

Protective effects of red ginger extract (RG) on oxidative stress markers in a zebrafish model of gestational diabetes mellitus

Efek Protektif Ekstrak Jahe Merah terhadap Penanda Stres Oksidatif Pada Model Ikan Zebra dengan Diabetes Melitus Gestasional

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ABSTRACT

Background: Gestational diabetes mellitus (GDM) results from pregnancy-induced insulin resistance and increases oxidative stress, marked by decreased Superoxide Dismutase (SOD) and elevated Malondialdehyde (MDA). Current therapies, including Insulin and Metformin, have limitations during pregnancy. Red ginger extract (RG) (*Zingiber officinale* var. *rubrum*) contains antioxidant compounds and may reduce oxidative stress.

Objective: This study aimed to analyze the effect of red ginger extract (RG) on SOD and MDA levels in zebrafish larvae as hyperglycemia-induced oxidative stress relevant to GDM.

Methods: This study used a true experimental laboratory design with a post-test only control group approach conducted from January to April 2026 at the Pharmacology Laboratory. A total of 450 zebrafish embryos were allocated into five groups consisting of three biological replicates of embryos each (90 embryos/group): a control group, 3% glucose, and three treatment groups receiving red ginger extract (RG) at 5, 10, and 15 µg/mL. Hyperglycemia was induced from 2–72 hpf, and SOD and MDA levels were measured at 6 dpf using ELISA.

Results: The 3% glucose group exhibited reduced SOD and increased MDA levels compared with controls. Red ginger extract (RG) at all doses tended to increase SOD levels, although the differences were not statistically significant. MDA levels were significantly reduced in all treatment groups, with the most optimal effect observed at a dose of 10 µg/mL. SOD and MDA levels showed a significant negative correlation with moderate strength.

Conclusion: Red ginger extract (RG) may serve as a potential complementary antioxidant agent.

Keywords: gestational diabetes, malondialdehyde, superoxide dismutase, zebrafish, *zingiber officinale*

ABSTRAK

Latar Belakang: Diabetes melitus gestasional (GDM) disebabkan oleh resistensi insulin yang diinduksi kehamilan dan peningkatan stres oksidatif, yang ditandai dengan penurunan Superoksida Dismutase (SOD) dan peningkatan Malondialdehida (MDA). Terapi saat ini, termasuk Insulin dan Metformin, memiliki keterbatasan selama

kehamilan. Jahe merah (*Zingiber officinale* var. *rubrum*) mengandung senyawa antioksidan dan dapat mengurangi stres oksidatif.

Tujuan: Penelitian ini bertujuan untuk menganalisis efek ekstrak jahe merah pada kadar SOD dan MDA pada larva ikan zebra sebagai model GDM.

Metode: Penelitian ini menggunakan desain *true experimental laboratory dengan post-test only control group* yang dilakukan dari Januari hingga April 2026 di Laboratorium Farmakologi, dan menggunakan pengambilan *simple random sampling* ke dalam lima kelompok yang masing-masing terdiri dari tiga replikasi biologis embrio (90 embrio/kelompok): kelompok kontrol, glukosa 3%, dan tiga kelompok perlakuan yang menerima ekstrak jahe merah pada konsentrasi 5, 10, dan 15 µg/mL. Hiperglikemia diinduksi dari 2–72 hpf, dan kadar SOD serta MDA diukur pada 6 dpf menggunakan ELISA.

Hasil: Kelompok glukosa 3% menunjukkan penurunan kadar SOD dan peningkatan kadar MDA dibandingkan dengan kelompok kontrol. Ekstrak jahe merah pada semua dosis cenderung meningkatkan kadar SOD, meskipun perbedaannya tidak signifikan secara statistik. Kadar MDA menurun secara signifikan pada semua kelompok perlakuan, dengan efek paling optimal diamati pada dosis 10 µg/mL. SOD dan MDA berkorelasi negatif dengan kekuatan sedang.

Kesimpulan: Ekstrak jahe merah berpotensi menjadi agen antioksidan pelengkap.

Kata kunci: *diabetes gestasional, malondialdehida, superoksida dismutase, zebrafish, zingiber officinale*

INTRODUCTION

Gestational diabetes mellitus (GDM) is a metabolic disorder characterized by glucose intolerance that is first detected during pregnancy and is associated with increased insulin resistance and pancreatic β -cell dysfunction.¹ The prevalence of GDM in the world ranges from 7%-15% of all pregnancies annually.² This condition causes uncontrolled hyperglycemia and contributes to various maternal and fetal complications.³ Hyperglycemia in GDM is closely associated with increased oxidative stress due to excessive free radical production, which disrupts the balance of the body's antioxidant system.⁴ Oxidative stress plays a key role in the pathogenesis of GDM and contributes to cellular damage in both the mother and fetus. Hyperglycemia in GDM triggers increased production of Reactive Oxygen Species (ROS), resulting in an imbalance between free radicals and the antioxidant defense system.⁵ This condition results in damage to various cellular components, including lipids, proteins, and DNA.⁶ Excessive ROS triggers lipid peroxidation in cell membranes and produces malondialdehyde (MDA), a biomarker of oxidative damage.^{7,8} Increased ROS also decreases the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), thereby exacerbating oxidative stress.^{4,9} Increased MDA levels and decreased SOD activity indicate a significant redox imbalance, which contributes to cell damage and apoptosis.

Current treatments for GDM begin with lifestyle changes, including maintaining a healthy diet and physical activity patterns. Additionally, pharmacological therapy, including insulin injections, is available. It has been reported that lifestyle changes are the first-line treatment for GDM; however, if these are ineffective, insulin injections are the recommended pharmacological treatment option.¹⁰ Furthermore, medications such as metformin are effective in lowering glucose levels. However, their use during pregnancy has limitations, potentially causing adverse effects on the fetus due to their ability to cross the placenta and potentially cause long-term side effects.² Furthermore, metformin has been shown to prevent increased intestinal permeability but fails to

prevent increased oxidative stress associated with hyperglycemia.¹¹ Therefore, safer, antioxidant-rich alternative therapies are needed to address oxidative stress that conventional GDM therapies do not adequately address. Red ginger extract (RG), which contains active compounds such as gingerol and shogaol, possesses antioxidant activity that may serve as a potential alternative therapy to address oxidative stress in GDM.

Clinical research also shows that ginger administration can help lower blood glucose levels in patients with GDM.¹² Therefore, interventions that can effectively suppress oxidative stress are needed. One potential approach is the use of natural antioxidant compounds, such as red ginger extract (RG) (*Zingiber officinale* var. *rubrum*), which contains gingerols, shogaols, and flavonoids with strong antioxidant activity.^{13,14} In zebrafish, larval model of oxidative stress, ginger-derived compounds, including 6-gingerol, have been reported to increase SOD and CAT activities, reduce lipid peroxidation, and decrease ROS related cell death.¹⁵ Mechanistically, ginger bioactive compounds may enhance endogenous antioxidant defense through activation of the nuclear factor erythroid 2-related factor 2/ antioxidant response element (Nrf2/ARE) pathway, which promotes Nrf2 translocation into the nucleus and upregulates antioxidant and cytoprotective enzymes such as HO-1, NQO1, SOD, CAT, and Gpx.^{16,17} Through this mechanism, red ginger extract (RG) bioactive compounds may reduce ROS accumulation, suppress lipid peroxidation, decrease MDA formation, and improve redox balance under hyperglycemic conditions.

Experimental research requires animal models that can represent human metabolic conditions. Zebrafish (*Danio rerio*) are a widely used model due to their genetic and physiological similarities to humans and the possibility of directly observing embryonic development.^{18–20} The zebrafish GDM model can be induced by exposure of embryos to 3% glucose 2–72 hours post-fertilization (hpf), which mimics hyperglycemia and oxidative stress in humans.²¹ This condition causes decreased antioxidant enzyme activity and increased MDA levels.^{20,22} Although zebrafish larvae exposed to glucose do not fully replicate the complex maternal, placental, and hormonal features of human gestational diabetes mellitus, this model is useful for depicting the major consequences of gestational diabetes-induced hyperglycemia, particularly oxidative stress, impaired antioxidant defenses, and impaired development.²³ High glucose exposure during early embryogenesis induces a hyperglycemic environment that promotes ROS generation and lipid peroxidation, allowing for the evaluation of antioxidant interventions in a living vertebrate model²⁴. Therefore, in this study, the 3% glucose-induced zebrafish larval model was used as an *in vivo* model of hyperglycemia-induced oxidative stress relevant to gestational diabetes, rather than as a complete reproduction of the pathophysiology of human gestational diabetes.

SOD and MDA were selected as representative oxidative stress markers because they reflect two complementary aspects of redox imbalance. SOD represents endogenous enzymatic antioxidant defense against superoxide radicals, while MDA represents lipid peroxidation and oxidative damage to cell membranes.²⁵ Therefore, measuring both SOD and MDA allowed this study to evaluate the balance between antioxidant response and oxidative injury in glucose-exposed zebrafish larvae. Other oxidative stress parameters, such as CAT, GPx, GSH, and direct ROS levels, were not assessed because this study focused on two primary markers and was limited by the amount of biological material available from zebrafish larvae. Future studies should assess additional antioxidant and pro-oxidant markers to provide a more comprehensive mechanistic evaluation of redox homeostasis in this model. However, to our knowledge, few studies have investigated the simultaneous effect of red ginger extract (RG) on both

SOD and MDA levels using zebrafish larvae model of GDM induced by glucose exposure at 2-72 hpf. This gap underscores the need for an *in vivo* study that evaluates these oxidative stress parameters concurrently. It was found that ginger extract administration can significantly reduce MDA and increase SOD, GPx, and CAT activities.²⁶ However, the study used a mammalian model rather than zebrafish. In addition, it has also been reported that red ginger extract (RG) has been shown to have antioxidant, antihyperlipidemic, antihypertensive, and immunomodulatory activities, but this study was a literature review study and did not specifically focus on SOD, MDA parameters, and hyperglycemia-induced oxidative stress relevant to GDM.²⁷ Therefore, to date, there are still limited studies that specifically evaluate the effects of red ginger extract (RG) on SOD and MDA parameters simultaneously in hyperglycemia-induced oxidative stress relevant to GDM.

Based on the description above, this study aims to analyze the effect of red ginger extract (RG) on SOD and MDA levels in zebrafish larvae as a model of hyperglycemia-induced oxidative stress relevant to gestational diabetes mellitus. This study aims to provide scientific evidence on the modulation of SOD and MDA levels in a zebrafish GDM model, thereby contributing to the development of biomedical and obstetric sciences by increasing insight into the potential of red ginger extract (RG) in increasing antioxidant levels, reducing prooxidant levels, and improving physiological functions of the body in gestational diabetes mellitus. Unlike previous studies that evaluated individual oxidative stress biomarkers or used mammalian models, this study simultaneously evaluated SOD and MDA in a glucose-induced zebrafish model relevant to gestational diabetes, thereby providing preliminary evidence regarding the antioxidant activity of red ginger extract (RG).

METHODS

Study design

This study used a true experimental laboratory design with a post-test only control group approach conducted from January to April 2026 at the Pharmacology Laboratory.²⁸ The study consisted of five groups. (1) control group (embryonic medium) representing embryos developing under normal physiological conditions without any glucose or extract exposure. (2) 3% glucose, which embryos were induced with hyperglycemia but received non-therapeutic intervention, thereby validating the hyperglycemia-induced oxidative stress relevant to GDM. (3) The 3% glucose + RG 5 µg/mL, (4) 3% glucose + RG 10 µg/mL, and (5) 3% glucose + RG 15 µg/mL were treatment groups, each receiving simultaneous GDM induction and red ginger extract (RG) at three escalating dose levels.

Data source and sampling procedure

Sampling was conducted using a simple random sampling method. Zebrafish embryos aged 0-2 hpf were selected as experimental subjects based on the following inclusion criteria: transparent embryos, clear chorion, and no visible fungal contamination. Each group consisted of 30 embryos per replicate. This study employed three biological replicates per group, yielding a total of 90 embryos per group. For biochemical analysis, samples from each replicate were pooled into a single biological replicate. Therefore, the statistical analysis for biochemical parameters was performed using three biological replicate values per group ($n=3$), not individual embryo counts ($n=90$), to avoid pseudoreplication. The study consisted of five groups of 90 embryos each, the total number of embryos used in this study was 450. The sample size was justified based on zebrafish larvae and the amount of biological material required for ELISA measurements. Because individual zebrafish larvae provide limited tissue

material, 30 larvae were pooled to form one biological replicate. Three independent biological replicates were used within each group to allow for statistical comparisons between groups. This approach was chosen to obtain sufficient supernatant volume for SOD and MDA measurements while maintaining independent biological replicates, representing a robust and statistically sound experimental design for zebrafish developmental toxicity and pharmacological studies.^{29,30} Formal power analysis was not performed because this study followed commonly accepted experimental designs for pooled zebrafish biochemical assays. The sample size was determined based on biological replication and sufficient tissue requirements for ELISA analysis.

Variables of the study

The independent variable in this study was the dose of red ginger extract (RG) (*Zingiber officinale* var. *rubrum*) at concentrations of 5 µg/mL, 10 µg/mL, and 15 µg/mL. The dependent variables were superoxide dismutase (SOD) and malondialdehyde (MDA) levels measured in zebrafish larvae at 6 dpf.

Measurement and instruments

Superoxide dismutase (SOD) and malondialdehyde (MDA) levels in 6 dpf zebrafish larvae were measured using ELISA kits (SOD ELISA kit ELK8859 and MDA ELISA kit ELK10947). Each group consisted of three independent biological replicates, and each biological replicate was prepared by pooling 30 larvae. For ELISA measurement, each pooled biological sample was analyzed in technical triplicate. The technical triplicate values were averaged to obtain one value for each biological replicate. Therefore, statistical analysis was conducted using biological replicate values, not technical replicate readings. Samples were obtained by homogenizing larvae in lysis buffer on ice, then centrifuging at $\pm 13,000 \times g$ at 5 °C for 10 minutes to obtain the supernatant. All reagents, standards, and samples were prepared at room temperature and analyzed in triplicate. A standard curve was prepared through serial dilution within the appropriate concentration range of each kit. SOD examination was carried out through gradual incubation with biotinylated antibody and streptavidin-HRP, while MDA was examined using a biotinylated conjugate. After addition of Tetramethylbenzidine substrate and stop solution, absorbance was measured at 450 nm using a microplate reader, and concentrations of SOD and MDA were calculated based on standard curve (ng/mL).

Data collection

The test material used in this study was a 70% ethanolic crude extract of red ginger (RG) (*Zingiber officinale* var. *rubrum*). Red ginger (RG) simplicia was obtained from the UPT Laboratorium Herbal Materia Medica Batu, East Java, Indonesia. Based on the certificate of simplicia production and quality testing No. 400.7.29.2/4778/102.20/2025, the plant material was identified as red ginger (RG) (*Zingiber officinale* var. *rubrum* Theilade), batch No. 250528.JMH.L.MMB.001. The certified simplicia had a drying loss of 6.2% and a simplicia yield of 17.4%. The dried red ginger (RG) simplicia was powdered and then extracted at the laboratory by maceration using 70% ethanol as the solvent. A total of 100 gram of red ginger (RG) simplicia powder was macerated in 1,000 mL of 70% ethanol at room temperature for 24 hours. The filtrate was separated using a double-layer Whatman No. 1 filter paper and then concentrated using a rotary evaporator under reduced pressure at a temperature not exceeding 50°C to obtain a thick extract. The extract was then dried in an oven at a temperature of 55°C until a dry RG extract paste was obtained. Phytochemical profiling of the 70% ethanol extract of red ginger extract (RG) using LC-MS/MS detected 6-gingerol as one of the bioactive compounds present, with retention time of 12.33 and 21.52 min, molecular formula $C_{17}H_{26}O_4$, and a calculated molecular weight of approximately 294.18. This finding supports the presence of

gingerol-related bioactive compounds in the extract used in this study. However, quantitative standardization of gingerol and shogaol concentrations was not performed and is acknowledged as a limitation of this study. Although 6-gingerol was identified qualitatively, quantitative standardization of gingerol and shogaol contents was beyond the scope of this study and should be included in future studies to improve reproducibility.

Embryonic medium (EM) was prepared as a 10× stock solution consisting of 0.25 g CaCl_2 , 0.15 g KCl, 5 g NaCl, and 0.815 g MgSO_4 dissolved in 500 mL Water For Injection (WFI).³¹ Prior to use, the stock solution was diluted to a 1× working concentration with WFI. A stock solution of red ginger extract (RG) was prepared at a concentration of 1,000 µg/mL (1 mg/mL) using dimethyl sulfoxide (DMSO) as the solvent.³² Based on toxicity considerations, the final DMSO concentration in all treatment groups was maintained at 0.1%.³³ A 0.1% DMSO vehicle control group was omitted from the experimental design based on well-established literature demonstrating that DMSO concentrations up to 1% induce no increase in lethality or malformations in animal models. Including this redundant group would unnecessarily increase animal use. Therefore, in strict accordance with the 3Rs principles (Reduction and Refinement), this group was excluded to minimize the total number of animals sacrificed and to prevent unnecessary experimental distress, without compromising the statistical or scientific validity of the study.³³ The doses selected for this study were 5 µg/mL, 10 µg/mL, and 15 µg/mL, based on previously reported toxicity data of ginger extract in zebrafish, where the LC50 was reported to be 16.3 µg/mL.³⁴ Each treatment well contained a final volume of 8 mL, and the treatment medium was freshly prepared and replaced every 24 hours. The hyperglycemic induction medium was prepared by dissolving the 10× embryonic medium stock into a 3% glucose solution, which was then further diluted with 3% glucose at a ratio of 1:9 to yield a 1× working medium containing 3% glucose. This 3% glucose solution was prepared by mixing 300 mL of 5% dextrose solution with 200 mL of WFI to obtain 500 mL of the final solution.

A gestational diabetes mellitus (GDM) model was induced by exposing zebrafish embryos to medium containing 3% glucose from 2 hpf to 72 hpf. This hyperglycemic exposure was intended to serve as an *in vivo* model of hyperglycemia-induced oxidative stress relevant to gestational diabetes mellitus.^{18,31} Embryos in the red ginger extract (RG) treatment groups were simultaneously exposed to 3% glucose and the respective extract concentrations within the same time window. Embryos were maintained from 3 to 6 dpf in six well plates, with each well containing 8 mL of embryonic medium or treatment medium according to group allocation. Glucose exposure and red ginger extract (RG) treatment were administered from 2 to 72 hpf with the medium replaced every 24 hours. Following the 72 hpf treatment period, larvae were rinsed and transferred to fresh embryonic medium, then maintained until 6 dpf prior to sampling for biochemical analysis.

Ethical considerations

All experimental procedures were approved by the Ethics Committee of the Faculty of Medicine, Universitas Brawijaya, No. 18/EC/KEPK-S2/01/2026.

Data analysis

Data analysis was performed using IBM SPSS Version 27 with a 95% confidence level and a significance threshold of $p < 0.05$. Normality of data distribution was assessed using the Shapiro-Wilk test. Comparative statistical analyses were performed using one-way ANOVA or the Kruskal–Wallis test, as appropriate. For MDA levels, LSD post hoc analysis was used after a significant one-way ANOVA because the analysis was exploratory and aimed to identify mean differences between 3% glucose group and each

treatment group. For SOD levels, planned pairwise comparisons were performed using the Mann-Whitney U test with Bonferroni correction (adjusted $\alpha=0.0125$). Correlation analysis of SOD and MDA levels was carried out using the Spearman correlation test. Data are presented as mean $\pm$ standard deviation (SD). Experimental workflow is shown in Figure 1.

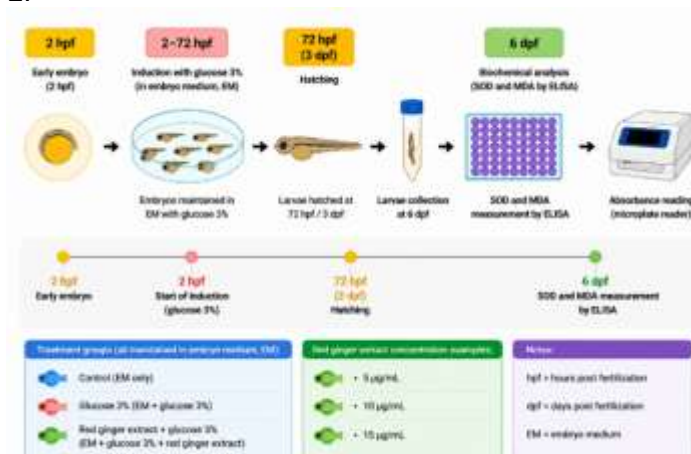


Figure 1. Experimental Workflow of Glucose Induction, RG Treatment, and Biochemical Analysis in Zebrafish Larvae.

Embryos were maintained in embryonic medium and exposed to 3% glucose from 2-72 hpf. Red ginger extract (RG) was administered at concentrations of 5, 10, and 15 $\mu\text{g/mL}$. At 6 dpf, larvae were collected for quantification of SOD and MDA using ELISA, with absorbance measured at 450 nm

RESULT

Effect of red ginger extract (RG) on SOD levels

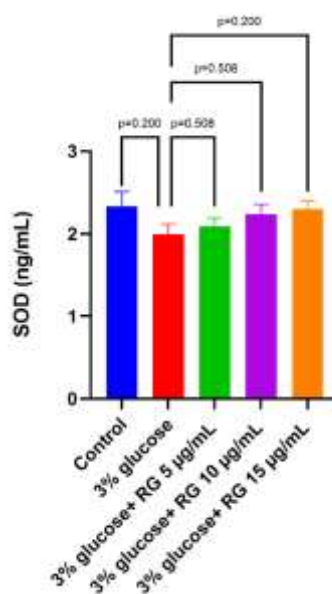


Figure 2. Average SOD (ng/mL) Levels Across Treatment Groups

Superoxide dismutase (SOD) levels were assessed to evaluate antioxidant activity in zebrafish larvae exposed to hyperglycemic conditions. SOD is an antioxidant enzyme

that catalyzes the dismutation of superoxide radicals (O_2^-) into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2). SOD is sensitive to any slight change in the redox state of the cell, and its activity declines under conditions of elevated cellular oxidative stress as the enzyme becomes depleted or overwhelmed by excessive free radical production.

The lowest average SOD levels were found in the 3% glucose group (1.99 ± 0.12) compared to the control group (2.33 ± 0.18). Meanwhile, the 3% glucose + RG 5 $\mu\text{g/mL}$ (2.09 ± 0.10), 3% glucose + RG 10 $\mu\text{g/mL}$ (2.24 ± 0.12), and 3% glucose + RG 15 $\mu\text{g/mL}$ (2.30 ± 0.10) groups showed a tendency to increase SOD levels compared to the 3% glucose group, with the highest value in the 3% glucose + RG 15 $\mu\text{g/mL}$ group (2.30 ± 0.10) approaching the control group value.

However, the Kruskal-Wallis test showed no statistically significant difference in SOD levels among groups ($H=8.600$; $df=4$; $p=0.072$). Planned pairwise comparisons between the 3% glucose group and all other groups were performed using the Mann-Whitney U test with Bonferroni correction (adjusted $\alpha=0.0125$). No significant differences were found: 3% glucose vs control ($p=0.200$), 3% glucose vs RG 5 $\mu\text{g/mL}$ ($p=0.508$), 3% glucose vs RG 10 $\mu\text{g/mL}$ ($p=0.508$), and 3% glucose vs RG 15 $\mu\text{g/mL}$ ($p=0.200$). The mean rank distribution supported this trend, with the highest mean rank observed in the control group (11.67), followed by 3% glucose + RG 15 $\mu\text{g/mL}$ group (11.00), 3% glucose + RG 10 $\mu\text{g/mL}$ group (9.00), 3% glucose + RG 5 $\mu\text{g/mL}$ group (5.67), and the 3% glucose group as the lowest (2.67).

This finding indicates that although red ginger extract (RG) tended to increase SOD levels in glucose exposed zebrafish larvae, the difference was not statistically significant. The differences in SOD levels between groups are shown in Figure 2.

Red ginger extract (RG) can reduce MDA levels

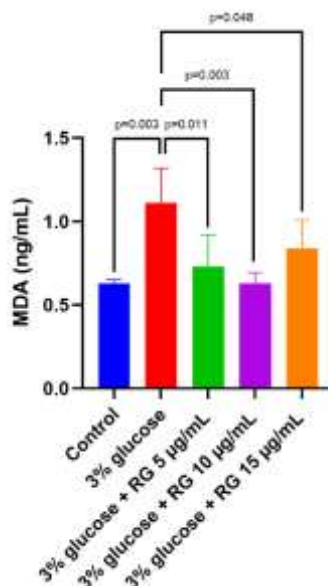


Figure 3. Mean MDA levels (ng/mL) across treatment groups (Data were analyzed based on three biological replicates per group, each biological replicate consisted of pooled samples from 30 larvae).

Malondialdehyde (MDA) levels are an indicator of pro-oxidant activity in zebrafish larvae exposed to hyperglycemic conditions. MDA is one of the final products of polyunsaturated fatty acid peroxidation in cells and commonly known as a marker of oxidative stress. The highest mean MDA level was observed in the 3% glucose group

(1.11 ± 0.21) compared to the control group (0.63 ± 0.02). Administration of red ginger extract (RG) to the treatment group showed a tendency to decrease MDA levels, 3% glucose + RG 5 $\mu\text{g/mL}$ (0.73 ± 0.19), 3% glucose + RG 10 $\mu\text{g/mL}$ (0.63 ± 0.06), and 3% glucose + RG 15 $\mu\text{g/mL}$ (0.84 ± 0.17), with the lowest MDA value among treatment groups was observed in the 3% glucose + RG 10 $\mu\text{g/mL}$ (0.63 ± 0.06).

The One-way ANOVA test showed a significant difference in MDA levels between groups ($F=5.387$; $p=0.014$). Post hoc analysis using the LSD method showed that MDA levels in the 3% glucose group were significantly different from all other groups. MDA levels in 3% glucose group were significantly higher than those in the control group ($p=0.003$), 3% glucose + RG 5 $\mu\text{g/mL}$ group ($p=0.011$), 3% glucose + RG 10 $\mu\text{g/mL}$ group ($p=0.003$), and 3% glucose + RG 15 $\mu\text{g/mL}$ group ($p=0.048$). Meanwhile, no significant differences were found in the other group pairs ($p>0.05$). These findings indicate that RG extract significantly reduced MDA levels in glucose exposed zebrafish larvae compared with the 3% glucose group. However, the effect was not dose dependent, as the lowest MDA level was found at 10 $\mu\text{g/mL}$ rather than 15 $\mu\text{g/mL}$. Therefore, 10 $\mu\text{g/mL}$ appeared to be the most effective concentration for reducing MDA levels in this study. The effect of RG extract on MDA levels is shown in Figure 3.

Negative correlation between SOD and MDA levels

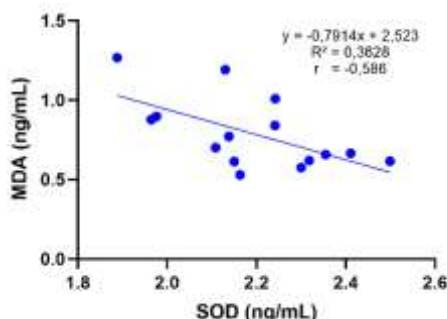


Figure 4. Q-Q Plot Between SOD and MDA levels. A Moderate Negative Correlation Was Found Between SOD and MDA Levels ($r = -0.586$; $p = 0.022$)

A correlation analysis was conducted to determine the relationship between SOD and MDA levels as parameters reflecting antioxidant activity and oxidative stress across all treatment groups. MDA serves as an oxidative radical marker, while SOD plays a role as an endogenous antioxidant, and their inverse relationship is expected under conditions where antioxidant defense is active. The Spearman correlation test indicated a significant negative relationship between SOD and MDA levels ($p=0.022$; $r=-0.586$), reflecting a moderate negative correlation between the two variables. The significant negative correlation between SOD and MDA levels indicates an inverse relationship between antioxidant defenses and lipid peroxidation in all treatment groups. This means that higher SOD levels tend to be associated with lower MDA levels. However, this correlation does not prove that red ginger extract (RG) directly causes a decrease in MDA through an increase in SOD. Therefore, this correlation should be interpreted as supporting evidence for changes in redox balance rather than as confirmation of the antioxidant mechanism of red ginger extract (RG). Further mechanistic studies measuring Nrf2/ARE, CAT, GPx, GSH, and ROS signaling are needed to confirm the pathways involved. The relationship between SOD and MDA levels among groups is shown in Figure 4.

DISCUSSION

To our knowledge, few studies have investigated the effects of red ginger extract (RG) on SOD and MDA levels simultaneously in a glucose-induced zebrafish larval model of hyperglycemia-induced oxidative stress related to GDM. Previous studies have shown that ginger has the potential to improve metabolic, inflammatory, and oxidative stress pathways associated with gestational diabetes, but red ginger extract (RG) has also been reported to have antioxidant and other biological activities.²⁶ However, there is limited evidence on the simultaneous evaluation of SOD and MDA after red ginger extract (RG) in glucose-exposed zebrafish larvae.³⁵ Therefore, the present study was conducted to provide preliminary *in vivo* evidence regarding the antioxidant effect of red ginger extract (RG) in this model. Our study showed that red ginger extract (RG) tended to increase SOD levels in the treatment groups compared to the 3% glucose group, although the Kruskal Wallis test showed no statistically significant difference among groups. This is consistent with findings reporting that a diet containing ginger did not produce a significant change in SOD activity, although improvements were observed in other parameters such as immunological and antioxidant markers.³⁶ It was reported that ginger supplementation did not show a significant effect on SOD activity, although other parameters such as MDA showed improvement.³⁷

These findings are broadly comparable with several previous studies. Luo et al. reported that ginger may improve gestational diabetes related pathways involving nutrient metabolism, oxidative stress, inflammation, and WNT/ β -catenin/GSK3 β signaling, supporting the biological relevance of ginger in GDM related mechanism.²⁶ Khotimah et al. showed that antioxidant intervention in high-glucose-induced zebrafish embryos improved oxidative stress-related outcomes, supporting the use of zebrafish as a glucose-induced oxidative stress model.³⁸ Ahmadifar et al. found that dietary ginger in zebrafish did not consistently increase SOD activity, which is in line with the nonsignificant SOD trend observed in the present study.³⁶ Matin et al. demonstrated that ginger supplementation in mice regulated antioxidant defense in a dose- and age-dependent manner, supporting the possibility of nonlinear antioxidant responses.³⁷ In contrast, clinical evidence from Hajimoosayi et al. demonstrated that ginger may reduce blood glucose in women with GDM, suggesting that ginger has translational potential, although clinical effects cannot be directly equated with biochemical changes in zebrafish larvae.³⁹

The more pronounced reduction in MDA compared with the change in SOD may indicate that red ginger extract (RG) acts through both non-enzymatic and enzymatic antioxidant mechanisms. This suggests that the antioxidant effect of ginger operates more dominantly through non-enzymatic pathways, whereby its antioxidant effect is detected faster through a decrease in MDA levels compared to an increase in SOD.⁴⁰ Furthermore, zingerone, a bioactive compound in ginger has demonstrated catalytic behavior similar to SOD enzymes, acting as a SOD mimic by scavenging superoxide radicals into hydrogen peroxide and molecular oxygen.⁴¹ The results of this study demonstrated that red ginger extract (RG) significantly reduced MDA levels in zebrafish larvae as a hyperglycemia-induced oxidative stress relevant to gestational diabetes mellitus. This finding is consistent with the mechanism by which hyperglycemia triggers an uncontrolled increase in ROS production.¹¹ This leads to an imbalance between oxidants and antioxidants characterized by increased MDA as a product of lipid peroxidation.^{42–44}

At the molecular level, the decrease in MDA levels can be attributed to the bioactive compounds 6-gingerol and 6-shogaol, which act as free radical scavengers against ROS. These compounds also activate the Nrf2/ARE (Nuclear factor erythroid 2-related factor 2 / Antioxidant Response Element) pathway which is the main regulator expression of antioxidant genes including SOD, GPx and CAT.^{13,14} The more pronounced decrease in MDA levels compared to the increase in SOD in our study reflects the faster kinetics of non-enzymatic antioxidants, where phenolic compounds directly inhibit lipid peroxidation at the cell membrane level before enzymatic compensatory mechanisms are fully activated.⁴⁰ The proposed mechanism of red ginger extract (RG) in reducing hyperglycemia-induced oxidative stress is illustrated in Figure 5.

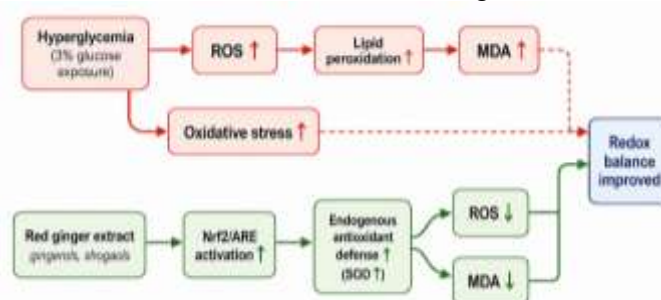


Figure 5. Mechanism of Red Ginger Extract (RG) In Reducing Hyperglycemia-Induced Oxidative Stress.

In addition to the Nrf2/ARE pathway, hyperglycemia-induced oxidative stress in GDM is also closely linked to inflammatory and metabolic stress pathways, including NF- κ B, AGE-RAGE, and PKC signaling. Persistent hyperglycemia promotes the formation of advanced glycation end products (AGEs), which interact with RAGE and increase ROS production, inflammatory cytokine release, and cellular oxidative injury. Hyperglycemia may also activate PKC through increased diacylglycerol formation, contributing to vascular dysfunction and further oxidative stress. ROS can, in turn, activate NF- κ B signaling, amplifying inflammation and oxidative damage.^{4,24} Bioactive compounds in red ginger extract (RG), particularly gingerols and shogaols, may help restore redox balance through direct radical scavenging, activation of Nrf2/ARE-mediated antioxidant responses, and suppression of inflammation-related oxidative pathways.^{16,45} However, these pathways were not directly measured in this study and should be confirmed in future mechanistic research.

MDA levels in the 3% glucose group were significantly higher than those in the control group, while no significant differences were found among the other groups. These results are in line with research reporting elevated MDA levels in prediabetic individuals compared with controls, indicating greater oxidative stress in the prediabetic condition.⁴⁶ In addition, it has also been reported that red ginger extract (RG) has the potential to lower MDA levels and increase activity antioxidants, which supports the role of these compounds in reducing lipid peroxidation and enhancing immune response.⁴⁷

In this study, both doses 5 μ g/mL and 10 μ g/mL showed a significant decrease in MDA levels compared to the 3% glucose group, indicating the potential of red ginger extract (RG) in reducing oxidative stress in hyperglycemia-induced oxidative stress relevant to GDM. Interestingly, the reduction in MDA levels did not follow a strictly linear, dose-dependent pattern. Although all treatment groups receiving red ginger extract (RG) showed lower MDA levels than the 3% glucose group, the lowest MDA level was observed at 10 μ g/mL, whereas the 15 μ g/mL dose did not produce a greater reduction

than 10 µg/mL. This result suggests that the antioxidant effect of red ginger extract (RG) has a threshold rather than a continuously increasing dose-response pattern. The weaker response at 15 µg/mL could reflect a biphasic or hormetic response, in which moderate concentrations provide the most effective antioxidant protection, while higher concentrations do not necessarily enhance the effect.⁴⁸

This nonlinear pattern could also be related to the saturation of antioxidant pathways, reduced cellular tolerance, or possible prooxidative effects of phenolic compounds at higher concentrations.³⁷ Therefore, the dose of 10 µg/mL could represent the most effective concentration for reducing lipid peroxidation in this zebrafish model. The 10 µg/mL dose appeared to be the most effective concentration because it produced the lowest MDA level among the red ginger extract (RG) treatment groups. However, this finding should not be interpreted as a strictly dose-dependent effect, because the 15 µg/mL dose did not produce a greater reduction than 10 µg/mL.¹³ In addition, it was also reported that higher doses of ginger extract can reduce oxidative stress parameters such as MDA which are increased in gestational diabetes mellitus.⁴³ This interpretation is supported by the hormesis concept, which explains that natural bioactive antioxidants may exert biphasic or apparent non dose dependent effects, where higher concentrations are not always associated with stronger biological protection.⁴⁸

These correlation findings reinforce the redox balance interpretation discussed above and further support the potential clinical relevance of red ginger extract (RG) supplementation in mitigating hyperglycemia-induced oxidative stress^{25,49} as shown in Figure 4. The findings of this study have implications for obstetric practice by strengthening evidence-based education on natural antioxidant consumption in pregnant women at risk for gestational diabetes mellitus. Furthermore, the optimal dose of 10 µg/mL identified in the zebrafish model requires further investigation in mammalian models to determine an equivalent therapeutic dose for human clinical settings.

This study has several limitations. First, blood glucose levels were not measured to confirm hyperglycemia. Second, histopathological examination was not performed, so tissue level damage could not be directly evaluated. Third, only SOD and MDA were evaluated without additional oxidative stress markers such as CAT, GPx, GSH, ROS, and Nrf2 signaling. Fourth, quantitative standardization of gingerol and shogaol was not performed. Fifth, pooled larval samples may reduce individual variability and statistical power, and a separate 0.1% DMSO vehicle control was not included. Therefore, further studies using standardized extracts, vehicle controls, blood glucose confirmation, histopathological assessment, broader oxidative stress markers, mammalian models, and clinical validation are needed before translation to human GDM.

CONCLUSION

Red ginger extract (RG) (*Zingiber officinale* var. *rubrum*) significantly reduced malondialdehyde (MDA) levels in zebrafish larvae (*Danio rerio*) as hyperglycemia-induced oxidative stress relevant to GDM, with a significant moderate negative correlation between SOD and MDA, indicating improved oxidative stress status. These findings demonstrate the extract as a potential complementary antioxidant agent for reducing hyperglycemia-associated oxidative stress. The findings of this study have implications for midwifery practice through strengthening evidence-based education regarding the consumption of natural antioxidants in pregnant women at risk of gestational diabetes mellitus. Further studies are recommended to investigate safety profile and mechanism of action of red ginger extract (RG) in mammalian models and clinical settings before its application in human subjects can be established.

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