The Effect of Curcumin on Reactive Oxygen Species (ROS) Levels in the Hearts of Wistar Rats (Rattus norvegicus) as a Preeclampsia Model

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Abstract

Reactive Oxygen Species (ROS) play a pivotal role in the pathogenesis of preeclampsia through mechanisms of oxidative stress that impair endothelial function and contribute to multiorgan injury, including cardiac tissue. Curcumin, a polyphenolic compound derived from Curcuma longa, possesses strong antioxidant and anti-inflammatory properties that may reduce ROS production and protect maternal organs during preeclampsia. This study investigated the effect of curcumin on cardiac ROS levels in a Wistar rat model of preeclampsia induced by L-NAME. This true experimental study used a post-test only control group design involving 25 pregnant Wistar rats randomly assigned to five groups (n = 5/group): negative control, positive control (L-NAME 125 mg/kgBW), and three treatment groups receiving curcumin at doses of 30, 50, and 100 mg/kgBW. Curcumin was administered orally from gestational day 13 to 19. Cardiac ROS levels were measured using a fluorometric flow cytometry assay. Data were analyzed using one-way ANOVA followed by Tukey post-hoc test, and dose–response correlation was evaluated using Pearson analysis. L-NAME induction significantly increased cardiac ROS levels compared to the negative control ($p < 0.05$). Curcumin administration reduced ROS levels in a dose-dependent manner. The highest dose (100 mg/kgBW) resulted in ROS levels that were not significantly different from the negative control, indicating restoration of near-physiological oxidative status. A strong negative correlation was found between curcumin dose and ROS levels ($r = –0.831$; $p < 0.001$). Curcumin effectively reduces cardiac ROS levels in a dose-dependent manner in a preeclampsia rat model, with the highest dose demonstrating substantial antioxidant protection. These findings highlight the potential of curcumin as an adjunctive therapy for mitigating oxidative stress–mediated cardiac injury in preeclampsia.

Keywords: Curcumin, Reactive Oxygen Species, Preeclampsia, Oxidative Stress, Antioxidant

INTRODUCTION

Preeclampsia is a pregnancy-specific clinical syndrome that develops after 20 weeks of gestation and is characterized by elevated blood pressure (≥140/90 mmHg), which may be accompanied by proteinuria and/or dysfunction of vital organs, including the kidneys, liver, hematologic system, and central nervous system (Mohseni et al., 2021). This condition represents a multisystem disorder originating from impaired trophoblast invasion into the maternal spiral arteries, leading to inadequate placental perfusion and chronic placental hypoxia (Phanaka et al., 2020). Placental hypoxia subsequently stimulates the release of inflammatory and antiangiogenic factors into the maternal circulation, triggering systemic

oxidative stress, endothelial dysfunction, and immune activation. These pathological processes ultimately result in damage to multiple maternal target organs, including the kidneys, brain, liver, and heart (Chiarello et al., 2020). Clinically, preeclampsia is classified into early-onset (before 34 weeks of gestation) and late-onset (after 34 weeks), with early-onset preeclampsia generally associated with more severe maternal and fetal outcomes (Mohseni et al., 2021).

Globally, preeclampsia remains a major contributor to maternal and perinatal mortality. The World Health Organization estimates that preeclampsia is responsible for approximately 70,000 maternal deaths and 500,000 neonatal deaths annually. The prevalence ranges from 1.3% to 6% in developed countries and may reach 8%–18% in developing regions. In Indonesia, the prevalence is reported to be approximately 5.3%, accounting for nearly 128,000 cases per year and contributing to 33% of maternal deaths. National health data further indicate that hypertensive disorders of pregnancy were the leading cause of maternal mortality in 2023, with 412 recorded cases (Rahmatullah et al., 2024; Rohaeni & Simanjuntak, 2024).

Increasing evidence indicates that preeclampsia is closely linked to impaired cardiovascular adaptation during pregnancy. Endothelial dysfunction, a hallmark of preeclampsia, disrupts the balance between vasodilatory and vasoconstrictive mediators, resulting in systemic vasoconstriction, elevated blood pressure, and reduced uteroplacental blood flow. Importantly, endothelial injury also affects maternal cardiac structure and function, thereby increasing the risk of cardiovascular complications during pregnancy and elevating long-term cardiovascular risk in the postpartum period (Chiarello et al., 2020). These findings highlight the heart as a critical target organ in the pathophysiology of preeclampsia.

Oxidative stress, particularly excessive production of Reactive Oxygen Species (ROS), plays a central role in the development and progression of preeclampsia. Under physiological conditions, ROS are involved in normal cellular processes such as signal transduction, regulation of cell proliferation, and immune defense. However, in preeclampsia, placental hypoxia and systemic inflammation lead to excessive ROS generation that exceeds the capacity of endogenous antioxidant defense systems, resulting in pathological oxidative stress (Wu et al., 2015). This imbalance induces lipid peroxidation, protein oxidation, DNA damage, and endothelial dysfunction, thereby exacerbating vascular injury and organ damage. Moreover, elevated ROS levels activate proinflammatory signaling pathways, including nuclear factor kappa B (NF-κB), which enhances the production of proinflammatory cytokines and further amplifies oxidative and inflammatory injury in maternal tissues, including the heart (Zhang et al., 2016; Chiarello et al., 2020).

Given the pivotal role of ROS in mediating cardiovascular injury in preeclampsia, therapeutic strategies targeting oxidative stress have gained increasing attention. Curcumin, a natural polyphenolic compound derived from the rhizome of Curcuma longa (turmeric), is widely recognized for its potent antioxidant and anti-inflammatory properties. Curcumin exerts its antioxidant effects by directly scavenging ROS, inhibiting lipid peroxidation, and upregulating endogenous antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) (Rizky et al., 2022; Sarma et al., 2022) In addition, curcumin suppresses inflammatory signaling pathways, including NF-κB and p38 mitogen-activated protein kinase (MAPK), which are closely associated with oxidative stress–induced tissue injury. Due to its natural origin, low toxicity, wide availability, and demonstrated efficacy in various inflammatory and degenerative disease models, curcumin has emerged as a promising adjuvant candidate for managing pregnancy-related disorders characterized by oxidative stress, including preeclampsia.

METHODS

This true experimental study used a post-test only control group design to evaluate the effect of curcumin on cardiac reactive oxygen species (ROS) levels in a preeclampsia rat model. The study was conducted at the Physiology Laboratory, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia, during a 1–2 month. The inclusion criteria were pregnant female Wistar rats (Rattus norvegicus) aged 10–12 weeks, weighing 150–200 g, with no prior exposure to experimental treatments or chemical agents. Only animals in healthy condition, characterized by normal activity, intact fur, and baseline systolic blood pressure between 90 and 120 mmHg prior to intervention, were included in the study. The exclusion criteria comprised animals that died before treatment initiation, exhibited hypertension prior to intervention, failed to show signs of pregnancy after mating, presented with physical abnormalities or congenital defects that could affect data validity, or had body weights outside the predefined range (<150 g or >300 g, according to protocol). Additionally, rats that showed no physiological response following L-NAME administration were excluded from the analysis.

Total twenty-five pregnant Wistar rats were housed under controlled temperature and a 12-hour light/dark cycle with ad libitum access to food and water. The animals were randomly assigned into five groups (n = 5/group): a healthy negative control group, a positive control group induced with L-NAME, and three treatment groups receiving curcumin at doses of 30, 50, and 100 mg/kgBW. Preeclampsia was induced using NG-nitro-L-arginine methyl ester (L-NAME) at 125 mg/kgBW/day, administered orally via intragastric gavage from gestational day

13 to 19. Curcumin (C0434, TCI, Japan) was prepared as a suspension in 1% carboxymethylcellulose and administered orally twice daily at the designated doses during the same gestational period.

On gestational day 20, all rats were anesthetized with ketamine–xylazine, euthanized, and cardiac tissue was immediately collected. The left ventricle was rinsed with cold phosphate-buffered saline, homogenized, and processed for ROS analysis. Cardiac ROS levels were quantified using a fluorometric flow cytometry assay with 2',7'-dichlorofluorescein diacetate (DCFH-DA) as the fluorescent probe, and results were expressed as mean fluorescence intensity (MFI). Data were evaluated for normality and homogeneity using Shapiro–Wilk and Levene's tests, followed by one-way ANOVA with Tukey's post-hoc test for group comparisons. Pearson correlation analysis was conducted to examine the dose–response relationship between curcumin administration and ROS levels. Statistical significance was defined as $p < 0.05$. All experimental procedures adhered to institutional animal care guidelines and were approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Brawijaya (Approval No. 103/EC/KEPK-S2/04/2025).

RESULTS

L-NAME induction significantly increased cardiac ROS levels compared with the negative control group ($p < 0.05$), confirming successful establishment of the preeclampsia model. Administration of curcumin resulted in a dose-dependent reduction in ROS levels. The 30 mg/kgBW dose produced a moderate decrease compared with the positive control, whereas the 50 mg/kgBW dose showed a more pronounced reduction ($p < 0.05$). The highest dose of curcumin (100 mg/kgBW) demonstrated the strongest antioxidant effect, yielding ROS levels that were not significantly different from the negative control group, indicating near-restoration of physiological oxidative balance.

Table 1. Cardiac ROS levels among study groups (Mean ± SD)

Group	Shapiro Wilk p-value	Distribution	Levene's Test p-value
Negative Control	0.227	Normal	
Positive Control	0.073	Normal	
Treatment 1	0.297	Normal	0.053*
Treatment 2	0.175	Normal	
Treatment 3	0.057	Normal	

The ANOVA test revealed significant differences among groups ($p < 0.05$), and Tukey's post-hoc analysis confirmed that all curcumin-treated groups differed significantly from the positive control, whereas only the 100 mg/kgBW group approached the negative control level. Pearson correlation analysis demonstrated a strong negative relationship between

curcumin dosage and ROS levels ($r = –0.831$, $p < 0.001$), indicating that increased curcumin dosage was associated with progressively lower ROS concentrations.

Table 2. Post-hoc Tukey analysis of differences in cardiac ROS levels among groups (Mean ± SD)

Groups	n	Mean ± SD	p-value one way anova
Negative Control	5	2.89 ± 0.52^{a}	
Positive Control	5	50.9 ± 9.51^{d}	
Treatment 1	5	35.5 ± 12.0^{c}	0.000
Treatment 2	5	5.25 ± 1.97^{b}	
Treatment 3	5	2.43 ± 0.39^{a}	

Table 3. Pearson corelation between curcumin dosage and cardiac ROS Levels

Variable	Correlation	p-value
ROS levels with dose groups	-0.831	0.000

The histogram illustrates a clear dose-dependent decline in cardiac ROS levels following curcumin administration. The positive control group shows the highest fluorescence intensity, while the negative control exhibits the lowest. Curcumin at 30 and 50 mg/kgBW produces a gradual reduction in ROS, and the 100 mg/kgBW group demonstrates a marked decrease, visually approaching the negative control. The graphical pattern supports the statistical findings, confirming the progressive antioxidant effect of curcumin in this preeclampsia model.

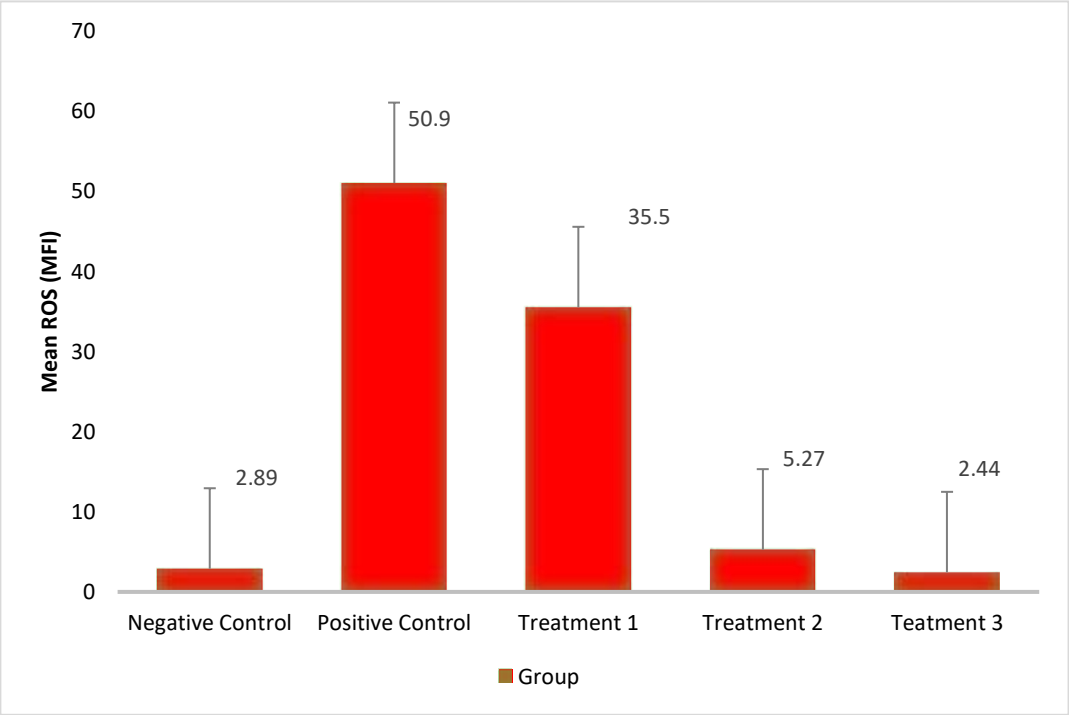

Figure 1. Histogram of cardiac ROS levels across all study groups

*Description : The histogram shows a dose-dependent reduction in ROS levels following curcumin administration, with the highest dose approaching the negative control.

DISCUSSION

This study demonstrated that curcumin effectively reduced cardiac reactive oxygen species (ROS) levels in pregnant rats induced with L-NAME as a model of preeclampsia. The findings showed that L-NAME administration markedly increased cardiac ROS, confirming the establishment of oxidative stress. Curcumin treatment attenuated this increase in a dose-dependent manner, with the highest dose restoring ROS levels close to those of the negative control. These results indicate that curcumin has a strong antioxidant effect capable of counteracting oxidative injury in cardiac tissue during preeclampsia. (Juan-reyes et al., 2020).

The significant elevation of ROS in the positive control group supports existing evidence that inhibition of nitric oxide synthase (NOS) by L-NAME leads to endothelial dysfunction, reduced nitric oxide (NO) bioavailability, and increased oxidative stress. Elevated ROS contributes to lipid peroxidation, protein oxidation, and cellular damage in maternal organs, including the heart. Cardiac tissue is especially vulnerable to oxidative injury due to its high mitochondrial density and constant metabolic demand. This vulnerability explains the marked increase in ROS observed in the L-NAME group (Conti-ramsden et al., 2019; Sanchez et al., 2020; Wollert & Kempf, 2012; Romus, 2022).

Curcumin's capacity to reduce cardiac ROS aligns with its known biochemical mechanisms. Curcumin acts as a direct scavenger of free radicals, inhibits ROS-producing enzymes such as NADPH oxidase, and enhances endogenous antioxidant systems. Furthermore, curcumin activates the Nrf2–Keap1 signaling pathway, which promotes the expression of antioxidant response elements (ARE) and strengthens cellular defenses against oxidative stress. These mechanisms collectively support the dose-dependent decline in ROS observed in this study (Susilawati, 2020; Kehrer, 2000). However, ROS not only damage cellular structures but also induce the expression of signaling molecules that promote progressive injury in organs such as the heart.

The reduction of ROS, particularly at the 100 mg/kgBW dose, suggests that curcumin may mitigate oxidative cardiac injury associated with preeclampsia. Previous studies have demonstrated curcumin's ability to enhance endogenous antioxidant enzyme expression, including SOD, GPx, and CAT, through activation of the Nrf2 signaling pathway, thereby significantly reducing intracellular ROS levels (Rapti et al., 2024; Yang et al., 2021; Gorabi et al., 2020).

The strong negative correlation between curcumin dosage and ROS levels further reinforces the dose–response relationship. This trend indicates that higher doses of curcumin produce more pronounced antioxidant effects, which is consistent with pharmacological studies

suggesting that curcumin's bioactivity increases with concentration, despite its limited systemic bioavailability. The ability of the highest dose to nearly normalize ROS levels highlights its potential as an adjunctive therapy.

This study has several strengths, including the use of a well-established preeclampsia model, standardized curcumin dosing, and objective ROS measurement via fluorometric flow cytometry. However, some limitations should be acknowledged. First, this study assessed ROS levels only at a single time point, which may not fully capture temporal changes in oxidative stress. Second, curcumin's bioavailability was not measured; therefore, its pharmacokinetic profile in this model remains unclear. Third, this study focused solely on cardiac tissue, while preeclampsia affects multiple organ systems that could also benefit from curcumin's antioxidant properties (Hafer et al., 2018; Su et al., 2019).

Future research should explore the molecular pathways underlying curcumin's cardioprotective effects, such as NOX4 inhibition, mitochondrial regulation, and anti-fibrotic signaling. Studies evaluating histopathological changes in cardiac tissue, systemic oxidative markers, and functional cardiovascular parameters would further clarify curcumin's therapeutic potential. Additionally, evaluating curcumin nanoformulations may help overcome bioavailability limitations and enhance clinical applicability. Overall, the findings of this study support curcumin as a promising antioxidant agent capable of reducing cardiac oxidative stress in a preeclampsia model. Its dose-dependent effect highlights its potential role as an adjunctive therapy to prevent maternal organ damage associated with oxidative imbalance.

CONCLUSIONS

This study demonstrates that curcumin effectively reduces cardiac reactive oxygen species (ROS) levels in an L-NAME–induced preeclampsia rat model. Curcumin exhibits a clear dose-dependent antioxidant effect, with the highest dose restoring ROS levels close to physiological conditions. These findings highlight curcumin's potential as an adjunctive therapeutic agent for mitigating oxidative cardiac injury associated with preeclampsia. Further studies are needed to explore its molecular mechanisms, evaluate its effects on other maternal organs, and improve its bioavailability for potential clinical application. Additionally, given the multifactorial pathophysiology of preeclampsia, further investigations should explore the potential synergistic effects of curcumin when combined with standard antihypertensive agents or antioxidant therapies.

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