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Effects of *Nigella sativa* Extract on Renal Tubular Necrosis and TNF-α Expression in a Preeclampsia Mouse Model

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ABSTRACT

Introduction: Preeclampsia is associated with endothelial dysfunction, systemic inflammation, and renal injury, including proximal tubular necrosis mediated by pro-inflammatory cytokines such as TNF-α. *Nigella sativa* possesses anti-inflammatory properties that may attenuate these pathologic processes. This study evaluated the effect of ethanolic *Nigella sativa* seed extract on proximal renal tubular cell necrosis and TNF-α expression in a pregnant mouse (*Mus musculus*) model of preeclampsia.

Material and Methods: Using a true experimental post-test design with 24 mice (4/group, 6 groups including negative control, preeclampsia positive control, and treatment groups), this study administered ethanolic *Nigella sativa* extract orally at 500-2000 mg/kg/day on gestational days 15-20, after which necrosis was assessed via H&E staining and TNF-α expression via immunohistochemistry.

Results: Administration of the ethanol extract of *Nigella sativa* seeds showed a trend toward reducing the percentage of proximal tubular cell necrosis and TNF-α expression in a preeclampsia mouse model. The lowest values were observed at 2000 mg/kg (necrosis: 18.84±2.89%; TNF-α: 19.80±12.98%). Necrosis differed significantly between groups ($p<0.005$); however, TNF-α expression did not differ significantly between groups ($p>0.005$). Linear regression showed a negative dose-dependent association with necrosis ($R^2=0.358$; $p=0.002$; coefficient −0.005).

Conclusion: Ethanolic *Nigella sativa* seed extract improved proximal tubular injury significantly, but did not significantly reduce TNF-α expression, showing only a trend toward lower TNF-α levels in a preeclampsia model, with the most evident effect at 2000 mg/kg body weight/day.

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INTRODUCTION

Hypertension in pregnancy affects approximately 5-10% of pregnancies and is a major cause of maternal mortality, with increasing prevalence [1]. Gestational hypertension, preeclampsia, chronic hypertension, and chronic hypertension with superimposed preeclampsia are the four categories into which ACOG classified hypertensive disorders of pregnancy in 2019 [2]. After 20 weeks of gestation, preeclampsia, a pregnancy-specific condition marked by vasospasm, endothelial activation, and decreased organ perfusion, manifests in previously normotensive women as blood pressure ≥140/90 mmHg with proteinuria [3,4]. Preeclampsia affects 5-7% of pregnancies worldwide, resulting in over 70,000 maternal and 500,000 fetal fatalities each year, and can progress from mild to severe conditions. 30-40% of maternal mortality in

Indonesia is caused by severe preeclampsia and eclampsia, making preeclampsia a recurring obstetric care problem [5].

During pregnancy, hemodynamic adaptations support maternal-fetal blood and nutrient exchange through remodeling of the uterine spiral arteries by trophoblast cells. Impaired remodeling may lead to relative placental ischemia, increased vasoconstriction, and elevated maternal blood pressure as fetal oxygen demands rise [6]. Placental hypoxia in preeclampsia disrupts the equilibrium between pro-angiogenic and anti-angiogenic factors, causing mediators, including sFlt-1, sEng, STBM, TNF-α, IL-6, AT1-AA, and reactive oxygen species (ROS), to be released. These mediators impair vascular function and cause systemic endothelial damage [7,8]. Endothelial dysfunction impairs the production of vasodilators, including nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarizing factors, thereby increasing oxidative stress, inflammation, and coagulation. Pro-inflammatory cytokines, particularly TNF-α and IL-6, play important roles in the immunological and inflammatory responses associated with preeclampsia [9].

Hypertension in preeclampsia can cause significant kidney injury. Elevated levels of sFlt-1 inhibit VEGF activity, leading to glomerular and podocyte damage, kidney dysfunction, and proteinuria. Inflammatory cytokines, such as TNF-α, IL-6, and IL-17, further enhance vasoconstriction, reducing renal blood flow and impairing the kidney's filtration capacity [7,8]. The severity of renal damage depends on both the magnitude and duration of hypertension, with prolonged elevated blood pressure increasing the risk of complications [10]. Preeclampsia-induced kidney injury is characterized by glomerular damage and swelling of proximal tubular epithelial cells, which can narrow or occlude the tubular lumen. Because the proximal tubule is highly susceptible to hypoxia and toxic injury and is responsible for 60-80% of renal reabsorption, it is a key target of renal damage and the focus of this study. Early prevention and intervention are essential, as untreated kidney injury may progress to chronic kidney disease requiring dialysis or kidney transplantation. Understanding renal adaptations and pathological alterations during pregnancy is critical for early detection, diagnosis, and therapy of preeclampsia [10,11].

Nigella sativa (black seed) contains anti-inflammatory, antioxidant, and antihypertensive effects and has long been used to treat a variety of ailments, including diarrhea, asthma, cough, and dermatological diseases. Thymoquinone, the major active ingredient, reduces inflammation by inhibiting cyclooxygenase (COX), lipoxygenase (LOX), NF-κB, and TNF-α enzymes. Additionally, thymoquinone, dithymoquinone, and thymol act as antioxidants by reducing free radicals and contribute to blood pressure reduction [9]. Due to these anti-inflammatory and antioxidant properties, *Nigella sativa* may inhibit key mechanisms involved in the pathophysiology of preeclampsia, including inflammation and oxidative stress. This study evaluated the effect of the ethanol extract of *Nigella sativa* seeds on necrosis and TNF-α expression in the proximal tubular cells in the kidneys in a preeclampsia mouse model (*Mus musculus*).

MATERIAL AND METHODS

This study used an in vivo true experimental, post-test-only controlled group design. Six groups of mice were included: a negative control (no preeclampsia induction or *Nigella sativa* extract), a positive control (preeclampsia induction only), and four treatment groups receiving oral ethanol extract of *Nigella sativa* at doses of 500, 1000, 1500, or 2000 mg/kg/day from gestational days 15-20. The study was conducted from March to June 2024. Serum from women with severe preeclampsia was obtained from Dr. Saiful Anwar General Hospital, Malang. Experimental animals were sourced from Maulana Malik Ibrahim State Islamic University and housed at the Bioscience Laboratory, Brawijaya University. Assessment of preeclampsia manifestations and renal histopathology was performed at the Physiology Laboratory, Faculty of Medicine, Brawijaya University, Malang.

The study population consisted of female *Mus musculus* mice induced to develop a preeclampsia model via intraperitoneal injection of serum from women with severe preeclampsia. Human serum

donors were aged 20-35 years, primigravida, diagnosed with severe preeclampsia before 34 weeks of gestation, and free from cardiovascular, metabolic, and infectious diseases. The sample comprised healthy pregnant female *Mus musculus* mice aged 10-12 weeks and weighing 150-200 g.

The Federer formula was used to determine the sample size, which was 4 mice per group, for a total of 24 mice across 6 groups. Inclusion criteria included healthy adult mice with confirmed pregnancy and successful induction of preeclampsia, defined as systolic blood pressure ≥140 mmHg and positive proteinuria following serum injection. Mice with pre-existing illness, infection, severe diarrhea, or prior exposure to potentially confounding substances were excluded. Dropout criteria included spontaneous abortion, death, premature delivery, or any procedural failure affecting data validity. The independent variable was the ethanol extract of *Nigella sativa*, while the dependent variables were proximal renal tubular cell necrosis and TNF-α expression.

Black cumin (*Nigella sativa*) seeds were obtained from UPT Laboratory Materia Medica Batu, East Java, and processed into an ethanol extract at the Pharmacology Laboratory, Faculty of Medicine, Brawijaya University. Female *Mus musculus* mice were acclimatized for 7 days under controlled environmental conditions with ad libitum access to food and water. Pregnancy was confirmed by the presence of sperm in vaginal smears. A preeclampsia model was established by intraperitoneal injection of 0.1 cc serum from women with severe preeclampsia on gestational days 10 and 11. Blood pressure was measured using a tail-cuff volume-pressure recording system, and urinary protein levels were assessed by dipstick analysis of urine collected in metabolic cages. Ethanolic extract of *Nigella sativa* was administered orally according to treatment-group doses from gestational days 15 to 20.

Kidney tissues were gathered, fixed in 10% formalin, paraffin-embedded, sectioned, and stained for histological analysis at the conclusion of the experiment. Hematoxylin-eosin (H&E) staining was used to evaluate renal structural alterations, namely degeneration and necrosis of proximal tubular cells. Immunohistochemical staining using anti-TNF-α antibodies and an HRP-DAB detection system was conducted to evaluate TNF-α expression in proximal renal tubular cells.

Histopathological and immunohistochemical preparations were examined under a light microscope, and TNF-α expression was assessed based on the intensity and distribution of positive staining. Data were analyzed using SPSS. The Shapiro-Wilk test was used to determine normality ($p > 0.05$ indicates normal distribution). Group differences were evaluated using Welch ANOVA for normally distributed but non-homogeneous data, followed by post hoc analysis when significant differences were detected. To evaluate the associations between the variables, Pearson correlation analysis was used. Statistical significance was defined as a p-value of less than 0.05.

Ethical approval for this study was obtained from the Health Research Ethics Commission of General Hospital Dr. Saiful Anwar (Approval No. 400/325/K.3/102.7/2024) and the Ethical Committee of the Medical Faculty at Brawijaya University (Approval No. 32/EC/KEPK-PPDS/02/2025). All animal-related procedures were carried out in compliance with institutional guidelines.

RESULTS

In this study, a total of 24 samples were subjected to experimental tests on female white mice (*Mus musculus*). After surgery, fixation, and tissue sectioning, hematoxylin and eosin staining were performed to observe the percentage of proximal tubular cell necrosis, and immunohistochemistry was used to examine TNF-α expression (Table 1).

Significant differences in proximal tubular cell necrosis and TNF-α expression were observed among the study groups. The preeclampsia group showed substantially higher tubular necrosis than the normal group (31.04 ± 5.53% vs. 3.28 ± 1.93%). Administration of *Nigella sativa* extract reduced necrosis in a dose-dependent manner, with the greatest reduction observed at 2000 mg/kg BW (18.84 ± 2.89%), followed by 1000 mg/kg BW (22.52 ± 2.18%) and 500 mg/kg BW

Table 1. Results of the Measurement of the Percentage of Proximal Tubular Cell Necrosis and TNF-α Expression

Variable	Dosage	Analysis Result		
		Mean ± SD	Median (Min-Max)	CI 95%
Proximal Tubule Cell Necrosis (%)	NC	3.28 ± 1.93	3.40 (0.40-5.80)	0.87-5.68
	PC	31.04 ± 5.53	31.20 (25.80-39.80)	24.16-37.91
	500 mg/kg	23.92 ± 7.00	26.00 (16.00-30.40)	15.22-32.61
	1000 mg/kg	22.52 ± 2.18	22.60 (19.80-25.20)	19.80-25.23
	1500 mg/kg	23.04 ± 5.58	22.40 (15.20-30.40)	16.10-29.97
	2000 mg/kg	18.84 ± 2.89	17.20 (16.80-23.60)	15.24-22.43
TNF-α (%)	NC	1.60 ± 1.14	2.00 (0.00-3.00)	0.18-3.01
	PC	27.00 ± 9.32	30.00 (15.00-38.00)	15.41-38.58
	500 mg/kg	26.20 ± 13.88	33.00 (8.00-40.00)	8.96-43.43
	1000 mg/kg	24.60 ± 7.63	25.00 (15.00-35.00)	15.11-34.08
	1500 mg/kg	25.80 ± 13.10	25.00 (5.00-38.00)	9.52-42.07
	2000 mg/kg	19.80 ± 12.98	25.00 (5.00-36.00)	3.67-35.92

Note: NC = negative control; PC = positive control; mg/kg = milligrams/kilogram; CI = confidence interval.

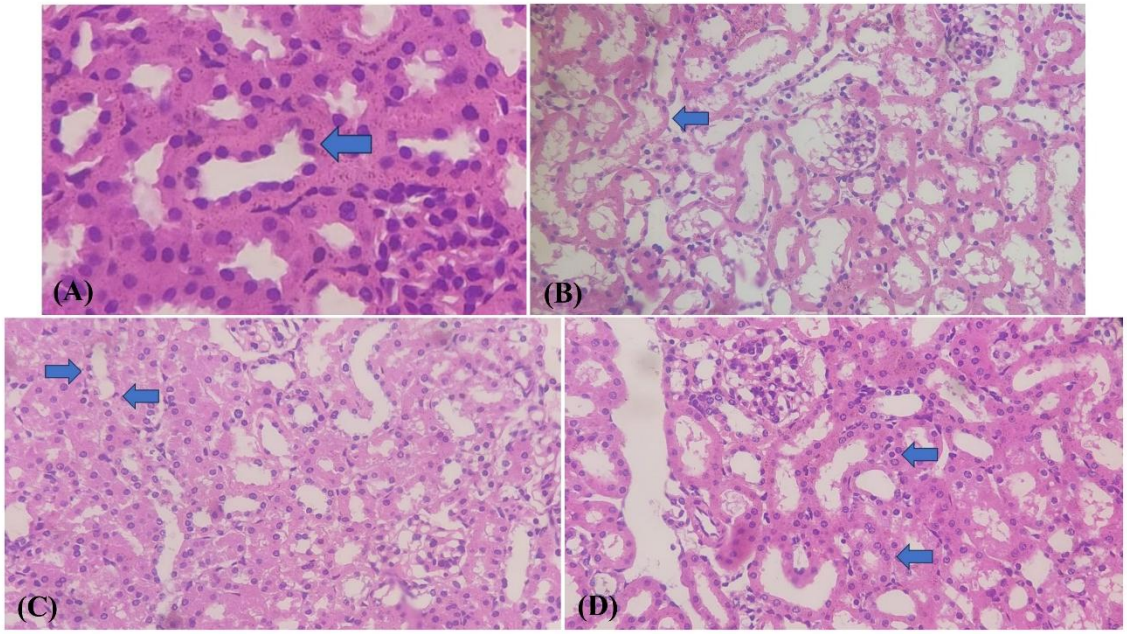


Figure 1. Hematoxylin and Eosin Staining of Kidney at 400x Magnification. (**A**) negative control; (**B**) positive control; (**C**) pycnotic tubular cells, karyorexis; (**D**) tubular cells with round-oval nuclei improvement.

(23.92 ± 7.00%). Similarly, TNF-α expression was lowest in the normal group (1.60 ± 1.14%) and highest in the untreated preeclampsia group (27.00 ± 9.32%). Increasing doses of *Nigella sativa* extract progressively decreased TNF-α expression, with the 2000 mg/kg BW dose producing the greatest reduction (19.80 ± 12.98%; median 25.00%, range 5.00-36.00%; 95% CI: 3.67-35.92%).

The Shapiro-Wilk test demonstrated a normal distribution of proximal tubular cell necrosis data in all groups ($p > 0.05$). However, Levene's test indicated unequal variances among groups ($p = 0.046$), violating the homogeneity assumption. Therefore, group differences were analyzed using Welch ANOVA. The Welch test revealed a significant difference in proximal tubular cell necrosis among treatment groups ($p = 0.000$). Post hoc pairwise comparisons revealed a substantial reduction in necrosis in the 2000 mg/kg Nigella sativa group compared with the positive control group ($p = 0.033$). In contrast, the 500, 1000, and 1500 mg/kg groups did not differ significantly

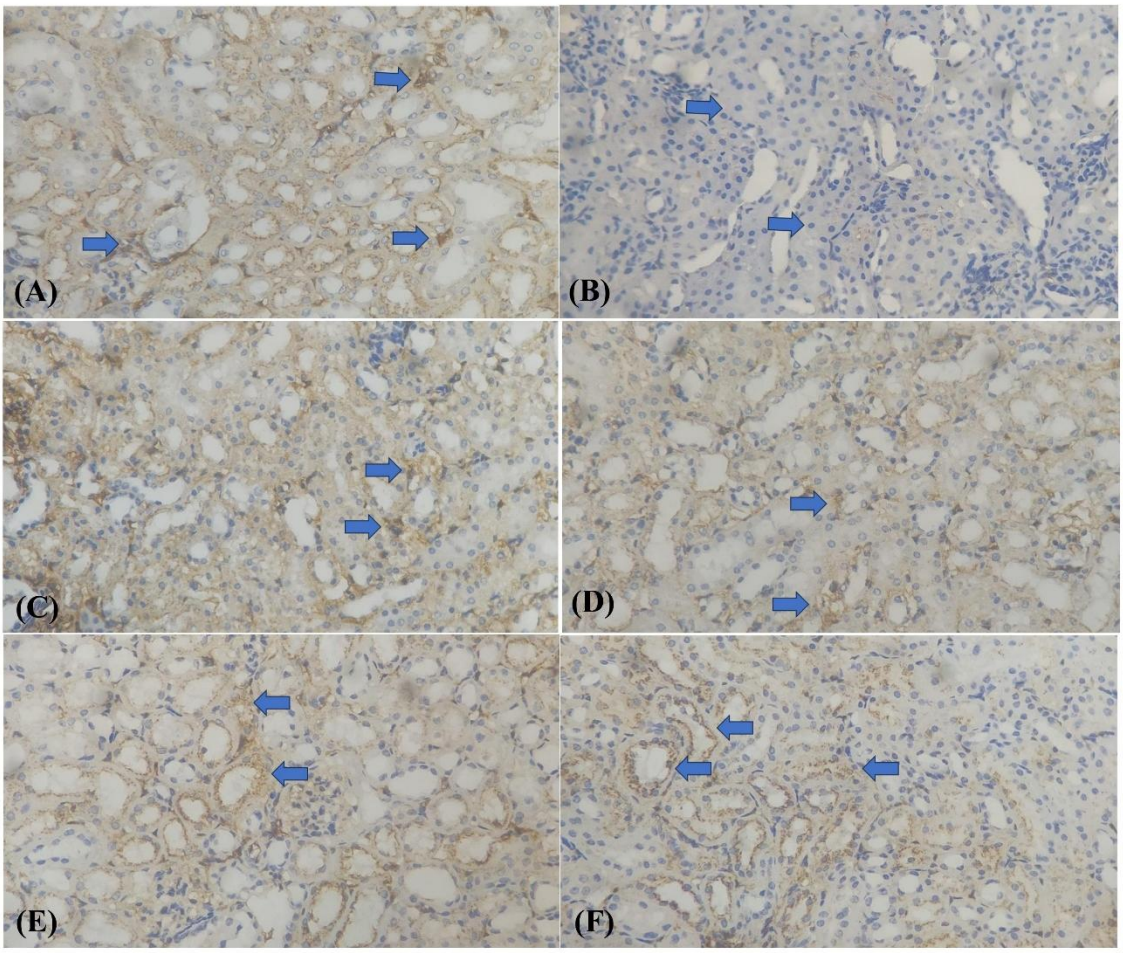


Figure 2. Immunohistochemical Staining TNF-α Magnification 400x. (**A**) Positive control; (**B**) Negative control; (**C**) 500 mg/kg; (**D**) 1000 mg/kg; (**E**) 1500 mg/kg; (**F**) 2000 mg/kg.

from the positive control ($p > 0.05$), although all treatment groups exhibited lower necrosis percentages (Table 2).

Regression analysis demonstrated a significant negative relationship between *Nigella sativa* dose and proximal tubular cell necrosis. The coefficient of determination ($R^2 = 0.358$) indicated that 35.8% of the variation in necrosis was explained by extract dosage, while the remaining 64.2% was attributable to other factors not included in the model. The linearity analysis further showed that increasing doses of *Nigella sativa* were associated with progressive reductions in proximal tubular cell necrosis (Table 3).

The Shapiro-Wilk test indicated that the TNF-α expression data from all groups were normally distributed ($p > 0.05$). For group comparisons, Welch ANOVA was required because Levene's test revealed heterogeneity of variances ($p = 0.028$). TNF-α expression varied significantly between groups, according to Welch ANOVA ($p = 0.000$). Nevertheless, post hoc pairwise comparisons showed no significant differences between the positive control group and any treatment group (500, 1000, 1500, or 2000 mg/kg; $p > 0.05$), although all treatment groups demonstrated lower TNF-α expression than the positive control group (Table 4).

Regression analysis yielded an R^2 value of 0.038, indicating that only 3.8% of the variation in TNF-α expression was explained by *Nigella sativa* dosage, while 96.2% was attributable to other factors. The linearity analysis showed a weak negative trend between extract dose and TNF-α expression, suggesting that increasing doses were associated with a slight reduction in TNF-α

Table 2. Results of the post-hoc Games-Howell test for the percentage of Proximal Tube Cell Necrosis (%) between groups

Pairwise Comparison	Mean Difference	Std. Error	p-value[1]	95% Confidence Interval
NC vs PC	-27.76	2.62	0.001	-38.98 – -16.54
NC vs 500 mg/kg	-20.64	3.25	0.012	-35.01 – -6.27
NC vs 1000 mg/kg	-19.24	1.31	<0.001	-24.03 – -14.45
NC vs 1500 mg/kg	-19.76	2.64	0.005	-31.08 – -8.44
NC vs 2000 mg/kg	-15.56	1.56	<0.001	-21.46 – -9.66
PC vs 500 mg/kg	7.12	3.99	0.525	-7.67 – 21.91
PC vs 1000 mg/kg	8.52	2.66	0.132	-2.64 – 19.68
PC vs 1500 mg/kg	8.00	3.52	0.302	-4.85 – 20.85
PC vs 2000 mg/kg	12.20	2.79	0.033	1.11 – 23.29
500 mg/kg vs 1000 mg/kg	1.40	3.28	0.997	-12.89 – 15.67
500 mg/kg vs 1500 mg/kg	0.88	4.01	1.000	-13.95 – 15.71
500 mg/kg vs 2000 mg/kg	5.08	3.39	0.680	-9.02 – 19.18
1000 mg/kg vs 1500 mg/kg	-0.52	2.68	1.000	-11.78 – 10.74
1000 mg/kg vs 2000 mg/kg	3.68	1.62	0.310	-2.36 – 9.72
1500 mg/kg vs 2000 mg/kg	4.20	2.81	0.681	-6.99 – 15.39

Note: [1]Games-Howell post-hoc test (used because the assumption of homogeneity of variances was not met). Differences are considered significant if $p < 0.05$, NC = negative control; PC = positive control; mg/kg = milligrams/kilogram

Table 3. Analysis of Proximal Tubule Cell Necrosis Regression Test

TNF-α Expression (mean±SD)	Regression Model		Regression Coeffient			
	R^2	p-value	Constant.	p-value	Coeff.	p-value
23.87 ± 6.09	0.358 (35.8%)	0.002	28.928	0.000	-0.005	0.000

expression. However, the regression results indicated that this relationship was not statistically significant (Table 5 and Figure 3).

DISCUSSION

This study evaluated the effects of the ethanol extract of *Nigella sativa* on proximal tubular cell necrosis and TNF-α expression in a preeclampsia mouse model. Female *Mus musculus* were assigned to a negative control group, a positive control group, and four treatment groups receiving *Nigella sativa* extract at doses of 500, 1000, 1500, and 2000 mg/kgBW. Proximal tubular necrosis was assessed using Hematoxylin-Eosin staining, while TNF-α expression was evaluated by immunohistochemistry. The untreated preeclampsia group exhibited the highest levels of proximal tubular necrosis (31.04 ± 5.53%) and TNF-α expression (27.00 ± 9.32%), indicating substantial renal injury and inflammation.

Administration of *Nigella sativa* extract produced dose-dependent reductions in both proximal tubular necrosis and TNF-α expression. The greatest improvement was observed at the highest dose (2000 mg/kg BW), which resulted in the lowest mean necrosis percentage (18.84 ± 2.89%) and TNF-α expression (19.80 ± 12.98%) among the treatment groups. Overall, these findings suggest that preeclampsia increases renal inflammation and tubular injury, while *Nigella sativa* extract may attenuate these pathological changes, particularly at higher doses.

The present study demonstrated that the ethanol extract of *Nigella sativa* significantly reduced proximal tubular cell necrosis in the kidneys of preeclampsia model mice. Untreated preeclampsia mice exhibited the highest percentage of tubular necrosis (31.04 ± 5.53%), whereas treatment with *Nigella sativa* produced a dose-dependent reduction, with the greatest effect observed at 2000 mg/kg BW (18.84 ± 2.89%). Histopathological examination supported these

Table 4. Results of the post-hoc Games-Howell test for the percentage of TNF- α (%) between groups

Pairwise Comparison	Mean Difference	Std. Error	p-value[1]	95% Confidence Interval
NC vs PC	-25.40	4.20	0.020	-45.02 – -5.78
NC vs 500 mg/kg	-24.60	6.23	0.088	-53.93 – 4.73
NC vs 1000 mg/kg	-23.00	3.45	0.014	-39.00 – -7.00
NC vs 1500 mg/kg	-24.20	5.88	0.077	-51.87 – 3.47
NC vs 2000 mg/kg	-18.20	5.83	0.174	-45.63 – 9.23
PC vs 500 mg/kg	0.80	7.48	1.000	-27.54 – 29.14
PC vs 1000 mg/kg	2.40	5.39	0.997	-17.49 – 22.29
PC vs 1500 mg/kg	1.20	7.19	1.000	-25.81 – 28.21
PC vs 2000 mg/kg	7.20	7.15	0.902	-19.62 – 34.02
500 mg/kg vs 1000 mg/kg	1.60	7.09	1.000	-26.26 – 29.46
500 mg/kg vs 1500 mg/kg	0.40	8.54	1.000	-30.82 – 31.62
500 mg/kg vs 2000 mg/kg	6.40	8.50	0.968	-24.70 – 37.50
1000 mg/kg vs 1500 mg/kg	-1.20	6.78	1.000	-27.57 – 25.17
1000 mg/kg vs 2000 mg/kg	4.80	6.74	0.974	-21.35 – 30.95
1500 mg/kg vs 2000 mg/kg	6.00	8.25	0.973	-24.15 – 36.15

Note: [1]Games-Howell post-hoc test (used because the assumption of homogeneity of variances was not met). Differences are considered significant if $p < 0.05$, NC = negative control; PC = positive control; mg/kg = milligrams/kilogram

Table 5. Analysis of TNF- α Expression Regression Test

TNF-α Expression (mean±SD)	Regression Model		Regression Coefficent			
	R^2	p-value	Constant.	p-value	Coeff.	p-value
24.68 ± 10.94	0.038 (3.8%)	0.350	27.640	0.000	-0.003	0.000

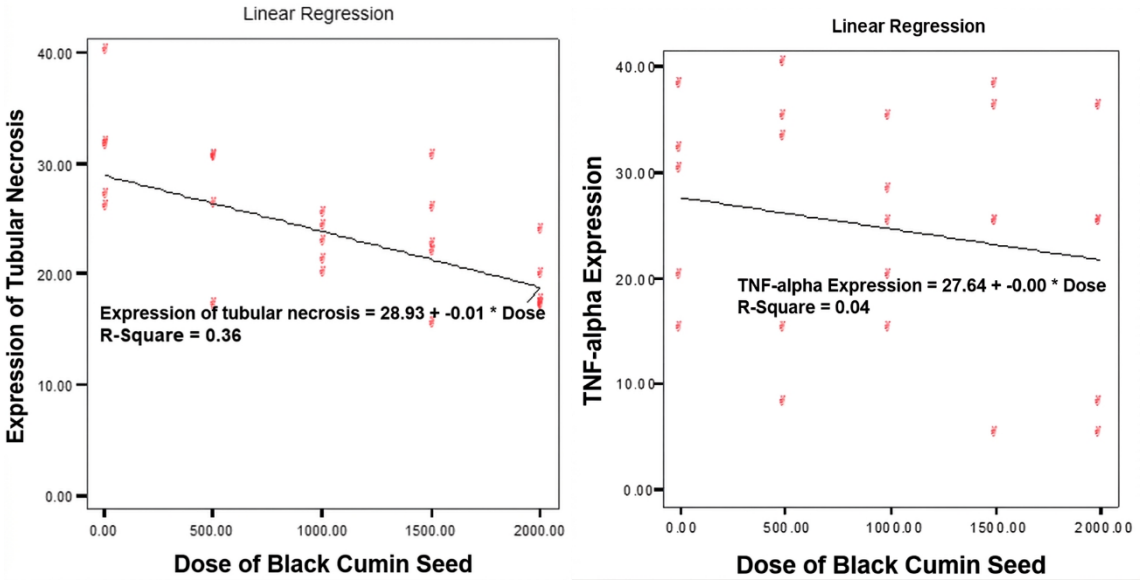


Figure 3. Graph of Dose Linearity with Percentage of Proximal Tubule Cell Necrosis and TNF-α Expression

findings (Figure 1), showing fewer pyknotic and karyorrhectic nuclei and improved tubular morphology in treated groups. Regression analysis further confirmed a significant negative association between *Nigella sativa* dose and tubular necrosis ($R^2 = 0.358$, $p = 0.002$), indicating that higher doses were associated with reduced renal injury.

The renoprotective effect of *Nigella sativa* is likely mediated through its antioxidant and anti-inflammatory properties, primarily attributed to thymoquinone. Preeclampsia causes oxidative stress and activation of NF-κB, resulting in IL-6 and IL-8. These cytokines can cause endothelial dysfunction, decreased renal perfusion, and tubular damage [12]. It has been demonstrated that thymoquinone inhibits NF-κB activation, reduces the production of inflammatory cytokines, and strengthens endogenous antioxidant defenses, including glutathione, catalase, superoxide dismutase, and glutathione peroxidase. These actions minimize tubular cell necrosis by reducing oxidative damage and lipid peroxidation. The results are consistent with other research showing that Nigella sativa enhances renal perfusion, reduces inflammatory markers, and protects against kidney damage in models of nephrotoxicity, ischemia-reperfusion injury, and preeclampsia. Collectively, these results suggest that *Nigella sativa* exerts a dose-dependent renoprotective effect and may be a promising therapeutic candidate for preventing preeclampsia-associated renal injury [13–15].

The present study demonstrated that the reduction in TNF-α expression was not statistically significant, although a tendency was observed at the highest dose (2000 mg/kgBW; 19.80 ± 12.98%) compared with the untreated preeclampsia group (27.00 ± 9.32%). Post hoc analysis did not detect any statistically significant differences between treatment groups and the positive control group, despite a dose-dependent reduction trend. Importantly, regression analysis revealed a weak and non-significant relationship between *Nigella sativa* dosage and TNF-α expression ($R^2 = 0.038$, $p = 0.350$). Given this lack of statistical significance, the role of TNF-α modulation in mediating the potential protective effects of *Nigella sativa* should be interpreted with caution and requires further investigation. Nevertheless, immunohistochemical evaluation (Figure 2) revealed qualitative changes in TNF-α localization, with cytoplasmic staining predominating in the untreated group and a shift toward membrane and subnuclear staining at higher doses, particularly at 2000 mg/kg BW. Although these qualitative changes suggest a possible biological modulation of inflammatory activity, the absence of significant quantitative differences (as indicated by the non-significant regression analysis) suggests that TNF-α may not be the primary driver of the observed effects.

Through endothelial dysfunction, organ damage, and NF-κB-mediated inflammatory signaling, TNF-α is a crucial pro-inflammatory cytokine involved in the pathophysiology of preeclampsia [12]. *Nigella sativa*'s anti-inflammatory actions are primarily due to thymoquinone, which suppresses NF-κB activation and reduces the production of TNF-α and other inflammatory mediators [14,16]. Previous studies have reported significant reductions in TNF-α, IL-8, iNOS, and ET-1 expression following *Nigella sativa* administration in preeclampsia models, although the present study demonstrated a more modest effect [17,18]. This discrepancy may be related to biological variability, limited sample size, or the complex regulation of TNF-α in vivo. Importantly, TNF-α also participates in tissue repair and regeneration, and its presence may reflect both inflammatory and recovery processes [19,20]. Therefore, the observed shift in TNF-α localization from predominantly cytoplasmic to membrane-associated and subnuclear regions may indicate a transition from active inflammation to cellular recovery [21]. Overall, although Nigella sativa did not significantly reduce renal TNF-α expression, the observed downward trend and qualitative histological changes support its potential as an adjunctive therapy to mitigate renal inflammation and injury in preeclampsia [9,12,14,16].

Several mechanisms may explain why reduced tubular necrosis was observed despite non-significant changes in TNF-α expression. First, alternative anti-inflammatory pathways may predominate. A meta-analysis indicated that renal protection via IL-10 and M2 macrophage polarization can suppress inflammation and promote tissue repair without requiring significant reductions in TNF-α levels at specific time points. Second, non-TNF pathways may be involved. For instance, the protective molecule Apelin-13 has been proposed to ameliorate tubular damage through mechanisms that are not necessarily linear with TNF-α concentrations. In this context, NF-κB, a downstream target of multiple inflammatory signals, not solely TNF-α, may

represent a primary target. Reduced tubular necrosis could result from successful NF-κB inhibition by molecules such as Apelin-13, while TNF-α levels might be independently influenced by feedback mechanisms or distinct cellular sources [22,23].

Third, the complex nature of TNF-α itself should be considered. As reviewed by Hubbi et al. (2025), TNF-α is not invariably detrimental; TNF-α derived from tubular epithelial cells may have different functions compared to that from immune cells. If the cellular source of TNF-α shifts or remains unactivated, tubular necrosis may decrease even when total TNF-α levels appear unchanged. Furthermore, TNF-α acts rapidly (within hours) to trigger an inflammatory cascade and then returns to baseline. Tubular necrosis represents an accumulated outcome that occurs later; thus, a single time-point measurement may not capture the peak biological activity of TNF-α that initiated early injury. Finally, activation of distinct TNF receptors (TNF-R1 vs. TNF-R2) leads to divergent effects, pro-inflammatory versus protective, respectively. Taken together, the absence of significant quantitative changes in TNF-α does not exclude its potential role in modulating tubular necrosis, but alternative pathways and the temporal–spatial complexity of TNF-α signaling warrant further investigation [24].

This study suggests that *Nigella sativa* ethanol extract has potential as a complementary therapy for preeclampsia by reducing proximal tubular cell necrosis and TNF-α expression, indicating renoprotective and anti-inflammatory effects likely mediated by thymoquinone. These findings contribute to understanding the molecular mechanisms of *Nigella sativa* and support its potential development as a phytopharmaceutical candidate for hypertensive disorders during pregnancy. A significant strength of this study is the simultaneous examination of histological and inflammatory markers; however, its interpretation is limited by several factors, including the small sample size, biological variability, short intervention period, assessment of only one inflammatory biomarker (TNF-α), and the use of an animal model which may not fully reflect human physiology.

The authors acknowledge that the small sample size (n=4 per group) is a major limitation, as it increases the risk of false negatives (Type II errors), which is the failure to detect an effect that actually exists. The low statistical power due to the small sample size makes the study more susceptible to statistically non-significant results, even when clinically or biologically meaningful differences may be present. Therefore, the non-significant findings on the TNF-α parameter in this study are likely attributable to insufficient power, and it cannot be concluded that no effect exists. These findings must be interpreted with caution, and further studies with larger sample sizes are required to confirm or refute the research hypothesis. Moreover, the generalizability of the findings from this study is limited because a small sample cannot adequately represent broader population variability. Consequently, additional studies with larger samples, more diverse biomarkers, and eventual clinical trials are needed to confirm these findings.

Future research is encouraged to evaluate more thorough biomarkers of renal injury and other inflammation in order to address this restriction. Neutrophil Gelatinase-Associated Lipocalin (NGAL) and Kidney Injury Molecule-1 (KIM-1) are two examples of possible biomarkers for proximal tubular injury in preeclampsia. The increase in KIM-1 levels, which is a specific marker of tubular injury, has been shown to correlate strongly with complement activation and the degree of kidney damage in this condition. Combination biomarker studies have proven that the use of several markers simultaneously can increase the sensitivity and specificity of kidney injury diagnosis to over 98%, thus providing much stronger evidence of nephroprotective mechanisms compared to relying solely on TNF-α and tubular necrosis morphology[25–28].

CONCLUSION

Ethanol extract of *Nigella sativa* significantly reduced the percentage of proximal tubular cell necrosis in the kidneys of preeclampsia model mice in a dose-dependent manner. The extract also reduced renal TNF-α expression as the dose increased; however, this effect was not statistically significant. Future studies should include larger

sample sizes to improve statistical power and result reliability. Additional inflammatory, oxidative stress, and renal function biomarkers should be evaluated to better elucidate the mechanisms of *Nigella sativa*. Further research is also needed to determine the optimal dosage and duration of treatment and to assess its potential clinical application.

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CONFLICT OF INTEREST

The authors assert that there are no conflicts of interest pertaining to the publishing of this paper.

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