Iranian Journal of Basic Medical Sciences
ijbms.mums.ac.ir

Mitigation of sepsis-associated acute kidney injury by thymoquinone via targeting pyroptosis and attenuation of inflammation in C57BL/6 mice

Niloofar Izadi [1], Mahdieh Taheri [2], Mehrdad Roghani [2] *

[1] Faculty of Medicine, Shahed University, Tehran, Iran
[2] Neurophysiology Research Center, Shahed University, Tehran, Iran

ARTICLE INFO

Article type:
Original

Article history:
Received: Oct 26, 2025
Accepted: Feb 22, 2026

Keywords:
Acute kidney injury
Inflammation
Lipopolysaccharide
Oxidative stress
Pyroptosis
Thymoquinone

ABSTRACT

Objective(s): Acute kidney injury (AKI) is a severe, life-threatening complication of sepsis that often leads to renal failure. Thymoquinone, the main bioactive component of Nigella sativa, exhibits potent antioxidant and anti-inflammatory effects, protecting heart, liver, and kidney tissues in experimental studies. This study aimed to investigate the protective effects of thymoquinone on lipopolysaccharide (LPS)-induced AKI in mice by assessing oxidative stress, inflammatory mediators, pyroptosis factors, and renal function markers via modulation of oxidative stress and pyroptosis pathways.

Materials and Methods: Thirty-two male C57BL/6 mice (20–25 g) were randomized into four groups: control, LPS, and two LPS groups treated with thymoquinone solubilized in Cremophor (1 or 10 mg/kg). AKI was induced by intraperitoneal injection of LPS (10 mg/kg). After 24 hr, blood samples were collected for blood urea nitrogen (BUN), cystatin C, and serum creatinine assays. Kidney homogenates were analyzed for malondialdehyde (MDA), superoxide dismutase (SOD), nitrite, interleukin-6 (IL-6), nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3 (NLRP3), and caspase-1 levels.

Results: Thymoquinone (10 mg/kg; PO) significantly decreased BUN ($P<0.05$), cystatin C ($P<0.05$), and serum creatinine ($P<0.05$) levels. It also reduced MDA concentration ($P<0.05$) and increased SOD activity ($P<0.05$), while nitrite levels remained unchanged. Furthermore, IL-6, NLRP3, and caspase-1 were significantly lower (for all factors, $P<0.05$) in thymoquinone-treated mice compared to the LPS group.

Conclusion: Thymoquinone effectively attenuates LPS-induced AKI, likely by reducing oxidative stress, inhibiting pyroptosis and inflammation, and enhancing antioxidant defenses.

▶ Please cite this article as:
Izadi N, Taheri M, Roghani M. Mitigation of sepsis-associated acute kidney injury by thymoquinone via targeting pyroptosis and attenuation of inflammation in C57BL/6 mice. Iran J Basic Med Sci 2026; 29:

Introduction

Acute kidney injury (AKI) is a complex, multifactorial condition that affects a significant portion of hospitalized patients, especially those in intensive care units. It is characterized by a sudden decline in kidney function, which not only increases short-term risks such as mortality and complications but also leads to persistent outcomes such as chronic renal impairment and cardiovascular complications. AKI is notably more prevalent in low- and middle-income countries and imposes substantial healthcare burdens. Treatment primarily focuses on supportive care, managing underlying causes, preventing further kidney damage, and addressing complications (1). AKI is characterized pathologically by damage to renal tubular epithelial cells and the microvasculature, primarily driven by inflammatory responses, sepsis, or exposure to nephrotoxic agents (2).

LPS, a key component of Gram-negative bacterial membranes, initiates septic acute kidney injury by activating multiple inflammatory signaling pathways and promoting oxidative stress. LPS stimulation triggers Toll-like receptor 4 (TLR4) on renal tubular cells, resulting in the release of pro-inflammatory cytokines and the production of reactive oxygen species (ROS), which together cause renal cell apoptosis and tubular damage. Despite various therapeutic approaches, effective treatments targeting these molecular mechanisms remain limited, stressing the need for further research to develop novel interventions to mitigate LPS-induced kidney injury (3). LPS plays a crucial role in the development of AKI by inducing strong inflammatory responses that damage renal tissues and impair kidney function. This has been demonstrated in several studies, including one highlighting that LPS administration in mice led to kidney tissue damage and dysfunction, which could be alleviated by certain treatments (4). Moreover, LPS has been reported to stimulate tubular cell inflammation and injury, contributing to AKI pathogenesis through innate immune signaling pathways such as STING and NLRP3 inflammasome activation (5). One of the key mechanisms

***Corresponding author:** Mehrdad Roghani. Neurophysiology Research Center, Shahed University, Tehran, Iran. Email: mroghani@shahed.ac.ir, mehjour@yahoo.com

by which LPS exacerbates kidney damage is pyroptosis, a programmed inflammatory cell death involving caspase-1 and gasdermin D cleavage, resulting in the release of inflammatory cytokines and further renal tubular epithelial cell injury (6, 7).

Natural compounds that suppress inflammatory signaling and counteract oxidative stress offer promising therapeutic options for the effective management of AKI (8).

Thymoquinone, chemically known as 2-methyl-5-isopropyl-1,4-benzoquinone, is a bioactive compound derived from *Nigella sativa*, commonly known as black cumin, which has been employed in traditional remedies for hundreds of years. The seeds and extracted oil of this plant possess a broad range of pharmacological effects, including cancer prevention, blood sugar regulation, blood pressure reduction, antimicrobial properties, pain relief, immune system modulation, anti-inflammatory effects, muscle relaxation, and protective actions for the liver, kidneys, gastrointestinal tract, lungs, as well as strong anti-oxidant capabilities (9). Thymoquinone suppresses the activity of genes controlled by the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), including cyclooxygenase-2 (COX-2), vascular endothelial growth factor (VEGF), matrix metalloproteinase-9 (MMP-9), cellular myelocytomatosis oncogene (c-Myc), and cyclin D1. This targeted down-regulation significantly reduces NF-kB activation, positioning thymoquinone as a promising agent for reducing inflammation, cell growth, and tissue invasion (10).

However, the regulatory role of thymoquinone on AKI remains largely unknown. The novelty of our study lies in comprehensively evaluating the protective effects of thymoquinone against LPS-induced acute kidney injury by simultaneously assessing pyroptosis-related factors, oxidative stress, inflammatory mediators, and classical renal function markers in mice. Unlike previous research, which primarily focused on inflammation and oxidative stress pathways (11), this study highlights the involvement of pyroptosis in thymoquinone's renoprotective mechanism, offering deeper insights into its therapeutic potential against septic AKI.

Materials and Methods

Animals

The present experimental investigation was conducted on 32 male C57BL/6 mice weighing 20–25 grams, obtained from the Pasteur Institute of Iran. The animals were maintained under standard laboratory conditions with a 12-hour light/dark cycle and allowed to acclimate to the laboratory environment for one week prior to the experiment. They were housed under consistent environmental conditions and randomly assigned to five groups: Control, LPS, LPS with thymoquinone at 1 mg/kg, and LPS with thymoquinone at 10 mg/kg (12).

Experimental procedure

Thirty-two male C57BL/6 mice (20–25 g) were randomized into four groups (n=8 per group): (1) Control, (2) LPS, (3) LPS + thymoquinone (1 mg/kg), and (4) LPS + thymoquinone (10 mg/kg). One hour prior to LPS (Cat # L2630, Sigma-Aldrich, USA) injection (10 mg/kg, IP) (13), the thymoquinone (Cat # 274666, Sigma-Aldrich, USA) was administered to the treatment groups by oral gavage. Twenty-four hours after lipopolysaccharide administration, the mice were anesthetized with a combination of ketamine (100 mg/kg) and xylazine (10 mg/kg), after which blood samples were obtained through cardiac puncture, and the kidneys were immediately harvested. Finally, animals were euthanized in accordance with ethical guidelines.

Tissue homogenate preparation

The kidney tissue was homogenized at a w/v ratio of 2.5 % in cold lysis buffer using a rotary homogenizer (IKA Co., Germany). Following homogenization, the samples were centrifuged, and the clear supernatant was carefully collected and stored at −70 °C for subsequent biochemical assessments.

Blood biochemistry assay

Serum samples were collected one day after LPS administration by centrifuging whole blood at 3000 rpm for 10 min. The obtained serum was used to measure renal function markers, including BUN and creatinine, utilizing commercial diagnostic kits from Pars Azmun Co. (Tehran, Iran). Additionally, serum cystatin C levels were quantified using an ELISA-based immunoassay. These biomarkers collectively provide a comprehensive evaluation of kidney function in experimental animals.

Measurement of oxidative stress markers and anti-oxidant enzyme activity, including MDA, nitrite, and SOD in renal samples

MDA concentrations were measured using the thiobarbituric acid reactive substances (TBARS) method, with samples incubated in boiling water for 80 min, then centrifuged, absorbance was measured at 532 nm against a tetraethoxypropane standard curve.

Nitrite concentrations in kidney tissue were measured using the Griess reaction, with absorbance at 540 nm after incubation with reagents; levels were calculated from a sodium nitrite standard curve.

SOD activity was evaluated based on inhibition of nitro blue tetrazolium (NBT) reduction mediated by the xanthine-xanthine oxidase system, recording absorbance at 550 nm every 30 seconds over 5 min; enzyme activity was derived from inhibition percentages fitted to a standard curve.

Determination of IL-6 in renal tissue

The concentration of interleukin-6 in kidney tissue homogenates was quantified using a sandwich enzyme-linked immunosorbent assay (ELISA) with IL-6-specific antibodies from Santa Cruz Biotechnology, USA.

Assessment of renal pyroptosis via NLRP3 and caspase-1 quantification

NLRP3 levels were quantified in kidney tissue homogenates using a commercial NLRP3 kit (Cat # MBS920134, MyBiosource, USA) following the manufacturer's protocol. Kidney samples were processed to extract proteins, and equal amounts of lysate were applied to antibody-coated plates. After incubation and washing, the target protein was detected via colorimetric measurement using a microplate reader. This method provides a sensitive and specific quantification of NLRP3 expression in renal tissues.

The enzymatic activity of caspase-1 was assessed using a commercial kit (Abcam) following the prescribed instructions. In brief, a reaction mixture comprising 100 µl of buffer with 10 mM dithiothreitol was prepared, and 25 µl of sample supernatant was added under ice-cold conditions. Subsequently, 10 µL of a 2 mM YVAD-p-NA substrate was added, and the mixture was incubated in the dark at 37 °C for 60 min. The resulting product formation was quantified by measuring absorbance at 405 nm via microplate spectrophotometry.

Protein assay

Protein content was estimated by the Bradford assay using Coomassie Brilliant Blue reagent, with absorbance measured at 595 nm; assay sensitivity ranged from 5 to 100 µg protein.

Statistical analysis

Statistical analyses were performed using one-way ANOVA followed by Tukey's *post hoc* test. The results are shown as mean ± SEM. Analyses and graphing were performed using GraphPad Prism version 10.4. Statistical significance was accepted at $P<0.05$.

Results

Thymoquinone suppressed the biochemical indicators of kidney impairment induced by LPS

The administration of LPS caused a marked and statistically significant increase in BUN, creatinine, and cystatin C levels one day post-injection in comparison with the control group ($P<0.001$ for BUN and creatinine, $P<0.01$ for cystatin C) (Figure 1A-C). Administration of thymoquinone at 10 mg/kg effectively attenuated the LPS-induced rise in these renal function parameters, resulting in a significant reduction ($P<0.05$). However, BUN and creatinine levels were significantly increased in the LPS-exposed groups treated with thymoquinone at doses of 1 and 10 mg/kg compared with the control group ($P<0.001$, $P<0.05$, respectively), whereas cystatin C showed a significant elevation only in the group receiving 1 mg/kg thymoquinone relative to the control group ($P<0.05$).

Thymoquinone inhibited LPS-induced oxidative stress

LPS administration significantly increased MDA levels ($P<0.01$) and nitrite production ($P<0.05$) while markedly reducing SOD activity ($P<0.001$) relative to the control group (Figure 2 A-C). Conversely, pretreatment with thymoquinone at 10 mg/kg effectively diminished MDA concentration ($P<0.05$) and restored SOD enzymatic activity ($P<0.05$), indicating its protective anti-oxidant role against LPS-induced oxidative damage. No significant effect on nitrite levels was observed. In addition, administration of thymoquinone at a dose of 1 mg/kg to the injured group resulted in a significant increase in malondialdehyde and nitrite levels, along with a reduction in superoxide dismutase activity compared with the control group. Also, the group receiving thymoquinone at 10 mg/kg showed a significant

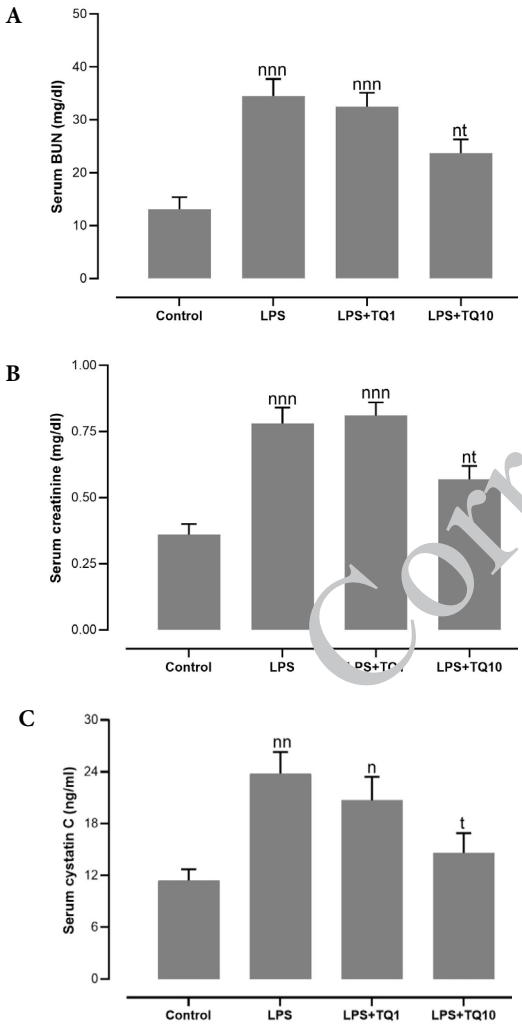

Figure 1. Mouse serum blood urea nitrogen (BUN) (A), creatinine (B), and cystatin C (C) levels in control, lipopolysaccharide (LPS)-induced acute kidney injury (AKI), and thymoquinone-treated groups.
Data are shown as mean ± SEM. Significant differences indicated by n, t $P<0.05$, nn $P<0.01$, nnn $P<0.001$, (n vs control group, t vs LPS group) (n=7 /each group).

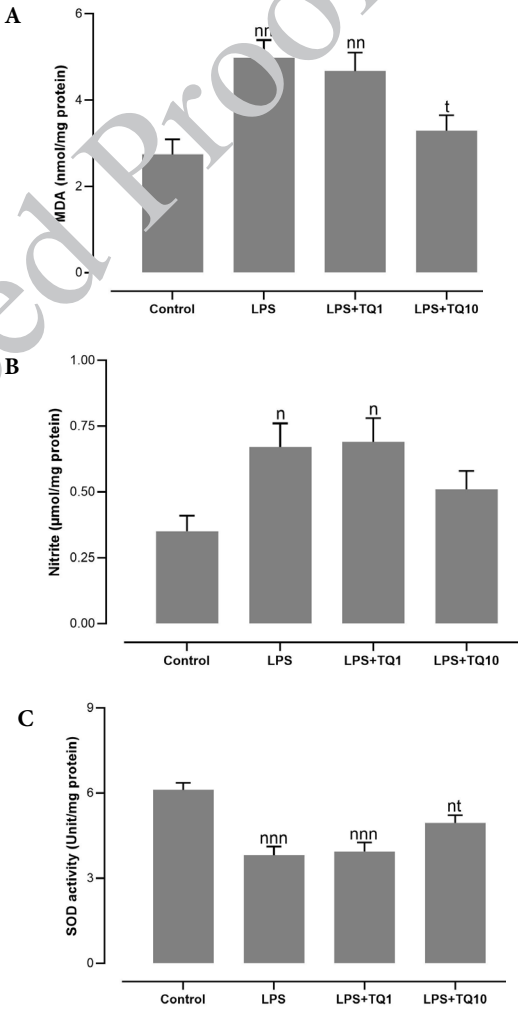

Figure 2. Mouse renal homogenate levels of malondialdehyde (MDA) (A), nitrite (B) and superoxide dismutase (SOD) activity (C) in control, lipopolysaccharide (LPS)-induced acute kidney injury (AKI), and thymoquinone-treated groups
Data are shown as mean ± SEM. Significant differences indicated by n, t $P<0.05$, nn $P<0.01$, nnn $P<0.001$, (n vs control group, t vs LPS group) (n=7 /each group).

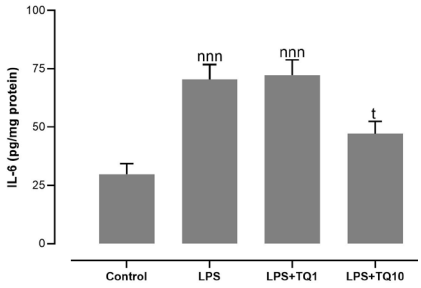

Figure 3. Renal homogenate levels of interleukin-6 (IL-6) in control, lipopolysaccharides (LPS)-induced acute kidney injury (AKI), and thymoquinone (TQ)-treated mouse groups
Data are shown as mean ± SEM. Significant differences indicated by nnn $P<0.001$, t $P<0.05$, (n vs control group, t vs LPS group) (n=7 /each group).

decrease in anti-oxidant enzyme activity compared with the injured group ($P<0.05$).

Thymoquinone inhibited LPS-induced inflammation

Inflammation was assessed by measuring IL-6 levels in kidney tissue (Figure 3). In LPS-challenged and injured mice treated with thymoquinone at 1 mg/kg, IL-6 levels were significantly increased compared with control mice (significant in both groups; $P<0.01$). However, thymoquinone (10 mg/kg) significantly reduced IL-6 levels ($P<0.05$).

Thymoquinone inhibited LPS-induced pyroptosis

NLRP3 inflammasome is a multiprotein complex that serves as a critical sensor of cellular stress and infection. Upon activation, it initiates caspase-1, which in turn promotes the processing and release of pro-inflammatory cytokines, orchestrating the body's innate immune response (14). The present study investigated the impact of thymoquinone on LPS-induced pyroptosis within renal tissue. As illustrated in Figures 4A and 4B, LPS administration significantly elevated the expression levels of NLRP3 and caspase-1 ($P<0.001$ for both). Conversely, pretreatment with thymoquinone at 10

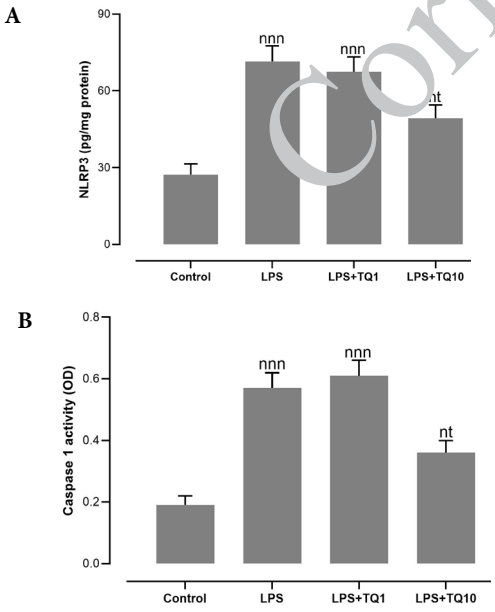

Figure 4. Renal homogenate levels of Nod-like receptor protein 3 (NLRP3) (A) and caspase-1 (B) in control, lipopolysaccharide (LPS)-induced acute kidney injury (AKI), and thymoquinone (TQ)-treated mouse groups
Data are shown as mean ± SEM. Significant differences indicated by n, t $P<0.05$, nnn $P<0.001$, (n vs control group, t vs LPS group) (n=7 /each group).

mg/kg reduced these elevations ($P<0.05$), demonstrating its modulatory effect on the pyroptotic pathway in the kidney. In addition, an increase in these two parameters was evident in the lipopolysaccharide-exposed groups that received thymoquinone at 1 and 10 mg/kg, compared with the control group ($P<0.001$, $P<0.05$, respectively).

Discussion

AKI represents a significant global health challenge, affecting over 13 million individuals each year (15). Oxidative stress, a critical factor in AKI pathogenesis, leads to excessive ROS production, which amplifies inflammation and oxidative damage, ultimately causing tissue injury through lipid peroxidation of cellular membranes (16). Following filtration into the renal tubules and cellular uptake, LPS initiates multiple harmful signaling pathways (17), leading to the up-regulation of pro-inflammatory mediators and increased oxidative stress (18). LPS contributes to oxidative disequilibrium by impairing endogenous anti-oxidant defense systems, including glutathione (GSH), SOD, and total anti-oxidant capacity (TAC) (19). Concurrently, it increases oxidative stress markers, including MDA, protein carbonyl (PC), and myeloperoxidase (MPO). Moreover, LPS directly induces tubulointerstitial nephropathy by promoting neutrophil infiltration and macrophage accumulation in renal tissues, thereby releasing pro-inflammatory mediators. (20). Our findings demonstrate that LPS exacerbates oxidative stress while impairing the kidney's anti-oxidant defenses. This was evidenced by significant reductions in SOD activity, alongside notable elevations in MDA and nitrite levels.

Inflammasomes are multiprotein complexes composed of pattern recognition receptors (PRRs) that detect damage-associated molecular patterns (DAMPs). This detection triggers their assembly and the recruitment of the adaptor protein ASC (also known as PYCARD), which possesses both PYD and CARD structural domains, along with the effector enzyme caspase-1. Activated caspase-1 then processes the inactive pro-interleukin-1β (pro-IL-1β) into its active form, IL-1β. Among the NOD-like receptor (NLR) family, the NLRP3 inflammasome is the most widely characterized (21). Caspase-1 is a proteolytic enzyme involved in inflammation, which becomes activated through inflammasome assembly triggered by pathogen-associated molecular patterns (PAMPs). Upon activation, caspase-1 enzymatically processes the precursor form of the proinflammatory cytokine IL-1β into its biologically active state (22).

As established, inflammation is a primary driver of AKI. In line with the central focus of this study on the NLRP3-caspase-1 axis, our results demonstrated that LPS challenge specifically and significantly up-regulated the key components of this inflammasome pathway, namely NLRP3 and caspase-1, in renal tissues compared to the control group. This activation was functionally coupled with a marked increase in IL-6 expression, a pivotal proinflammatory cytokine. The critical role of inflammatory mediators, including IL-1β, IL-6, and TNF-α, in the pathophysiology of sepsis-induced organ injury is well established (23-25). Importantly, our findings align with and extend previous reports indicating that LPS triggers systemic and local inflammation by elevating TNF-α, IL-6, and IL-1β, a process mechanistically linked to NLRP3 inflammasome activation (26). This inflammasome complex is a known

master regulator of pyroptosis, culminating in the cleavage of caspase-1 and GSDMD (27).

Our results indicated that LPS administration led to elevated levels of BUN, cystatin C and creatinine compared to the control group. These observations are partially consistent with findings from previous studies (28-30).

Thymoquinone has been studied for its therapeutic potential in kidney diseases (31, 32) owing to its strong capacity to counteract oxidative stress and suppress inflammatory responses. Evidence indicates its protective effects against kidney damage induced by oxidative stress, inflammation-mediated renal impairment, and dysfunction associated with various pathological conditions (33). The kidney-protective properties of thymoquinone have been validated across multiple experimental animal models of renal disorders, which have recognized thymoquinone as a multitarget agent with kidney-protective effects, attributed to its anti-oxidant, anti-inflammatory, and anti-apoptotic properties (34, 35). Like our study, previous research reported that thymoquinone treatment improved renal function by reducing BUN, cystatin C, and creatinine levels (36, 37).

In this study, thymoquinone treatment in the LPS-induced AKI group reduced the lipid peroxidation index (MDA) and increased anti-oxidant activity (SOD). Therefore, our findings are consistent with previous studies demonstrating that thymoquinone alleviates gentamicin-induced kidney dysfunction in rats by reducing MDA levels and enhancing the activities of anti-oxidant enzymes, including glutathione peroxidase-1 and SOD (38). Evidence has been presented demonstrating thymoquinone's protective role in kidney function against prolonged sodium nitrite-induced toxicity in mouse models (34). Another study reported that thymoquinone protected mouse kidneys from arsenic-induced oxidative stress by decreasing MDA levels and enhancing SOD, catalase, and glutathione peroxidase activities in renal tissue (39). The finding of this study showed that treatment of the lipopolysaccharide-induced acute kidney injury group with thymoquinone had no significant effect on the nitrite parameter, while previous studies reported that thymoquinone reduced this factor (40, 41). In our research, we observed that thymoquinone lowered IL-6 levels. This result aligns with findings from another study that examined how thymoquinone protects against methotrexate-induced testicular damage. That study found thymoquinone decreased MDA, which is important for preventing lipid damage, as well as reduced levels of biochemical markers like TNF-α, IL-1β, and IL-6 (42). In addition, a study examining the cardioprotective effects of thymoquinone in septic BALB/c mice revealed a significant reduction in plasma cTnT concentration and an increase in ATP levels. Moreover, thymoquinone markedly decreased the expression of inflammatory and pyroptosis-related markers, including p62, NLRP3, caspase-1, IL-1β, IL-18, IL-6, TNF-α, and MCP-1, while boosting the production of beclin 1 and the anti-inflammatory cytokine IL-10 (43). Thymoquinone was found in another study to markedly suppress the expression of CALR, NLRP3 subunits, and IL-1β, as well as to decrease the extracellular levels of IL-1β and soluble PD-L1 proteins associated with pro-tumorigenic breast cancer markers (44). These findings further confirm that thymoquinone attenuates lipopolysaccharide-induced pyroptosis by inhibiting caspase-1 and NLRP3 activation. In recent years, several studies have explored the suppressive role of thymoquinone on the NLRP3 signaling pathway (31, 45). Our findings extend those of previous studies (11) by demonstrating that thymoquinone significantly inhibits pyroptosis pathways through down-regulation of NLRP3 inflammasome and caspase-1 activity, thereby providing comprehensive renal protection in LPS-induced acute kidney injury. This study offers a novel understanding of thymoquinone's role in combating acute kidney injury. Our findings indicate that thymoquinone mitigates oxidative damage and inflammatory responses, thereby providing significant renal protection. Nevertheless, additional research is required to clarify its impact on molecular pathways, including apoptotic processes.

Conclusion

This study demonstrated that oral administration of a compound specifically targeting oxidative stress and inflammasome pathways exerted protective effects in mice with LPS-induced AKI. This was evidenced by marked improvement in elevated BUN and creatinine levels, restoration of oxidative balance, and inhibition of proinflammatory cytokine release alongside suppression of pyroptosis-related factors. These findings underscore thymoquinone's therapeutic potential in managing AKI.

Acknowledgment

The authors sincerely thank Shahed University, Iran, for its financial support. The reported findings constitute a segment of a medical dissertation.

Data Availability statement

The datasets can be obtained from the corresponding author if requested for a valid reason.

Authors' Contributions

N I and M R designed the experiments; N I and M R performed the experiments and collected the data; N I, M T, and MR discussed the results and strategy; M R supervised, directed, and managed the study; N I, M T, and M R approved the final version for publication.

Conflicts of Interest

The authors declare no competing interests.

Declaration

We have not used any AI tools or technologies to prepare this manuscript.

References

1. Ostermann M, Lumlertgul N, Jeong R, See E, Joannidis M, James M. Acute kidney injury. Lancet 2025; 405:241-256.
2. Bellomo R, Kellum JA, Ronco C, Wald R, Martensson J, Maiden M, *et al.* Acute kidney injury in sepsis. Intensive Care Med 2017; 43:816-828.
3. Hato T, Dagher PC. Molecular Mechanisms of sepsis-associated acute kidney injury. J Am Soc Nephrol 2025; 36:2259-2268.
4. Cui Y, Gao H, Han S, Yuan R, He J, Zhuo Y, *et al.* Oleuropein attenuates lipopolysaccharide-induced acute kidney injury *in vitro* and *in vivo* by regulating Toll-like receptor 4 dimerization. Front Pharmacol 2021; 12:617314.
5. Cao Y, Chen X, Zhu Z, Luo Z, Hao Y, Yang X, *et al.* STING contributes to lipopolysaccharide-induced tubular cell inflammation and pyroptosis by activating endoplasmic reticulum

stress in acute kidney injury. Cell Death Dis 2024; 15:217.
6. Deng J, Tan W, Luo Q, Lin L, Zheng L, Yang J. Long Non-coding RNA MEG3 promotes renal tubular epithelial cell pyroptosis by regulating the miR-18a-3p/GSDMD pathway in lipopolysaccharide-induced acute kidney injury. Front Physiol 2021; 12:663216.
7. Zhou Z, Li Q. The role of pyroptosis in the pathogenesis of kidney diseases. Kidney Dis (Basel) 2023; 9:443-458.
8. Kang HG, Lee HK, Cho KB, Park SI. A review of natural products for prevention of acute kidney injury. Medicina (Kaunas) 2021; 57.
9. Asaduzzaman Khan M, Tania M, Fu S, Fu J. Thymoquinone, as an anticancer molecule: From basic research to clinical investigation. Oncotarget 2017; 8:51907-51919.
10. Khan FZ, Mostaid MS, Apu MNH. Molecular signaling pathway targeted therapeutic potential of thymoquinone in Alzheimer's disease. Heliyon 2022; 8:e09874.
11. Althafar ZM, Al-Gabri N, Alnomasy SF. Ameliorative impacts of interleukin 35 or thymoquinone nanoparticles on lipopolysaccharide-induced renal injury in rats. Int Immunopharmacol 2024; 135:112249.
12. Alenezi SK. The ameliorative effect of thymoquinone on vincristine-induced peripheral neuropathy in mice by modulating cellular oxidative stress and cytokine. Life (Basel) 2022; 13:101.
13. Shahmohammadi A, Golchoobian R, Mirahmadi SM, Rousta AM, Ansari F, Sharayeli M, *et al.* Scutellarin alleviates lipopolysaccharide-provoked septic nephrotoxicity via attenuation of inflammatory and oxidative events and mitochondrial dysfunction. Immunopharmacol Immunotoxicol 2023; 45:295-303.
14. Valenzuela PL, Ruilope LM, Santos-Lozano A, Wilhelm M, Kränkel N, Fiuza-Luces C, *et al.* Exercise benefits in cardiovascular diseases: From mechanisms to clinical implementation. Eur Heart J 2023; 44:1874-1889.
15. Perico N, Remuzzi G. Acute kidney injury in low-income and middle-income countries: No longer a death sentence. Lancet Glob Health 2016; 4:e216-217.
16. Yang Y, Song M, Liu Y, Liu H, Sun L, Peng Y, *et al.* Renoprotective approaches and strategies in acute kidney injury. Pharmacol Ther 2016; 163:58-73.
17. Kalakeche R, Hato T, Rhodes G, Dunn KW, El-Achkar TM, Plotkin Z, *et al.* Endotoxin uptake by S1 proximal tubular segment causes oxidative stress in the downstream S2 segment. J Am Soc Nephrol 2011; 22:1505-1516.
18. Gomez H, Ince C, De Backer D, Pickkers P, Payen D, Hotchkiss J, *et al.* A unified theory of sepsis-induced acute kidney injury: Inflammation, microcirculatory dysfunction, bioenergetics, and the tubular cell adaptation to injury. Shock 2014; 41:3-11.
19. Plotnikov EY, Pevzner IB, Zorova LD, Chernikov VP, Prusov AN, Kireev, II, *et al.* Mitochondrial damage and mitochondria-targeted antioxidant protection in LPS-induced acute kidney injury. Antioxidants (Basel) 2019; 8:176.
20. Du J, Jiang S, Hu Z, Tang S, Sun Y, He J, *et al.* Vitamin D receptor activation protects against lipopolysaccharide-induced acute kidney injury through suppression of tubular cell apoptosis. Am J Physiol Renal Physiol 2019; 316:F1068-F1077.
21. Yang Y, Wang H, Kouadir M, Song H, Shi F. Recent advances in the mechanisms of NLRP3 inflammasome activation and its inhibitors. Cell Death Dis 2019; 10:128.
22. Sun Q, Scott MJ. Caspase-1 as a multifunctional inflammatory mediator: Noncytokine maturation roles. J Leukoc Biol 2016; 100:961-967.
23. Li G, Jia J, Ji K, Gong X, Wang R, Zhang X, *et al.* The neutrophil elastase inhibitor, sivelestat, attenuates sepsis-related kidney injury in rats. Int J Mol Med 2016; 38:767-775.
24. Yu C, Qi D, Sun JF, Li P, Fan HY. Rhein prevents endotoxin-induced acute kidney injury by inhibiting NF-κB activities. Sci Rep 2015; 5:11822.
25. Dirkes S. Sepsis and inflammation: Impact on acute kidney injury. Nephrol Nurs J 2013; 40:125-132;
26. Li Z, Pan H, Yang J, Chen D, Wang Y, Zhang H, *et al.* Xuanfei Baidu formula alleviates impaired mitochondrial dynamics and activated NLRP3 inflammasome by repressing NF-κB and MAPK pathways in LPS-induced ALI and inflammation models. Phytomedicine 2023; 108:154545.
27. Wu QR, Yang H, Zhang HD, Cai YJ, Zheng YX, Fang H, *et al.* IP3R2-mediated Ca(2+) release promotes LPS-induced cardiomyocyte pyroptosis via the activation of NLRP3/Caspase-1/GSDMD pathway. Cell Death Discov 2024; 10:91.
28. Zhang X, Su C, Zhao S, Li J, Yu F. Combination therapy of ulinastatin with thrombomodulin alleviates endotoxin (LPS) - induced liver and kidney injury via inhibiting apoptosis, oxidative stress and HMGB1/TLR4/NF-κB pathway. Bioengineered 2022; 13:2951-2970.
29. Yu YY, Li XQ, Hu WP, Cu SC, Dai JJ, Gao YN, *et al.* Self-developed NF-κB inhibitor 270 protects against LPS-induced acute kidney injury and lung injury through improving inflammation. Biomed Pharmacother 2022; 147:112615.
30. Luo RR, Yang J, Sun YL, Zhou BY, Zhou SX, Zhang GX, *et al.* Dexmedetomidine attenuates ferroptosis by Keap1-Nrf2/HO-1 pathway in LPS-induced acute kidney injury. Naunyn Schmiedebergs Arch Pharmacol 2024; 397:7785-7796.
31. Guo LP, Liu SX, Yang Q, Li HY, Xu LL, Hao YH, *et al.* Effect of Thymoquinone on acute kidney injury induced by sepsis in BALB/c mice. Biomed Res Int 2020; 2020:1594726.
32. Hannan MA, Zaman M, 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; 22:9078.
33. Al Fayi M, Omar H, Alshyarba M, Dera AA, Rajagopalan P. Thymoquinone and curcumin combination protects cisplatin-induced kidney injury, nephrotoxicity by attenuating NFκB, KIM-1 and ameliorating Nrf2/HO-1 signalling. J Drug Target 2020; 28:913-922.
34. Elsherbiny NM, Maysarah NM, El-Sherbiny M, Al-Gayyar MM. Renal protective effects of thymoquinone against sodium nitrite-induced chronic toxicity in rats: Impact on inflammation and apoptosis. Life Sci 2017; 180:1-8.
35. Hammad FT, Lubbad L. The effect of thymoquinone on the renal functions following ischemia-reperfusion injury in the rat. Int J Physiol Pathophysiol Pharmacol 2016; 8:152-159
36. Mahmood NN, Rashid BM, Abdulla SK, Marouf BH, Hamaamin KS, Othman HH. Effects of zofenopril and thymoquinone in cyclophosphamide-induced urotoxicity and nephrotoxicity in rats; the value of their anti-inflammatory and antioxidant properties. J Inflamm Res 2025; 18:3657-3676.
37. Hosseini A, Mehri S, Aminifard T, Ghasemzadeh Rahbardar M, Nouripor S, Khajavi Rad A, *et al.* Renoprotective effect of thymoquinone against rhabdomyolysis-induced acute kidney injury in the rat model. Iran J Basic Med Sci 2024; 27:552-559.
38. Alsharidah M, Abdel-Moneim A-MH, Alsharidah AS, Mobark MA, Rahmani AH, Shata A, *et al.* Thymoquinone, but not metformin, protects against gentamicin-induced nephrotoxicity and renal dysfunction in rats. Applied Sciences 2021; 11:3981
39. Sener U, Uygur R, Aktas C, Uygur E, Erboga M, Balkas G, *et al.* Protective effects of thymoquinone against apoptosis and oxidative stress by arsenic in rat kidney. Ren Fail 2016; 38:117-123.
40. Faisal Lutfi M, Abdel-Moneim AH, Alsharidah AS, Mobark MA, Abdellatif AAH, Saleem IY, *et al.* Thymoquinone lowers blood glucose and reduces oxidative stress in a rat model of diabetes. Molecules 2021; 26:2348.
41. Hofni A, Ali FEM, Ibrahim ARN, Aboubaker EM. Renoprotective effect of thymoquinone against streptozotocin-induced diabetic nephropathy: Role of NOX2 and Nrf2 signals. Curr Mol Pharmacol 2023; 16:905-914.
42. Kaymak E, Ceylan T, Akın T, Kuloğlu N, Sayan M, Değer N, *et al.* Effect of thymoquinone on NRF2/NF-kB/MAPK pathway in methotrexate-induced rat testis injury. Iran J Basic Med Sci 2024; 27:1410-1416.
43. Liu H, Sun Y, Zhang Y, Yang G, Guo L, Zhao Y, *et al.* Role of Thymoquinone in cardiac damage caused by sepsis from BALB/c

mice. Inflammation 2019; 42:516-525.
44. Elgohary S, Eissa RA, El Tayebi HM. Thymoquinone, a novel multi-strike inhibitor of pro-tumorigenic breast cancer (BC) markers: CALR, NLRP3 pathway and sPD-L1 in PBMCs of HR+ and TNBC patients. Int J Mol Sci 2023; 24:14254.
45. Pei ZW, Guo Y, Zhu HL, Dong M, Zhang Q, Wang F. Thymoquinone protects against hyperlipidemia-induced cardiac damage in low-density lipoprotein receptor-deficient (LDL-R(-/-)) mice via its anti-inflammatory and antipyroptotic effects. Biomed Res Int 2020; 2020:4878704.