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Exploring the anti-inflammatory properties of zingiber officinale var. rubrum in preventing polycystic ovary syndrome: In Silico and in Vivo studies

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Abstract: The increasing incidence of polycystic ovary syndrome (PCOS), will cause oxidative stress due to hyperglycemia, which subsequently elevates expression levels of proinflammatory cytokines, particularly tumor necrosis factor-alpha (TNF-α), contributing to a marked rise in infertility, observed in up to 89.6% cases. This study aimed to investigate the potential of Zingiber officinale var. rubrum in preventing PCOS. The in vivo laboratory-based experimental study utilized Rattus norvegicus, with 5 rats per group, divided into five experimental groups. The intervention consisted of Zingiber officinale var. rubrum extract for PCOS prevention. Statistical analysis was performed using one-way ANOVA. The silico study began with the preparation of activator ligands 6-Shogaol and 6-Gingerol, along with target receptors TNF-α and KEP1, NF-κβ. The in silico analysis showed that compounds from Zingiber officinale var. rubrum exhibited binding interactions with TNF-α at sites similar to the control ligands, showing greater affinity compared to interactions with SOD. The in vivo analysis showed that significantly elevated TNF-α expression levels and induced folliculogenesis abnormalities characteristic of PCOS ($p < 0.05$) compared to negative controls. The findings indicate that the bioactive compounds 6-Shogaol and 6-Gingerol present in Zingiber officinale var. rubrum may exert preventive effects against PCOS by modulating inflammatory pathways.

Keywords: *Anti-inflammatory, PCOS prevention, Zingiber officinale var rubrum.*

1. Introduction

Polycystic ovary syndrome (PCOS) is commonly characterized by insulin resistance, particularly in women with obesity. Diagnosis is typically based on the Rotterdam criteria, which require the presence of at least two of the following: oligo- or anovulation, clinical or biochemical signs of hyperandrogenism, and polycystic ovarian morphology on ultrasound. The prevalence of PCOS among women of reproductive age ranges from 5% to 21%. Among women with PCOS, the prevalence of insulin resistance is estimated at 50–75%, with a 35–40% reduction in insulin sensitivity compared to women with obesity but without PCOS. PCOS is associated with infertility in approximately 61% to 89.6% of cases [1]. Insulin resistance will cause oxidative stress due to hyperglycemia and elevated levels of free fatty acids, which subsequently elevates TNF-α expression levels [2]. Experimental PCOS models in rats have been successfully developed using subcutaneous or intraperitoneal administration of testosterone propionate for 21 days. Selection of rats that exhibit hyperinsulinemia is critical for modeling PCOS accurately [3]. Addressing hyperinsulinemia in PCOS can improve folliculogenesis, thereby increasing ovulation and pregnancy rates. Research has shown elevated expression levels of proinflammatory cytokines,

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particularly TNF-α, and KEAP1,NF-κβ and other cytokines, in women with PCOS [4, 5]. The compounds shogaol and gingerol, present in Zingiber officinale var. rubrum (red ginger), possess documented anti-inflammatory, antioxidant, and antibacterial properties. Further clinical trials are warranted to determine optimal dosing, efficacy, and safety for using Zingiber officinale var. rubrum in preventive applications [6]. The bioactive compounds gingerol and shogaol exert pharmacological effects by modulating inflammation and oxidative stress. These phenolic compounds inhibit the activity of proinflammatory cytokines, particularly TNF-α, and KEAP1,NF-κβ [7]. Gingerol also modulate the production of inflammatory cytokines by downregulating proinflammatory mediator expression, thereby reducing persistent inflammation. Additionally, gingerol inhibits the activation of KEAP1,NF-κβ a key transcription factor that regulates genes involved in immune responses and inflammation, including TNF-α [8]. Previous studies have confirmed the anti-inflammatory activity of shogaol and gingerol, as evidenced by reduced TNF-α expression levels [9]. Zingiber officinale var. rubrum has been shown to possess superior anti-inflammatory properties compared to other ginger varieties [10]. However, studies specifically examining whether the anti-inflammatory effects of Zingiber officinale var. rubrum can prevent the onset of PCOS remain limited and warrant further exploration.

2. Methods

2.1. Study Design and Setup

In silico study was conducted to investigate the anti-inflammatory properties of Zingiber officinale var. rubrum. The study began with the preparation of control activator ligands, 6-Shogaol (PubChem ID: 5281794) and 6-Gingerol (PubChem ID: 442793), and target receptor proteins: TNF-α (PDB ID: 2AZ5) and KEAP1, NF-κβ (PDB ID: 5CGJ). Molecular docking analyses were performed to predict the binding affinities of these compounds to the target proteins [11]. To validate these findings, in vivo laboratory-based experimental study was conducted utilizing female Rattus norvegicus at the Laboratory of the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia. The rats were divided into five experimental groups. The study protocol consisted of the following phases: a) Acclimatization (Days 1–7): All rats were acclimatized to laboratory conditions, b) Estrous Cycle Observation (Days 8–20): Vaginal smears were collected daily to monitor the estrous cycle, c) Pre-treatment Phase (Days 21–23): Rats in the treatment groups received Zingiber officinale var. rubrum extract at predetermined doses, d) Treatment Phase (Days 24–45): The five groups were treated as follows: (a) Group A (Negative Control): Received standard food and water orally without any treatment; (b) Group B (Positive Control): Normal rats received standard food and water orally and were administered testosterone propionate at 100 mg/kg body weight via daily intraperitoneal injection; (c) Group C (Treatment Group 1): Normal rats were given standard food and water orally, administered Zingiber officinale var. rubrum at 200 mg/kg body weight twice daily, followed by intraperitoneal injection of testosterone propionate (100 mg/kg body weight) one hour later each day; (d) Group D (Treatment Group 2): Similar to Group C but with a Zingiber officinale var. rubrum at 400 mg/kg body weight; (e) Group E (Treatment Group 3): Similar to Group C but with a Zingiber officinale var. rubrum at 800 mg/kg body weight. On day 46, all rats were euthanized under anesthesia. Blood samples and ovarian tissues were collected. Histological preparations of ovarian tissues were examined under a binocular microscope. TNF-α expression levels were assessed through immunohistochemical staining. Insulin levels were assessed through an ELISA assay, and blood glucose levels were also assessed. Statistical analysis was performed using one-way ANOVA.

In Silico Study to Assess the Anti-Inflammatory Effects of Zingiber officinale var. rubrum in Preventing Polycystic Ovary Syndrome (PCOS)

In the present study, the compounds 6-Shogaol (PubChem ID: 5281794) and 6-Gingerol (PubChem ID: 442793) were downloaded from the PubChem database to assess their predicted binding affinities for the target proteins TNF-α, insulin receptor substrate (IRS),NF-κβ, and KEAP1. These analyses were performed using molecular docking techniques [12].

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2.2. Preparation of Ligands for Molecular Docking Analysis

The structures of the control activator ligands, 6-shogaol and 6-gingerol, were identified using SMILES (Simplified Molecular-Input Line-Entry System) notation. The 3D structures of both compounds were obtained from the PubChem database. These compounds were further analyzed using the Way2Drug PASS (Prediction of Activity Spectra for Substances) online tool (http://www.pharmaexpert.ru/passonline/predict.php) to assess their potential as preventive and therapeutic agents for polycystic ovary syndrome (PCOS). The Way2Drug PASS online tool utilizes Structure-Activity Relationship (SAR) analysis to compare input compounds with a database of known bioactive compounds. The degree of structural similarity determines the likelihood that a compound will exhibit a specific biological activity. The Way2Drug PASS online tool outputs a Probability to be Active (Pa) value, which indicates the predicted potential of a compound. A Pa value greater than 0.7 indicates a high likelihood of biological activity (e.g., anti-inflammatory), due to a high similarity with known bioactive compounds in the database. A Pa value greater than 0.5 is recommended as a cut-off threshold for considering predictive relevance. The higher the Pa value, the greater the confidence in the predicted functional activity of the compound [13, 14].

2.3. Preparation of Receptor Proteins for Molecular Docking Analysis

Target proteins relevant to Zingiber officinale var. rubrum were identified using the Comparative Toxicogenomics Database (https://ctdbase.org) [14]. Target proteins relevant to PCOS were identified using the Open Targets Database (https:/ /www.opentargets.org/) and DisGeNET Database (www.disgenet.com) [15]. These target datasets—those relevant to Zingiber officinale var. rubrum and those relevant to PCOS—were compared (mapped) using a Venn diagram generated via the Venny tool (https://bioinfogp.cnb.csic.es/tools/venny/) to identify shared molecular targets (targets intersection). Subsequently, target gene annotation was performed using the DAVID webserver (https://david.ncifcrf.gov/) utilizing Gene Ontology (GO) terms and KEGG pathway, with a significance threshold of $p < 0.05$ [16]. Protein structure preparation was performed using PyMOL version 2.5.2. Ligand structure minimization was performed using the Universal Force Field (UFF) energy model via Open Babel software integrated in PyRx version 1.0. Molecular docking was performed using AutoDock Vina, also integrated in PyRx version 1.0 [11]. Targeted (specific) docking methodology was performed, where the grid box was defined based on the amino acid residues known to bind control inhibitors or activators as established by previous studies, namely: TNF-α control inhibitor: Position 307 (PDB ID: 2AZ5), KEAP1, NF-κβ control activator: RA839 (PDB ID: 5CGJ). Molecular docking results were reported in terms of binding affinity (or binding energy), representing the strength of interaction between each ligand and its target protein. These interactions were further visualized and analyzed using BIOVIA Discovery Studio 2024 software.

2.4. Molecular Docking Visualization Techniques

Based on data from the Comparative Toxicogenomics Database (CTD), gingerol has been shown to reduce serum insulin and leptin levels [17] while shogaol has been shown to elevate nuclear factor erythroid 2–related factor 2 (NF-κβ), a gene regulator for antioxidants, thereby inhibiting damage caused by free radicals [18].

Molecular docking results were reported in terms of binding affinity (or binding energy), representing the strength of interaction between each ligand and its target protein. These interactions were further visualized and analyzed using BIOVIA Discovery Studio 2024 software. Molecular docking results were validated using the following criteria: (1) Root Mean Square Deviation (RMSD), where validation is performed by comparing the docking pose (redocking) to crystallographic (experimental) structures. A docking result with an RMSD value < 2 Å (angstroms) is considered acceptable, as it indicates conformational similarity between the docked ligand and the experimental control structures; (2) the docking position of the comparison ligand should fall within the same binding

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pocket as the control ligand; (3) the comparison ligand should share at least one critical interacting amino acid residue with the control ligand to suggest a similar binding mechanism and functional role [11, 12].

2.5. Analysis of Molecular Docking Results and Interpretation

One of the criteria that must be met so that molecular docking results can be considered valid is to have an RMSD value < 2 Å (angstroms). In this study, molecular docking analysis has RMSD values of 0.595 Å for TNF-α and 0.405 Å for KEAP1, confirming that the docking simulations have met the criteria (valid).

Due to the unavailability of the NF-κβ structure, its binding partner KEAP1 was used for docking simulations. KEAP1 (Kelch-like ECH-associated protein 1) binds NF- κβ and the activator RA839 (PDB ID 5CGJ). Activation of the transcription factor NF-E2-related factor 2 (NF- κβ) maintains cellular homeostasis in response to oxidative stress through the regulation of several cytoprotective genes. Without stressors, NF- κβ activity is inhibited by its interaction with KEAP1 (Kelch-like ECH-associated protein 1). The small molecule RA839 (PDB ID: 5CGJ) acts as a KEAP1 activator, allowing NF- κβ to dissociate and translocate to the nucleus [12]. Figure 1 illustrates that the comparison ligand interacts with KEAP1 at a site proximal to the RA839 binding site, although not at the identical position. This positional discrepancy may contribute to the lower binding efficiency of the gingerol-based compounds compared to the control ligand RA839. Binding affinity results revealed that gingerol had the highest binding affinity among the comparison ligands at −6.5 kcal/mol, followed by shogaol at −6.2 kcal/mol (Table 1). A more negative binding affinity indicates stronger ligand–protein interaction. However, both values are lower than the control ligand RA839, which exhibited a binding affinity at −10.8 kcal/mol.

An analysis of amino acid interactions showed that gingerol did not share any interacting residues with the control ligand at the KEAP1 binding site, suggesting it may not effectively activate NF- κβ via KEAP1 binding. In contrast, shogaol showed an interaction with KEAP1 via the amino acid residue ALA556 through hydrophobic interactions, consistent with those observed for the control ligand (Table 2). Hydrophobic interactions are critical for ligand–protein stability and play a role in minimizing drug degradation. Therefore, shogaol may have greater potential than gingerol in interacting with KEAP1 [11, 12].

In Vivo Study to Assess the Anti-Inflammatory Effects of Shogaol-Gingerol from Zingiber officinale var. rubrum in Preventing Polycystic Ovary Syndrome (PCOS)

2.6. Experimental Animals

In this study, 8-week-old female *Rattus norvegicus* mice, weighing between 100–150 grams, were used. The animals were obtained from the Veterinary Farm Center (Pusvetma), Surabaya, Indonesia. All mice underwent a one-week acclimatization period before the initiation of the experiment. They were housed in plastic cages under controlled environmental conditions at a temperature of approximately 26 ± 2°C, with ad libitum access to standard food and water throughout the study duration.

2.7. Polycystic Ovary Syndrome (PCOS) Induction

In this study, PCOS was induced in rats via intraperitoneal injection of testosterone propionate at 100 mg/kg body weight daily for 21 consecutive days. Blood samples were collected via the lateral tail vein 24 hours after the final testosterone injection. Blood glucose levels were assessed using an Accu-Chek glucometer. Rats exhibiting blood glucose levels of approximately 200 mg/dL were included in the study as PCOS models.

2.8. Experimental Design

A total of 25 rats were randomly assigned to five groups. The study protocol consisted of the following phases: a) Acclimatization (Days 1–7): All rats were acclimatized to laboratory conditions,

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b) Estrous Cycle Observation (Days 8–20): Vaginal smears were collected daily to monitor the estrous cycle, c) Pre-treatment Phase (Days 21–23): Rats in the treatment groups received Zingiber officinale var. rubrum extract at predetermined doses, d) Treatment Phase (Days 24–45): The five groups were treated as follows: (a) Group A (Negative Control): Received standard food and water orally without any treatment; (b) Group B (Positive Control): Normal rats received standard food and water orally, during Days 24–45, they were also administered testosterone propionate (100 mg/kg body weight) via daily intraperitoneal injection; (c) Group C (Treatment Group 1): Normal rats were given standard food and water orally, administered Zingiber officinale var. rubrum at 200 mg/kg body weight twice daily, during Days 24–45, one hour after extract administration, they were also administered testosterone propionate (100 mg/kg body weight) via intraperitoneal injection; (d) Group D (Treatment Group 2): Similar to Group C but with a Zingiber officinale var. rubrum at 400 mg/kg body weight; (e) Group E (Treatment Group 3): Similar to Group C but with a Zingiber officinale var. rubrum at 800 mg/kg body weight. On day 46, all rats were euthanized under anesthesia. Blood samples and ovarian tissues were collected. Histological preparations of ovarian tissues were examined under a binocular microscope to assess TNF-α, KEAP1, NF-κβ expression levels. Insulin levels were assessed through an ELISA assay, and blood glucose levels were also assessed. Statistical analysis was performed using one-way ANOVA.

2.9. Preparation of Ovarian Histological Preparations

Histological preparations of ovarian tissues were prepared through the following phases. First, in the fixation stage, ovaries were immersed in 10% formalin solution for 24 hours, with fixation repeated twice in separate formalin baths to ensure optimal preservation. Second, in the dehydration stage, the fixed ovaries were sequentially dehydrated in 70% ethanol for 1 hour, followed by immersion in 80% ethanol, then 100% ethanol twice, and finally in absolute ethanol for 1 hour, repeated twice using different batches of absolute ethanol. Third, in the clearing stage, the dehydrated ovaries were cleared by immersion in xylene for 1.5 hours, followed by a second xylene bath for another 1.5 hours to remove residual ethanol and prepare the tissue for embedding. Fourth, in the embedding stage, the ovaries were placed in embedding cassettes and infiltrated with molten paraffin at 60°C. The paraffin were then allowed to harden and stored in a freezer for more or less one hour. Fifth, in the sectioning stage, the hardened ovarian tissues were removed from the cassettes and sectioned using a microtome at a thickness of 5 microns. The sections were floated on a water bath at 40°C to flatten, then mounted vertically onto glass slides and air-dried. Sixth, in the staining stage, hematoxylin and eosin (H&E) staining was performed through a series of steps: slides (preparations) were immersed in xylene I for 10 minutes, followed by xylene II for 5 minutes. They were then passed through absolute ethanol for 5 minutes, 96% ethanol for 30 seconds, and 50% ethanol for 30 seconds, followed by rinsing in running tap water for 5 minutes. Slides were then stained with Mayer's hematoxylin for 1–5 minutes and rinsed again in running tap water for 2–3 minutes. Subsequently, slides were immersed in eosin solution for 1–5 minutes, followed by dehydration through 75% ethanol for 5 seconds and absolute ethanol for 5 seconds, repeated three times with fresh ethanol. Finally, slides were cleared through xylene III for 5 minutes, xylene IV for 5 minutes, and xylene V for 10 minutes. Once dried, coverslips were mounted using deck glass for microscopic observation.

2.10. Observation of Ovarian Histological Preparations

Histological analysis of ovarian microanatomy from polycystic ovary syndrome (PCOS) model rats was performed under a Nikon E100 binocular microscope at 400× magnification (10×40). Photomicrographs were taken for documentation. Ovarian tissues from treated mice, which received Zingiber officinale var. rubrum extract at respective doses twice daily along with administration of testosterone propionate via intraperitoneal injection (100 mg/kg body weight/day), were similarly examined. To evaluate the therapeutic effect of Zingiber officinale var. rubrum extract, several histological parameters were assessed, including the number of primary follicles, secondary follicles, tertiary follicles,

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and Graafian (de Graaf) follicles, as well as the number of corpus lutea and the thickness of the theca cell layer. Quantitative analysis was conducted by counting each parameter in five randomly selected fields of view per histological slide.

2.11. Data Analysis

The number of primordial follicles, primary follicles, secondary follicles, tertiary follicles, Graafian follicles, corpus lutea, the thickness of the theca cell layer, and ovarian weight were analyzed using analysis of variance (ANOVA), followed by Duncan's multiple range test to determine statistically significant differences between groups at a significance level of $\rho < 0.05$. Statistical analysis was conducted using Jamovi software, version 2.4.

3. Results

In Silico Study of the Anti-Inflammatory Effects of Zingiber officinale var. rubrum in Preventing Polycystic Ovary Syndrome (PCOS)

Shogaol and gingerol were used to predict the binding affinity to the TNF-α target protein. To prepare the ligands control, 6-shogaol, and 6-gingerol for molecular docking, geometry optimization of their 3D structures was performed to minimize energy and obtain the most stable conformations. The primary output of molecular docking is the binding mode, which indicates the orientation and positioning of shogaol and gingerol within the active binding site of TNF-α. In the 3D visualization, shogaol and gingerol are displayed as stick models. Amino acid residues shared with the control are colored green, whereas the same (identical) residues with different types of interaction are colored red.

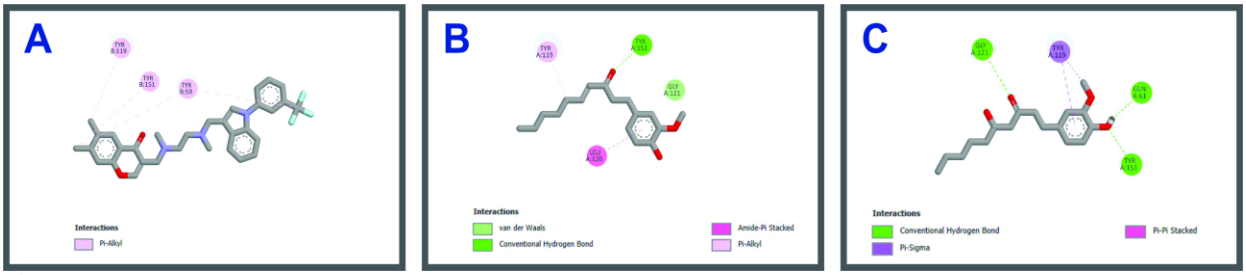

Figure 1.
Interactions TNF-α with control, shogaol, gingerol. **(A)** Interaction of TNF-α with control inhibitor 307, **(B)** Interaction of TNF-α with shogaol, **(C)** Interaction of TNF-α with gingerol.

The target protein structures for the ligands control, 6-shogaol, and 6-gingerol are displayed as ribbon models that highlight the coiled-coil motifs (Figure 2). Redocking procedures were performed to validate the reliability of the downloaded protein data bank (PDB) files, ensuring the accuracy of molecular docking results for shogaol and gingerol. The principal validation criterion for redocking was the Root Mean Square Deviation (RMSD) value, which compares the spatial similarity between the native ligand and the redocked ligand. An RMSD value < 2.0 Å is considered acceptable for TNF-α (Figure 2).

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Figure 2.
Interactions of TNF-α with ligands: control (red), 6-shogaol (green), and 6-gingerol (blue). (A) Interaction of TNF-α with ligands: control (red), (B) Interaction of TNF-α with ligands:, 6-shogaol (green), and (C) Interaction of TNF-α with ligands: 6-gingerol (blue).

In Figure 2, it can be seen that the ligands control, 6-shogaol, and 6-gingerol interact at positions close to the binding site of compound 307 with TNF-α, which serves as the inhibitor control. Based on the binding affinity values, 6-gingerol exhibits the highest binding affinity among the comparison ligands (tested liglands) with a score of –6.5 kcal/mol, compared to 6-shogaol, which has a binding affinity of –6.1 kcal/mol (Table 3). A more negative binding affinity indicates stronger ligand–protein interaction. Although the ligands from Zingiber officinale var. rubrum show relatively good binding affinities, their interaction strength is still lower than that of compound 307, which has a binding affinity of –8.9 kcal/mol.

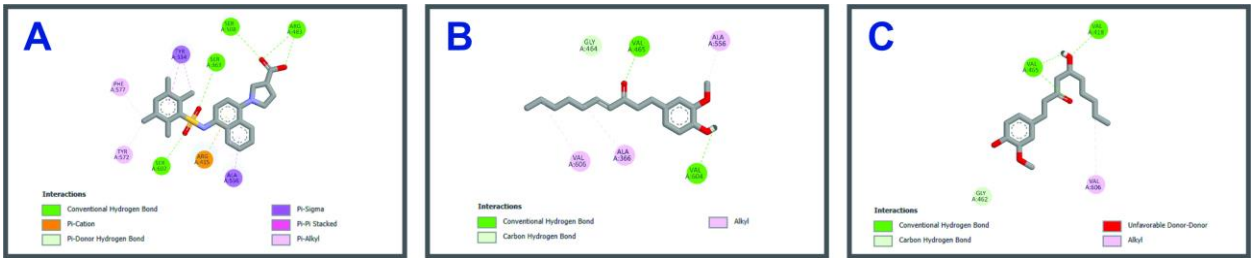

Figure 3.
Interactions of KEAP1 with control activator RA839, with shogaol, with gingerol (A) Interaction of KEAP1 with control activator RA839, (B) Interaction of KEAP1 with shogaol, and (C) Interaction of KEAP1 with gingerol.

The target protein structures for the ligands NF-κβ and KEAP1, are displayed as ribbon models that highlight the coiled-coil motifs. Redocking procedures were performed to validate the reliability of the downloaded protein data bank (PDB) files, ensuring the accuracy of molecular docking results for shogaol and gingerol. The principal validation criterion for redocking was the Root Mean Square Deviation (RMSD) value. For each protein, the RMSD between the native ligand and the redocked ligand was calculated, with an RMSD value < 2.0 Å considered acceptable for NF-κB and KEAP1.

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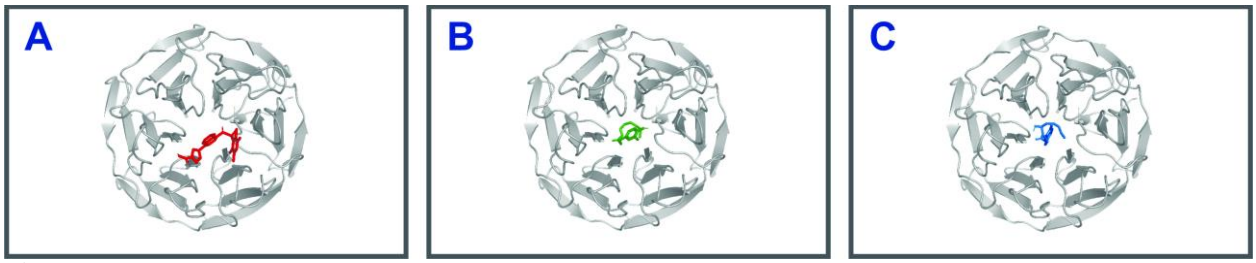

Figure 4.
Interactions of NF-κβ and KEAP1 with ligands: control (red), 6-shogaol (green), and 6-gingerol (blue). (A) Interaction of NF-κB and KEAP1 with ligands control (red), (B) Interaction of NF-κβ and KEAP1 with ligands 6-shogaol (green), and (C) Interaction of NF-κβ and KEAP1 with ligands: 6-gingerol (blue).

In Figure 4, it can be seen that the ligands—control, 6-shogaol, and 6-gingerol are able to interact at positions close to the binding site of compound 307 with NF-κβ and KEAP1, which serves as the inhibitor control. Based on the binding affinity values, 6-gingerol again exhibits the highest affinity among the comparison ligands (tested liglands) with a score of −6.5 kcal/mol, compared to 6-shogaol, which has a binding affinity of −6.1 kcal/mol. A more negative binding affinity indicates stronger ligand–protein interaction. While the interaction of gingerol is relatively favorable, it is still lower than that of compound 307, which has a binding affinity of −8.9 kcal/mol.

Table 1.
Binding energy of molecular interactions between TNF-α, NFkB, KEAP1 with control activator 6-shogaol and 6 gingerol.

Ligan	Binding	Hydrophobic	Hydrogen	Van der waals
TNF-α				
6-Shogaol	-6.1	TYR151	TYR119, LEU120	GLY121
6-Gingerol	-6.5	TYR119	TYR151, GLN61	
Control	-8.9	TYR119, TYR151	TYR151	
NFkB, KEAP1				
6-Shogaol	-6.2	VAL606, ALA366	VAL465, 418	GLY464
6-Gingerol	-6.5	VAL606, GLY62	VAL465, VAL604	
Control	-10.8	PHE577, TYR572	SER602, SER363	

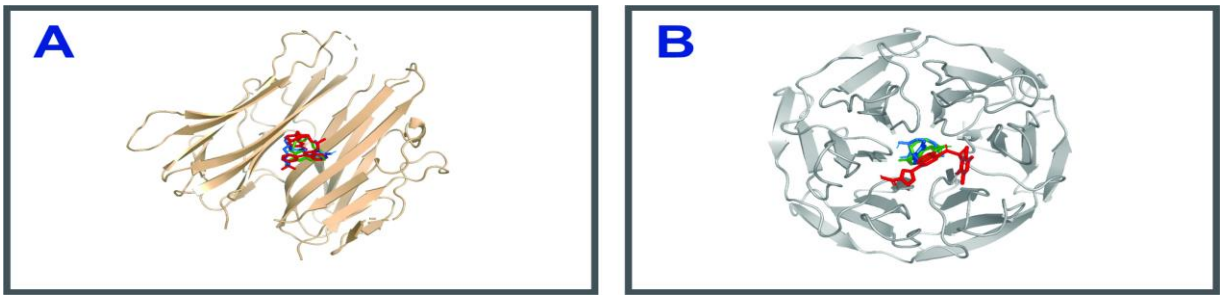

Figure 5.
Interaction of TNF-α, NF-κβ, and KEAP1 with ligands: control (red), 6-shogaol (green), and 6-gingerol (blue). (A) Interaksi TNF dengan ligand control activator (red), shogaol (green), gingerol (blue). Terlihat bahwa ligand jahe mampu berinteraksi pada posisi yang sama seperti interaksi control. (B) Interaksi KEAP1 dengan ligand control activator (red), shogaol (green), gingerol (blue). Meski tidak sama persis berada pada sisi pengikatan control dan KEAP1, namun ligand jahe mampu berada pada lokasi yang similar seperti interaksi KEAP1 dan control.

In Figure 5, the molecular interaction of TNF-α, NF-κβ, and KEAP1 shows that these proteins are able to bind at similar sites to the control, shogaol, and gingerol. Both gingerol and shogaol exhibit interactions with the same amino acid residues as compound 307. Specifically, gingerol interacts with

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TNF-α at TYR119 via hydrophobic interactions and at TYR151 via hydrogen interactions. Shogaol also interacts with TYR119 and TYR151, although the types of interactions differ from those of gingerol. Thus, all three ligands—control, shogaol, and gingerol—target the same amino acid residues (TYR119 and TYR151). Hydrogen interactions are critical for the binding affinity between ligand and protein, while hydrophobic interactions are critical for ligand–protein stability, preventing rapid degradation.

The effect of Zingiber officinale var. rubrum on expression levels of TNF-α, NF-κβ, and KEAP1 in testosterone propionate-induced rats

The activity of the pro-inflammatory cytokine TNF-α during ovarian folliculogenesis increased significantly following induction with testosterone propionate. However, treatment with Zingiber officinale var. rubrum at 800 mg/kg body weight (BW) significantly reduced TNF-α activity. These findings emphasize the protective effect of Zingiber officinale var. rubrum in the prevention of polycystic ovary syndrome (PCOS). Furthermore, expression levels of TNF-α, NF-κβ, and KEAP1 were significantly elevated in PCOS-induced rats compared to normal (healthy) controls. Administration of Zingiber officinale var. rubrum at 800 mg/kg BW markedly modulated expression levels of TNF-α, NF-κβ, and KEAP1 compared to the untreated PCOS group. These results suggest that Zingiber officinale var. rubrum exerts anti-inflammatory effects by downregulating pro-inflammatory cytokines.

Table 2.
Effect of Zingiber officinale var rubrum on the expression of TNF-α, NF-κβ and KEAP1 in PCOS rat model.

	Expression presentation (%)		
GROUP	**TNF-α (Mean ± SD)**	**NFKB (Mean ± SD)**	**KEAP1 (Mean ± SD)**
Health mice	4.54 ± 0.64^{a}	4.92 ± 0.47^{a}	3.66 ± 0.39^{a}
Untreated PCOS mice	5.62 ± 0.44^{a}	6.89 ± 1.30^{b}	6.22 ± 0.54^{b}
PCOS mice treated with zingiber officinale var rubrum 200 mg/kg BW	3.36 ± 0.16^{b}	3.44 ± 0.24^{b}	3.30 ± 0.10^{b}
PCOS mice treated with zingiber officinale var rubrum 400 mg/kg BW	3.26 ± 0.11^{c}	3.24 ± 0.12^{c}	3.02 ± 0.31^{c}
PCOS mice treated with zingiber officinale var rubrum 800 mg/kg BW	2.30 ± 0.20^{c}	2.62 ± 0.19^{c}	2.40 ± 0.12^{c}

3.1. TNF-α; Nuklir faktor kappa B; NF-κβ; KEAP1

^{ac} Huruf yang berbeda antara kelompok menunjukkan perbedaan yang signifikan antara kelompok ($p<0,05$)

TNF-α: Tumor necrosis factor-alpha; NF-κβ: Nuclear factor kappa B; KEAP1: Kelch-like ECH-associated protein 1

^{ac} Different superscript letters indicate statistically significant differences between groups ($p < 0.05$). Description (a) Rats (C-): negative controls, untreated rats; (b) Rats (C+): positive controls, rats injected with testosterone propionate; (c) Rats (T1): rats treated with red ginger extract 200 mg/kg BW followed by testosterone propionate; (d) Rats (T2): rats treated with red ginger extract 400 mg/kg BW followed by testosterone propionate; (e) Rats (T3): rats treated with red ginger extract 800 mg/kg BW followed by testosterone propionate.

The Effect of Gingerol-Shogaol on Testosterone Propionate-Induced Inflammation during Ovarian Folliculogenesis

The data show a significant increase in folliculogenesis-associated inflammation in the positive control group (testosterone propionate-induced) compared to the negative (normal) control group ($p < 0.05$). Administration of Zingiber officinale var. rubrum at 400 mg/kg and 800 mg/kg body weight (BW) significantly reduced the percentage of inflammation. These findings indicate that Zingiber officinale var. rubrum is effective in preventing inflammation during ovarian folliculogenesis induced by testosterone propionate.

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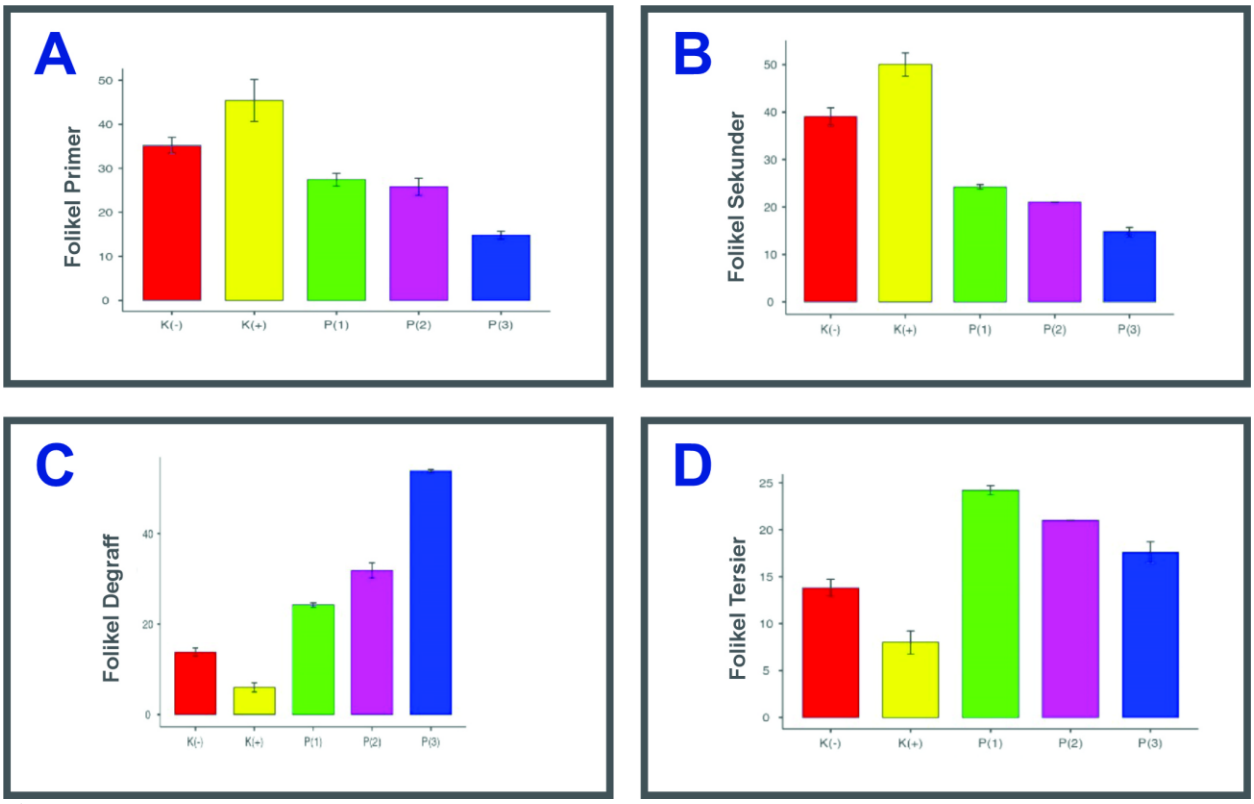

Figure 6.
Mean values of primary follicles, secondary follicles, tertiary follicles, and Graafian follicles in testosterone propionate-induced rats. Each experimental group consisted of five animals (rats). (A) Mean values of primary follicles approximately ten percent of the follicels degraft. (B) Mean values of secondary follicles approximately fifteen percent of the follicels degraft. (C) Mean values of Graafian follicles approximately fifty percent of the follicels degraft. (D) Mean values of tertiary follicles approximately twenty percent of the follicels degraft.

Table 3.
The effect of Zingiber officinale var. rubrum on Inflammation during Ovarian Folliculogenesis in testosterone propionate-induced rats.

	Mean ± SD number of folliculogenesis %			
GROUP	**Primary follicles**	**Secondary follicles**	**Tertiary follicles**	**Degraff follicles**
Health mice	35.2 ± 4.09^{A}	39.0 ± 4.18^{A}	13.8 ± 2.05^{A}	13.8 ± 2.05^{A}
Untreated PCOS mice	45.4 ± 10.7^{B}	50.0 ± 5.48^{B}	8.00 ± 2.74^{B}	6.00 ± 2.94^{B}
PCOS mice treated with zingiber officinale var rubrum 200 mg/kg BW	27.7 ± 3.29^{B}	24.2 ± 1.10^{B}	24.2 ± 1.10^{B}	24.2 ± 1.10^{B}
PCOS mice treated with zingiber officinale var rubrum 400 mg/kg BW	25.8 ± 4.38^{c}	21.0 ± 0.00^{c}	21.0 ± 0.00^{c}	31.8 ± 3.83^{c}
PCOS mice treated with zingiber officinale var rubrum 800 mg/kg BW	14.8 ± 2.05^{c}	14.8 ± 2.51^{c}	17.6 ± 2.51^{c}	53.8 ± 0.84

Source: Folikel primer, folikel sekunder, folikel tersier, folikel degraff acHuruf yang berbeda antar kelompok menunjukkan perbedaan yang signifikan antar kelompok ($p<0$).

Primary follicles, secondary follicles, tertiary follicles, graafian follicles.

[ac] Different superscript letters indicate statistically significant differences between groups ($p < 0.05$). Description (a) Rats (C-): negative controls, untreated rats; (b) Rats (C+): positive controls, rats injected with testosterone propionate; (c) Rats (T1): rats treated with red ginger extract 200 mg/kg BW followed by testosterone propionate; (d) Rats (T2): rats treated with red ginger extract

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400 mg/kg BW followed by testosterone propionate; (e) Rats (T3): rats treated with red ginger extract 800 mg/kg BW followed by testosterone propionate.

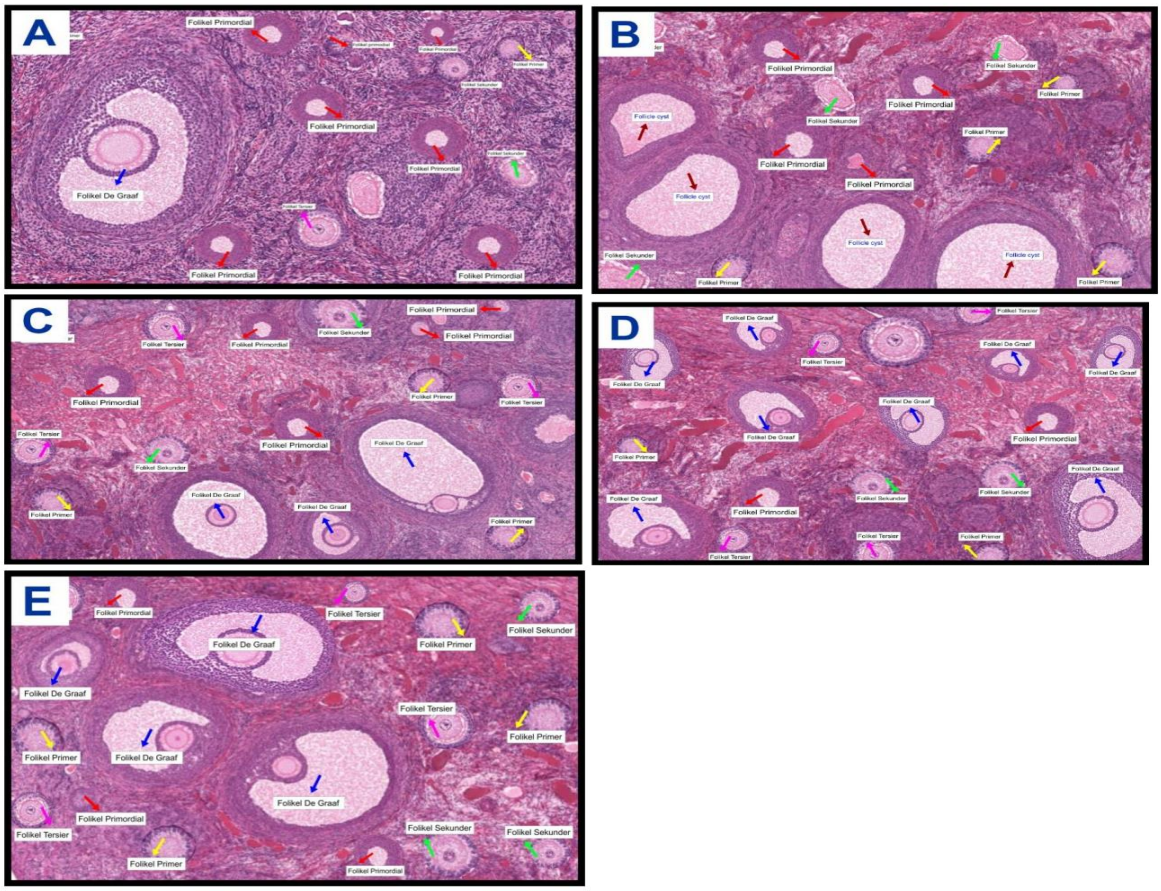

Figure 7.
Number of ovarian follicles across experimental groups: (A) number of follicles in both ovaries of the negative control group (normal rats); (B) number of follicles in both ovaries of the positive control group (rats injected with testosterone propionate); (C) number of follicles in both ovaries of rats treated with 200 mg/kg BW red ginger followed by testosterone propionate; (D) number of follicles in both ovaries of rats treated with 400 mg/kg BW red ginger followed by testosterone propionate; (E) number of follicles in both ovaries of rats treated with 800 mg/kg BW red ginger followed by testosterone propionate.

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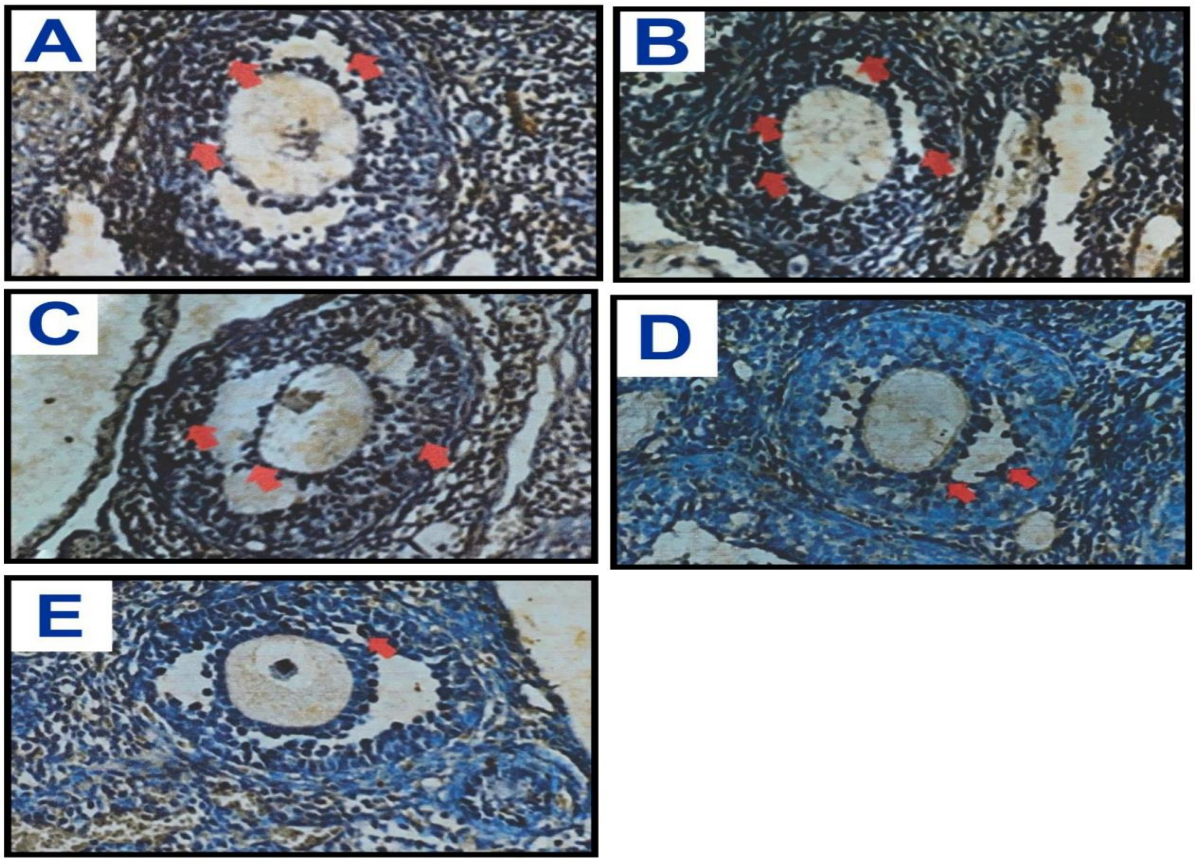

Figure 8.
TNF-α expression levels in ovarian follicles: (A) negative control group (normal rats); (B) positive control group (rats injected with testosterone propionate); (C) rats treated with 200 mg/kg BW red ginger followed by testosterone propionate; (D) rats treated with 400 mg/kg BW red ginger followed by testosterone propionate; (E) rats treated with 800 mg/kg BW red ginger followed by testosterone propionate. Each group consisted of five animals (rats).

4. Discussion

The protective effect of Zingiber officinale var. rubrum in reducing inflammation during ovarian folliculogenesis supports its potential role as part of a comprehensive strategy for preventing polycystic ovary syndrome (PCOS).

Zingiber officinale var. rubrum exerts anti-inflammatory effects, which were shown by a reduction in the expression levels of tumor necrosis factor-alpha (TNF-α) and Kelch-like ECH-associated protein 1 (KEAP1) in ovarian follicular cells following induction with testosterone propionate. The testosterone propionate-induced PCOS model remains a valuable tool in PCOS research, offering insight into the pathophysiology of the syndrome in animals and enabling the evaluation of potential therapeutic interventions [19]. A systematic review and meta-analysis of randomized controlled trials (RCTs) examining Zingiber officinale var. rubrum reported that oral consumption of the extract significantly reduced inflammatory biomarkers in serum, including TNF-α and KEAP1 [20].

Inflammatory cytokines such as TNF-α and KEAP1 may be modulated by shogaol, a bioactive compound in Zingiber officinale var. rubrum, which is known to interact with 113 protein targets, 88 of which are associated with PCOS. Among these, five key proteins have been identified as both targets of Zingiber officinale var. rubrum and as being closely associated with PCOS pathophysiology. These

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include BCL2 (B-cell lymphoma 2, involved in apoptosis), PPARG (Peroxisome proliferator-activated receptor gamma, involved in inflammatory regulation), LEP (Leptin, associated with body weight and metabolism), and YAP1 (Yes-associated protein 1, involved in apoptosis regulation). Additionally, therapeutic targets such as TNF-α, KEAP1, and insulin may also be influenced by Zingiber officinale var. rubrum. Data obtained from the Comparative Toxicogenomics Database (CTD) further support that gingerol and shogaol can downregulate TNF-α expression levels and modulate the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway—a key transcription factor that regulates genes involved in immune responses and inflammation, including TNF-α [21, 22].

The findings of this study indicate that oral administration of Zingiber officinale var. rubrum significantly reduced pro-inflammatory factors, specifically TNF-α and KEAP1, thereby protecting folliculogenesis from damage under conditions of insulin resistance. Chronic inflammation frequently leads to the activation of NF-κβ, which subsequently stimulates the production of inflammatory cytokines such as TNF-α, NF-κβ, and KEAP1—major contributors to the complications associated with insulin resistance, including impaired folliculogenesis [7]. The protective effects of Zingiber officinale var. rubrum are likely attributable to its anti-inflammatory and antioxidant properties [23]. Previous studies have shown that Zingiber officinale var. rubrum inhibits the production of inflammatory cytokines, including TNF-α and KEAP1, and possesses antioxidant activity that reduces oxidative stress [24].

This study further proves that Zingiber officinale var. rubrum exhibits anti-inflammatory effects on folliculogenesis under polycystic ovary syndrome (PCOS) conditions. The anti-inflammatory properties of Zingiber officinale var. rubrum are mediated, in part, through its bioactive compounds 6-gingerol and 6-shogaol, which act as key regulatory agents. Zingiber officinale var. rubrum supports cellular integrity essential for the survival of folliculogenesis in PCOS-prone conditions. Five protein targets of Zingiber officinale var. rubrum that are associated with PCOS have been identified: BCL2, PPARG, LEP, and YAP1 (21,22).

5. Conclusion

Zingiber officinale var. rubrum can prevent the onset of polycystic ovary syndrome (PCOS) induced by testosterone propionate through the modulatory effects of its active constituents, 6-shogaol and 6-gingerol. This is evidenced by a reduction in the expression levels of TNF-α and KEAP1. Furthermore, Zingiber officinale var. rubrum exhibits anti-inflammatory activity by downregulating the production of TNF-α and KEAP1 in ovarian follicular cells.

Institutional Review Board Statement:

This study protocol was reviewed and approved by the Animal Research Ethics Committee, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia (Approval Number: 212/EC/KEPK/FKUA/2024).

Transparency:

The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

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References

[1] D. Lizneva, L. Suturina, W. Walker, S. Brakta, L. Gavrilova-Jordan, and R. Azziz, "Criteria, prevalence, and phenotypes of polycystic ovary syndrome," *Fertility and Sterility*, vol. 106, no. 1, pp. 6-15, 2016.

[2] V. S. A. R. Velaga, N. Suryadevara, L. L. Chee, and N. E. Ismail, "Phytochemical analysis and immune-modulatory effect of Moringa oleifera flowers," *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 9, no. 6, p. 24, 2017.

[3] H. S. Taylor, L. Pal, and E. Sell, *Speroff's clinical gynecologic endocrinology and infertility*. Philadelphia, PA: Lippincott Williams & Wilkins, 2019.

[4] T. Zuo, M. Zhu, and W. Xu, "Roles of oxidative stress in polycystic ovary syndrome and cancers," *Oxidative Medicine and Cellular Longevity*, vol. 2016, no. 1, p. 8589318, 2016. https://doi.org/10.1155/2016/8589318

[5] M.-X. Wang, Q. Yin, and X. Xu, "A rat model of polycystic ovary syndrome with insulin resistance induced by letrozole combined with high fat diet," *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research*, vol. 26, pp. e922136-1, 2020. https://doi.org/10.12659/MSM.922136

[6] R. D. Supu, A. Diantini, and J. Levita, "Red ginger (Zingiber officinale var. rubrum): Its chemical constituents, pharmacological activities and safety," *Fitofarmaka Jurnal Ilmiah Farmasi*, vol. 8, no. 1, pp. 25-31, 2018.

[7] A. M. Diapeti, H. Merlita, and W. Doti, *The effect of the combination of zingiber officinale var. Rubrum and Imperata cylindrica decoction on serum TNF-α levels in osteoarthritis rats*. Fakultas Kedokteran: Universitas Malang, 2019.

[8] R. J. Riduan, "The effect of administering red ginger extract on the histopathological appearance of the pancreas induced by alloxan," *Majority*, vol. 4, no. 8, pp. 11-16, 2015.

[9] D. F. Rachman, "The effect of a combination of Zingiber officinale var rubrum rhizome decoction and Imperata cylindrica rhizome on serum superoxide dismutase (SOD) and serum malondialdehyde (MDA) levels in osteoarthritis rats," *Jurnal Bio Komplementer Medicine*, vol. 6, no. 3, 2019.

[10] I. Indrawati, M. Miranti, and I. a. R. Mayfi, "Antibacterial activity of ethanolic extracts of rhizome from three ginger varieties against acne isolated bacteria," *Nusantara Bioscience*, vol. 9, no. 1, pp. 92-96, 2017.

[11] O. Trott and A. J. Olson, "Auto dock vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading," *Journal of Computational Chemistry*, vol. 31, no. 2, pp. 455-461, 2010.

[12] A. F. Winkel *et al.*, "Characterization of RA839, a noncovalent small molecule binder to Keap1 and selective activator of NF-kB signaling," *Journal of Biological Chemistry*, vol. 290, no. 47, pp. 28446-28455, 2015.

[13] D. A. Filimonov *et al.*, "Prediction of the biological activity spectra of organic compounds using the pass online web resource," *Chemistry of Heterocyclic Compounds*, vol. 50, no. 3, pp. 444-457, 2014. https://doi.org/10.1007/s10593-014-1496-1

[14] S. DAVID, Brad T *et al.*, "A web server for functional enrichment analysis and functional annotation of gene lists (2021 update)," *Nucleic Acids Research*, vol. 50, no. W1, pp. W216-W221, 2022. https://doi.org/10.1093/nar/gkac194

[15] J. Piñero *et al.*, "The disgenet knowledge platform for disease genomics: 2019 update," *Nucleic Acids Research*, vol. 48, no. D1, pp. D845-D855, 2019. https://doi.org/10.1093/nar/gkz1021

[16] B. T. Sherman *et al.*, "DAVID: A web server for functional enrichment analysis and functional annotation of gene lists (2021 update)," *Nucleic Acids Research*, vol. 50, no. W1, pp. W216-W221, 2022. https://doi.org/10.1093/nar/gkac194

[17] M. Okamoto *et al.*, "Synthesis of a new [6]-gingerol analogue and its protective effect with respect to the development of metabolic syndrome in mice fed a high-fat diet," *Journal of Medicinal Chemistry*, vol. 54, no. 18, pp. 6295-6304, 2011.

[18] K.-K. Mak *et al.*, "Synthesis of new shogaol analogues as NF-kB activators and evaluation of their anti-inflammatory activity, modes of action and metabolic stability," *Antioxidants*, vol. 12, no. 2, p. 475, 2023. https://doi.org/10.3390/antiox12020475

[19] G. Russo *et al.*, *Oxidative stress and diseases*. London, UK: IntechOpen, 2012, pp. 757–772.

[20] M. Morvaridzadeh *et al.*, "Effect of ginger (Zingiber officinale) supplementation on oxidative stress parameters: A systematic review and meta-analysis," *Journal of Food Biochemistry*, vol. 45, no. 2, p. e13612, 2021.

[21] Y. Jia, X. Li, X. Meng, J. Lei, Y. Xia, and L. Yu, "Anticancer perspective of 6-shogaol: Anticancer properties, mechanism of action, synergism and delivery system," *Chinese Medicine*, vol. 18, no. 1, p. 138, 2023.

[22] Ç. Yücel *et al.*, "Immunomodulatory and anti-inflammatory therapeutic potential of gingerols and their nanoformulations," *Frontiers in Pharmacology*, vol. 13, p. 902551, 2022.

[23] D. Chakraborty, A. Mukherjee, S. Sikdar, A. Paul, S. Ghosh, and A. R. Khuda-Bukhsh, "[6]-Gingerol isolated from ginger attenuates sodium arsenite induced oxidative stress and plays a corrective role in improving insulin signaling in mice," *Toxicology Letters*, vol. 210, no. 1, pp. 34-43, 2012.

[24] N. Das *et al.*, "Antioxidant activities of ethanol extracts and fractions of crescentia cujete leaves and stem bark and the involvement of phenolic compounds," *BMC Complementary and Alternative Medicine*, vol. 14, no. 1, p. 45, 2014.

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