ACCSCIENCE PUBLISHING

Eurasian Journal of Medicine and Oncology

ORIGINAL RESEARCH ARTICLE

Nephroprotective effects of melatonin and *Nigella sativa* against renal ischemia-reperfusion injury

Yasmeen Ali Hussein[1,2*], Salim Kadhim[1], Lafta Fayez Kadhim[3], and Faten Ali Albarki[3]

[1]Department of Pharmacology, College of Pharmacy, University of Alkafeel, Najaf, Iraq

[2]Alkafeel Research Center for Pharmaceutical Medical Science, University of Alkafeel, Najaf, Iraq

[3]Department of Pharmacology, College of Pharmacy, Altoosi University, Najaf, Iraq

Abstract

Introduction: Renal ischemia-reperfusion (IR) injury is a major cause of acute kidney injury and is strongly associated with oxidative stress, inflammation, and apoptosis. Melatonin and *Nigella sativa* possess well-documented antioxidant and anti-inflammatory properties that may provide renoprotective effects against IR-induced renal damage.

Objective: The present study aimed to evaluate the potential nephroprotective effects of melatonin and *Nigella sativa*, individually and in combination, against renal IR injury in rats.

Methods: Experimental renal IR injury was induced in rats by bilateral renal pedicle clamping followed by reperfusion. Animals were divided into experimental groups: Sham, IR, melatonin-treated, *Nigella sativa*-treated, and combined treatment. Biomarkers of renal function, oxidative stress, inflammatory cytokines, apoptotic markers, and kidney injury molecule-1 (KIM-1) were analyzed. Histopathological changes of the renal tissue were examined using hematoxylin & eosin staining.

Results: In the renal IR injury group, serum urea, creatinine, tumor necrosis factor-alpha, interleukin-6, and KIM-1 levels, as well as renal malondialdehyde and B-cell lymphoma 2-associated X protein levels, were significantly higher, whereas renal tissue reduced glutathione and B-cell lymphoma 2 levels were significantly lower, compared with those of the Sham group. Melatonin and *Nigella sativa* treatment significantly reduced these biochemical and histopathological changes. Importantly, the combination therapy was associated with stronger renoprotective effects than either therapy alone, as reflected by improved renal function, lower levels of oxidative stress and inflammatory responses, restoration of apoptotic balance, and better preservation of renal histological architecture.

Conclusion: The results of the present study suggest that melatonin and *Nigella sativa* exert potent nephroprotective effects against IR injury via their modulation of oxidative stress, inflammation, apoptosis, and KIM-1 protein levels. Co-treatment with combined administration resulted in more powerful protective effects and could be a potential therapeutic option for the prevention of IR-associated renal damage.

Keywords: Renal ischemia-reperfusion injury; Oxidative stress; Inflammation; Apoptosis; Kidney injury molecule-1; Melatonin; *Nigella sativa*

***Corresponding author:**
Yasmeen Ali Hussein
(Yasmeen.alamri@alkafeel.edu.iq)

Citation: Hussein YA, Kadhim S, Kadhim LF, Albarki FA. Nephroprotective effects of melatonin and *Nigella sativa* against renal ischemia-reperfusion injury. *Eurasian J Med Oncol*. 2026;10(5):026210229. doi: 10.36922/EJMO026210229

Received: May 21, 2026

Revised: July 9, 2026

Accepted: July 28, 2026

Published online: August 17, 2026

Copyright: © 2026 Author(s). This is an Open-Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, provided the original work is properly cited.

Publisher's Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

Acute kidney injury (AKI) is a common clinical condition associated with the rapid deterioration of renal function with the onset of metabolic waste accumulation and impairment in fluid/electrolyte and acid–base regulation.[1] Renal ischemia-reperfusion (IR) injury is believed to be one of the most important causes of AKI and is often observed in renal transplantation, septic shock, cardiovascular surgery, and severe hypotension. Despite advances in supportive treatment, renal IR injury remains associated with high morbidity and mortality due to the complexity of its underlying pathophysiological mechanisms.[2]

The pathogenesis of renal IR injury involves multiple interconnected mechanisms, including oxidative stress, inflammation, mitochondrial dysfunction, and apoptotic cell death. Reperfusion following ischemia leads to excessive generation of reactive oxygen species (ROS), causing lipid peroxidation, DNA damage, and cellular dysfunction.[3] In addition, inflammatory mediators such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) contribute to tubular epithelial injury and endothelial dysfunction.[4] Apoptosis also plays a crucial role in renal damage through modulation of apoptotic regulatory proteins, including B-cell lymphoma 2-associated X protein (Bax) and B-cell lymphoma 2 (Bcl-2), where an increased Bax/Bcl-2 ratio promotes mitochondrial membrane permeability and activation of caspase-dependent pathways.[5]

Kidney injury molecule-1 (KIM-1) is a transmembrane glycoprotein highly expressed in proximal tubular epithelial cells following renal injury.[6] KIM-1 has emerged as an early, sensitive, and specific biomarker for detecting acute kidney injury and evaluating the severity of tubular damage. Elevated KIM-1 levels are strongly associated with ischemic and inflammatory renal injury, making it a valuable marker for assessing nephroprotective interventions.[7]

Nigella sativa, commonly known as black seed, is a medicinal plant widely used in traditional medicine because of its diverse pharmacological activities. Thymoquinone, the major bioactive constituent of *Nigella sativa*, possesses well-documented antioxidant, anti-inflammatory, and anti-apoptotic properties.[8] Previous experimental studies have shown that *Nigella sativa* possesses significant antioxidant and anti-inflammatory properties that may attenuate renal tissue injury and improve histopathological alterations following ischemic damage.[9]

Melatonin is an endogenous indoleamine secreted mainly by the pineal gland and has demonstrated significant antioxidant and anti-apoptotic effects in an experimental renal IR injury model.[10] Melatonin exerts renoprotective effects through scavenging ROS, preserving mitochondrial function, suppressing inflammatory signaling pathways, and inhibiting apoptosis. Several studies have demonstrated the beneficial effects of melatonin against renal IR injury and oxidative renal damage.[11]

Both melatonin and *Nigella sativa* have demonstrated renoprotective effects against renal IR injury, but there are limited data on their combined therapeutic efficacy. In addition, the possible synergism observed between the different agents on oxidative stress, inflammation, apoptosis, and renal injury markers, including KIM-1, has not been extensively studied in one experimental model. Hence, the present study aimed to assess the nephroprotective effect of combined *Nigella sativa* and melatonin treatment compared to their individual treatment in reducing oxidative stress, inflammatory mediators, apoptosis, and KIM-1 protein concentration in renal IR injury in a rat model. Given their complementary mechanisms of action, we hypothesized that the combination therapy would have greater nephroprotective effects than either agent alone.

2. Materials and methods

2.1. Experimental animals

Adult male Wistar albino rats aged 8–10 weeks old, weighing 200–250 g (n = 40), were used in the present experimental study. Animals were obtained from the animal house of the University of Alkafeel and maintained under standard laboratory conditions, including controlled temperature (22 ± 2 °C), relative humidity (55 ± 5%), and a 12-h light/dark cycle with free access to standard pellet diet and water ad libitum. All experimental procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Alkafeel (AU-IACUC-2024-039).

2.2. Chemicals and reagents

Melatonin powder was purchased from Sigma-Aldrich (United States). All biochemical assay kits used to determine oxidative stress and inflammatory markers were commercially available, analytical-grade kits obtained from certified manufacturers.

2.3. Preparation of *Nigella sativa* extract

Nigella sativa seeds were procured from a certified local herbal supplier in Najaf, Iraq, and authenticated by a pharmacognosy specialist. The seeds were cleaned, air-dried, and mechanically ground into a fine powder. Hydroalcoholic extraction was performed using 70% ethanol in a Soxhlet apparatus for 24 h. The resulting extract

was concentrated under reduced pressure at 40 °C using a rotary evaporator and dried to obtain a semisolid extract. Before administration, the extract was freshly dissolved in distilled water and orally administered by gastric lavage to the experimental animals at the 200 mg/kg dose.[12]

2.4. Experimental design and grouping

For the study, 53 male Wistar rats were initially obtained. During the acclimatization period and induction of anesthesia, 13 animals were lost and were thus excluded from the experimental protocol. The 40 healthy rats were assigned to the five experimental groups (eight rats per group). Each rat was assigned a unique identification number, and simple randomization was performed by randomly drawing the numbered animals until each group contained eight rats. All animals in the experimental groups were included in biochemical, histopathological, and statistical analyses:

(i) Group I (Sham): Rats underwent an operation without renal ischemia.
(ii) Group II (IR): Rats subjected to renal IR injury without treatment.
(iii) Group III (NS + IR): Rats treated with *Nigella sativa* extract before renal IR injury.
(iv) Group IV (Mel + IR): Rats treated with melatonin before renal IR injury.
(v) Group V (NS + Mel + IR): Rats treated with combined *Nigella sativa* and melatonin before renal IR injury.

The study was planned with eight rats in each experimental group as stipulated in the experimental protocol. No formal a priori power analysis was conducted prior to the start of the study, which would be considered a limitation.

2.5. Induction of renal ischemia-reperfusion injury

Renal IR injury was induced according to previously established protocols.[13] Briefly, rats were anesthetized using ketamine (80 mg/kg) and xylazine (10 mg/kg) intraperitoneally. Following a midline abdominal incision, both renal pedicles were carefully isolated and occluded using non-traumatic vascular clamps for 45 min to induce ischemia. Reperfusion was initiated by removing the clamps and restoring blood flow for 24 h. Successful ischemia and reperfusion were confirmed visually by color changes in renal tissues.[13]

2.6. Drug administration

Melatonin was administered intraperitoneally at a dose of 10 mg/kg body weight, while *Nigella sativa* hydroalcoholic extract was orally administered at a dose of 200 mg/kg body weight once daily for seven consecutive days before ischemia induction. The selected doses were based on previous experimental studies.[14,15]

2.7. Sample collection

At the end of the reperfusion period, blood samples were collected via cardiac puncture under anesthesia for biochemical analysis. Rats were then sacrificed, and kidney tissues were rapidly excised, washed with cold saline, and divided into portions for biochemical, molecular, and histopathological analyses.

2.8. Assessment of renal function

Serum creatinine (E-BC-K188-M, Elabscience, United States) and serum urea levels (E-BC-K183-M, Elabscience, United States) were measured using commercially available diagnostic kits according to the manufacturer's instructions to evaluate renal function.

2.9. Oxidative stress markers

Renal tissue malondialdehyde (MDA) levels were determined as an index of lipid peroxidation (E-BC-K025-M, Elabscience, United States), whereas reduced glutathione (GSH) levels (E-BC-K030-M, Elabscience, United States) were assessed to evaluate antioxidant defense status.

2.10. Inflammatory markers

Serum concentrations of TNF-α (E-EL-R2856, Elabscience, United States) and IL-6 (E-EL-R0015, Elabscience, United States) were measured using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's protocols.

2.11. Apoptosis assessment

Protein levels of Bax (E-EL-R0098, Elabscience, United States) and Bcl-2 (E-EL-R0096, Elabscience, United States) were quantified in renal tissue homogenates using commercially available ELISA kits according to the manufacturer's protocols.

2.12. Assessment of kidney injury molecule-1 concentration

Serum KIM-1 protein concentration was quantified using a commercially available ELISA kit (E-EL-R0586, Elabscience, United States) based on the manufacturer's instructions.

2.13. Histopathological examination

Kidney tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4–5 μm thickness, and stained with hematoxylin and eosin (H&E). A pathologist blinded to the treatment-group assignments evaluated the histopathological changes, such as tubular necrosis,

interstitial edema, inflammatory infiltration, and tubular dilation, using light microscopy (Eclipse E200, Nikon Corporation, Japan).

2.14. Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). GraphPad Prism version 10 (GraphPad Software, United States) was used for statistical analyses. One-way analysis of variance was conducted after the Shapiro–Wilk test was used to determine the normality of the data. One-way analysis of variance and Tukey's multiple comparisons test were used to analyze differences between groups. Statistically significant differences were determined using adjusted p-values calculated using Tukey's test. p-values < 0.05 were deemed to be statistically significant.

3. Results

The present study investigated the potential renoprotective effects of melatonin, *Nigella sativa*, and their combined administration against renal IR-induced injury in experimental rats. Renal function parameters, oxidative stress biomarkers, inflammatory mediators, apoptotic markers, and kidney injury markers were evaluated among the studied groups.

3.1. Effect of treatments on renal function parameters

3.1.1. Effect of treatments on serum urea levels

Serum urea levels were significantly elevated in the IR group compared with the Sham group, indicating severe renal dysfunction following IR injury. Treatment with melatonin or *Nigella sativa* significantly reduced serum urea levels compared with the IR group. The combined treatment (IR+Mel+NS) demonstrated the greatest reduction in serum urea levels, indicating enhanced renal protective efficacy. The mean serum urea level was higher in the IR group (91.29 mg/dL) than in the IR+Mel group (42.43 mg/dL), IR+NS group (38.21 mg/dL), and IR+Mel+NS group (29.46 mg/dL). Compared with the IR group, the combined treatment reduced serum urea levels by 67.7%, whereas melatonin alone and *Nigella sativa* alone reduced serum urea levels by 53.5% and 58.1%, respectively (Figure 1).

3.1.2. Effect of treatments on serum creatinine levels

Serum creatinine levels showed a significant increase in the IR group compared with the Sham group. Administration of melatonin or *Nigella sativa* significantly attenuated creatinine elevation, while combined treatment restored creatinine levels closer to normal values. The mean serum creatinine was higher in the IR group (1.793 mg/dL) than in the IR+Mel group (1.198 mg/dL), IR+NS group (1.094 mg/dL), and IR+Mel+NS group (0.840 mg/dL). The serum creatinine level in the combined treatment group was decreased by about 53.2%, which is higher than the reductions in serum creatinine level in the melatonin and *Nigella sativa* groups alone (39.0% and 33.2%, respectively) (Figure 2).

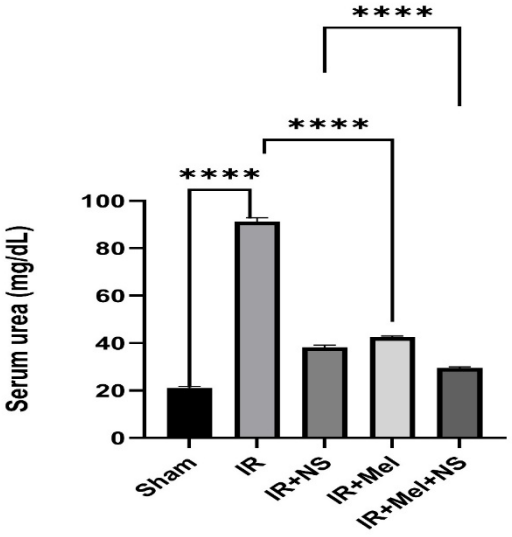


Figure 1. Effect of different treatments on serum urea levels in experimental groups. Data are expressed as mean ± standard error of the mean. Statistical significance: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.
Abbreviations: IR: Ischemia-reperfusion; Mel: Melatonin; NS: *Nigella sativa*.

3.2. Effect of treatments on oxidative stress biomarkers

3.2.1. Effect of treatments on renal malondialdehyde levels

The level of MDA in the IR group was significantly elevated, indicating elevated lipid peroxidation and oxidative stress. Combination therapy resulted in the greatest decrease in renal MDA, while significant decreases were observed for the melatonin and *Nigella sativa* treatments. The mean renal MDA level was lower in the IR+Mel group (32.84 µmol/g), the IR+NS group (27.84 µmol/g), and the IR+Mel+NS group (24.90 µmol/g) than in the IR group (51.45 µmol/g). Compared with the IR group, the combined treatment reduced renal MDA levels by 51.6%, whereas melatonin alone and *Nigella sativa* alone reduced renal MDA levels by 36.2% and 45.9%, respectively (Figure 3).

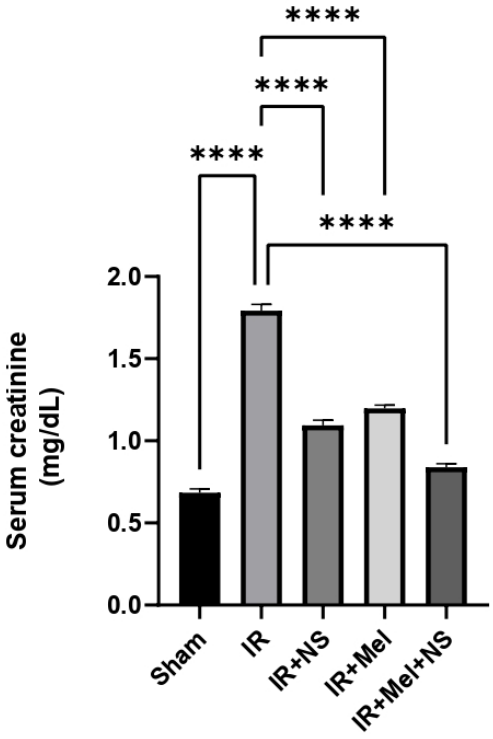


Figure 2. The effect of various treatments on the serum creatinine level in experimental groups. Data are expressed as mean ± standard error of the mean. Statistical significance: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

Abbreviations: IR: Ischemia-reperfusion; Mel: Melatonin; NS: *Nigella sativa*.

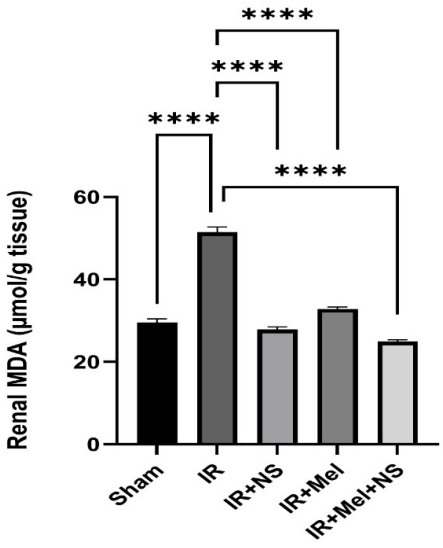


Figure 3. Effect of different treatments on renal malondialdehyde (MDA) levels in experimental groups. Data are expressed as mean ± standard error of the mean. Statistical significance: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

Abbreviations: IR: Ischemia-reperfusion; Mel: Melatonin; NS: *Nigella sativa*.

3.2.2. Effect of treatments on renal glutathione levels

Renal reduced GSH levels were significantly decreased in the IR group compared with the Sham group. Treatment with melatonin and *Nigella sativa* significantly restored GSH levels, with the combined treatment showing the greatest antioxidant effect. The mean renal GSH level was elevated to 1.996 mmol/g in the IR+Mel group, 2.148 mmol/g in the IR+NS group, and 2.489 mmol/g in the IR+Mel+NS group, compared with 1.149 mmol/g in the IR group. Compared with the IR group, the combined treatment increased renal GSH levels by 116.6%, whereas melatonin alone and *Nigella sativa* alone increased renal GSH levels by 73.7% and 87.0%, respectively (Figure 4).

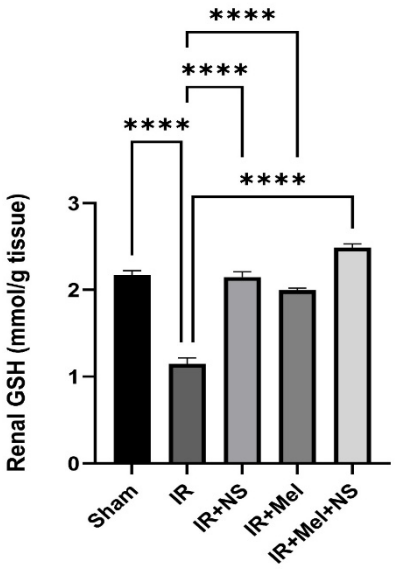


Figure 4. Effect of different treatments on renal glutathione (GSH) levels in experimental groups. Data are expressed as mean ± standard error of the mean. Statistical significance: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

Abbreviations: IR: Ischemia-reperfusion; Mel: Melatonin; NS: *Nigella sativa*.

3.3. Effect of treatments on inflammatory markers

3.3.1. Effect of treatments on serum tumor necrosis factor-alpha levels

Serum TNF-α levels were markedly elevated in the IR group compared with the Sham group, indicating a severe inflammatory response induced by renal IR injury. Treatment with melatonin or *Nigella sativa* significantly reduced TNF-α levels, whereas combined treatment exerted the strongest anti-inflammatory effect. The mean serum TNF-α concentration showed a higher value of 353.5 pg/mL in the IR group than 224.4 pg/mL in the IR+Mel group, 242.5 pg/mL in the IR+NS group, and 187.7 pg/mL in the IR+Mel+NS group. Compared with the IR group,

the combined treatment reduced TNF-α levels by 46.9%, whereas melatonin alone and Nigella sativa alone reduced TNF-α levels by 36.5% and 31.4%, respectively (Figure 5).

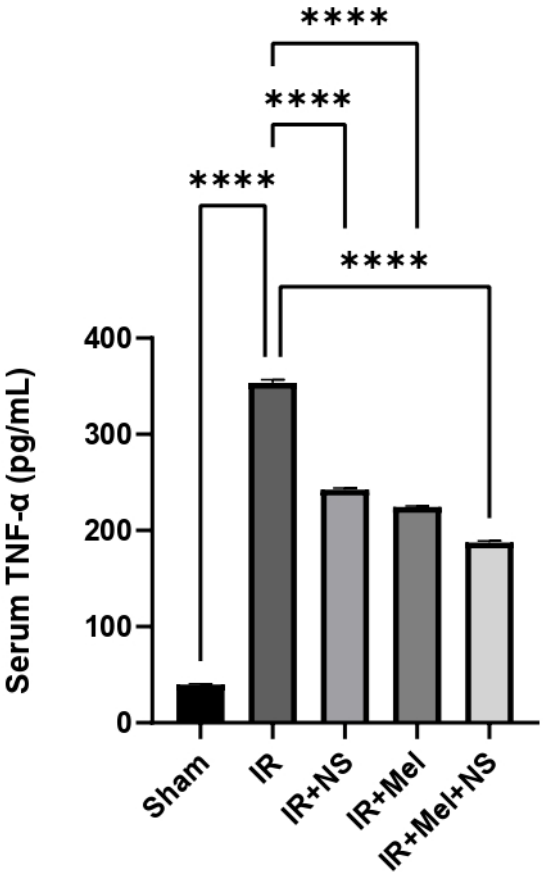


Figure 5. Effect of different treatments on serum tumor necrosis factor alpha (TNF-α) levels in experimental groups. Data are expressed as mean ± standard error of the mean. Statistical significance: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.
Abbreviations: IR: Ischemia-reperfusion; Mel: Melatonin; NS: *Nigella sativa*.

3.3.2. Effect of treatments on serum interleukin-6 levels

Serum IL-6 levels were significantly increased in the IR group compared with the Sham group. Administration of melatonin or *Nigella sativa* significantly attenuated IL-6 elevation, while combined treatment restored IL-6 levels closer to normal values. The mean serum IL-6 levels were lower in the IR+Mel group (4,461 pg/mL), the IR+NS group (4,843 pg/mL), and the IR+Mel+NS group (3,656 pg/mL) than in the IR group (7,054 pg/mL). Compared with the IR group, the combined treatment reduced IL-6 levels by 48.2%, whereas melatonin alone and *Nigella sativa* alone reduced IL-6 levels by 36.8% and 31.3%, respectively (Figure 6).

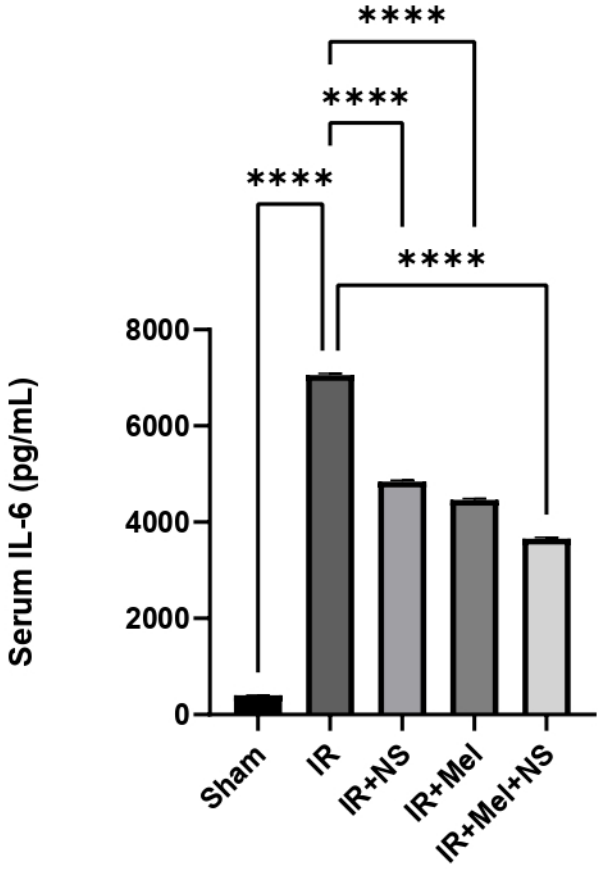


Figure 6. Effect of different treatments on serum interleukin-6 (IL-6) levels in experimental groups. Data are expressed as mean ± standard error of the mean. Statistical significance: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.
Abbreviations: IR: Ischemia-reperfusion; Mel: Melatonin; NS: *Nigella sativa*.

3.4. Effect of treatments on apoptotic markers

3.4.1. Effect of treatments on B-cell lymphoma 2-associated X protein level

Renal Bax level was significantly upregulated in the IR group compared with the Sham group, indicating enhanced apoptotic activity. Treatment with melatonin or *Nigella sativa* significantly reduced Bax levels, while the combined treatment demonstrated the greatest anti-apoptotic effect. The mean renal Bax level in the IR group was higher (3.330 pg/mg) than in the IR+Mel group (1.076 pg/mg), IR+NS group (1.289 pg/mg), and IR+Mel+NS group (0.879 pg/mg). The combined treatment significantly reduced Bax level by about 73.6%. In comparison, melatonin alone reduced Bax level by about 67.7%, and *Nigella sativa* alone by about 61.3%, compared to the IR group (Figure 7).

3.4.2. Effect of treatments on B-cell lymphoma 2 level

Renal Bcl-2 level was significantly reduced in the IR group compared with the Sham group. Treatment with melatonin or *Nigella sativa* significantly increased Bcl-2 level, whereas combined treatment restored Bcl-2 level to near normal levels. The mean renal Bcl-2 level was 0.955 pg/mg in the IR group, 2.230 pg/mg in the IR+Mel group, 1.799 pg/mg in the IR+NS group, and 2.925 pg/mg in the IR+Mel+NS group. Compared with the IR group, Bcl-2 level increased by 133.5% following melatonin treatment, 88.4% following *Nigella sativa* treatment, and 206.3% following combined treatment, with the highest concentration observed in the combined treatment group (Figure 8).

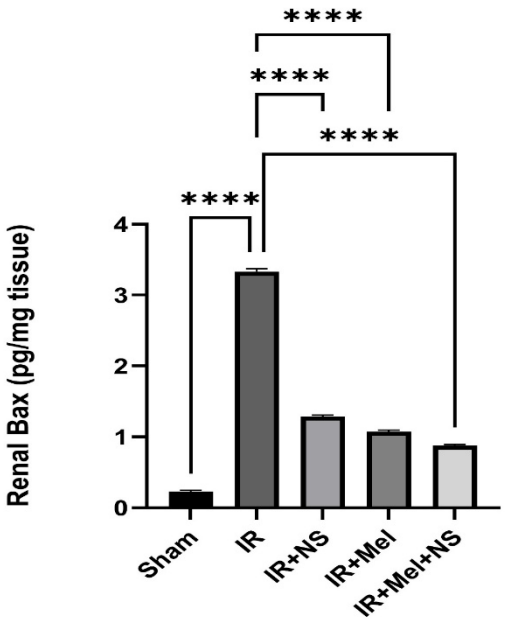


Figure 7. Effect of different treatments on renal B-cell lymphoma 2-associated X protein (Bax) levels in experimental groups. Data are expressed as mean ± standard error of the mean. Statistical significance: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.
Abbreviations: IR: Ischemia-reperfusion; Mel: Melatonin; NS: *Nigella sativa*.

3.5. Effect of treatments on renal injury marker

3.5.1. Effect of treatments on serum kidney injury molecule-1 levels

Serum KIM-1 levels were significantly elevated in the IR group compared with the Sham group, indicating severe tubular epithelial injury. Treatment with melatonin or *Nigella sativa* significantly reduced KIM-1 levels, whereas combined treatment demonstrated the greatest reduction, suggesting marked protection against renal tubular damage. The mean serum KIM-1 level increased from 74.31 ng/L in the Sham group to 126.70 ng/L in the IR group. KIM-1 levels were 92.88 ng/L in the IR+Mel group, 88.36 ng/L in the IR+NS group, and 79.94 ng/L in the IR+Mel+NS group. Melatonin, *Nigella sativa*, and combined treatment showed lower KIM-1 levels by 26.7%, 30.3%, and 36.9%, respectively, compared with the IR group, with the combined treatment showing the largest reduction (Figure 9).

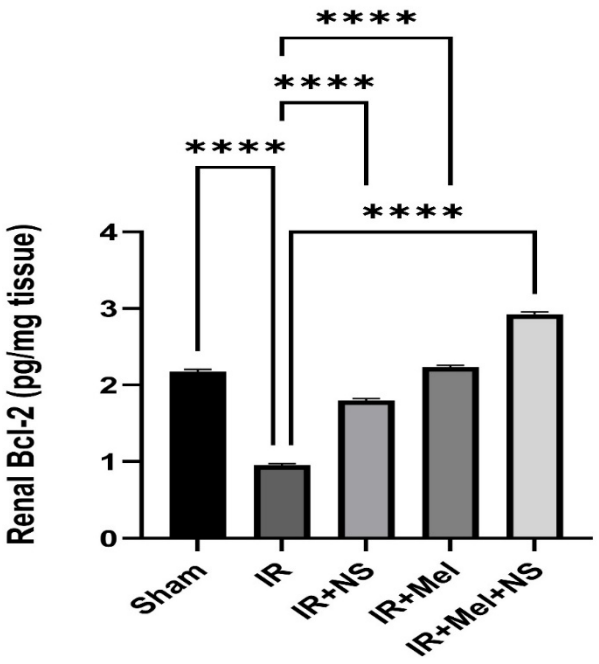


Figure 8. Effect of different treatments on renal B-cell lymphoma 2 (Bcl-2) levels in experimental groups. Data are expressed as mean ± standard error of the mean. Statistical significance: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.
Abbreviations: IR: Ischemia-reperfusion; Mel: Melatonin; NS: *Nigella sativa*.

3.5.2. Comparison between melatonin and Nigella sativa monotherapy

The melatonin (IR+Mel) and *Nigella sativa* (IR+NS) monotherapy groups were compared directly using Tukey's multiple comparisons test, which showed that the mean serum urea in the IR+Mel group was significantly higher than in the IR+NS group, while the mean serum KIM-1 in the IR+NS group was significantly lower than in the IR+Mel group. There were no significant differences between the two monotherapies for serum creatinine or renal GSH. Overall, these results suggest that both agents demonstrate different but complementary nephroprotective properties and that the synergistic treatment showed the highest nephroprotective activity across all parameters evaluated. No significant differences were found between the two monotherapy groups in serum creatinine and renal GSH

levels (Tukey's adjusted $p > 0.05$), but significant differences were observed in serum urea, renal MDA, serum TNF-α, serum IL-6, renal Bax, renal Bcl-2, and serum KIM-1 levels.

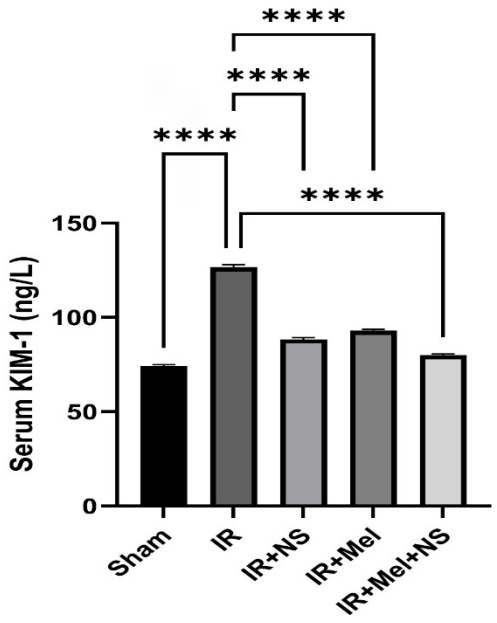


Figure 9. Effect of different treatments on serum kidney injury molecule-1 (KIM-1) levels in experimental groups. Data are expressed as mean ± standard error of the mean. Statistical significance: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.
Abbreviations: IR: Ischemia-reperfusion; Mel: Melatonin; NS: *Nigella sativa*.

3.6. Histopathological findings

Histopathological examination of renal tissues from the Sham group revealed normal renal architecture with intact glomeruli and renal tubules. In contrast, renal tissues from the IR group demonstrated marked tubular degeneration, tubular dilatation, epithelial cell necrosis, vascular congestion, inflammatory cell infiltration, and interstitial edema. Treatment with melatonin or *Nigella sativa* partially ameliorated these histopathological alterations. Notably, combined treatment markedly preserved renal tissue architecture with minimal tubular damage and reduced inflammatory infiltration, indicating substantial nephroprotective activity (Figure 10).

4. Discussion

Renal IR injury remains one of the major causes of AKI and contributes significantly to morbidity and mortality in both experimental and clinical settings.[16] The pathophysiology of renal IR injury is complex and interlinked with oxidative stress, inflammatory responses, endothelial dysfunction, mitochondrial impairment, and activation of apoptotic pathways.[17] Thus, the development of effective renoprotective agents capable of modifying renal tissue damage is a major therapeutic goal.

The present study aimed to assess the protective effect of melatonin, *Nigella sativa*, and their combination against renal IR-induced injury in rats. The current study showed that renal IR caused a marked decrease in renal function, elevated levels of oxidative stress, inflammatory markers, activation of apoptotic pathways, and histopathological changes in renal tissues. In contrast, the administration of melatonin, *Nigella sativ*a, and especially the combination of both substantially prevented the onset of these renal pathological changes.

Serum urea and creatinine levels were significantly elevated in the IR group, suggesting severe renal dysfunction after an IR injury. The present results are consistent with those obtained from previous experimental studies[11], which showed that renal IR injury causes severe renal tubular epithelial injury and glomerular dysfunction, leading to high levels of serum creatinine and blood urea nitrogen.

Melatonin and *Nigella sativa* treatment showed significantly lower levels of serum urea and creatinine compared to the untreated IR rat group, indicating attenuation of renal dysfunction. Dun *et al.*[11] also reported an anti-inflammatory effect of melatonin on IR-induced renal damage, while Caskurlu *et al.*[18] showed that *Nigella sativa* is protective against renal IR injury. Interestingly, combined treatment with melatonin and *Nigella sativa* had the greatest reduction in renal function biomarkers, suggesting an enhanced renoprotective effect compared with monotherapy.

The enhanced renoprotection achieved by the combination may be due to interactions among the different pharmacological mechanisms rather than a shared mechanism. The antioxidant properties of *Nigella sativa*, primarily its ability to scavenge free radicals via thymoquinone, and of melatonin, mainly its ability to protect mitochondrial function and limit oxidative injury, are key factors in their anti-inflammatory effects and in boosting endogenous antioxidant capacity. Concomitant modulation of these interrelated mechanisms may explain the improved renal function observed in the combined treatment group.

Melatonin is a powerful endogenous antioxidant, mainly produced by the pineal body. It has free-radical-scavenging activity and exhibits a protective effect against oxidative tissue damage.[19] Previous studies have shown that melatonin is a direct scavenger of hydroxyl radicals,

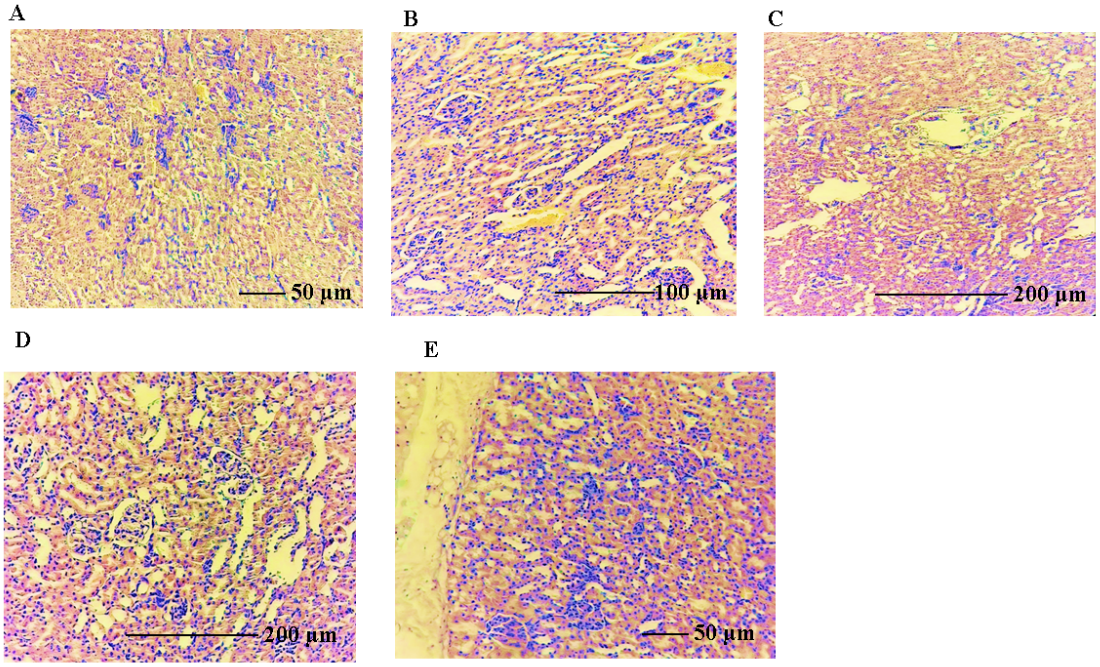


Figure 10. Histopathological examination of renal tissue sections stained with hematoxylin and eosin in different experimental groups. The Sham group showed normal renal architecture, whereas the IR group demonstrated marked renal tissue injury. Treatment with NS or melatonin improved renal histological changes, while the combined treatment group (IR+Mel+NS) exhibited the greatest renoprotective effect with near-normal renal morphology. (A) Sham group. Scale bar: 50 µm; magnification: 400×. (B) IR group. Scale bar: 100 µm; magnification: 200×. (C) IR + NS group. Scale bar: 200 µm; magnification: 100×. (D) IR + Mel group. Scale bar: 200 µm; magnification: 100×. (E) IR + Mel + NS group. Scale bar: 50 µm; magnification: 400×. Abbreviations: IR: Ischemia-reperfusion; Mel: Melatonin; NS: *Nigella sativa*.

hydrogen peroxide, and peroxynitrite, and upregulates the antioxidant defense systems. Additionally, melatonin affects mitochondrial membranes, helping to preserve their integrity and the function of the electron transfer chain, which prevents ROS production during reperfusion injury.[20] These mechanisms could have played a role in the significant biochemical improvements observed in the present study.

Likewise, *Nigella sativa* has attracted considerable attention due to its antioxidant, anti-inflammatory, and anti-apoptotic effects. The main active component, thymoquinone, has been demonstrated to have anti-inflammatory activities, including inhibition of lipid peroxidation, enhancement of antioxidant enzyme activity, and suppression of the production of inflammatory cytokines.[21] Accordingly, the protective properties of *Nigella sativa* observed in the present study might be mainly due to its antioxidative and anti-inflammatory action.

Accordingly, the protective properties of *Nigella sativa* observed in the present study might be mainly due to its antioxidant and anti-inflammatory activities. These molecular mechanisms could also contribute to the protective activity of *Nigella sativa* against renal ischemia-reperfusion injury.

Oxidative stress is a key component of renal IR injury pathogenesis. During reperfusion, excess ROS are generated, injuring cellular lipids, proteins, and DNA, resulting in renal tubular injury and cell death.[22] The present study revealed that renal MDA was significantly increased in the IR group, suggesting increased lipid peroxidation. At the same time, the renal GSH level was significantly decreased, indicating a loss of endogenous antioxidant capacity.

Melatonin and *Nigella sativa* supplementation significantly lowered MDA levels and restored GSH levels. These findings are consistent with previous studies indicating that both compounds had the most potent antioxidant effect when they were used together in renal IR injury.[15,23] Combined treatment produced the greatest antioxidant effect among all treated groups. These results indicate that both agents can similarly suppress oxidative stress in renal IR injury.

Excessive ROS generation can trigger downstream inflammatory and apoptotic pathways such as nuclear

factor kappa B (NF-κB) activation, downstream cytokine production, and mitochondrial apoptotic cascades, which are some of the earliest events that occur after reperfusion. Thus, the decrease in lipid peroxidation and recovery of the endogenous antioxidant capacity may be attributed, at least in part, to the subsequent improvement in inflammatory and apoptotic markers in the treated groups.

Tumor necrosis factor-alpha is considered one of the key pro-inflammatory cytokines involved in renal tissue damage during IR, as it promotes leukocyte infiltration, endothelial dysfunction, and apoptotic signaling. IL-6 also contributes significantly to inflammatory amplification and progression of tissue injury.[24] Renal IR markedly increased serum TNF-α and IL-6 levels, confirming activation of inflammatory pathways following IR injury.

Administration of melatonin or *Nigella sativa* significantly reduced TNF-α and IL-6 levels compared with the IR group, while combined therapy demonstrated the most pronounced anti-inflammatory effect. Similar anti-inflammatory effects of melatonin and *Nigella sativa* were previously documented in experimental renal IR studies through the suppression of inflammatory cytokines and NF-κB signaling pathways.[23,25] These results may be explained by inhibition of NF-κB activation and suppression of pro-inflammatory cytokine production by both melatonin and thymoquinone.[23,25]

In recent years, systems pharmacology studies have confirmed that natural bioactive compounds can control more than one molecular target that is associated with kidney inflammation and cell survival. Kaya *et al.*[26] found that these genes include *TNF*, *AKT1* and *SRC*, and noted that the phosphoinositide 3-kinase/protein kinase B signaling pathway plays a key role in regulating inflammatory responses, oxidative stress, and tissue repair. Thus, the decrease in TNF-α and IL-6 production in the present study might reflect a more general modulation of interconnected signaling pathways, in addition to cytokine inhibition.[26]

Apoptosis represents another important mechanism underlying renal IR-induced tissue injury. In the present study, Bax levels were significantly increased in the IR group, whereas Bcl-2 levels were significantly reduced. Bax is a pro-apoptotic protein that promotes mitochondrial membrane permeability and cytochrome c release. Conversely, Bcl-2 is an anti-apoptotic protein that preserves mitochondrial integrity and inhibits programmed cell death.[27]

Treatment with melatonin or *Nigella sativa* significantly downregulated Bax protein levels while increasing Bcl-2 levels, suggesting suppression of apoptotic pathways. These findings are supported by previous reports indicating that melatonin and thymoquinone inhibit mitochondrial apoptotic signaling and reduce renal tubular cell apoptosis following IR injury.[11,28] The combination therapy showed superior anti-apoptotic activity compared with either treatment alone. These findings support the hypothesis that the protective effects of melatonin and *Nigella sativa* involve the modulation of mitochondrial apoptotic signaling.

Intracellular survival pathways (such as the phosphoinositide 3-kinase/protein kinase B pathway) are a significant determinant of the balance between Bax and Bcl-2, favoring cell survival and inhibiting mitochondrial apoptosis. Thus, reestablishment of this balance with the combined treatment group could be one of the most important mechanisms in maintaining the viability of renal tubular cells after IR injury.

Kidney injury molecule-1 is considered one of the most sensitive biomarkers for detecting renal tubular injury.[29] In the present study, serum KIM-1 levels were markedly elevated in the IR group, indicating severe tubular epithelial damage following IR injury. Treatment with melatonin or *Nigella sativa* significantly reduced KIM-1 levels, while combined treatment produced the greatest reduction, supporting the observed findings and the renoprotective effects of these agents.

The observed reduction in KIM-1 levels is consistent with the overall attenuation of oxidative stress, inflammation, and apoptosis observed in the present study. These findings suggest that the combined treatment may suppress these pathological processes, thereby contributing to the preservation of renal tubular integrity.

The biochemical results were corroborated by histopathological examination. Renal tissue of the Sham group had a normal renal architecture with intact glomeruli and tubules. The IR group, however, showed significant changes in tubular degeneration, tubular dilatation, inflammatory cell infiltration, vascular congestion, and necrotic changes. These pathological changes are the hallmark of ischemic reperfusion injury to the kidney.

Melatonin and *Nigella sativa* supplementation partially reduced histopathologic damage and slowed deterioration of renal tissue architecture. Remarkably, the combined treatment group showed near-normal renal morphology with minimal tissue injury, confirming the better protective effect of combined therapy.

The protective effect observed after the combination of melatonin and *Nigella sativa* may be attributed to their complementary mechanism(s). Melatonin is mainly known for its direct scavenging effect on free radicals

and mitochondrial protection, whereas *Nigella sativa* and its constituent thymoquinone modulate inflammatory cytokines and cellular oxidative pathways. Therefore, the combination may provide broader protection against renal ischemia-reperfusion injury.

In summary, these results indicate that the superior effectiveness of the combined treatment is not likely to be attributed to one mechanism. Concurrently targeting oxidative stress, inflammatory signaling, mitochondrial dysfunction, and apoptosis, however, might provide greater protection against renal IR injury than either targeting alone. Nevertheless, more mechanistic studies that directly involve phosphoinositide 3-kinase/protein kinase B, NF-κB, and mitogen-activated protein kinase signaling pathways are warranted to validate these potential molecular interactions.

A limitation of the present study is that histopathological changes were analyzed descriptively and without the use of a standardized semi-quantitative injury scoring system, such as the Jablonski score. The histological results matched the biochemical results, and although this supports the evaluation of renal tissue injury and treatment efficacy, quantitative histopathological scoring should be included in future studies.

Nigella sativa was prepared as a hydroalcoholic extract following a previously published extraction protocol, using the same standardized laboratory conditions. However, the present study did not include analytical characterization of the thymoquinone content and the detailed phytochemical profile of the extract. Therefore, it is impossible to rule out any batch-to-batch variability. For further standardization of the extract and to enhance reproducibility, future studies should include complete phytochemical characterization, such as high-performance liquid chromatography analysis. No formal a priori power analysis was carried out before this study. Prospective sample size estimation should be used in future studies to strengthen the statistical design.

5. Conclusion

The present study concluded that melatonin and *Nigella sativa* effectively reduced renal IR injury in rats by inhibiting oxidative stress, inflammatory response, apoptotic pathways, and renal injury markers. Combined administration produced greater protective effects than either treatment alone, as evidenced by improved renal function parameters, reduced inflammatory and oxidative markers, restoration of apoptotic balance, and preservation of renal histological architecture. These findings suggest the potential therapeutic value of combined melatonin and *Nigella sativa* administration in preventing IR-associated renal injury.

Acknowledgments

None.

Funding

None.

Conflict of interest

The authors declare they have no competing interests.

Author contributions

Conceptualization: Yasmeen Ali Hussein, Salim Kadhim
Formal analysis: Lafta Fayez Kadhim
Investigation: Yasmeen Ali Hussein, Faten Ali Albarki
Methodology: Yasmeen Ali Hussein
Writing – original draft: Yasmeen Ali Hussein
Writing – review & editing: Yasmeen Ali Hussein, Salim Kadhim

Ethics approval and consent to participate

The experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Alkafeel, Iraq (AU-IACUC-2024-039). All animal experimental procedures were performed in accordance with institutional guidelines for the care and use of laboratory animals.

Consent for publication

Not applicable.

Availability of data

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Further disclosure

Artificial intelligence tools were used only for language editing and grammatical refinement. All scientific content, study design, data analysis, interpretation, and conclusions were performed and verified by the authors.

References

1. Kellum JA, Romagnani P, Ashuntantang G, Ronco C, Zarbock A, Anders HJ. Acute kidney injury. *Nat Rev Dis Primers.* 2021;7(1).

   doi: 10.1038/s41572-021-00284-z

2. Nieuwenhuijs-Moeke GJ, Pischke SE, Berger SP, *et al.* Ischemia and reperfusion injury in kidney transplantation: relevant mechanisms in injury and repair. *J Clin Med.* 2020;9(1):253.

   doi: 10.3390/jcm9010253

3. Kezić A, Stajic N, Thaiss F. Innate immune response in kidney ischemia/reperfusion injury: potential target for therapy. *J Immunol Res.* 2017;2017:1-10.

doi: 10.1155/2017/6305439

4. Zhang M, Liu Q, Meng H, *et al.* Ischemia-reperfusion injury: molecular mechanisms and therapeutic targets. *Sig Transduct Target Ther.* 2024;9(1).

doi: 10.1038/s41392-023-01688-x

5. Zhu Y, Huang J, Chen X, *et al.* Senkyunolide I alleviates renal Ischemia-Reperfusion injury by inhibiting oxidative stress, endoplasmic reticulum stress, and apoptosis. *Int Immunopharmacol.* 2022;102:108393.

doi: 10.1016/j.intimp.2021.108393

6. Brilland B, Boud'Hors C, Wacrenier S, *et al.* Kidney injury molecule 1 (KIM-1): a potential biomarker of acute kidney injury and tubulointerstitial injury in patients with ANCA-glomerulonephritis. *Clin Kidney J.* 2023;16(9):1521-1533.

doi: 10.1093/ckj/sfad071

7. Su Y, Yang X, Cheng WW, Shang XM, Wang HL, Shen HC. Kidney injury molecule 1 in the early detection of acute kidney injury—A systematic review and meta-analysis. *Front Med*. 2025;12.

doi: 10.3389/fmed.2025.1574945

8. Hannan MA, Zahan MS, Sarker PP, Moni A, Ha H, Uddin MJ. Protective effects of black cumin (Nigella sativa) and its bioactive constituent, thymoquinone against kidney injury: An aspect on pharmacological insights. *Int J Mol Sci.* 2021;22(16):9078.

doi: 10.3390/ijms22169078

9. Alrashdi BM, Ashry M, Germoush MO, *et al.* Anti-nephrotoxic, antioxidant, and anti-inflammatory efficiency of Nigella sativa ethanolic extract against CCl4-induced nephrotoxicity in rats. *Open Vet J.* 2025;15(1):402.

doi: 10.5455/ovj.2025.v15.i1.36

10. Panah F, Ghorbanihaghjo A, Argani H, *et al.* The effect of oral melatonin on renal ischemia–reperfusion injury in transplant patients: A double-blind, randomized controlled trial. *Transpl Immunol.* 2019;57:101241.

doi: 10.1016/j.trim.2019.101241

11. Dun R, Lan T, Tsai J, *et al.* Protective effect of melatonin for renal ischemia-reperfusion injury: a systematic review and meta-analysis. *Front Physiol.* 2022;12.

doi: 10.3389/fphys.2021.791036

12. Devadhe AS, Dighe S, Yadav SS, Bhawar SB, Ghogare RD. Antioxidant and hepatoprotective activity of Nigella sativa alcoholic extract in a CCl4-induced rat. *J Appl Pharm Res.* 2025;13(2):115-126.

doi: 10.69857/joapr.v13i2.985

13. Akdeniz S, Ulu M, Bakirtas M, Erdemir F. The renal protective efficacy of ketamine vs. propofol vs. ketofol against renal ischemia/reperfusion injury: An experimental rat study. *Med Sci.* 2022;11(3):1317.

doi: 10.5455/medscience.2022.04.097

14. Mashayekhi-Sardoo H, Rezaee R, Karimi G. Nigella sativa (black seed) safety: an overview. *Asian Biomed.* 2020;14(4):127-137.

doi: 10.1515/abm-2020-0020

15. Sezgin G, Öztürk G, Güney Ş, Sinanoğlu O, Tunçdemir M. Protective effect of melatonin and 1, 25-dihydroxyvitamin D3 on renal ischemia–reperfusion injury in rats. *Renal Fail.* 2013;35(3):374-379.

doi: 10.3109/0886022x.2012.760409

16. Shiva N, Sharma N, Kulkarni YA, Mulay SR, Gaikwad AB. Renal ischemia/reperfusion injury: An insight into in vitro and in vivo models. *Life Sci.* 2020;256:117860.

doi: 10.1016/j.lfs.2020.117860

17. Bhatia D, Capili A, Choi ME. Mitochondrial dysfunction in kidney injury, inflammation, and disease: Potential therapeutic approaches. *Kidney Res Clin Pract.* 2020;39(3):244-258.

doi: 10.23876/j.krcp.20.082

18. Caskurlu T, Kanter M, Erboga M, Erboga ZF, Ozgul M, Atis G. Protective effect of Nigella sativa on renal reperfusion injury in rats. *Iran J Kidney Dis.* 2016;10(3):135-143.

19. Hacışevki A, Baba B. An Overview of Melatonin as an Antioxidant Molecule: A Biochemical Approach. In: *Melatonin - Molecular Biology, Clinical and Pharmaceutical Approaches*. IntechOpen; 2018.

doi: 10.5772/intechopen.79421

20. Abdel-Razek H, Rizk MS, Amer G, Kora M, Afifi A, Donia S. Impact of combined ischemic preconditioning and melatonin on renal ischemia-reperfusion injury in rats. *Iran J Basic Med Sci.* 2023;26(2).

doi: 10.22038/ijbms.2022.67127.14722

21. Algheshairy RM, Alharbi HF, Almujaydil MS, Alhomaid RM, Ali HA. The protective effect of various forms of Nigella sativa against hepatorenal dysfunction: underlying mechanisms comprise antioxidation, anti-inflammation, and anti-apoptosis. *Front Nutr*. 2025;12.

doi: 10.3389/fnut.2025.1553215

22. Tejchman K, Kotfis K, Sieńko J. Biomarkers and mechanisms of oxidative stress—last 20 years of research with an emphasis on kidney damage and renal transplantation. *Int J Mol Sci.* 2021;22(15):8010.

doi: 10.3390/ijms22158010

23. Yildiz F, Coban S, Terzi A, Savas M, Bitiren M, Celik H,

et al. Protective effects of Nigella sativa against ischemia-reperfusion injury of kidneys. *Renal Fail.* 2010;32(1):126-131.

doi: 10.3109/08860220903367577

24. Aboelez MO, Ezelarab HAA, Alotaibi G, Abouzed DEE. Inflammatory setting, therapeutic strategies targeting some pro-inflammatory cytokines and pathways in mitigating ischemia/reperfusion-induced hepatic injury: a comprehensive review. *Naunyn Schmiedebergs Arch Pharmacol.* 2024;397(9):6299-6315.

doi: 10.1007/s00210-024-03074-y

25. Cho JH, Bhutani S, Kim CH, Irwin MR. Anti-inflammatory effects of melatonin: A systematic review and meta-analysis of clinical trials. *Brain Behav Immun.* 2021;93:245-253.

doi: 10.1016/j.bbi.2021.01.034

26. Kaya S, Gurer E, Dastan S. Network pharmacology and molecular docking-based insights into the anti-glomerulonephritis potential of Calendula officinalis. *Pak J Pharm Sci.* 2025;38(6):2474-2492.

doi: 10.36721/pjps.2025.38.6.reg.14742.1

27. Putri GFT. Bax/Bcl-2 ratio as the golden marker of apoptosis: molecular mechanisms and regulatory pathways. *Int J Cell Biomed Sci.* 2025;4(10):309-317.

doi: 10.59278/cbs.v4i10.65

28. Sadeghi E, Imenshahidi M, Hosseinzadeh H. Molecular mechanisms and signaling pathways of black cumin (Nigella sativa) and its active constituent, thymoquinone: a review. *Mol Biol Rep.* 2023;50(6):5439-5454.

doi: 10.1007/s11033-023-08363-y

29. Khonsha F, Valilo M, Nejabati H-R, Rahmati-Yamchi M, Mota A. Biomarkers for diabetic nephropathy with a focus on kidney injury molecule-1 (KIM-1). *Curr Diabetes Rev.* 2024;20(1).

doi: 10.2174/1573399819666230328151108