Avicenna Veterinary Research
Vol. 1, No. 3, Summer 2025: 1-5
Bu-Ali Sina University

ORIGINAL ARTICLE

Protective Effects of Hydroalcoholic Extract of *Nigella Sativa* on Enzymatic Activity and Kidney Histopathology in Mice Infected with *Plasmodium Berghei*

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Article info

Article history:
Received 2025-04-12
Received in revised form
2025-05-26
Accepted 2025-08-10

Keywords:
Plasmodium berghei
Malaria
Nigella sativa
Renal histology

Abstract

Nigella sativa is a medicinal plant traditionally used for the treatment of various diseases. The presence of essential fatty acids in *Nigella sativa* has contributed to its anticancer properties and its effectiveness in promoting skin health. This study aimed to evaluate the protective effect of the hydroalcoholic extract of *Nigella sativa* on enzymatic activity and renal histopathology in mice infected with *Plasmodium berghei*. A total of 35 adult male mice, were divided into 7 groups. The mice were infected with *P. berghei* to induce malaria. After confirmation of infection, the mice were treated with *Nigella sativa* extract for 4 consecutive days. At the end of the treatment period, blood samples were collected to measure serum levels of urea and creatinine. Additionally, kidney tissues were subjected to histopathological examination. The results demonstrated that the *Nigella sativa* extract at a dose of 400 mg/kg reduced serum levels of urea and creatinine compared to the negative control group (PBS). Histopathological assessment of the kidney tissues revealed no significant morphological differences between the control and treated groups. Furthermore, the lumen and epithelial lining of the renal convoluted tubules in the extract-treated group appeared similar to those in the negative control group, with no statistically significant changes observed ($p>0.05$). Based on these findings, further long-term studies are recommended to investigate the potential of *Nigella sativa* extract in reducing elevated urea and creatinine levels in various pathological conditions. This extract may serve as an effective agent in managing renal dysfunction associated with different diseases.

1. Introduction

Malaria is one of the most common and deadly parasitic diseases worldwide, particularly in tropical and subtropical regions. In experimental models, *Plasmodium berghei* is the primary parasite used to induce malaria in mice, where it causes severe and often fatal infections. Due to its high pathogenicity in rodents, *P. berghei* is widely employed as a model organism for studying malaria pathogenesis and potential treatments *in vivo* (Hajialiani et al., 2021; Elmi et al., 2019).

Current treatments for malaria include a range of antimalarial drugs such as artemisinin, primaquine, chloroquine, mefloquine, and lumefantrine. However,

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http://dx.doi.org/10.22084/AVR.2025.30802.1003

in recent years, the emergence of drug-resistant strains-especially resistance to chloroquine-has become a major public health concern in many developing countries (Elmi et al., 2020; Wicht et al., 2020). Moreover, the side effects associated with antimalarial drugs and the increasing resistance of parasites to these therapies have significantly limited their effectiveness and sustainability. As a result, there is an urgent need to explore alternative therapeutic strategies for malaria management.

Malaria infection can cause severe damage to various internal organs, including the kidneys. Renal failure is one of the most serious and life-threatening complications of severe malaria, emphasizing the importance of investigating potential protective interventions against malaria-induced renal injury (Mishra and Das, 2008; Das, 2022).

Iran's diverse geography and climate provide a rich habitat for various medicinal plants, including *Nigella sativa* (black seed), a flowering plant from the Ranunculaceae family with pale blue to deep blue flowers. Traditionally, *Nigella sativa* has been used to treat numerous conditions, including bronchitis, asthma, rheumatism, and various skin disorders.

The seeds of *Nigella sativa* contain a wide range of bioactive compounds, including volatile and fixed oils, proteins, alkaloids, phenolic compounds, flavonoids, saturated and unsaturated fatty acids, terpenoids, and saponins (Shafodino et al., 2022; Alberts et al., 2024). The plant is known for its diverse pharmacological properties, such as antioxidant, anti-inflammatory, anticancer, analgesic, antimicrobial, and nephroprotective effects.

Given the rich therapeutic potential of *Nigella sativa* and the lack of studies investigating its protective effects on enzymatic activity and kidney tissue in malaria-infected individuals, this study was designed to evaluate, for the first time, the protective role of the hydroalcoholic extract of *Nigella sativa* seeds on renal histopathology and biochemical parameters in mice infected with *Plasmodium berghei*.

2. Materials and Methods

2.1. Extraction of Nigella Sativa

Fresh and pure *Nigella sativa* seeds were purchased and prepared for extraction. The seeds were first ground into a fine powder using an electric grinder. The resulting powder was then soaked in methanol for 72 hours (Khanabadi et al., 2022). After the soaking period, the mixture was filtered, and centrifugation was performed to eliminate any remaining suspended particles. The supernatant was separated, and the solution was concentrated using a rotary evaporator. Finally, the concentrated extract was freeze-dried (lyophilized) to obtain a dry powder. Various concentrations of the extract were prepared from this powder for experimental use.

2.2. Animal Preparation and Grouping

In this experiment, 35 adult male Syrian mice with an average body weight of 23±2 grams were used. The mice were acclimatized to the laboratory environment for one week prior to the experiment. Animals were housed in individual cages under controlled conditions (temperature: 22-26°C, relative humidity: 40–70%, and a 12-hour light/dark cycle) with ad libitum access to food and water. The mice were then randomly divided into seven groups.

All groups except the seventh were infected with *Plasmodium berghei* to induce malaria. After confirmation of infection, the mice received treatment with *Nigella sativa* extract for four consecutive days (Elmi et al., 2022). The dosage of the extract was determined based on established therapeutic limits. Group 1 served as the negative control (PBS), Group 2 as the positive control (chloroquine), and Groups 3 through 6 received 50, 100, 200, and 400 mg/kg of *Nigella sativa* extract, respectively. Group 7 included healthy, untreated mice and was used to monitor baseline mortality during the study.

2.3. Sample Collection

At the end of the treatment period, the mice were anesthetized, and blood was collected directly from the heart. Following blood collection, the animals were euthanized by cervical dislocation. Blood samples were centrifuged at 3000 rpm for 5 minutes to separate the serum, which was then stored at –80°C for biochemical analysis. Kidney tissues were preserved in 10% formalin for histopathological evaluation.

2.4. Histological Section Preparation

Kidney samples were fixed in 10% buffered formalin at room temperature for histomorphometric analysis. The tissues were dehydrated through graded ethanol concentrations (70%, 80%, 90%), cleared in xylene, and embedded in molten paraffin for 4 hours. Paraffin blocks were sectioned using a microtome to obtain 5 μm thick slides, which were then stained with hematoxylin and eosin (H&E) (Elmi et al., 2019).

2.5. Histometric Analysis of Kidney Samples

Histological sections of the kidneys were examined for Bowman's capsule integrity, glomerular network structure, degeneration of proximal and distal tubules, vascular congestion, and interstitial edema. Histopathological alterations were scored on a scale from 0 to 3: 0 = no change, 1=mild changes, 2=moderate changes, and 3=severe changes.

2.6. Hematological and Biochemical Analyses

Biochemical parameters, including serum urea and creatinine levels, were measured using spectrophotometry, following the instructions provided with the commercial assay kits. Serum biochemical analyses were conducted using an automated analyzer.

2.7. Statistical Analysis

All data were analyzed using SPSS software version 22. One-way analysis of variance (ANOVA) and the Kruskal-Wallis test were employed for statistical comparisons. Data were presented as mean ± standard deviation (SD), and a p-value of less than 0.05 was considered statistically significant

3. Results

3.1. Body Weight of Mice

The analysis of body weight among different groups showed that the average weight of mice in the negative control group was 26.8 g, in the positive control group was 26.2 g, and in the group treated with 400 mg/kg of *Nigella sativa* extract was 27 g. According to the results, there was no statistically significant difference in body weight among the groups ($p>0.05$). These findings suggest that treatment with *Nigella sativa* extract had no significant effect on the body weight of mice (Fig. 1).

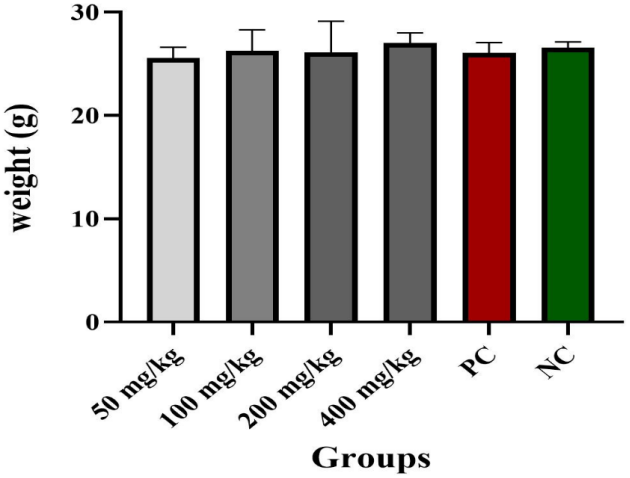

Fig. 1. Comparison of body weight in malaria-infected mice following treatment in negative control (NC), positive control (PC), and different doses of *Nigella sativa* extract.

3.2. Serum Urea Levels

Treatment with *Nigella sativa* extract at doses of 100, 200, and 400 mg/kg significantly reduced serum urea levels compared to the negative control group receiving PBS ($p<0.05$). The most prominent reduction was observed in the group treated with 400 mg/kg of the extract, where serum urea levels decreased from 29.06 mg/dL to 26.18 mg/dL (Fig. 2). This significant decrease in serum urea indicates a nephroprotective effect of the extract in improving kidney function and reducing uremic toxicity in malaria-infected mice.

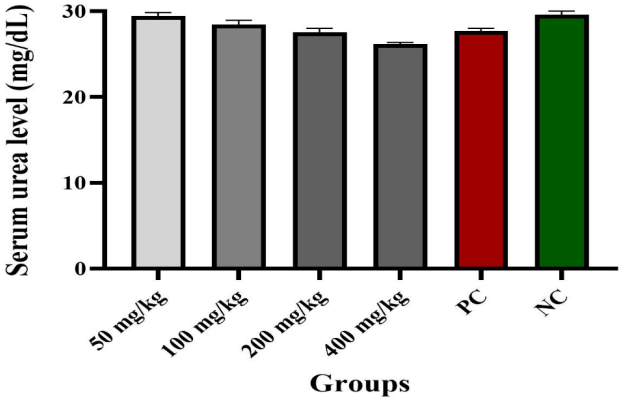

Fig. 2. . Serum urea levels in negative control (NC), positive control (PC), and groups treated with various doses of *Nigella sativa* extract.

3.3. Serum Creatinine Levels

Treatment with *Nigella sativa* extract at doses of 50, 100, and 200 mg/kg did not significantly reduce serum creatinine levels compared to the negative control group ($p>0.05$). However, the most notable decrease was observed at the dose of 400 mg/kg, where creatinine levels declined from 0.54 mg/dL to 0.42 mg/dL (Fig. 3).

3.4. Kidney Histopathology

Histological evaluations revealed no significant differences in renal tissue structure between the control and treated groups ($p>0.05$). The morphology of renal tubule lumens and epithelial lining in the extract-treated group was similar to that of the negative control, and no notable histological alterations were observed (Fig. 4).

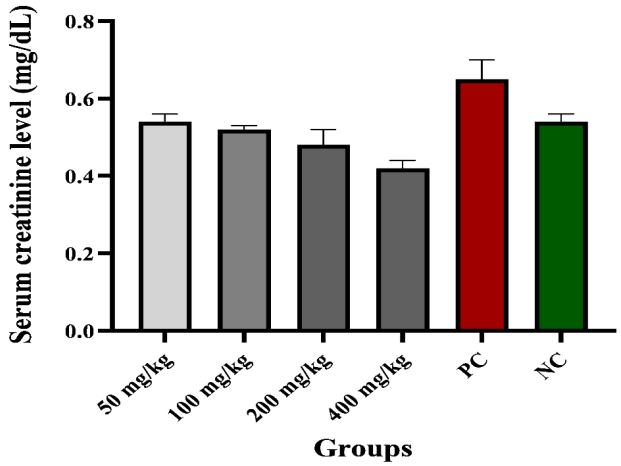

Fig. 3. Serum creatinine levels in negative control (NC), positive control (PC), and groups treated with various doses of *Nigella sativa* extract.

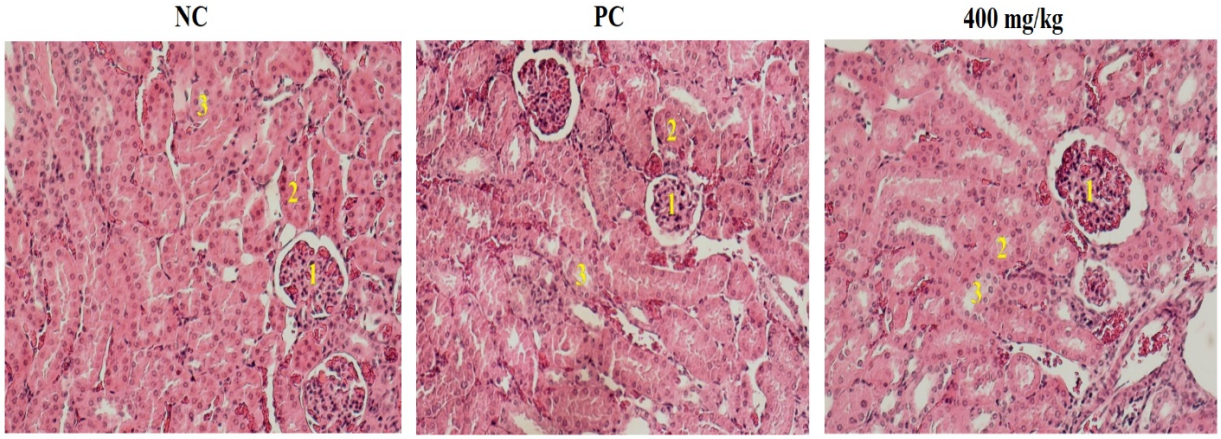

Fig. 4. Histopathological analysis of kidney tissue in negative control (NC), positive control (PC), and the group treated with 400 mg/kg of *Nigella sativa* extract. Labels 1, 2, and 3 indicate the renal corpuscle, proximal convoluted tubule, and distal convoluted tubule, respectively.

4. Discussion

Malaria, caused by *Plasmodium* parasites, remains one of the most detrimental parasitic diseases affecting human health, particularly in tropical regions. Due to the growing challenges of drug resistance and the adverse effects of conventional antimalarial drugs, research into alternative treatments has become increasingly important. Among these alternatives, medicinal plants have gained significant attention in recent years. In this study, the protective effects of the hydroalcoholic extract of *Nigella sativa* seeds on enzymatic activity and kidney tissue in mice infected with *Plasmodium berghei*-induced malaria were evaluated.

The results of the present study showed that administration of *Nigella sativa* extract at various doses did not have a significant effect on body weight. This finding is consistent with previous studies. For instance, Ali et al. (2012) reported that oral administration of *Nigella sativa* extract over a short period did not alter the body weight of rats, suggesting that the bioactive compounds of *Nigella sativa* may not significantly affect general metabolism or appetite within a similar timeframe (Ali et al., 2003).

However, other studies have reported either an increase or decrease in body weight, often in a dose-dependent manner and over longer periods. For example, a study by Bashir *et al.* (2023) demonstrated that *Nigella sativa* extract at various doses (100–400 mg/kg) improved conditions such as diet-induced obesity, non-alcoholic fatty liver disease (NAFLD), hyperlipidemia, and diabetic nephropathy compared to metformin (250 mg/kg) (Bashir et al., 2023). Notably, the 200 mg/kg dose significantly alleviated metabolic disorders induced by a high-fat diet, which is in line with some of the metabolic effects observed in the present study.

A key finding of this study was the significant reduction in serum urea levels, particularly at the 400 mg/kg dose of *Nigella sativa* extract. This effect may reflect the nephroprotective properties of the extract. Similar results were reported by Al-Ghamdi *et al.* (2003), where *Nigella sativa* conferred renal protection against drug-induced nephrotoxicity by lowering nitrogenous markers such as urea and blood urea nitrogen (BUN) (Al-Ghamdi, 2001).

From a pharmacological perspective, the active compound thymoquinone found in *Nigella sativa* possesses strong antioxidant and anti-inflammatory properties (Bordoni et al., 2019). These properties may help reduce oxidative stress in renal tubular cells, thereby improving renal excretory function. In animal models with drug- or infection-induced renal injury, thymoquinone has been shown to reduce urea levels by inhibiting inflammatory pathways such as NF-κB (Kordestani et al., 2020; Alkis et al., 2021).

In contrast to urea, a reduction in serum creatinine was observed only at the highest dose (400 mg/kg). This may suggest that creatinine is less sensitive to mild renal functional changes than urea, or that the extract did not exert a complete protective effect on glomerular structure and filtration rate.

Supporting this, Mehrdad et al. reported that ginger extract significantly reduced BUN levels in treated mice, with a non-linear relationship to the administered dose (Mehrdad et al., 2007). However, only minimal changes in serum creatinine levels were observed compared to the control group.

Histopathological findings in this study demonstrated preserved kidney architecture in the extract-treated groups, with no significant pathological alterations observed. These results are consistent with the biochemical findings, as the reduction in serum urea in the absence of severe histological damage may indicate early-stage kidney protection under disease conditions.

5. Conclusion

The results of this study indicate that *Nigella sativa* seed extract, particularly at a dose of 400 mg/kg, exerts protective effects against malaria-induced renal damage and may be considered as a potential adjunct therapy for patients with this condition. However, further studies are warranted to evaluate the long-term effects of this extract and to elucidate the precise mechanisms underlying its influence on kidney function and other organs.

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