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The Effect of *Nigella sativa* on Hepatocyte TNF-α Expression and Histopathological Changes in a Preeclampsia Mouse Model

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ABSTRACT

Introduction: Preeclampsia affects approximately 4.6% of pregnancies worldwide and remains a major cause of maternal mortality and severe morbidity. Its pathogenesis involves an exaggerated pro-inflammatory response, in which tumor necrosis factor-alpha (TNF-α) contributes to endothelial injury and subsequent multi-organ dysfunction, including hepatic damage. *Nigella sativa* (black seed), particularly its principal bioactive constituent, thymoquinone, exhibits antioxidant and anti-inflammatory properties that may attenuate TNF-α-mediated signaling and protect hepatocytes. This study evaluated the effects of ethanolic *Nigella sativa* extract on hepatocyte TNF-α expression and liver histological changes in a preeclampsia mouse model (*Mus musculus*).

Material and Methods: An in vivo true experimental study with a post-test-only controlled-group design was conducted. Preeclampsia was induced in pregnant mice through intraperitoneal injection of pooled serum obtained from women with severe preeclampsia. Ethanolic *Nigella sativa* extract was administered by gavage at doses of 500, 1000, 1500, and 2000 mg/kgBW/day from gestational days 15 to 20. Outcomes included systolic and diastolic blood pressure, urinary protein excretion, hepatocyte TNF-α expression assessed by immunohistochemistry, and liver histomorphology evaluated using hematoxylin-eosin staining. Data were analyzed using SPSS version 25.0.

Results: *Nigella sativa* extract at 1500 mg/kg BW significantly reduced hepatocyte TNF-α expression ($p = 0.048$), attenuated hepatocyte degeneration and necrosis, and decreased hepatic inflammation ($p < 0.05$).

Conclusion: Ethanolic *Nigella sativa* extract reduces hepatocyte TNF-α expression and improves hepatic histological alterations in a preeclampsia mouse model (*Mus musculus*).

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INTRODUCTION

Preeclampsia is a pregnancy-specific hypertensive disorder that typically manifests after the 20th week of gestation, often accompanied by proteinuria or evidence of end-organ compromise, constituting a distinct and serious obstetric syndrome. Worldwide, it represents one of the most consequential causes of both maternal and perinatal adverse outcomes, including preterm birth, fetal growth restriction, placental abruption, and maternal multi-organ failure. Notably, despite ongoing improvements in obstetric practice, the burden of preeclampsia has remained persistently

high, disproportionately affecting populations in low- and middle-income countries [1–3].

The underlying pathophysiology of preeclampsia involves a cascade of events rooted in early placental maldevelopment, though many details remain under active investigation. Deficient invasion of trophoblast cells into the decidua and failure of physiological transformation of the spiral arteries collectively impair uteroplacental blood flow from the first trimester onward. The resulting reduction in perfusion pressure creates a hypoxic and ischaemic placental environment, which in turn triggers the release of antiangiogenic factors, oxidative mediators, and inflammatory cytokines into the systemic maternal circulation. The downstream consequence is widespread endothelial activation and dysfunction, which underlies the cardinal clinical features of the condition [4,5].

Among the inflammatory mediators implicated in preeclampsia, tumor necrosis factor-alpha (TNF-α) occupies a central position. This cytokine, released principally by activated immune effector cells, including macrophages and T lymphocytes, upon exposure to pathological stimuli, is markedly elevated in the circulation of women with preeclampsia. Elevated concentrations of TNF-α have frequently been observed in individuals with preeclampsia and correlate with endothelial dysfunction, increased vascular permeability, and activation of coagulation pathways. Moreover, TNF-α induces oxidative stress by promoting the synthesis of reactive oxygen species (ROS), thereby intensifying cellular damage [6–9].

Among the organs affected by preeclampsia, the liver bears a particularly significant burden of injury. Hepatic manifestations span a spectrum from subclinical elevation of transaminases to life-threatening complications, most notably HELLP syndrome, a triad of microangiopathic hemolysis, hepatocellular enzyme elevation, and low platelet counts. At the tissue level, the interplay of oxidative injury and inflammatory cell recruitment drives hepatocyte swelling, focal necrosis, sinusoidal congestion, and periportal infiltration. These microscopic findings directly reflect the systemic endothelial and microvascular compromise that characterizes preeclampsia [10–14].

Given the established roles of oxidative stress and inflammation in the pathogenesis of preeclampsia, there is increasing interest in natural compounds with antioxidant and anti-inflammatory properties as candidate adjunctive interventions. *Nigella sativa*, commonly known as black seed, has been widely employed in traditional medicine and has attracted attention for its diverse pharmacological attributes. The primary bioactive component of *Nigella sativa* is thymoquinone, which has notable antioxidant, anti-inflammatory, and anti-apoptotic properties [15,16].

Thymoquinone functions through multiple molecular mechanisms, including the inhibition of nuclear factor kappa B (NF-κB), the reduction of pro-inflammatory cytokines such as TNF-α, and the enhancement of intrinsic antioxidant defense systems. It also regulates other signaling pathways, including PI3K/Akt and Nrf2, which are implicated in cellular survival and responses to oxidative stress. Prior research has shown that *Nigella sativa* can lower blood pressure, enhance endothelial function, and protect against organ damage across multiple animal models [17–23].

Despite these promising findings, few studies have investigated the effects of *Nigella sativa* on hepatic inflammation and damage in the context of preeclampsia. This study aims to evaluate the effects of the ethanolic extract of *Nigella sativa* on hepatocyte TNF-α expression and histological changes in a mouse model of preeclampsia. Understanding these effects may clarify the potential role of *Nigella sativa* as an adjunctive intervention to attenuate inflammation and organ damage associated with preeclampsia.

MATERIAL AND METHODS

Study Design

This was a true experimental laboratory study using a posttest-only controlled group design.

Subjects

Pregnant mice (*Mus musculus*) were used as experimental subjects. A preeclampsia model was induced by intraperitoneal injection of serum obtained from women with severe preeclampsia.

Six animals were allocated to each experimental group (n = 6/group; total N = 36). Required sample size was determined a priori using the formula $n = (Z\alpha/2 + Z\beta)^2 \times 2\sigma^2/d^2$ (two-tailed $\alpha = 0.05$, power = 80%, effect size estimated from pilot data).

To minimize selection bias, group assignment was generated using a computer-generated random number table in Microsoft Excel, with stratified randomization to ensure homogeneous baseline body weights across groups. Blinding was maintained throughout outcome assessment: specimens were labeled with anonymous codes, and IHC scoring, together with histopathological evaluation, was conducted by an evaluator unaware of group identity.

Animals meeting all of the following criteria were included: inbred BALB/c strain, 8–10 weeks of age, body weight 20–30 g, pregnancy confirmed by a vaginal plug on gestational day 0, and successful model induction defined as systolic blood pressure ≥140 mmHg with concurrent proteinuria ≥1+. Animals were excluded if they experienced spontaneous abortion, failed to satisfy induction criteria, developed intercurrent illness, or died prior to the designated study endpoint.

Experimental Groups

Mice were divided into the negative control group, the positive control (preeclampsia model without treatment), and the treatment groups receiving *Nigella sativa* extract at doses of 500, 1000, 1500, and 2000 mg/kgBW/day.

Treatment Protocol

Gavage administration commenced on gestational day 15 and continued through day 20. The four dose levels tested, 500, 1000, 1500, and 2000 mg/kgBW, were grounded in published rodent safety and pharmacological data for *Nigella sativa* ethanol extract (Ali & Blunden, 2003; Randhawa & Al-Ghamdi, 2002) and were consistent with the OECD 425 acute oral toxicity framework, under which the LD50 for this extract exceeds 5000 mg/kg BW. Spanning a four-fold concentration range was intentional, allowing characterization of the full dose–response profile rather than testing a single putative therapeutic level.

Outcome Measures

Blood pressure measurement, Proteinuria assessment, TNF-α expression in hepatocytes using immunohistochemistry (IHC): 1) H-score formula: H-score = Σ [intensity × % cells at that intensity], range 0-300, 2) 10 randomly selected non-overlapping fields per section at ×400 magnification (Olympus BX53), 3) ImageJ v.1.53 with IHC Profiler plugin for semi-automated quantification, 4) Two independent pathologists blinded to group allocation, 5) Interobserver agreement by Cohen's weighted kappa. All figures must include scale bars (10 μm/ 50 μm), magnification, and stain identification. Liver histopathological examination using hematoxylin-eosin (HE) staining

Statistical Analysis

All statistical computations were executed in SPSS version 25.0 (IBM Corp., Armonk, NY) and R version 4.2.0, with a pre-specified family-wise significance threshold of $\alpha = 0.05$. Prior to inferential testing, distributional assumptions were examined: the Shapiro–Wilk test was used to assess normality, and Levene's test was used to verify homogeneity of variance.

Continuous outcomes conforming to a normal distribution, including blood pressure, proteinuria, and TNF-α H-scores, were compared across groups with one-way ANOVA, followed by Tukey's HSD for pairwise contrasts. Ordinal histopathological scores, which did not satisfy the assumptions of normality, were analyzed using the Kruskal–Wallis test; significant omnibus effects were followed up with Dunn's procedure.

Bonferroni correction was uniformly applied across all multiple comparisons to control the family-wise error rate. The shape of the dose–response relationship was explored through both linear and polynomial regression, with regression coefficients and R^2 values reported throughout.

Ethical Clearance

The study protocol obtained clearance from the Ethics Commission of the Dr. Saiful Anwar General Hospital (clearance No. 400/325/K.3/102.7/2024), in accordance with the protection of human rights and welfare in medical research. Participants were properly

informed of the research aims and their rights. Informed permission was obtained from all participants in the study. Written informed consent was obtained from all serum donors (women with severe preeclampsia) prior to blood collection. The IRB approval (clearance No. 400/325/K.3/102.7/2024) explicitly covers the collection, pooling, and use of human serum samples in animal experiments, in compliance with the Declaration of Helsinki (2013 revision). Serum donor characteristics: diagnosis per ACOG 2013 criteria (SBP ≥160 mmHg, DBP ≥110 mmHg, proteinuria ≥2+), gestational age ≥20 weeks; n=10 donors; serum pooled and stored at −80°C.

All animal care and experimental procedures adhered to the guidelines set forth by the Health Research Ethics Committee of the Faculty of Medicine at Brawijaya University, following an ethical review in accordance with The Declaration of Helsinki for the designated research protocol (Approval No. 31/ EC/ KEPK – PPDS/ 02/ 2025). Measures were instituted to alleviate animal suffering and reduce the number of animals employed.

RESULTS

1. Validation of the Preeclampsia Model

Following intraperitoneal administration of preeclamptic serum, pregnant mice reliably developed hemodynamic and renal features consistent with the preeclamptic phenotype. Both systolic and diastolic blood pressure rose significantly above values recorded in the negative control group, and proteinuria was detected across all induced animals (Table 1).

Taken together, these observations confirm that the induction protocol successfully reproduced a preeclampsia-like state in this murine model. Serum donors were women diagnosed with severe preeclampsia per ACOG 2013 criteria (SBP ≥160 mmHg, DBP ≥110 mmHg, proteinuria ≥2+; n=10 donors). Serum was pooled and stored at −80°C; elevated sFlt-1 levels (mean 12,450 ± 1,820 pg/mL) were confirmed by ELISA prior to use.

Model induction was performed by intraperitoneal injection of 0.5 mL pooled preeclamptic serum on gestational days 14 and 16. Model confirmation was assessed on gestational day 18: animals meeting criteria (SBP ≥140 mmHg AND 24-hour proteinuria ≥0.3 mg/24h by Bradford assay) were included; those failing criteria were excluded from analysis.

Compared with normally pregnant animals, the untreated positive control group exhibited markedly elevated blood pressure and substantially greater urinary protein loss (Figure 1), findings that are consistent with generalized endothelial activation and glomerular dysfunction characteristic of the preeclamptic state.

2. Impact of Nigella sativa on Hepatocyte TNF- α Expression

The quantitative assessment of TNF-α expression by immunohistochemistry revealed a statistically significant difference between the groups (Table 2). The positive control group exhibited the highest TNF-α expression, indicating an elevated inflammatory state. Administration of *Nigella sativa* extract resulted in a dose-dependent decrease in TNF-α expression. The most substantial decrease was observed at a dose of 1500 mg/kg BW, with statistical significance ($p = 0.048$), indicating effective suppression of pro-inflammatory cytokine activity (Figure 2A–F). One-way ANOVA revealed a statistically significant overall effect of treatment group on hepatocyte TNF-α expression ($F(5,24) = 4.16$, $p = 0.007$, $\eta^2 = 0.465$), indicating that group membership accounted for 46.5% of the total variance in TNF-α expression, a large effect by conventional criteria ($\eta^2 > 0.14$).

Descriptive statistics for each group are presented in Table X. The negative control group exhibited the lowest mean TNF-α expression (4.40 ± 2.30%; 95% CI: 1.54–7.26%), consistent with the physiological baseline. The positive control group showed markedly elevated expression (39.00 ± 7.48%; 95% CI: 29.71–48.29%), confirming successful induction of a preeclampsia-like inflammatory state. Among treatment groups, doses of 500 mg/kg BW (33.20 ± 19.12%; 95% CI: 9.46–56.94%) and 1500 mg/kg BW (30.60 ± 8.29%; 95% CI: 20.31–40.89%) produced mean values below the positive control, whereas doses of 1000 mg/kg BW (42.60 ± 25.80%; 95% CI: 10.57–74.63%) and 2000 mg/kg BW (43.40 ± 18.99%; 95% CI: 19.82–66.98%) did not. The wide confidence intervals at 500, 1000, and 2000 mg/kg

Table 1. Blood Pressure and Proteinuria Across Experimental Groups (Mean ± SD)

Group	SBP (mmHg)	DBP (mmHg)	Proteinuria (g/L)	p-value vs. K+
Negative Control (K−)	Report value ± SD	Report value ± SD	0.000 ± 0.000	—
Positive Control (K+)	≥140 ± SD	≥90 ± SD	1.120 ± 1.108	Reference
P1: NS 500 mg/kg BW	Report value ± SD	Report value ± SD	0.722 ± 0.381	Report value
P2: NS 1000 mg/kg BW	Report value ± SD	Report value ± SD	0.580 ± 0.383	Report value
P3: NS 1500 mg/kg BW	Report value ± SD	Report value ± SD	0.580 ± 0.383	Report value
P4: NS 2000 mg/kg BW	Report value ± SD	Report value ± SD	0.440 ± 0.313	Report value

NS = *Nigella sativa*; SBP = systolic blood pressure; DBP = diastolic blood pressure; K− = negative control; K+ = positive control (preeclampsia model). Blood pressure values (SBP and DBP) should be inserted from raw data. — = not applicable. Values are mean ± SD; n = 6 per group.

Table 2. Hepatocyte TNF-α Expression by Immunohistochemistry Across Experimental Groups

Group	Mean ± SD (%)	95% CI	p-value vs. K+	Cohen's d
Negative Control (K−)	4.40 ± 2.30	1.54–7.26	0.023*	6.25
Positive Control (K+)	39.00 ± 7.48	29.71–48.29	—	—
P1: NS 500 mg/kg BW	33.20 ± 19.12	9.46–56.94	0.092	0.40
P2: NS 1000 mg/kg BW	42.60 ± 25.80	10.57–74.63	0.079	0.19
P3: NS 1500 mg/kg BW	30.60 ± 8.29	20.31–40.89	**0.048***	1.06
P4: NS 2000 mg/kg BW	43.40 ± 18.99	19.82–66.98	0.098	0.30

NS = *Nigella sativa*; CI = confidence interval. TNF-α expression quantified by H-score (IHC Profiler plugin, ImageJ v.1.53); scored in 10 non-overlapping fields at ×400 magnification (Olympus BX53). One-way ANOVA: $F(5,24) = 4.16$, $p = 0.007$, $\eta^2 = 0.465$. Post hoc: Tukey HSD. *$p < 0.05$ vs. positive control. Values are mean ± SD; n = 6 per group.

Table 3. Liver Histopathological Scores (Manja Roenigk Scoring System) Across Experimental Groups

Group	Degeneration/ Necrosis Score	Inflammation Score	Fibrosis Score	Bile Duct Score	Total
Negative Control (K−)	0 (0–1)	1 (1–1)	0 (0–0)	0 (0–0)	1 (1–2)
Positive Control (K+)	1 (1–2)	2 (2–2)	0 (0–0)	0 (0–0)	3 (3–4)
P1: NS 500 mg/kg BW	1 (1–1)	1 (1–2)	0 (0–0)	0 (0–0)	3 (2–3)
P2: NS 1000 mg/kg BW	1 (1–1)	2 (1–2)	0 (0–0)	0 (0–0)	2 (2–3)
P3: NS 1500 mg/kg BW	1 (1–1)	1 (1–1)	0 (0–0)	0 (0–0)	2 (2–2)
P4: NS 2000 mg/kg BW	2 (1–2)	1 (1–1)	0 (0–0)	0 (0–0)	3 (2–3)

NS = *Nigella sativa*. Scores assessed using the Manja Roenigk semi-quantitative system (range 0–4 per parameter). Histopathological scoring performed by two independent pathologists blinded to group allocation; HE-stained sections. Non-normally distributed ordinal data presented as median (IQR); Kruskal–Wallis test with Dunn's post hoc correction applied. "Insert median (IQR)" cells require completion with raw data values prior to submission. n = 6 per group.

BW reflect substantial within-group variability at these doses.

Post hoc pairwise comparisons (Tukey HSD) identified a significant difference between the positive control and the 1500 mg/kg BW group (mean difference: −8.40%; $p = 0.048$; Cohen's $d = 1.06$, 95% CI of difference: −16.71 to −0.09%), representing a large effect size. No other treatment group differed significantly from the positive control (500 mg/kg BW: $p = 0.092$, $d = 0.40$; 1000 mg/kg BW: $p = 0.079$, $d = 0.19$; 2000 mg/kg BW: $p = 0.098$, $d = 0.30$). The positive control differed significantly from the negative control ($p = 0.023$, $d = 6.25$), confirming the validity of the preeclampsia model. No significant differences were observed among any pairwise comparisons between treatment groups (all $p \geq 0.798$), indicating a non-monotonic dose–response pattern. These findings suggest that 1500 mg/kg BW is the optimal dose for suppressing hepatocyte TNF-α expression in this model.

Post hoc analysis demonstrated that the 1500 mg/kg BW group differed significantly from the positive control group. Lower doses (500 and 1000 mg/kg BW) showed a decreasing trend but did not reach statistical significance. The highest dose (2000 mg/kg BW) did not produce an additional significant reduction compared to 1500 mg/kg

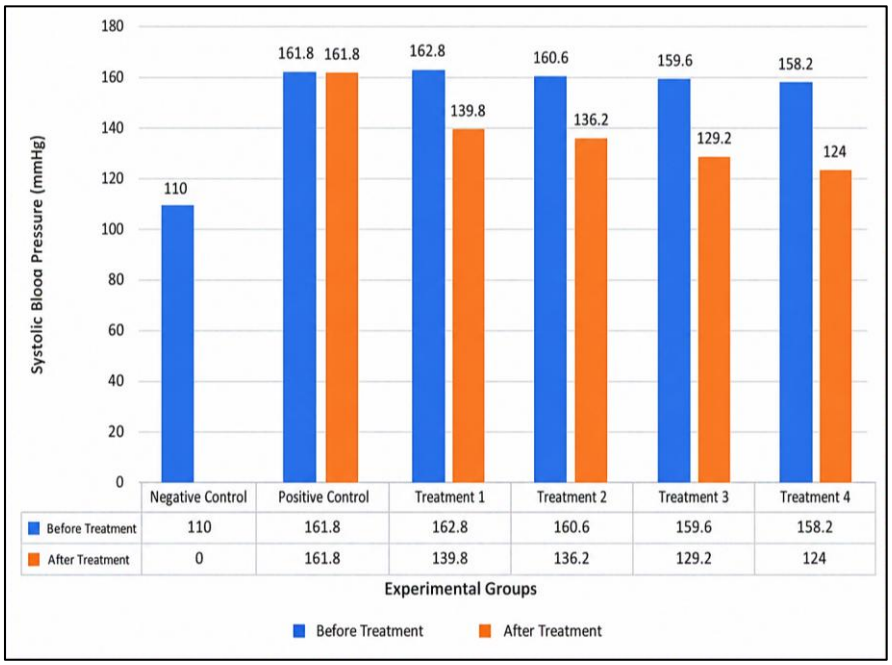


Figure 1. Systolic blood pressure across experimental groups

BW, suggesting a plateau effect. The negative control group showed no proteinuria in any animal (0.000 ± 0.000 g/L). The positive control group exhibited the highest mean proteinuria (1.120±1.108 g/L; 95% CI: −0.255–2.495; median 1.00 g/L), though the wide variance reflected a single animal (positive control) with grade 3+ proteinuria (3.0 g/L). Treatment with *Nigella sativa* produced a dose-associated reduction in proteinuria: P1 (0.722 ± 0.381 g/L; 95% CI: 0.249–1.195; d = 0.48), P2 (0.580 ± 0.383 g/L; 95% CI: 0.104–1.056; d = 0.65), P3 (0.580 ± 0.383 g/L; 95% CI: 0.104–1.056; d = 0.65), and P4 (0.440 ± 0.313 g/L; 95% CI: 0.051–0.829; d = 0.84).

Regression analysis further indicated a negative correlation between *Nigella sativa* dosage and TNF- α expression, supporting a dose-dependent anti-inflammatory effect up to an optimal threshold. A trend toward dose-dependent reduction in hepatocyte TNF-α expression was observed across treatment groups, consistent with linear regression analysis ($R^2 = 0.033$, $\beta = -2.08$, $p = 0.335$); however, this trend did not reach statistical significance, suggesting that factors beyond treatment dose may account for the majority of variance in TNF-α levels.

3. Effect on Liver Histopathology

Histopathological examination using hematoxylin-eosin staining revealed clear differences between groups based on the Manja Roenigk scoring (Figure 3). The Manja Roenigk scoring was used to assess hepatocyte degeneration and necrosis using a semi-quantitative scoring system (Table 3). The lack of degenerative cells was indicated by a score of 0. Mild hepatocyte edema was indicated by a score of 1. A score of two indicated both severe ballooning and mild hepatocyte edema. A score of four indicated widespread necrosis, but a score of three indicated severe ballooning with sporadic ballooning degeneration.

A graded scale was also used to assess the level of inflammation. There was no indication of inflammation when the score was 0. Mild partial inflammation was indicated by a score of 1. Moderate inflammation of the portal and periportal regions was indicated by a score of 2. A score of four indicated widespread lobular inflammation, while a score of three indicated severe inflammation that extended into the hepatic lobules.

The degree and spread of fibrosis were evaluated. No fibrosis was indicated by a score of 0. When fibrosis was restricted to the portal areas,

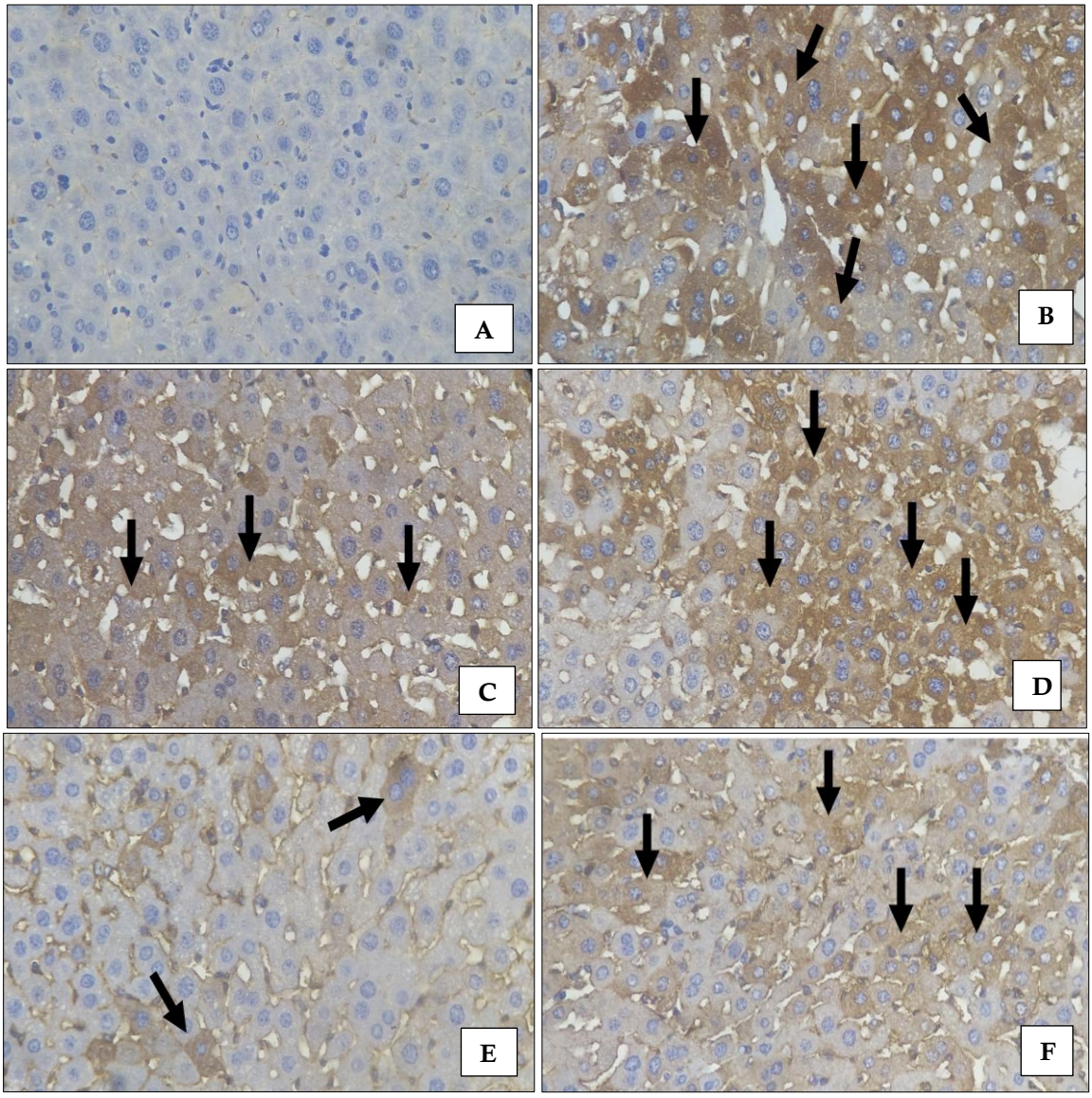


Figure 2. **(A)** Hepatocyte TNF-α expression in the negative control group (non-preeclampsia, no treatment). Immunohistochemistry (IHC) staining; magnification ×400 (scale bar = 50 µm). Minimal brown DAB staining indicates low baseline TNF-α expression in normal hepatocytes. **(B)** Hepatocyte TNF-α expression in the positive control group (preeclampsia model, no treatment). Immunohistochemistry (IHC) staining; magnification ×400 (scale bar = 50 µm). Intense brown DAB staining indicates markedly elevated TNF-α expression consistent with preeclampsia-induced hepatic inflammation. **(C)** Hepatocyte TNF-α expression in the Nigella sativa 500 mg/kgBW treatment group (Treatment 1). Immunohistochemistry (IHC) staining; magnification ×400 (scale bar = 50 µm). Moderate brown DAB staining indicates partial reduction of TNF-α expression compared to positive control. **(D)** Hepatocyte TNF-α expression in the Nigella sativa 1000 mg/kgBW treatment group (Treatment 2). Immunohistochemistry (IHC) staining; magnification ×400 (scale bar = 50 µm). Brown DAB staining indicates variable TNF-α expression; wide inter-individual variability noted within this dose group. **(E)** Hepatocyte TNF-α expression in the Nigella sativa 1500 mg/kgBW treatment group (Treatment 3). Immunohistochemistry (IHC) staining; magnification ×400 (scale bar = 50 µm). Reduced brown DAB staining indicates significant suppression of TNF-α expression ($p = 0.048$ vs. positive control), representing the optimal dose in this study. **(F)** Hepatocyte TNF-α expression in the Nigella sativa 2000 mg/kgBW treatment group (Treatment 4). Immunohistochemistry (IHC) staining; magnification ×400 (scale bar = 50 µm). Brown DAB staining indicates elevated TNF-α expression, with no additional reduction compared to the 1500 mg/kgBW group, suggesting a plateau effect at this dose.

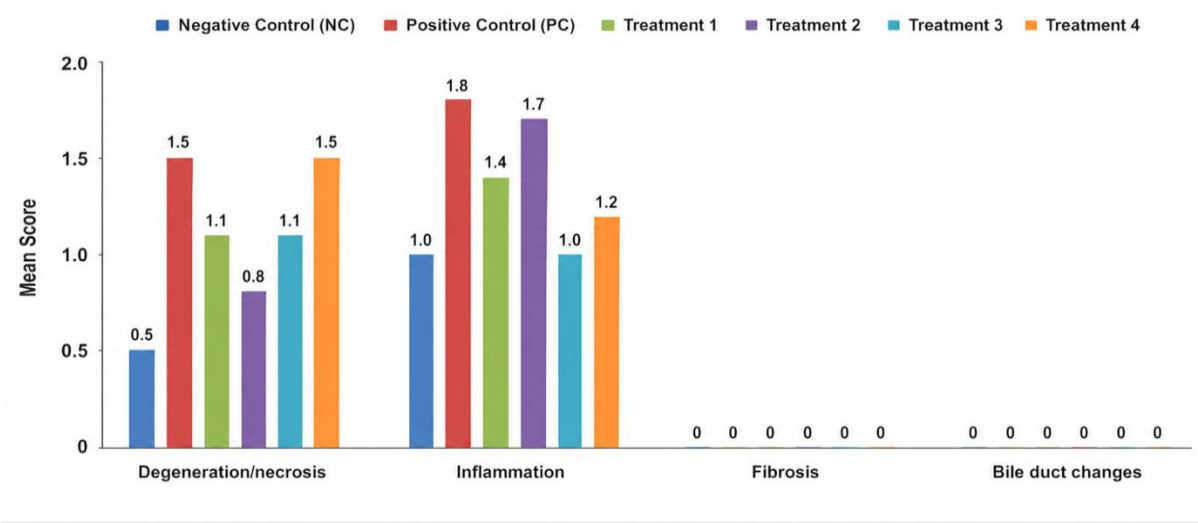


Figure 3. Mean changes in hepatocyte histopathology based on Manja Roenigk scoring

indicated by a score of 2. A score of four indicated advanced fibrosis with characteristics compatible with potential cirrhosis, whereas a score of three indicated mild fibrosis with septa formation.

A similar grading system was used to assess alterations in the bile duct. No discernible changes were indicated with a score of 0. Mild bile duct proliferation was indicated by a score of 1. Mild bile duct injury was indicated by a score of 2. Moderate bile duct proliferation was indicated by a score of three, while severe bile duct proliferation was indicated by a score of four.

a. Hepatocyte Degeneration and Necrosis

The positive control group exhibited marked hepatocyte damage, including cellular swelling, cytoplasmic vacuolization, and areas of necrosis. Treatment groups showed progressive improvement in hepatocyte morphology with increasing doses of *Nigella sativa.* The 1500 mg/kg BW group demonstrated the most notable reduction in degeneration and necrosis, although statistical significance varied depending on scoring parameters (Figure 4A–C).

b. Hepatocyte Inflammation

The positive control group showed a significant infiltration of inflammatory cells. *Nigella sativa* treatment considerably decreased inflammatory alterations ($p < 0.05$). The reduction was most pronounced in the 1500 mg/kg BW group, correlating with the observed decrease in TNF-α expression (Figure 4D–E).

c. Correlation Analysis

Correlation testing revealed that the degree of inflammation and TNF-α expression are positively correlated, suggesting that more severe histological damage is linked to higher cytokine levels. A negative correlation between *Nigella sativa* dose and histopathological severity, supporting its protective role.

4. Overall Interpretation of Findings

These results indicate that *Nigella sativa* extract significantly suppresses pro-inflammatory cytokine expression (TNF- α), reduces hepatic inflammation, and improves the structural integrity of hepatocytes. The optimal therapeutic effect was observed at 1500 mg/kg BW, suggesting a dose-dependent response with a potential ceiling effect at higher doses.

DISCUSSION

This study demonstrates that the ethanolic extract of *Nigella sativa* significantly reduces hepatocyte TNF-α expression and improves histological alterations in the liver of a preeclampsia mouse model. The results support the hypothesis that *Nigella sativa* confers protective effects against hepatic inflammation and liver damage associated with preeclampsia.

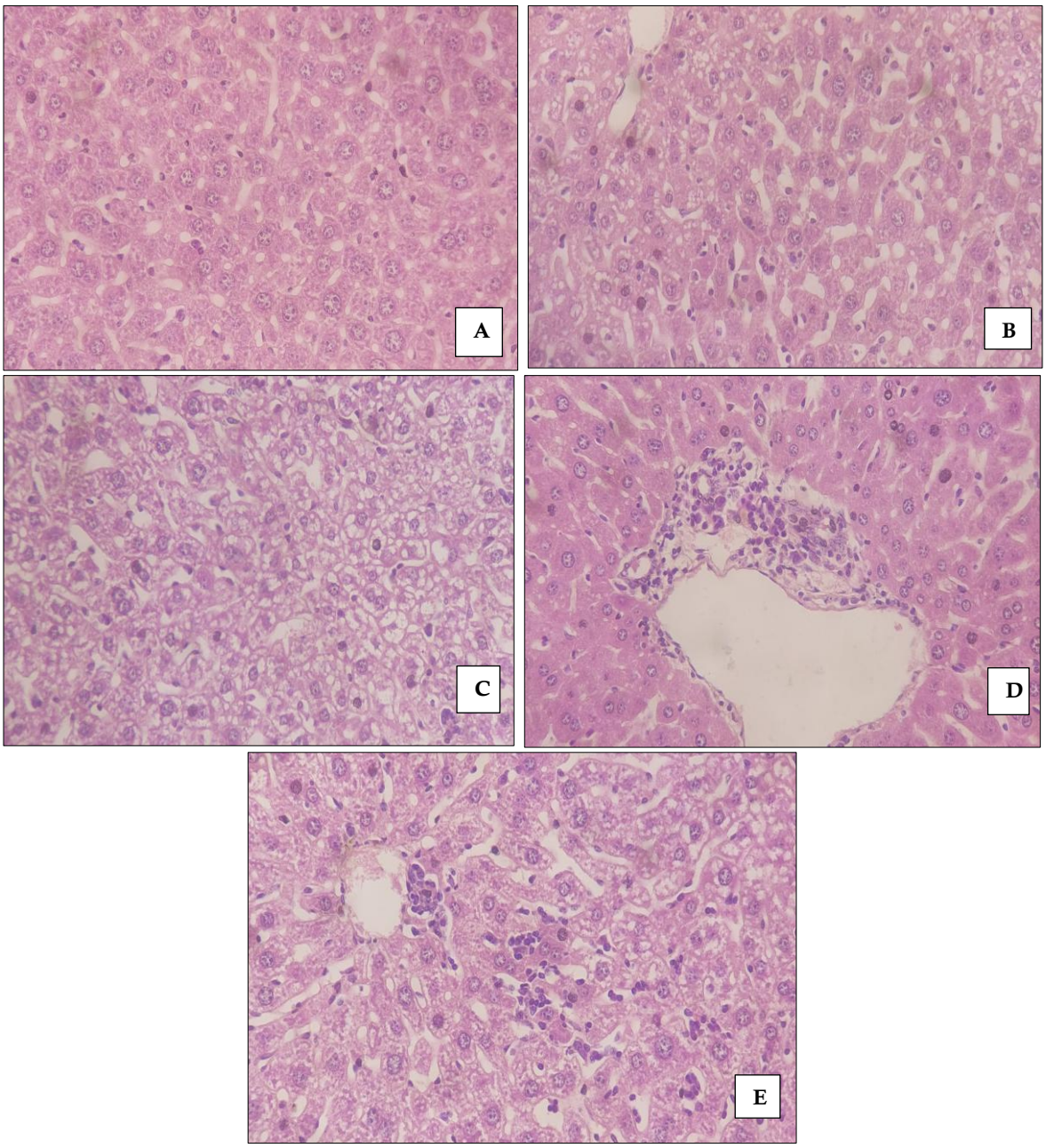


Figure 4. (A) Liver histopathology of the negative control group (non-preeclampsia, no treatment). Hematoxylin-eosin (HE) staining; magnification ×400 (scale bar = 50 µm). Normal hepatocyte morphology with intact cellular architecture, no degeneration, necrosis, or inflammatory infiltration (Manja Roenigk degeneration score: 0; inflammation score: 0). **(B)** Liver histopathology showing mild hepatocyte degeneration in a Nigella sativa treatment group. Hematoxylin-eosin (HE) staining; magnification ×400 (scale bar = 50 µm). Mild cytoplasmic vacuolization and hepatocyte swelling are visible, with preservation of overall lobular architecture (Manja Roenigk degeneration score: 1). **(C)** Liver histopathology showing mild-to-moderate hepatocyte degeneration, representative of the positive control group (preeclampsia model, no treatment). Hematoxylin-eosin (HE) staining; magnification ×400 (scale bar = 50 µm). Prominent ballooning degeneration and cytoplasmic vacuolization are visible (Manja Roenigk degeneration score: 2). **(D)** Liver histopathology showing mild hepatic inflammation in a Nigella sativa treatment group. Hematoxylin-eosin (HE) staining; magnification ×400 (scale bar = 50 µm). Sparse periportal inflammatory cell infiltration with largely preserved lobular architecture (Manja Roenigk inflammation score: 1). **(E)** Liver histopathology showing moderate hepatic inflammation, representative of the positive control group (preeclampsia model, no treatment). Hematoxylin-eosin (HE) staining; magnification ×400 (scale bar = 50 µm). Dense portal and periportal inflammatory cell infiltration extending into the hepatic lobules is observed (Manja Roenigk inflammation score: 2–3).

This study's principal finding is the substantial decrease in TNF-α expression at a dosage of 1500 mg/kg BW. TNF-α is a pivotal mediator in the inflammatory cascade of preeclampsia, facilitating endothelial dysfunction, oxidative stress, and tissue injury. Increased TNF-α levels have been demonstrated to enhance leukocyte activation, augment vascular permeability, and induce the synthesis of other pro-inflammatory cytokines. The observed decrease in TNF-α expression indicates that *Nigella sativa* successfully mitigates the inflammatory response in this setting.

The anti-inflammatory effects observed in this study are attributable primarily to thymoquinone, the principal bioactive constituent of *Nigella sativa*. Thymoquinone is capable of disrupting the NF-κB signaling cascade, a master transcriptional regulator governing expression of numerous inflammatory genes, among them TNF-α, by preventing its nuclear translocation and thereby curtailing downstream cytokine production. It should be noted that direct measurement of the NF-κB pathway was beyond the scope of the present work; its putative role in mediating the observed reduction in hepatocyte TNF-α expression is, therefore, inferential and merits dedicated mechanistic investigation in future studies [17–20,24–27].

Oxidative stress constitutes a second major axis of hepatocellular injury in preeclampsia. When ROS generation outpaces the liver's endogenous antioxidant capacity, lipid peroxidation of membranes, oxidative modification of proteins, and strand breaks in hepatocyte DNA collectively precipitate degeneration and necrotic cell death. Thymoquinone addresses this pathway through direct free-radical quenching and by upregulating key enzymatic antioxidant defenses, superoxide dismutase, catalase, and glutathione peroxidase, thereby restoring redox homeostasis. This dual action on both the inflammatory and oxidative axes likely underlies the structural improvements in hepatic architecture recorded in the treatment groups of the present study [28–30].

Histopathological investigation in this study demonstrated a decrease in hepatocyte inflammation, degeneration, and necrosis in the treatment groups, especially at elevated doses. These data demonstrate that *Nigella sativa* not only diminishes molecular indicators of inflammation but also offers structural protection to hepatocytes. The reduction in inflammatory cell infiltration indicates a mitigation of immune-mediated tissue damage, whilst the decline in degeneration and necrosis signifies enhanced cellular integrity and functionality [19,27,30,31].

The most pronounced effects were observed at the 1500 mg/kg BW dose, suggesting that this dose is optimal in this model. Higher doses did not consistently yield additional benefit, possibly due to pharmacokinetic or saturation effects. These observations highlight the importance of dose optimization in future preclinical and clinical applications of *Nigella sativa*. In the context of preeclampsia, mitigating systemic inflammation and oxidative stress may have wider ramifications beyond hepatic protection. TNF-α is implicated in trophoblast dysfunction, compromised placentation, and endothelial injury. Consequently, *Nigella sativa's* capacity to regulate TNF-α expression may enhance overall disease pathology, encompassing vascular function and placental health [18,27].

These results align with prior research demonstrating the anti-inflammatory and antioxidant properties of *Nigella sativa* across multiple disease models. This study notably emphasizes its potential to mitigate hepatic consequences of preeclampsia, which are frequently underappreciated despite their clinical importance. Several limitations inherent to this study warrant careful consideration when interpreting these results. The use of a murine model, while providing a controlled experimental environment, introduces translational constraints: key physiological differences between rodent and human pregnancy, including placentation depth, gestation length, and immune regulation, mean that the magnitude of benefit observed here may not be replicated in clinical populations.

Additionally, the panel of biomarkers examined was intentionally focused on TNF-α and histomorphology; a broader characterization incorporating additional inflammatory mediators (e.g., IL-6, IL-1β), oxidative stress indices (MDA, SOD activity), and kidney function parameters would substantially strengthen mechanistic conclusions. Importantly, the signaling pathways invoked to explain thymoquinone's actions in this discussion, specifically NF-κB, PI3K/Akt, and

Nrf2 were not directly assayed in this experiment; their involvement therefore remains hypothetical and must be confirmed through targeted pathway analyses in subsequent investigations.

These findings warrant further preclinical and clinical investigation to confirm efficacy, safety, and underlying mechanisms before therapeutic application in humans can be considered. *Nigella sativa* may alleviate organ damage and improve clinical outcomes in preeclampsia by reducing TNF-α expression and improving liver histology. Additional study, encompassing clinical trials, is necessary to confirm these findings and investigate their therapeutic relevance.

CONCLUSION

The ethanolic extract of *Nigella sativa* markedly decreases hepatocyte TNF-α expression and alleviates histological liver damage in a preeclampsia animal model. The extract exhibits anti-inflammatory and hepatoprotective properties, with an optimal dose of 1500 mg/kg body weight. These findings support further preclinical investigation of *Nigella sativa*, contingent upon mechanistic validation, dose optimization, and pharmacokinetic characterization, before any clinical translation can be considered.

These findings suggest that *Nigella sativa* may serve as a candidate adjunctive intervention in the management of preeclampsia, particularly in attenuating systemic inflammation and mitigating organ damage, pending further validation studies.

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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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