ORIGINAL RESEARCH

Curcumin reduces inflammation process in mice model preeclampsia

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ABSTRACT

Objective: Preeclampsia is one of the most frequently documented pregnancy complications, with a prevalence of approximately 2 to 15% of all pregnancies. Preeclampsia is a leading cause of maternal mortality. According to research in Indonesia, preeclampsia accounts for 66.8% of all cases of hypertension during pregnancy. This research aims to evaluate the impact of curcumin on serum levels of COX-2 and iNOS in a mouse model of preeclampsia.

Materials and Methods: This study employed a true experimental design with a post-test-only controlled group approach using pregnant *Rattus norvegicus* as a preeclampsia model. Curcumin was administered orally via a feeding tube after dissolving powdered tablets. Dosages were 30 mg/day, 50 mg/day, or 100 mg/day, adjusted for the rats' weight. Serum COX-2 and iNOS levels were measured using ELISA kits from Bioassay Technology Laboratory, with concentrations reported in pg/ml. Analysis was performed using SPSS for Windows 19.0.

Results: Serum COX-2 levels showed significant differences ($p < 0.05$) across groups. L-NAME treatment increased COX-2 levels compared to the negative control. Curcumin (50 and 100 mg/kgBW) reduced COX-2 levels significantly compared to the positive control, with no notable differences between curcumin doses. For iNOS levels, significant differences were also found ($p < 0.05$). Curcumin at 100 mg/kgBW significantly lowered iNOS levels compared to the positive control, with no significant differences between other treatment groups.

Conclusion: Curcumin administration effectively reduces COX-2 and iNOS levels in the serum of *Rattus norvegicus* with a preeclampsia model.

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Highlights:

1. Increased oxidative stress results in increased iNOS in pre-eclampsia.
2. COX-2 expression in the placenta of women suffering from preeclampsia increases and is associated with increased thromboxane production.

INTRODUCTION

Preeclampsia and eclampsia lead to over 50,000 maternal fatalities annually. Hypertensive disorders, including preeclampsia, show ethnic and racial disparities in incidence, particularly higher among African-American and Hispanic populations, accounting for roughly 26% of maternal deaths in these groups. The WHO reports that 800 pregnant women die daily from preventable causes, with 99% of these deaths occurring in developing countries, at a rate of 240 deaths per 100,000 births. Hypertensive pregnancy disorders, such as preeclampsia and eclampsia, significantly contribute to maternal mortality, especially in Africa and Asia, where they account for approximately 10% of deaths. At Befelatanana University Maternity Hospital (CHUGOB) in Antananarivo, Madagascar, the maternal mortality rate stands at 456 per 100,000 live births, with preeclampsia responsible for 41.7% of these deaths.[1] Risk factors for preeclampsia include familial history, genetic predisposition, length of sexual cohabitation, maternal smoking, parity, advanced maternal age, in vitro fertilization history, and underlying medical conditions such as pre-existing hypertension, diabetes, chronic kidney disease (CKD), and obesity.[2]

The pathogenesis of preeclampsia involves genetic, risk, and immunological factors. Key genetic factors include FLT1, SNPs, and trisomy 13. These contribute to poor placental implantation, leading to reduced placental perfusion in the first and second trimesters. During the third trimester, elevated levels of circulating sFLT1 and sENG result in vascular dysfunction, capillary leakage, and vasospasm. Additionally, COX converts arachidonic acid into prostaglandins like thromboxane (TXA2), which induces vasoconstriction and platelet aggregation.[3] Nitric oxide (NO) is synthesized by NOS in endothelial cells and platelets, with inducible NOS (iNOS) levels rising in response to inflammation and oxidative stress. In preeclampsia, iNOS is also found in the placenta, underscoring the involvement of pro-inflammatory cytokines in the condition.[4]

Turmeric (*Curcuma longa*), a plant related to the ginger family (Zingiberaceae), originates from India and is now cultivated in various regions worldwide, including Southeast Asia, China, and Latin America.[5] Turmeric is a spice commonly used in curry preparation in India and other Asian countries because of its taste and color.[6] India stands as the top producer and primary exporter of turmeric. Reports indicate that the global turmeric market, currently estimated at 1.7 million metric tons, is projected to grow substantially by 2027.[7] The global curcumin market was valued at around half a billion USD in 2016, with a projected compound annual growth rate (CAGR) of approximately 13% from 2018 to 2025. Clinical research suggests curcumin's potential anti-inflammatory benefits in treating conditions like inflammatory bowel disease, acute respiratory distress syndrome, post-operative inflammation, knee osteoarthritis, and chronic kidney disease. Although limited data exist on curcumin's use during pregnancy, phase I clinical trials have confirmed its safety for human consumption, even at high doses (up to 12 g/day).[8] Wistar and Sprague-Dawley strains of white rats (*Rattus norvegicus*) are commonly used in biomedical research, testing, and education. *Rattus norvegicus* is favored in studies due to its genetic similarities to humans, availability of various strains, and easy accessibility.[9] This study aims to examine the effect of curcumin on serum COX-2 and iNOS levels in a preeclampsia rat model, exploring its potential to influence these inflammatory markers.

MATERIALS AND METHODS

Research design

This research employed a true experimental laboratory design with an in vivo post-test-only controlled group, using preeclampsia model pregnant rats (*Rattus norvegicus*) treated with curcumin following L-NAME induction. The study's objective was to assess the impact of curcumin on serum COX-2 and iNOS levels in the preeclampsia rat model. The research process started with a 7-day acclimatization period for the rats, after which they were randomly assigned to 5 different groups: First, the negative control group, consisting of normal pregnant rat; second, the positive control group, comprising rats which received L-NAME injection (125 mg/kgBW/day) intraperitoneally on the 13th to 19th day of gestation.[10]; third, the treatment group 1, including rats which received subcutaneous injection of L-NAME (125 mg/kgBW/day) on the 13th to 19th day of gestation. Rats were also given 30 mg/kg/day of curcumin per rat on the 13th to 19th day of pregnancy; fourth, the treatment group 2, comprising rats received subcutaneous injection of L-NAME (125 mg/kgBW/day) on the 13th to 19th day of pregnancy. Rats also received 50 mg/kg/day of curcumin per rat on the 13th to the 19th day of pregnancy; and the last the treatment group 3 that included rats receiving subcutaneous injection of L-NAME (125 mg/kgBW/day) on the 13th to 19th day of pregnancy. Rats also received 100 mg/kg/day of curcumin per rat on the 13th to 19th day of pregnancy.

The experimental subjects in this study were pregnant Wistar strain *Rattus norvegicus* rats, which were obtained from the Bioscience Laboratory at Brawijaya University, Malang. The induction of L-NAME and

administration of curcumin to the rats, as well as their maintenance, were conducted at the Bioscience and Physiology Laboratory of the Faculty of Medicine, Brawijaya University. The study was carried out over a period of four months, from March to June 2023. The treatment of the experimental animals adhered to the ARRIVE guidelines (https://arriveguidelines.org/).

Inclusion and exclusion criteria

The study included pregnant female Wistar strain *Rattus norvegicus* rats aged between 10 and 15 weeks, with a body weight ranging from 150 to 200 grams. Eligible rats had no prior exposure to treatments or chemical substances and were in good health, displaying active movement and intact fur. Their systolic blood pressure ranged between 90 and 120 mmHg. Rats were excluded if they died before the treatment began, had previously been exposed to chemical treatments, or exhibited hypertension prior to the intervention. Dropout criteria for the study included giving birth during the research, death, or intrauterine fetal demise (IUFD).

Curcumin administration

Curcumin was administered by first grinding Helmig curcumin tablets into a powder, which was then dissolved and delivered orally using a feeding tube. Doses were based on the rats' estimated weight of 200 g (0.2 kg), with the following amounts administered: 6 mg/day for 30 mg dosage, 10 mg/day for 50 mg dosage, and 20 mg/day for 100 mg dosage. These doses were diluted in distilled water, resulting in 1.5 cc, 2.5 cc, and 5 cc solutions, respectively. The procedure involved loading the curcumin solution into a 5 cc syringe connected to a feeding tube, gently holding the rat's neck, and carefully inserting the tube through the rat's mouth into the esophagus. The solution was then delivered into the stomach before removing the tube and returning the rat to its cage.

COX-2 and iNOS levels examination

COX-2 and iNOS levels were measured in the serum (collected from the left cardiac blood vessels) of pregnant rats with a preeclampsia model after treatment according to their respective groups. The COX-2 and iNOS concentration was assessed using the ELISA Kit from Bioassay Technology Laboratory with an Elisa Reader, reported in pg/ml.

Statistical analysis

The data analysis was conducted in two primary steps. First, parametric assumptions were tested by assessing normality using the Shapiro-Wilk test and evaluating the homogeneity of variances. For data that were normally distributed, a One-Way ANOVA (F-test) was performed to compare means across multiple groups, using a significance level of 95% ($p < 0.05$). If the data did not meet the normality assumption, the Kruskal-Wallis test, a non-parametric alternative, was applied with the same 95% significance level. Following the ANOVA, if significant differences were found, post-hoc analysis using the Least Significant Difference (LSD) test was conducted to determine which specific group means were different. Additionally, Pearson's correlation test was applied to evaluate the linear relationship between variables when the data met the normality criteria. All statistical analyses were performed using SPSS for Windows version 19.0, and a significance level of $p < 0.05$ was maintained throughout.

Ethical clearance

This study had received ethical approval under number 400/064/K.3/102.7/2023 from the Ethics Committee of Dr. Saiful Anwar Regional General Hospital, Malang, Indonesia (March 30th, 2023).

RESULTS AND DISCUSSION

Preeclamptic model pregnant rats were induced by administering L-NAME at a dose of 125 mg/kg body weight from gestation days 13 to 19.[10] Preeclampsia is diagnosed by measuring blood pressure and urine protein levels. The condition is indicated by elevated blood pressure exceeding 140 mmHg systolic and urine protein levels of ≥ positive one (+).

Research was conducted on preeclampsia model rats to determine the effect of 30, 50 and 100 mg/KgBW/day of curcumin per rat on the 13th to 19th day of pregnancy on COX-2 and iNOS levels in preeclampsia model rats. Blood pressure and urine protein examinations were carried out at two times, namely at gestational age of 13 days (G13) and gestational age of 19 days (G19). The first examination at 13 days of gestational age was carried out to screen for symptoms of preeclampsia (hypertension and positive urine protein) before treatment. The second examination at 19 gestational age was carried out to evaluate the administration of L-NAME and curcumin.

The study revealed that the serum COX-2 level data were not normally distributed, prompting the use of one-way ANOVA for analysis. The ANOVA results indicated a significant difference ($p < 0.05$) (Table 1). Consequently, post hoc analysis was performed using the LSD test (Table 2).

Table 1. One-Way ANOVA test of serum COX-2 level.

Groups	Mean ± SD	p-value
NC	159.0 ± 18.25	
PC	591.5 ± 239.21	
T1	411.5 ± 147.73	<0.05
T2	206.5 ± 118.70	
T3	171.5 ± 95.35	

NC: Negative Control; PC: Positive Control:
T: Treatment; SD: Standard Deviation

Analysis revealed variations in serum COX-2 levels across different groups. Statistical tests indicated that L-NAME induction of preeclampsia led to significantly higher serum COX-2 levels in the positive control group compared to serum NO levels in the negative control group. Treatment groups 2 (50 mg/kg BW) and 3 (100 mg/kg BW) showed significantly lower serum COX-2 levels compared to the positive control. However, there were no significant differences in COX-2 levels among the treatment groups themselves (p >0.05).

This study showed that the serum iNOS level data had a non-normal distribution, so it was continued with one-way ANOVA analysis (Table 3). The results of analysis using one-way ANOVA showed that there were significant differences (p <0.05). Therefore, we continued using post hoc LSD analysis (Table 4).

Based on the analysis, it shows differences in serum iNOS levels for each group. The results of statistical tests show that administration of L-NAME to induce preeclampsia can cause serum iNOS levels in the positive control group to be significantly higher than serum NO levels in the negative control group. Treatment group 3 (100 mg/kgBW) had significantly lower serum iNOS levels than the positive control. iNOS levels between treatment groups 1 and 2 did not show a significant difference (p > 0.05). iNOS levels in treatment 3 were significantly lower than those in treatment 1 and treatment 2 (p < 0.05).

The results of producing preeclamptic rats showed symptoms of preeclampsia, namely increased blood pressure and urine protein. These symptoms were found in the positive control group, while the negative controls showed no symptoms. The results of this study showed an increase in blood pressure, namely >140/90 mmHg at 15 or 3 days of gestation after L-NAME injection. This is in line with research, that the positive control group also showed an increase in blood pressure that persisted until the 19th day of gestation.[11] Research by injecting L-NAME 125 mg/KgBW/day from the 13th day of gestation to the 21st day was also able to increase the systolic blood pressure of mice.[12] This condition shows success in creating a rat model of preeclampsia. Another study found that mice administered L-NAME exhibited symptoms including elevated urine protein, increased blood pressure, endothelial damage, and higher levels of sFlt-1 and PLGF.[13]

Table 2. LSD test of serum COX-2 level

	NC	PC	T1	T2	T3
NC		<0.05	<0.05	>0.05	>0.05
PC	<0.05		>0.05	<0.05	<0.05
T1	<0.05	>0.05		>0.05	>0.05
T2	>0.05	<0.05	>0.05		>0.05
T3	>0.05	<0.05	>0.05	>0.05	

NC: Negative Control; PC: Positive Control: T: Treatment

Table 3. One-Way ANOVA test of serum iNOS level.

Groups	Mean ± SD	p-value
NC	0.805 ± 0.507	
PC	5.683 ± 3.176	
T1	3.793 ± 2.591	<0.05
T2	2.817 ± 1.903	
T3	1.110 ± 0.609	

NC: Negative Control; PC: Positive Control:
T: Treatment; SD: Standard Deviation

Table 4. LSD test of serum iNOS level

	NC	PC	T1	T2	T3
NC		<0.05	<0.05	<0.05	>0.05
PC	<0.05		>0.05	>0.05	<0.05
T1	<0.05	>0.05		>0.05	<0.05
T2	<0.05	>0.05	>0.05		<0.05
T3	>0.05	<0.05	<0.05	<0.05	

NC: Negative Control; PC: Positive Control: T: Treatment

This study demonstrated that administering curcumin at a dose of 100 mg/kg BW lowered serum COX-2 levels. Preeclampsia is characterized by elevated hypoxia and inflammatory mediators, including COX-2. Increased COX-2 expression in the placenta of preeclamptic women correlates with higher thromboxane production. L-NAME (N(G)-nitro-L-arginine methyl ester) is a nitric oxide synthase (NOS) inhibitor that mimics the pathophysiological conditions of preeclampsia by reducing nitric oxide (NO) production. This reduction triggers oxidative stress, vasoconstriction, and endothelial dysfunction. In this study, curcumin demonstrated anti-inflammatory and antioxidant effects by significantly reducing serum COX-2 and iNOS levels in L-NAME-induced preeclampsia. Curcumin counters L-NAME's effects by suppressing COX-2-mediated inflammatory pathways and lowering iNOS expression, mitigating oxidative stress and vascular dysfunction. These results underscore curcumin's potential in alleviating inflammation and oxidative stress in preeclampsia models. Elevated thromboxane levels in the placenta contribute to vasoconstriction, playing a significant role in the development of pre-eclampsia.[14] COX-2 impacts the endothelium by heightening sensitivity to angiotensin II, stimulating the immune response, and elevating oxidative stress in preeclampsia.[15]

This study found that curcumin administration led to a reduction in serum iNOS levels. iNOS, an isoform of nitric oxide synthase (NOS), influences nitric oxide (NO) production.[16] NO, crucial for vascular homeo-stasis, is synthesized from L-arginine by NOS enzymes, including iNOS. Although NO levels are normally regulated, elevated NO can react with reactive oxygen species like superoxide to produce peroxynitrite. Increased oxidative stress in preeclampsia also elevates iNOS levels.[17] Increased oxidative stress in preeclamp-sia also elevates iNOS levels. This rise in iNOS can lower serum NO, leading to vasoconstriction and hypertension.[18]

These findings are consistent with previous research by Zhou et al. (2017), which demonstrated a reduction in inflammatory mediators in a preeclampsia mouse model induced by lipopolysaccharide. Similarly, Fu et al. (2014) reported a decrease in inflammatory mediators such as TLR4, NF-κB, IL-6, and MCP-1 following curcumin administration in lipopolysaccharide-induced mice. These results align with previous studies. Ben et al. (2011) found that curcumin administration enhances the degradation of iNOS, initiated by the inhibition of ERK 1/2, leading to reduced iNOS levels. Additionally, Streyczek et al. (2022) reported that curcumin lowers iNOS levels in cell cultures. Furthermore, Greish et al. (2020) demonstrated that curcumin decreases reactive oxygen species (ROS) in L-NAME-induced animals by increasing catalase activity and reducing lipid peroxidase levels.[19–23]

Preeclampsia is asymptomatic on first two trimesters pregnancy because the symptoms are widely varied. Preeclampsia frequently remained unclearly identified and thus, is dangerous. Preeclampsia depicted as a heterogenous multisystemic disturbance that may only be diagnosed during routine antenatal visit. Early indicators include high blood pressure, with systolic readings of ≥140 mmHg and diastolic readings of ≥90 mmHg, accompanied by proteinuria of ≥300 mg over 24 hours.[24] Preeclampsia is globally defined as newly developed gestational hypertension, marked by systolic blood pressure above 140 mmHg and/or diastolic pressure exceeding 90 mmHg. This condition is associated with the development of organ dysfunction, including proteinuria, maternal organ dysfunction (involving hepatic, neurological, hematologic, or renal systems), or uteroplacental dysfunction occurring after 20 weeks of gestation. Preeclampsia patients which received delayed treatment affected the mother and the carried fetus. On the mother, cerebral hemorrhage, heart failure with pulmonary edema, kidney failure and gastric reflux aspiration may occur during seizure. On the fetus, death may occur due to intrauterine hypoxia and preterm birth may also occur.

COX is essential in the synthesis of prostanoids (Figure 1). It begins by converting arachidonic acid into the intermediate prostaglandin (PG) G, which is then converted into PGH2. PGH2 is swiftly processed into thromboxane (TXA2), prostacyclin (PGI2), and other prostaglandins (PGE2, PGD2, and PGF2α) by TXA2 synthase, PGI2 synthase, and various isomerases. TXA2, primarily produced by platelets, promotes vasoconstriction, vascular remodeling, and platelet aggregation and adhesion.

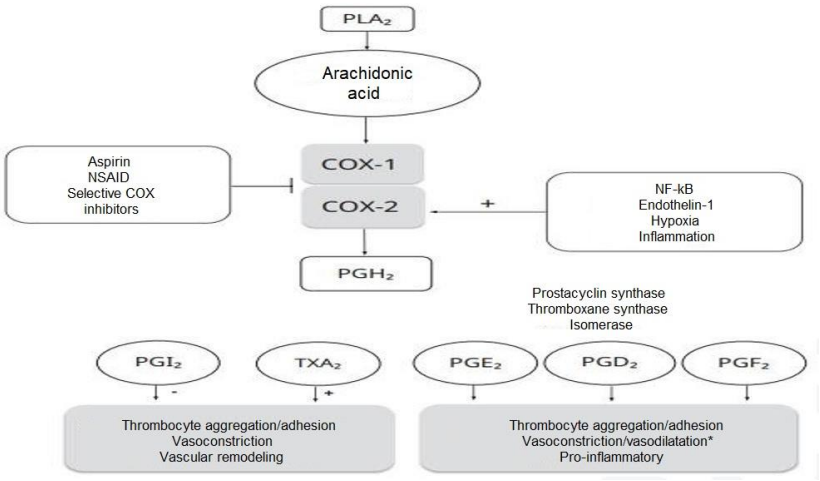

Figure 1. Aspirin and track biosynthesis prostanoids.

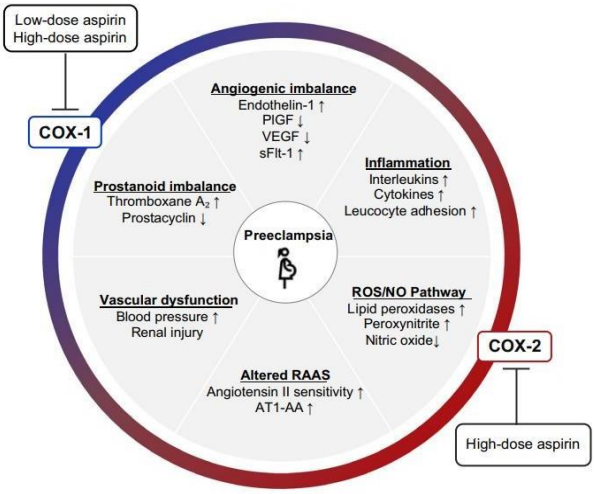

Figure 2. COX-1 mechanism and COX-2.[25]

There are two isoforms of COX: COX-1 and COX-2. COX-1 is constitutively expressed in everywhere and produce part big prostanoids during situation physiological. In contrast, constitutive COX-2 expression was low and mainly limited in the brain, thymus, intestines, kidneys, and placenta. Inflammatory mediators (e.g., nuclear-κB (NF-κB), hyperosmolality, endothelin (ET)-1 and hypoxia) is the main pusher to increase the regulation of inducible COX-2 (Figure 2).

Nitric oxide (NO) is produced by NO synthase (NOS) in the endothelium and platelets. Inducible NOS (iNOS) levels rise during platelet aggregation, oxidative stress, and macrophage infiltration driven by pro-inflammatory factors. iNOS is also found in the placenta of hypertensive and preeclamptic pregnancies. Proinflammatory cytokines are likely key in endothelial activation in preeclampsia. NO, a potent vasodilator, is crucial for blood pressure control in preeclampsia and is generated by three NOS forms: neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). Both iNOS and eNOS are present in the placenta. Unlike the other NOS forms, iNOS operates in-dependently of calcium or calmodulin. iNOS expression has been identified in villous mesenchymal cells using iNOS-specific monoclonal antibodies and can also be detected in vascular endothelial cells under inflamma-tory conditions. Pregnancy, even without complications, is associated with oxidative stress, where reactive oxygen species interact with NO, forming peroxynitrite during preeclampsia development. iNOS contributes to NO production, thereby participating in oxidative stress,

which may be linked to the onset of preeclampsia. Pregnancy increases iNOS expression in smooth muscle cells, indicating a possible role for iNOS in regulating uterine muscle tone.[26] N(G)-nitro-L-arginine methyl ester (L-NAME) is metabolized by cellular esterases into bioactive NG-nitro-L-arginine, which inhibits NOS activity in the vascular endothelium by displacing its usual substrate, L-arginine.[27] L-NAME has a higher affinity for eNOS and can also inhibit other NOS isoforms at elevated concentrations. Its synthetic availability makes L-NAME a common NO antagonist in animal model studies.[28]

Strengths and limitations of the study

This study provides significant insights into the impact of curcumin on COX-2 and iNOS levels in a pre-eclampsia mouse model, demonstrating a thorough evaluation of its anti-inflammatory effects. Conducted under ethical oversight, the study adheres to ethical guidelines and aligns with previous research, which reinforces its validity. The methodology, including ELISA for accurate measurement and body weight-based dosing, contributes to the study's credibility.

Nevertheless, the study has limitations. The lack of funding may have constrained resources for more extensive experiments or advanced analyses. Using only a mouse model may not fully represent the complexities of human preeclampsia. Additionally, the short study duration might not account for the long-term effects of curcumin or its impact across different preeclampsia stages. The research also focused solely on COX-2 and iNOS, without investigating other potentially relevant inflammatory mediators or pathways influenced by curcumin.

CONCLUSION

The administration of curcumin can reduce COX-2 and iNOS level in the serum of Wistar rats (*Rattus norvegicus*) a model of preeclampsia. In order to support the conclusions of this research, further research is needed to analyze peroxynitrite and iNOS levels which play a role in the mechanism of preeclampsia and to analyze the effect of combining curcumin with antihypertensives on COX-2 and iNOS levels.

DISCLOSURES

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Conflict of interest

The authors report no conflicts of interest.

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Author contribution

All authors contributed significantly to all stages of this research, including study design, data acquisition and analysis, manuscript preparation, and final approval for publication.

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