-) differed significantly from the positive control group (K+). Additionally, the positive control group (K+) showed significant divergence from all treatment groups, including P1, P2, and P3.

Influence of administration of Zingiber officinale var. rubrum on the parameter IRS

Immunohistochemical IRS data underwent initial normality assessment. Shapiro-Wilk testing confirmed normality across all experimental groups ($p > 0.05$), thereby permitting subsequent homogeneity testing and intergroup comparisons via ANOVA. Given that the analysis of variance yielded statistically significant differences among the groups ($p < 0.05$), further post hoc testing was conducted to pinpoint the precise loci of these disparities. Results Post Hoc Tests show that IRS on the control group negative (K-) is different in a significant way from the group control positive (K+), and all group treatments, group positive control (K+), are different in a significant way from the group control negative (K-) and all group treatments.

Table 2

Results of the test of differences in IRS expression between groups ($p < 0.05$)

Group	N	Mean $\pm$ SD	p-value
K-	6	10.3 $\pm$ 21.0	< 0.001*
K+	6	4.34 $\pm$ 6.89	< 0.001*
P1	6	21.1 $\pm$ 35.92	< 0.001*
P2	6	50.1 $\pm$ 38.65	< 0.001*
P3	6	74.7 $\pm$ 23.75	< 0.001*

(*): significant $\alpha=0.05$

As shown in Table 2, immunohistochemical analysis of IRS expression in *Rattus norvegicus* revealed a statistically significant difference between the negative control group (K-) and the positive control group (K+), with a p-value of < 0.001. Furthermore, both treatment groups 1 (P1) and 2 (P2) demonstrated significant divergence from treatment group 3 (P3), with p-values < 0.001.

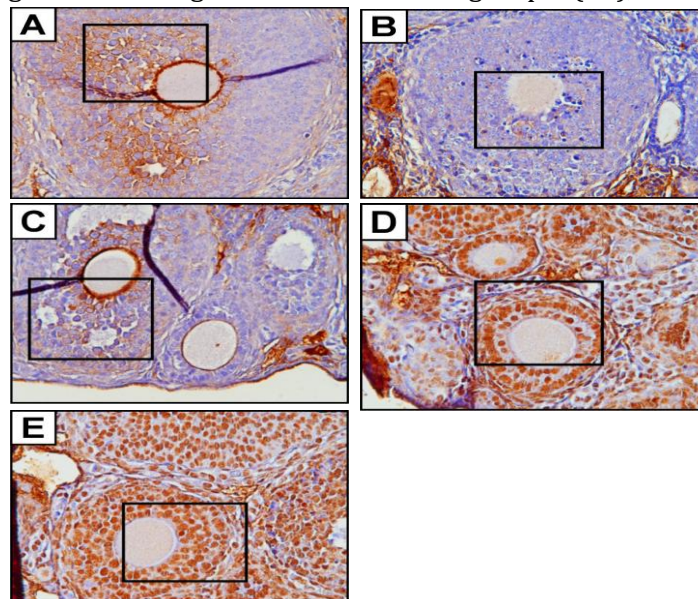


Figure 2.

Results of immunohistochemical examination of IRS expression in granulosa cells. (A) negative control group (B), positive control group (C), treatment group 1 (D), treatment group 2 (E), treatment group 3.

ANOVA results indicated significant between-group variation in IRS expression ($p < 0.05$). Subsequent homogeneity testing and post hoc analysis established significant distinctions between the negative (K-) and positive (K+) control groups, as well as between the positive control (K+) and each treatment group (P1, P2, and P3).

The effect of administering *Zingiber officinale* var. *rubrum* to Ovarian follicles

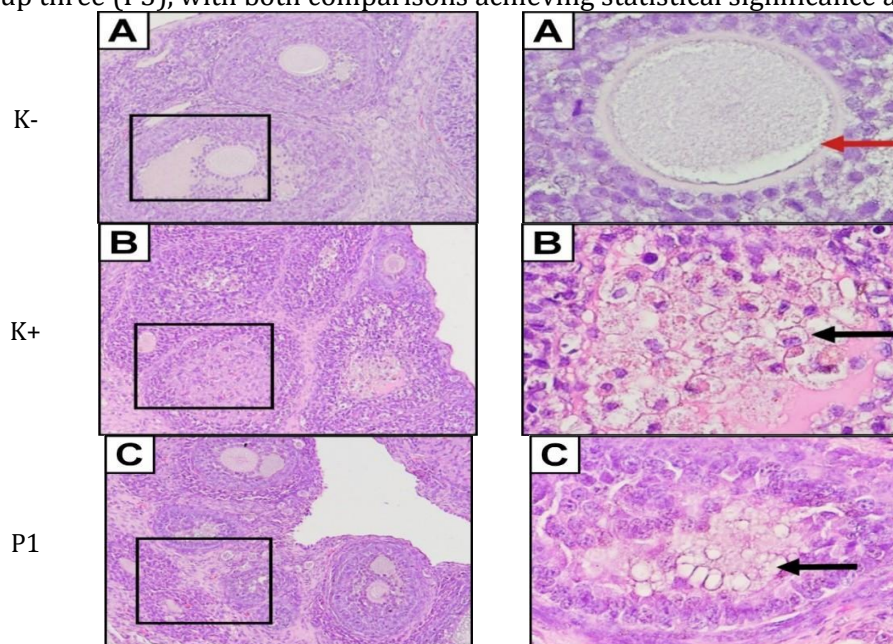
Homogeneity testing via ANOVA proved significant between-group variance in follicular characteristics ($p < 0.05$), prompting post hoc comparisons. The results established a reciprocal pattern of significant differences: the negative control (K-) was distinct from the positive control (K+) and all treatment groups, while the positive control (K+) was, in turn, distinct from the negative control (K-) and each treatment cohort. This investigation used ovarian samples derived from *Rattus norvegicus* granulosa cells, with microscopic examination performed at 400× and 1000× magnification across 10 microscopic fields.

Table 3
Results of the test of differences in follicle expression between groups ($p < 0.05$)

Group	N	Mean $\pm$ SD	p-value
K-	6	12.2 $\pm$ 3.54	< 0.001*
K+	6	26.6 $\pm$ 4.35	< 0.001*
P1	6	19.5 $\pm$ 3.35	< 0.001*
P2	6	11.8 $\pm$ 1.94	< 0.001*
P3	6	9.50 $\pm$ 1.05	< 0.001*

(*): significant $\alpha=0.05$

Table 3 presents immunohistochemical findings indicating markedly divergent follicular expression between negative (K-) and positive (K+) control cohorts ($p < 0.001$). Additionally, significant disparities were observed between treatment groups one and two (P1, P2) relative to treatment group three (P3), with both comparisons achieving statistical significance at $p < 0.001$.



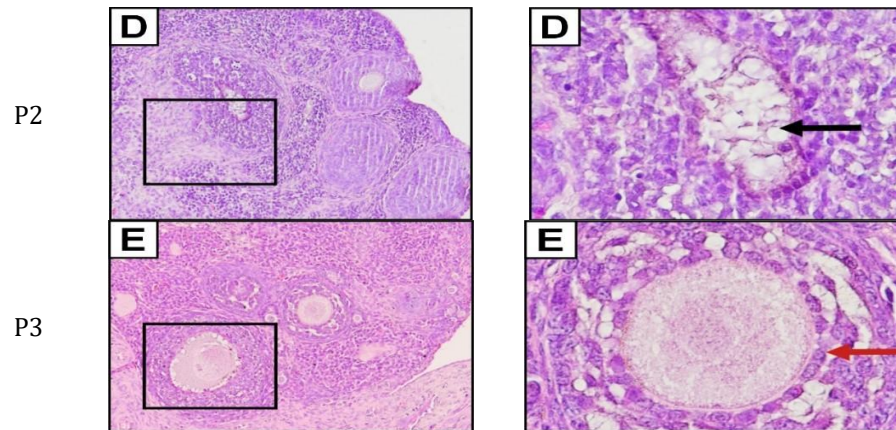


Figure 3.

Hematoxylin-Eosin (HE) staining of ovarian tissue sections at 400× (left column) and 1000× (right column) magnification. (A) K- (negative control): black box indicates region of interest; red arrow indicates a normal ovarian follicle. (B) K+ (positive control, PCOS model): black box and black arrow indicate polycystic follicular morphology. (C) P1 (200 mg/kg BW red ginger extract): black box and black arrow indicate polycystic follicular morphology. (D) P2 (400 mg/kg BW red ginger extract): black box and black arrow indicate polycystic follicular morphology. (E) P3 (800 mg/kg BW red ginger extract): black box indicates region of interest; red arrow indicates a recovering ovarian follicle.

Further analysis via ANOVA confirmed statistically significant intergroup heterogeneity in follicular expression ($p < 0.05$). Subsequent post hoc comparisons revealed that the negative control group (K-) differed significantly from the positive control (K+). Moreover, the positive control group (K+) was significantly distinct from all treatment groups, namely P1, P2, and P3.

Discussion

PCOS is characterized by a proinflammatory state, as demonstrated by elevated TNF- α levels; clinical studies have consistently reported increased TNF- α concentrations in affected individuals (Baptiste et al., 2010; Chlipala et al., 2020). Hyperinsulinemia occurs in 50-70% of women with PCOS, resulting in decreased insulin sensitivity due to post-binding abnormalities in the insulin receptor substrate (IRS, with decreased insulin production. Insulin acts through several sites to increase androgens. Increased insulin in the periphery results in high serum insulin levels, which increase theca cell androgen production. Excess intra-ovarian androgen plays a role in ovulation by acting directly on the ovaries and causing follicular atresia (De Leo et al., 2016; Muttaqin, 2009).

Herbal ingredients are medicines containing active ingredients derived from traditional plants, commonly used by local communities to treat diseases. The category of herbal medicinal products encompasses organic chemical compound mixtures obtained through the processing of partial or complete traditional plant materials. In Indonesia, where such products are culturally designated as jamu and applied for nutritional and systemic health purposes, their administration requires structured therapeutic protocols (Chakraborty et al., 2012; Lizneva et al., 2016). Phytochemicals are also a major focus of the government in efforts to overcome drug imports. The advantage of herbal ingredients compared to modern medicines is that herbal ingredients are believed to have fewer side effects than modern medicines, and herbal ingredients contain various active substances that work synergistically to produce various benefits in a single extract (Chlipala et al., 2020; Mareta & Amran, 2018; Sam, 2019).

While prior investigations have examined related fields, no in vivo studies to date have specifically evaluated the efficacy of *Zingiber officinale* var. *rubrum* ethanol extract in preventing

PCOS in *Rattus norvegicus*. This gap exists despite the extract's well-documented content of bioactive constituents with antioxidant and anti-inflammatory activities (Mudrikatin et al., 2026). The rationale for employing *Zingiber officinale* var. *rubrum* in this research stems from in vitro studies documenting its comparatively superior anti-inflammatory activity compared with other varieties (Rachman et al., 2019; Sam, 2019; Tosi et al., 2017). *Zingiber officinale* var. *rubrum* was selected based upon HPLC evidence indicating elevated 6-gingerol and 6-shogaol content compared with elephant ginger and emprit ginger counterparts (Rachman et al., 2019; Riduan, 2015).

TNF- α expression in *Rattus norvegicus* induces the release of cytokines and then stimulates insulin resistance by interfering with IRS serine phosphorylation (Pertiwi et al., 2022; Rachman et al., 2019; Zeng et al., 2019). Significant differences were observed between the negative control and both the positive control and the treatment group three (P3). The 800 mg/kg/bw dosage of *Zingiber officinale* var. *rubrum* ethanol extract exhibited significant distinctions from all treatment groups (P1, P2, P3), thereby substantiating its effectiveness in PCOS prophylaxis via TNF- α and IRS pathway modulation (Chakraborty et al., 2012; Supu et al., 2018).

The active substance content of the ethanol extract of *Zingiber officinale* var. *rubrum* was examined in the pharmacology laboratory at Brawijaya University, Malang. It showed that *Zingiber officinale* var. *rubrum* contains active compounds with potential anti-inflammatory activity, including 6-shogaol and 6-gingerol. In *Zingiber officinale* var. *rubrum*, after oral administration, it interacts with insulin receptors, acting as an insulinomimetic agent that disrupts the signal cascade to reduce circulating glucose. Protein interactions trigger insulin receptor autophosphorylation and phosphorylation of IRS-associated regulatory proteins. This molecular event indirectly stimulates insulin secretion, affecting a biochemical cascade that attenuates blood glucose levels (Gaytán et al., 2002; Rani et al., 2011; Wahyuni et al., 2023; Zeng et al., 2019). In vivo investigations demonstrated that the ethanol extract of *Zingiber officinale* var. *rubrum* possesses immunomodulatory, anti-inflammatory, and antioxidant properties. These properties were observed through modulation of gene expression across three functional categories: immune system signaling, anti-inflammatory cytokines, and antioxidant mechanisms (Rachman et al., 2019; Rani et al., 2011; Zeng et al., 2019).

The findings of this in vivo study demonstrate that the ethanol extract of *Zingiber officinale* var. *rubrum* effectively modulates TNF- α , supporting its potential use in PCOS prophylaxis. This approach is pathophysiologically justified by the proinflammatory nature of PCOS, which is characterized by elevated TNF- α and documented increases in proinflammatory cytokines such as TNF- α and IL-1 among patients (Hu et al., 2011; Li et al., 2012; Nazir et al., 2014). *Zingiber officinale* extract has been demonstrated to enhance insulin secretion from pancreatic β -cells in murine models. Glucose tolerance testing further established that *Zingiber officinale* var. *rubrum* extract elevates circulating plasma insulin concentrations, thereby reducing blood glucose levels (Hu et al., 2011; Li et al., 2012; Nazir et al., 2014). One of the components of *Zingiber officinale* var. *rubrum*. The most important component in lowering blood glucose levels is gingerol. This component shows a protective effect on pancreatic β cells in diabetic rats and restores plasma insulin levels (Gaytán et al., 2002; Hu et al., 2011). Unlike alternative mechanisms, this component reduces blood glucose by potentiating insulin, reducing serum glucose, and lowering fructosamine levels. This antidiabetic combination further improves antioxidant status and ameliorates oxidative damage in renal and pancreatic tissues of diabetic rat models (Chakraborty et al., 2012; Li et al., 2012; Liguori et al., 2018). *Zingiber officinale* var. *rubrum*, commonly known as red ginger, possesses chemical compounds with documented anti-inflammatory and antioxidant efficacy in the management of diverse pathologies; these therapeutic attributes are mediated by bioactive components, notably 6-gingerol and 6-shogaol (Morvaridzadeh et al., 2021; Rachman et al., 2019). In this study, ovarian granulosa cells treated with *Zingiber officinale* var. *rubrum* showed immunohistochemical results, while the positive control group experienced Polycystic Ovary Syndrome (PCOS). This shows that *Zingiber officinale* var. *rubrum* protects ovarian granulosa cells.

Previous research has established that intraperitoneal administration of testosterone propionate for twenty-one days reliably induces a macroscopic phenotype consistent with Polycystic Ovary Syndrome (PCOS). *Zingiber officinale* var. *rubrum* is known to exert anti-inflammatory effects via distinct mechanisms. In the present experimental protocol, the ethanol extract was administered at graded doses: 200 mg/kg body weight to group 1 (P1), 400 mg/kg to group 2 (P2), and 800 mg/kg to group 3 (P3). Testosterone propionate was administered for no more than twenty-one days, as prolonged administration may cause ovarian damage that cannot be reversed. Immunohistochemical analysis of ovarian cell flow in this study indicates the presence of Polycystic Ovary Syndrome (PCOS) in all treatment groups that received *Zingiber officinale* var. *rubrum*, as well as in the negative control group, which did not receive *Zingiber officinale* var. *rubrum*. This outcome may also be attributed to insufficient testosterone administration, an inadequate dose of *Zingiber officinale* var. *rubrum*, and the duration of *Zingiber officinale* var. *rubrum* administration, which also plays a role.

Conclusion

Administration of *Zingiber officinale* var. *rubrum* was proven to exert a significant effect on inflammatory parameters, oxidative stress, and ovarian follicular development in *Rattus norvegicus* as a PCOS model. Significant differences were found between groups across all tested parameters, namely TNF- α , Immunoreactive Remmele Score (IRS), and ovarian follicle count/expression ($p < 0.001$). TNF- α expression showed significant differences between the negative control group (K-; 3.00 ± 1.27) and the positive control group (K+; 10.5 ± 1.34), as well as all treatment groups (P1, P2, P3), with the greatest reduction trend observed in group P3 (2.50 ± 0.84). For the IRS (Immunoreactive Remmele Score) parameter, significant increases were observed between groups ($p < 0.001$), with the highest value in group P3 (74.7 ± 23.75) compared to P2 (50.1 ± 38.65), P1 (21.1 ± 35.92), K- (10.3 ± 21.0), and K+ (4.34 ± 6.89). These changes indicate an improvement in immunohistochemical activity in ovarian tissue. Additionally, follicle count and expression showed significant differences ($p < 0.001$), with the greatest increase observed in group P3 (9.50 ± 1.05) compared to the other groups. Overall, the 800 mg/kg BW dose (P3) demonstrated the greatest improvement in TNF- α , IRS, and folliculogenesis parameters.

This study has several limitations, including a relatively small sample size ($n=6$ per group), the use of an animal model that does not fully reflect the complexity of PCOS in humans, and the absence of further molecular mechanism analysis (e.g., the NF- κ B or insulin signaling pathways).

The results of this study indicate the potential of *Zingiber officinale* var. *rubrum* as a phytotherapy agent in modulating inflammation and improving ovarian function under PCOS-like conditions. Future research is recommended to explore more specific molecular mechanisms, long-term toxicity testing, and clinical trials in humans to confirm the effectiveness and safety of the optimal dose.

Acknowledgments

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