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Molecular Mechanism of Red Ginger Ethanol Extract (*Zingiber officinale var. rubrum*) through Ovarian Cell Flow Cytometry Results: MDA, NF-κβ and Blood Glucose Levels as a Protector Against Polycystic Ovary Syndrome (PCOS) in *Rattus norvegicus* Rats

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting 5–21% of women of reproductive age. Insulin resistance is present in 50–75% of cases, resulting in a 35–40% reduction in insulin sensitivity compared to healthy women. Mareta's study reported that 92.2% of 249 women with PCOS were obese, with infertility rates of 61–89.6%, and an 8.5-fold higher risk of infertility compared to women without PCOS. In experimental models, intraperitoneal administration of 100 mg/kg body weight of testosterone propionate elevates testosterone levels, induces ovarian inflammation, and disrupts the balance between protective and damaging cellular factors, leading to PCOS. This investigation endeavored to appraise the influence of red ginger (Zingiber officinale var. rubrum) ethanol extract on ovarian cellular flow-cytometric parameters—MDA, NF-κB, and glycemic indices—in mitigating the onset of polycystic ovary syndrome (PCOS) in Rattus norvegicus. In this laboratory experiment, a randomized post-test–only control group framework was utilized to examine ovarian cell MDA and NF-κB expression through flow cytometry, as well as blood glucose concentrations. Thirty Rattus norvegicus subjects were randomly assigned to five groups: K1 (negative control), K2 (positive control), P1 (200 mg/kg BW), P2 (400 mg/kg BW), and P3 (800 mg/kg BW). Treatment groups received red ginger extract for three days, followed by 21 days of combined extract and testosterone propionate (100 mg/kg BW, intraperitoneally). On day 22, rats were euthanized, and blood and ovarian tissues were collected. Ethical approval was obtained from Universitas Airlangga (No. 2.KEH.59.04.2025). Flow cytometry analysis of ovarian cells demonstrated that treatment with red ginger extract significantly reduced MDA and NF-κB expression, as well as blood glucose levels, compared to the positive control group. All treatment groups (P1, P2, and P3) showed significant improvements, with $p = 0.001$ for each parameter. One-way ANOVA confirmed these findings, indicating highly significant differences among groups ($p = 0.001$). The ethanol extract derived from red ginger (Zingiber officinale var. rubrum) was shown to diminish MDA and NF-κB expression in ovarian cells and to decrease blood glucose levels, suggesting its potential as a protective agent against polycystic ovary syndrome (PCOS).

KEYWORDS: Ethanol Extract of Red Ginger, Ovarian Cell Flow Cytometry Results, Testosterone Propionate

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INTRODUCTION

Women with polycystic ovary syndrome (PCOS) who exhibit insulin resistance frequently present with elevated insulin levels (hyperinsulinemia). This condition reduces the effectiveness of ovulation induction therapy because elevated levels of luteinizing hormone (LH) impair oocyte quality. Conventional ovulation-inducing agents are unable to suppress insulin levels, or reduce the elevated concentrations of LH and circulating androgens. Management of hyperinsulinemia in PCOS patients has been shown to improve folliculogenesis, which consequently increases the likelihood of ovulation and pregnancy [1, 2].

PCOS is also identified as a condition with pronounced inflammatory activity, marked by heightened malondialdehyde (MDA) levels. Numerous investigations have demonstrated that individuals with PCOS tend to show increased amounts of pro-inflammatory cytokines, including MDA [3].

Polycystic ovary syndrome (PCOS) is characterized by the presence of ovulatory disturbances and excessive androgen levels. The

condition is also closely linked to insulin resistance, a metabolic issue that is particularly common in women who are overweight or obese [4]. At present, PCOS is diagnosed using the Rotterdam criteria, which require the presence of at least two of the following: (a) infrequent or absent ovulation, (b) clinical or biochemical evidence of hyperandrogenism, and (c) polycystic ovarian features detected through ultrasound. The condition is estimated to affect approximately 5% to 21% of women in their reproductive years [5]. The administration of testosterone propionate has been widely used as an experimental approach to induce a PCOS animal model, as it reproduces pathophysiological features similar to those observed in women with PCOS [6, 7].

Red ginger (*Zingiber officinale* var. *rubrum*) is one of the most widely used medicinal plants in Indonesia, traditionally consumed to alleviate various health conditions. Its bioactive compounds possess both anti-inflammatory and antioxidant properties. Further clinical studies, however, are required to determine its optimal dosage, efficacy, and safety as a preventive agent against PCOS [5, 6]. The main active constituents, gingerols and shogaols, exhibit pharmacological activities relevant to inflammation and oxidative stress [8, 9]. Both shogaol and gingerol are phenolic compounds that suppress inflammatory activity by reducing cytokine production associated with MDA [8, 10]. Specifically, gingerol inhibits cytokine-mediated activation of inflammatory cells by downregulating the expression of pro-inflammatory mediators, which are responsible for persistent inflammation [11, 12]. Moreover, gingerol suppresses NF-κB activation—one of the key transcription factors that regulate genes involved in immunity and inflammation—thereby reducing MDA levels [13].

Building upon this background, the current research seeks to investigate the molecular action of red ginger (Zingiber officicinale var. rubrum) ethanol extract by examining ovarian cells through flow cytometry, with particular attention to MDA, NF-κB, and blood glucose levels as indicators of its protective effects against polycystic ovary syndrome (PCOS) in Rattus norvegicus.

METHOD

Study Setting and Ethical Approval

The experiment took place in April 2025 at the Laboratory of the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya. Approval for the study's ethical procedures was issued by the Faculty's Ethics Committee on April 28, 2025, under the authorization number 2.KEH.59.04.2025.

Study Design

The research employed a laboratory-based experimental design using a randomized post-test only control group design. This approach allows for evaluating the effects of red ginger ethanol extract on induced polycystic ovary syndrome (PCOS) without baseline measurement bias.

Experimental Animals

Thirty healthy female Rattus norvegicus (weighing 100–200 g) were used as experimental subjects. Prior to the intervention, all animals underwent a 14-day acclimatization period under controlled environmental conditions (temperature 22–25 °C, 12-hour light/dark cycle) with free access to standard feed and drinking water.

Grouping and Treatment Protocols

The rats were randomly assigned into five groups (n = 6 per group):

- K1 (Negative control): received only standard food and water.
- K2 (Positive control): received testosterone propionate at a dose of 100 mg/kg body weight (BW) intraperitoneally for 21 consecutive days, followed by euthanasia on day 22.
- P1 (Treatment 1): pre-treated with ethanol extract of red ginger at 200 mg/kg BW orally for 3 days, then co-administered red ginger extract (200 mg/kg BW) with testosterone propionate (100 mg/kg BW) for 21 days, followed by euthanasia on day 22.
- P2 (Treatment 2): pre-treated with red ginger extract at 400 mg/kg BW orally for 3 days, then co-administered red ginger extract (400 mg/kg BW) with testosterone propionate (100 mg/kg BW) for 21 days, followed by euthanasia on day 22.
- P3 (Treatment 3): pre-treated with red ginger extract at 800 mg/kg BW orally for 3 days, then co-administered red ginger extract (800 mg/kg BW) with testosterone propionate (100 mg/kg BW) for 21 days, followed by euthanasia on day 22.

Sample Collection and Processing

On the 22nd day, all animals were euthanized under anesthesia. The ovarian tissues were then excised with care and placed in phosphate-buffered saline (PBS) to maintain cellular integrity. Flow cytometric evaluation of malondialdehyde (MDA) and NF-κB expression in ovarian cells was subsequently conducted at the Laboratory of the Faculty of Medicine, Universitas Brawijaya (UB), Malang.

Blood Glucose Measurement

Blood samples were collected immediately following euthanasia. Blood glucose levels were determined using a validated glucometer based on the glucose oxidase-peroxidase method.

RESULT

Flow cytometric assessment of ovarian cells showed notable variations in malondialdehyde (MDA) concentrations across the treatment groups. As presented in Table 1, the negative control group (K1) displayed the lowest MDA levels, reflecting normal ovarian function. Conversely, the positive control group (K2), which was administered testosterone propionate, exhibited a substantial increase in MDA, indicating successful induction of oxidative stress characteristic of polycystic ovary syndrome (PCOS). Treatment with ethanol extract of red ginger (Zingiber officinale var. rubrum) produced a clear protective effect in all intervention groups. The P1 group (200 mg/kg BW) showed a moderate reduction in MDA compared to K2, while the P2 group (400 mg/kg BW) demonstrated a greater reduction, indicating a dose-dependent response. The P3 group (800 mg/kg BW) exhibited the most pronounced decrease in MDA, with levels approaching those observed in the healthy control group (K1).

These findings suggest that administration of red ginger ethanol extract effectively reduces oxidative stress in ovarian cells, with the highest dose (800 mg/kg BW) yielding the strongest protective effect. Statistical analysis confirmed significant differences between treatment groups and the positive control ($p < 0.05$).

Table 1: Flow cytometry results of ovarian cell MDA levels in control and treatment groups

Malondealdehyde (MDA)			
Group	**Mean**	**± SD**	**ρ**
K1 (negative)	22.6	± 26.10	
K2 (positive)	40.20	± 22.10	<0.001*
P1	10.7	± 1.92	
P2	4.73	± 3.43	
P3	1.36	± 0.61	
(*): significant α=0.05			

The results presented in Table 1 show that malondialdehyde (MDA) levels in ovarian cells were significantly different across groups. The negative control group (K1), which represented healthy animals, showed significantly lower MDA concentrations than the positive control group (K2), with a highly significant statistical difference ($p = 0.001$). A similarly strong difference ($p = 0.001$) was observed when comparing K1 with the treatment group receiving the highest dose of red ginger extract (P3; 800 mg/kg BW). These results suggest that, although the red ginger extract effectively reduces oxidative stress in ovarian tissue, MDA levels in the P3 group had not fully returned to the normal baseline seen in healthy controls.

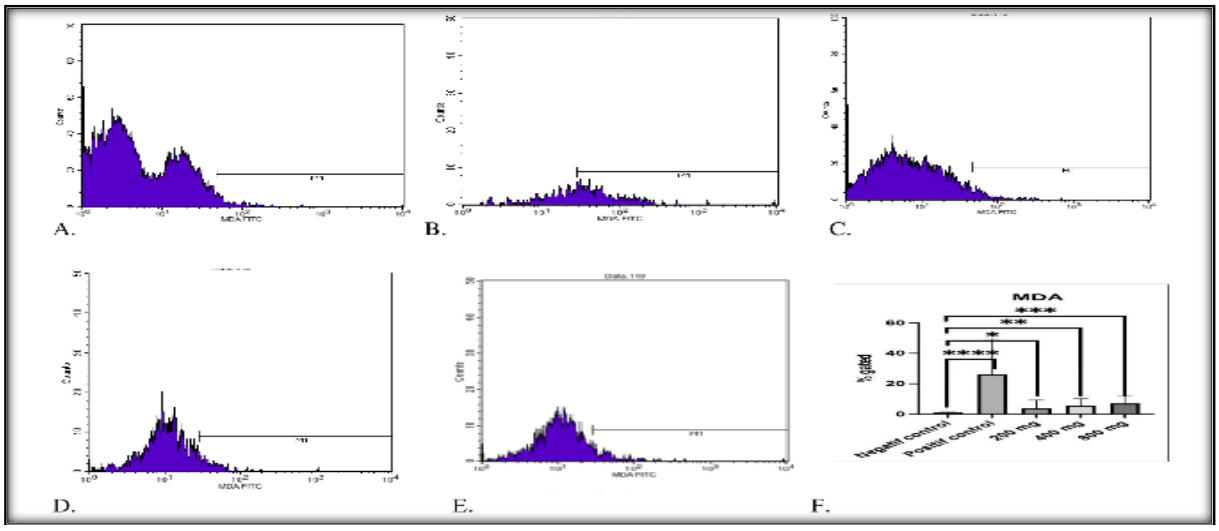

Figure 1: Flow cytometry analysis of ovarian cell MDA levels in control and treatment groups of Rattus norvegicus

Flow cytometry analysis of ovarian cells demonstrated significant differences in malondialdehyde (MDA) expression across all experimental groups (Figure A–F). Quantitative analysis revealed that the negative control group (K1) had a mean MDA level of 22.6 ± 26.1, whereas the positive control group (K2), induced with testosterone propionate, exhibited markedly elevated levels (40.20 ± 22.10), confirming oxidative stress induction associated with PCOS.

Administration of ethanol extract of red ginger (Zingiber officinale var. rubrum) resulted in a progressive, dose-dependent reduction in MDA levels. The P1 group (200 mg/kg BW) demonstrated a decrease to 10.70 ± 1.92, while the P2 group (400 mg/kg BW) showed a further reduction (4.73 ± 3.43). The P3 group (800 mg/kg BW) exhibited the lowest MDA level (1.36 ± 0.61), approaching near-normal values compared to the healthy control group.

Statistical analysis using one-way ANOVA confirmed that all treatment groups (P1, P2, P3) differed significantly from the positive control (K2) ($p = 0.001$). Furthermore, pairwise comparisons indicated that the high-dose treatment group (P3) showed significantly greater improvements compared to both P1 and P2, underscoring the dose-dependent antioxidant effect of red ginger ethanol extract. Figure A–F. Flow cytometry histograms of malondialdehyde (MDA) expression in ovarian cells. (A) Negative control (K1), (B) Positive control (K2), (C) Treatment group P1 (200 mg/kg BW), (D) Treatment group P2 (400 mg/kg BW), (E) Treatment group P3 (800 mg/kg BW), (F) Bar graph summary of mean ± SD values and statistical comparisons.

Flow cytometry analysis of nuclear factor kappa β (NF-κB) expression in ovarian cells revealed significant variations across the experimental groups (Table 2). The negative control group (K1) demonstrated the lowest NF-κB levels, reflecting normal inflammatory status. In contrast, the positive control group (K2) exhibited a marked elevation in NF-κB expression, confirming that testosterone propionate induction successfully triggered inflammatory responses associated with PCOS. Administration of ethanol extract of red ginger (Zingiber officinale var. rubrum) significantly reduced NF-κB expression in a dose-dependent manner. The P1 group (200 mg/kg BW) showed a moderate reduction, the P2 group (400 mg/kg BW) exhibited a greater decrease, and the P3 group (800 mg/kg BW) had the lowest NF-κB levels among all treatment groups. Statistical analysis confirmed that all treatment groups differed significantly from the positive control ($p < 0.05$), with the P3 group showing the strongest protective effect, approaching values observed in the healthy control group.

Table 2: NF-κB Expression in Ovarian Cells of Rattus Norvegicus Across Control and Treatment Groups.

Nuclear Factor Kappa B (NF -κβ)			
Group	**Mean**	**± SD**	**ρ**
K1 (negative)	10.10	± 6.09	
K2 (positive)	13.10	± 4.51	
P1	3.98	± 4.34	<0.001*
P2	3.95	± 2.81	
P3	1.41	± 0.99	
(*): significant α=0.05			

As shown in Table 2, nuclear factor kappa B (NF-κB) expression in ovarian cells varied significantly across the experimental groups. The negative control group (K1) displayed markedly lower NF-κB levels than the positive control group (K2) ($p = 0.001$), verifying that the inflammatory state characteristic of the PCOS model was successfully induced. In addition, the group treated with 800 mg/kg BW of red ginger extract (P3) showed a highly significant decline in NF-κB expression compared with both the P1 (200 mg/kg BW) and P2 (400 mg/kg BW) groups ($p = 0.001$). These results suggest that the highest dosage of the extract produced the most pronounced anti-inflammatory response, reducing NF-κB expression to levels approaching those of the healthy control group.

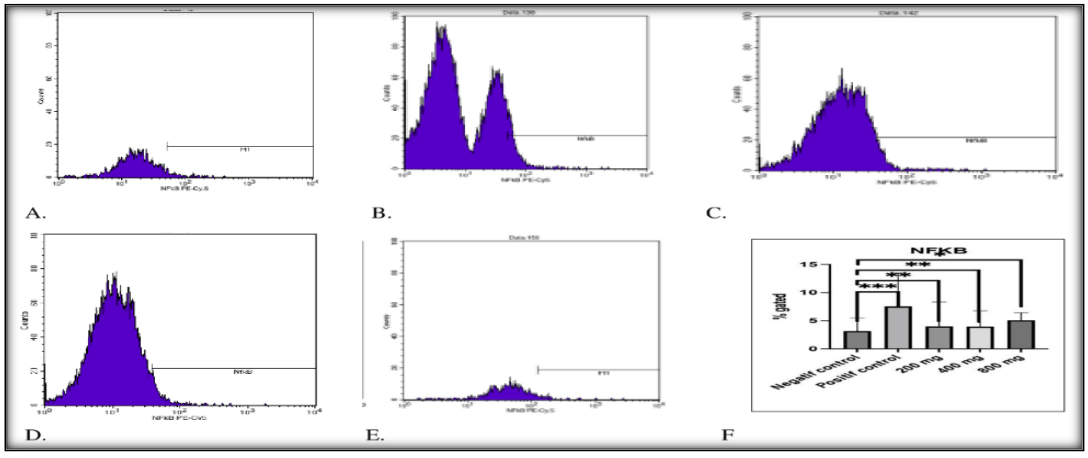

Figure 2: NF-κB expression in ovarian cells of Rattus norvegicus across control and treatment groups.

Flow cytometry analysis of nuclear factor kappa B (NF-κB) expression in ovarian cells demonstrated significant differences among groups (Figure 2). The negative control group (K1) showed mean NF-κB levels of 10.10 ± 6.09, while the positive control group (K2) exhibited higher expression (13.10 ± 4.51), indicating successful induction of inflammatory pathways in the PCOS model. Treatment with ethanol extract of red ginger markedly reduced NF-κB expression in a dose-dependent manner. The P1 group (200 mg/kg BW) had levels of 3.98 ± 4.34, the P2 group (400 mg/kg BW) showed 3.95 ± 2.81, and the P3 group (800 mg/kg BW) recorded the lowest expression at 1.41 ± 0.99, approaching values seen in healthy controls. Statistical analysis using one-way ANOVA confirmed that all treatment groups differed significantly from the positive control ($p < 0.05$).

Flow cytometry analysis of NF-κB expression in ovarian cells showed that all treatment groups differed significantly from the positive control group. This result clearly indicates that administration of red ginger extract exerted a measurable anti-inflammatory effect on ovarian tissue subjected to PCOS induction. The reduction in NF-κB expression observed in the treatment groups demonstrates that red ginger extract was effective in modulating inflammatory pathways that are typically activated in the disease state.

Among the three treatment groups, the P3 group (800 mg/kg BW) demonstrated the greatest reduction in NF-κB expression, showing a much stronger suppressive effect compared to both the P1 group (200 mg/kg BW) and the P2 group (400 mg/kg BW). This progressive decrease across the treatment groups reflects a consistent dose-dependent trend, where higher doses of the extract were associated with greater inhibition of NF-κB. The results therefore suggest that the highest dose of red ginger ethanol extract provided the most substantial protective effect against the inflammatory changes induced by PCOS, approaching the condition observed in the negative control group.

Analysis of blood glucose levels revealed clear differences among the experimental groups (Table 3). The negative control group (K1) displayed normal glucose values, consistent with healthy physiological conditions. In contrast, the positive control group (K2) showed elevated blood glucose levels, confirming the successful induction of metabolic disturbances associated with PCOS. Treatment with ethanol extract of red ginger (Zingiber officinale var. rubrum) resulted in a dose-dependent reduction of blood glucose. The P1 group (200 mg/kg BW) demonstrated a modest decrease compared to the positive control, the P2 group (400 mg/kg BW) showed a greater reduction, and the P3 group (800 mg/kg BW) exhibited the lowest blood glucose levels among all treatment groups. Statistical analysis confirmed that all treatment groups differed significantly from the positive control ($p < 0.05$), with the most pronounced improvement observed in the P3 group, approaching values seen in healthy controls.

Table 3: Blood glucose levels in control and treatment groups of Rattus norvegicus

Glucose in the blood			
Group	**Mean**	**± SD**	**ρ**
K1 (negative)	74.7	± 4.46	
K2 (positive)	312	± 97.02	
P1	204	± 75.6	<0.001*
P2	238	± 28.0	
P3	86.6	± 10.6	
(*): significant α=0.05			

The results of blood glucose analysis are presented in Table 3. The negative control group (K1) showed normal glucose levels (74.66 ± 4.46 mg/dL), consistent with healthy baseline values. In contrast, the positive control group (K2) demonstrated a marked elevation (312 ± 97.02 mg/dL), confirming the development of hyperglycemia following testosterone propionate induction. Administration of ethanol extract of red ginger (Zingiber officinale var. rubrum) produced a significant, dose-dependent reduction in blood glucose levels. The P1 group (200 mg/kg BW) showed a decline to 204 ± 75.6 mg/dL, while the P2 group (400 mg/kg BW) experienced a further decrease to 128 ± 28.0 mg/dL. The most pronounced effect was found in the P3 group (800 mg/kg BW), which reached 86.8 ± 10.6 mg/dL, closely approaching the values of the healthy control group. One-way ANOVA analysis confirmed that these differences were highly significant ($p = 0.001$).

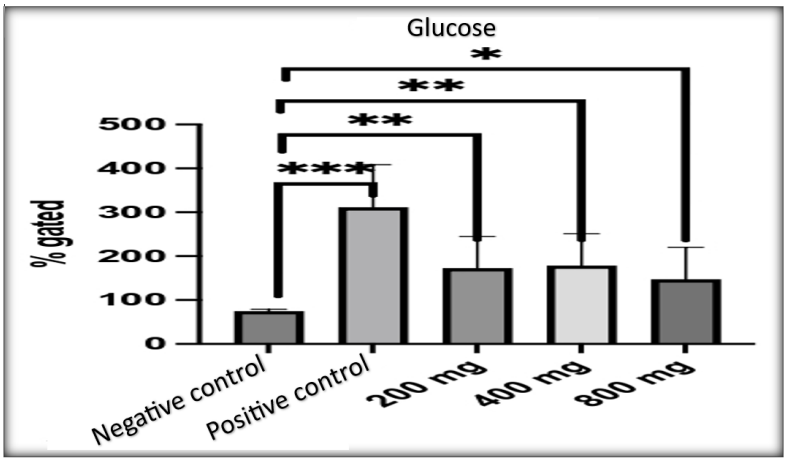

Figure 3: Blood glucose levels in control and treatment groups of Rattus norvegicus

The analysis of blood glucose levels revealed that the negative control group (K1), representing healthy rats, exhibited significantly lower glucose concentrations than the positive control group (K2) ($p = 0.001$). Furthermore, glucose levels in the P3 treatment group (800 mg/kg BW red ginger extract) were highly significantly different from those in the healthy control group, indicating that although red ginger extract markedly reduced hyperglycemia, glucose levels had not yet fully returned to baseline values.

DISCUSSION

Herbal medicines can be defined as therapeutic agents containing bioactive compounds derived from traditional plants that have long been used in local communities for the treatment of various diseases. Herbal medicinal products are generally mixtures of organic chemical compounds obtained from processing parts or the entirety of medicinal plants [7]. In Indonesia, the use of herbal products is commonly associated not only with disease management but also with nutritional support and overall health maintenance, thereby requiring further evaluation of their therapeutic potential [6]. Phytochemicals, in particular, have also become a strategic focus of government initiatives to reduce dependency on imported pharmaceuticals.

Compared to modern synthetic drugs, herbal medicines are believed to have fewer adverse side effects [14]. Moreover, herbal products often contain multiple active compounds that act synergistically to produce a variety of beneficial effects within a single extract [9]. This synergism is considered one of the key advantages of herbal therapies, as it may provide a broader spectrum of activity compared to drugs with single active ingredients [15].

To date, no in vivo studies have investigated the use of ethanol extract of red ginger (*Zingiber officinale* var. *rubrum*) for the prevention of polycystic ovary syndrome (PCOS) in Rattus norvegicus models. Red ginger contains bioactive compounds with potent antioxidant and anti-inflammatory properties, making it a promising candidate for further exploration. The selection of this ginger variety in the present study was supported by previous in vitro findings showing that *Zingiber officinale* var. *rubrum* exhibits stronger anti-inflammatory activity than other ginger varieties [14, 10].

This selection is further supported by phytochemical profiling studies demonstrating that red ginger contains higher concentrations of 6-gingerol and 6-shogaol compared to elephant ginger (*Zingiber officinale* var. *officinale*) and small ginger (*Zingiber officinale* var. *amarum*). These differences were confirmed through high-performance liquid chromatography (HPLC), which identified red ginger as the variety with the most abundant bioactive constituents [11]. These compounds are known to be potent antioxidants and modulators of inflammatory signaling pathways, thereby providing a strong mechanistic basis for their use in managing PCOS-related oxidative stress and inflammation.

Examination of the bioactive compounds in red ginger extract carried out at the Pharmacology Laboratory, Universitas Brawijaya, Malang, confirmed the presence of potent anti-inflammatory agents such as 6-shogaol and 6-gingerol. These compounds are known to inhibit the activation of protein kinase B (Akt) and nuclear factor kappa B (NF-κB), leading to an upregulation of anti-inflammatory cytokines and a concurrent reduction in pro-inflammatory cytokines. In vitro studies have demonstrated that red ginger extract exhibits immunomodulatory, anti-inflammatory, and antioxidant activities, as evidenced by alterations in the expression of gene groups involved in immune signaling, anti-inflammatory responses, and antioxidant pathways [17].

Findings from a systematic review and meta-analysis of randomized controlled trials (RCTs) further reinforce these observations, demonstrating that oral administration of red ginger significantly reduces inflammatory serum biomarkers, particularly tumor necrosis factor-alpha (TNF-α) [18]. Additionally, in vitro studies have shown that red ginger extract enhances insulin secretion from pancreatic β-cells in rats [19]. Results from glucose tolerance tests also indicate that red ginger extract increases plasma insulin levels, which subsequently contributes to a reduction in blood glucose concentrations [20]. One of the most critical bioactive components, gingerol, has been shown to exert protective effects on pancreatic β-cells in diabetic rat models, restoring plasma insulin concentrations [21, 22]. Moreover, red ginger contributes to lowering blood glucose by elevating serum insulin levels while simultaneously reducing serum glucose and fructosamine concentrations. When combined with antidiabetic drugs, ginger extract enhances antioxidant defenses and mitigates oxidative damage in the kidneys and pancreas of diabetic rat models [23].

Red ginger is thus recognized as a herbal plant rich in bioactive compounds with therapeutic benefits, particularly due to its 6-gingerols and 6-shogaol content, which contribute to its anti-inflammatory and antioxidant properties. In the present study, flow cytometry analysis of ovarian cells from rats treated with red ginger extract revealed intact ovarian morphology, in contrast to the positive control group, which developed ovarian alterations consistent with polycystic ovary syndrome (PCOS). This finding indicates that red ginger exerts a protective effect on ovarian cells.

Previous research has shown that intraperitoneal administration of testosterone propionate for 21 days consistently induces macroscopic features of PCOS. In this study, red ginger supplementation significantly attenuated these changes, demonstrating its role as an anti-inflammatory agent. However, it is important to note that prolonged administration of testosterone propionate beyond 21 days may cause irreversible ovarian damage, thereby preventing recovery. Flow cytometry of ovarian cells in the current study confirmed PCOS-like changes in all groups treated with red ginger (Groups III, IV, and V), whereas the most severe alterations were observed in the positive control group without red ginger supplementation. These outcomes may have been influenced by factors such as insufficient testosterone exposure, suboptimal red ginger dosage, or treatment duration, all of which could affect the degree of ovarian protection observed.

CONCLUSION

Administration of red ginger (*Zingiber officinale* var. *rubrum*) in combination with testosterone propionate (100 mg/kg BW) demonstrated a preventive effect against the development of polycystic ovary syndrome (PCOS) in Rattus norvegicus. The strongest protective response was observed in the P3 group (800 mg/kg BW), which exhibited greater efficacy than the P1 (200 mg/kg BW) and P2 (400 mg/kg BW) groups. Red ginger extract effectively reduced malondialdehyde (MDA) levels, NF-κB expression, and blood glucose concentrations.

These results suggest that the protective effects of red ginger are closely associated with its potent antioxidant activity, which reduces oxidative stress and subsequently suppresses inflammatory pathways. Therefore, ethanol extract of red ginger holds potential as a natural therapeutic agent for mitigating oxidative stress and inflammation linked to PCOS.

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