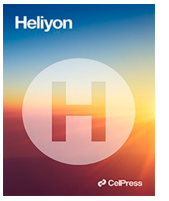
Heliyon 10 (2024) e24840

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Thymoquinone alleviates cisplatin-induced kidney damage by reducing apoptosis in a rat model

Shuai Li [a], Zhanxue Zhao [b,*]

[a] Department of Clinical Pharmacy, Qinghai University Affiliated Hospital, Xining, Qinghai Province, China
[b] Department of General Surgery, Qinghai Provincial People's Hospital, Xining, Qinghai Province, China

ARTICLE INFO

Keywords:
Thymoquinone
Cisplatin
Kidney damage
Apoptosis

ABSTRACT

Purpose: The aim of the study was to compare the ameliorating effects of thymoquinone at various dosages on cisplatin-induced renal toxicity, and to investigate its effects on cisplatin-induced nephrocyte apoptosis via the mitochondrial pathway in a rat model.
Methods: A rat model of cisplatin-induced renal damage was established, with thymoquinone treatment groups (receiving 1, 3, 5, 10, or 20 mg/kg of thymoquinone). We determined serum creatinine (Cr) and blood urea nitrogen (BUN), measured the expression of the anti-apoptotic protein Bcl-2, the pro-apoptotic protein Bax, caspase-3, kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL) in renal tissue. Additionally, we observed pathological changes in renal tissue and performed paller score for renal tubule injury.
Results: Relative to the control, the cisplatin group exhibited significantly elevated Bax, caspase-3, NGAL and KIM-1 expression, elevated serum Cr and BUN concentrations and significantly reduced Bcl-2 expression ($P < 0.05$). Histopathological examination of cisplatin-treated group revealed vacuolar degeneration, tubular epithelial cell swelling, and an absence of brush margins on renal tubules. Paller score was significantly elevated in the cisplatin group relative to the normal control group. Thymoquinone dose-dependently ameliorated these effects.
Conclusion: Thymoquinone at 1–20 mg/kg improved cisplatin-induced renal dysfunction in rats. This protective effect is related to the inhibition of mitochondria-mediated apoptosis.

1. Introduction

Cisplatin is a platinum-based complex widely used in clinical oncology. As a cell-cycle nonspecific drug with potent and broad-spectrum anticancer effects, it has strong inhibitory and lethal effects toward various solid tumors. However, its well-documented renal toxicity is one of the main limitations in its clinical application. The high concentration and long-term accumulation of cisplatin in kidney tissue are believed to form the basis of this nephrotoxicity. Cisplatin-induced oxidative stress, inflammatory reactions, and apoptosis are closely related to its nephrotoxicity [1,2]. In addition to limiting the cisplatin dosage to reduce damage to renal function, the development of prevention strategies, potentially including drug therapy, is indispensable in the clinical management of cisplatin-induced kidney damage.

Thymoquinone (2-isopropyl-5-methyl-1, 4-benzoquinone, molecular weight: 164.2 g/mol), is the primary active component of black grass seed *Nigella sativa*, accounting for 27.8–57 % of the volatile oil [3,4]. Many studies, some in clinical stages, are being

* Corresponding author.
E-mail addresses: yaoxuelishuai@sina.com (S. Li), zhaozhanxue1025@sina.com (Z. Zhao).

https://doi.org/10.1016/j.heliyon.2024.e24840
Received 12 July 2023; Received in revised form 15 January 2024; Accepted 15 January 2024
Available online 17 January 2024

conducted on its anticancer and antiinflammatory activity. It has a wide range of pharmacological effects, including anti-inflammatory, analgesic, anti-hyperglycemic, neuroprotective, anticancer, and antioxidant effects [5], as well as a good safety profile, with an LD_{50} in rats of 57.5 mg/kg intraperitoneally and 794.3 mg/kg orally [6]. Studies have shown that it can ameliorate drug-induced kidney damage caused by drugs such as antibiotics (including gentamicin [7] and vancomycin [8]), antitumor drugs (including doxorubicin [9], methotrexate [10], and cisplatin [11]), non-steroidal anti-inflammatories (including acetaminophen [12] and diclofenac [13]), and by heavy metals (including arsenic [14], lead [15], and cadmium [16]) and contrast agents.

Few studies that thymoquinone improved kidney damage induced by cisplatin were reported. The dosage of thymoquinone required to ameliorate cisplatin-induced renal damage is controversial. In a rat study, administering thymoquinone at 1.5 mg/kg achieved the greatest improvement; higher concentrations did not improve the curative effect, but reduced renal function [17]. In a vivo (rat) study, the highest dosage of thymoquinone used to improve cisplatin-induced kidney injury was 10 mg/kg [18], although another study confirmed that it could protect against cisplatin-induced nephrotoxicity at 20 mg/kg [19].

Cisplatin-induced kidney damage is closely associated with apoptosis. Nonetheless, few studies about thymoquinone have addressed the mechanisms of anti-apoptotic action, based on a PUBMED search [7,9,10,13,20]. Many preclinical studies have confirmed that it can ameliorate drug-induced kidney injury, with such research focusing on its antioxidant and anti-inflammatory mechanisms [21]. To address this, we created a rat model of cisplatin-induced kidney damage and observed the preventative and therapeutic effects of different dosages of thymoquinone on this condition, focusing on Bcl-2 (anti-apoptotic protein), Bax (pro-apoptotic protein), caspase-3 (end-cutting enzymes in the process of apoptosis), kidney injury molecule-1 (KIM-1, a marker of kidney injury) and neutrophil gelatinase-associated lipocalin (NGAL, a marker of kidney injury). These findings provide a theoretical basis for further research on the preventative and therapeutic effects of thymoquinone in cisplatin-induced kidney damage.

2. Materials and methods

2.1. Animals

Forty-two specific pathogen-free (SPF) healthy male Sprague Dawley (SD) rats aged 6–8 weeks and weighing 200 ± 20 g were obtained from the Hubei Experimental Animal Research Center [SCXK (E) 2020-0018](Wuhan, China). The animals were adaptively fed in a metabolic cage for one week. The experiment was approved by the research Ethics Committee of the Qinghai University Affiliated Hospital , China (P-SL-2021150).

2.2. Drugs and chemicals

Thymoquinone and cisplatin were purchased from MedChemExpress (NJ, USA). The following were used: β-Actin antibody kit (TDY051, Tiandeyue, Beijing, China), Bcl-2 antibody kit (ab196495, Abcam, Cambridge, Britain), Bax antibody Kit (#2772, CST, BO, USA), Cleaved caspase-3 antibody kit (AF7022, Affbiotech, OH, USA), GAPDH antibody kit (ab181602, Abcam, Cambridge, Britain), NGAL antibody kit (ab125075, Abcam, Cambridge, Britain), KIM-1 antibody kit (bs-21420r, BIOSS, Beijing, China), 0.45 μm polyvinylidene fluoride (PVDF) film (IPVH00010, Millipore, MA, USA), Eosin Y (alcohol soluble; D12621, Xiya, Sichuan, China), Hematoxylin (H9627-25G, Sigma Aldrich, MO, USA), Neutral balsam (G8590, Solarbio, Beijing, China), Creatinine assay kit (C011-2, Jiancheng, Nanjing, China) and Urea Nitrogen (BUN) colorimetric detection kit (C013-2, Jiancheng, Nanjing, China). All other reagents and assay test kits were obtained from ASPEN Biotechnology Co., Ltd. (Wuhan, China) or Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

2.3. Instruments and equipment

We used the following equipment made in China: a microplate reader (USCNK, SMR16.1), tabletop centrifuge (TGL-16c, Shanghai Anting), refrigerated centrifuge (TGL-16, Hunan Xingyi), ice machine (IMS-20, Changshu Xueke), water bath (HH–W-600, Jintan Jiangnan), constant-temperature incubator (GNP9160, Shanghai Jinghong), electric homogenizer (7495400000, Kimble), camera obscura (AX-II, Guangdong Yuehua), scanner (LiDE110, Canon), and an imaging system (MicroPublisher, Q-IMAGING). The electrophoresis apparatus, including tank and decolorization shaker, were purchased from Beijing Liuyi Instrument Factory. An optical microscope and photographic microscope (OLYMPUS) were used.

2.4. Experimental design

Cisplatin was dissolved into a 0.6 mg/mL solution with normal saline, and thymoquinone was prepared into a 2 mg/mL solution with corn oil. The 42 rats were randomly divided into the seven groups (n = six animals per group). Group A (the normal control group) was administered 2 mL corn oil intragastrically for 8 d, with a single intraperitoneal injection of normal saline (2 mL) on day 3. Group B was administered 2 mL corn oil intragastrically for 8 d, with a single intraperitoneal injection of cisplatin at 6 mg/kg (2 mL) on day 3. Groups C to G (thymoquinone treatment) were given intragastrically thymoquinone at 1, 3, 5, 10, or 20 mg/kg daily for 8 d, with a single intraperitoneal injection of 6 mg/kg (2 mL) cisplatin on day 3. It was worth noting that the selected doses of thymoquinone were below its oral LD50.

For cisplatin, the recommended human dose is 30–60 mg by intraperitoneal injection. Based on body surface area, the equivalent intraperitoneal injection cisplatin dosage is 2.7–5.4 mg/kg in rats. A single intraperitoneal injection of cisplatin at 6 mg/kg has

previously been used to create a rat model of kidney damage [19]. We therefore selected a cisplatin dosage of 6 mg/kg.

2.5. Biochemical analysis

Blood samples were collected on day 9 of the experiment, 24 h after the last treatment. The rats were anaesthetized using chloral hydrate (3.3 mL/kg intraperitoneally), the abdominal cavity was cut, and blood was sampled from the abdominal aorta. The sample was stored at room temperature (18–24 °C) for 1 h, after which the blood clotted; it was then centrifuged at 4 °C and 2000×*g* for 10 min. The supernatant was collected and stored at −20 °C or lower for further analysis. Serum Cr and BUN, indices of renal biochemistry, were measured using assay test kits, according to the manufacturers' protocol.

2.6. Protein expression measurements

Following blood sampling the kidney tissue was harvested, rinsed 2–3 times with pre-cooled PBS buffer (to remove substances such as blood or oil), then cut into small pieces and homogenized. Histone extraction reagent (ASPEN, Wuhan, China) was added to the homogenizer at a volume 10–20 times that of the tissue, and the mixture was thoroughly homogenized in an ice bath. The resulting mixture was centrifugated at 21367×*g* at 4 °C for 5 min, the supernatant was collected, the protein concentration was determined by using a BCA protein concentration assay kit (ASPEN, Wuhan, China) and the total protein-loading was at least 20 μg per well. Thereafter, 5 × protein-loading buffer was added, and the sample was bathed in boiling water at 95–100 °C for 5 min. After separation using SDS-PAGE gel electrophoresis, the sample was transferred to a PVDF membrane using a constant current at 300 mA. The membranes were then incubated at room temperature for 1 h. Primary antibodies against β-Actin, Bcl-2, Bax, cleaved caspase-3, GAPDH, NGAL and KIM-1 were diluted at ratios of 1:10000, 1:2000, 1:3000, 1:500, 1:10000, 1:10000 and 1:1000 respectively, and added to the membranes, which were then incubated overnight at 4 °C. The diluted primary antibody was recovered and the membranes were washed three times for 5 min each time with tris buffered saline (TBS) with 0.1 % Tween 20 detergent (TBST) buffer. Diluted secondary antibody (HRP-Goat anti-Rabbit Antibody kit) was added to the membrane, followed by incubation at room temperature for 30 min. After washing four times with TBST for 5 min each time at room temperature (18–24 °C), an enhanced chemiluminescence (ECL) solution was added. Protein imprinting was detected using an ECL chemiluminescence detection kit, and target band optical density was analyzed using AlphaEaseFC software (Alpha Innotech, Calif., USA). The relative expression level of the target protein was expressed as the ratio of the gray value of the target protein band to that of the β-Actin or GAPDH band.

2.7. Histopathology

Kidney tissue samples were fixed with 4 % paraformaldehyde for >24 h then embedded in paraffin. Paraffin sections (2–3 μm thick) were prepared for subsequent study. After dewaxing using xylene, anhydrous ethanol, and 75 % alcohol, the cores were stained with hematoxylin for 3–10 min, differentiated using hydrochloric acid, reverted to blue using ammonia, then stained with eosin dye solution for 1–3 min. After dehydration using anhydrous ethanol and xylene, the slices were sealed with neutral balsam. Histopathological examination was performed using a light microscope. Paller score was performed by observing the pathological changes of renal tubules (100 in total) in 10 consecutive fields under a 10 × 40 magnification microscope [22]. The total score was 5 points: obvious tubular dilatation (1point); brush border damage (1point) or shedding (2 points); exfoliated and necrotic cells (1point) or tubular type (2 points) in the lumen of renal tubules.

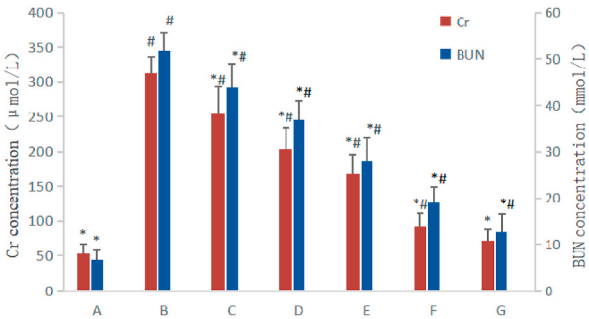


Fig. 1. Effect of thymoquinone on serum creatinine (Cr) and blood urea nitrogen (BUN) concentration in cisplatin induced nephrotoxicity in rats (n = 6)

*: Significantly different from group B; # : Significantly different from group A

Group A: normal control group, Group B: in rats treated with cisplatin only, Group C: in rats treated with cisplatin and 1 mg/kg thymoquinone, Group D: in rats treated with cisplatin and 3 mg/kg thymoquinone, Group E: in rats treated with cisplatin and 5 mg/kg thymoquinone, Group F: in rats treated with cisplatin and 10 mg/kg thymoquinone, Group G: in rats treated with cisplatin and 20 mg/kg thymoquinone.

2.8. Statistical analysis

Statistical analysis was performed using SPSS 25.0, and measurement data were expressed as mean ± standard deviation (mean ± SD). One-way ANOVA was used for inter-group comparisons, and the least significant difference (LSD) method was used for paired comparisons. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of thymoquinone on serum Cr and BUN concentration

The concentrations of serum Cr and BUN were significantly elevated in the cisplatin-treated group relative to the normal control. However, relative to the cisplatin group, the addition of thymoquinone significantly reduced the concentrations of serum Cr and BUN. Not only that, serum Cr and BUN concentrations of groups C to G decreased dose-dependently with increasing thymoquinone concentration. Serum Cr concentration did not differ significantly between groups F and G, and between the normal control group and group G. Every group differed significantly in serum BUN concentration with others. The reciprocal of serum BUN concentration exhibited a strong linear association with thymoquinone dosage in groups B to G. ($R^2 = 0.9958$) (Figs. 1 and 2).

3.2. Effects of thymoquinone on Bcl-2, Bax, and caspase-3 expression in rat kidney tissue

Relative to the normal control, Bcl-2 expression was significantly reduced and Bax and caspase-3 protein expression significantly elevated following cisplatin treatment. Relative to cisplatin treatment, Bcl-2 expression was significantly elevated and Bax and caspase-3 expression significantly reduced following thymoquinone and cisplatin treatment. Bcl-2, Bax, and caspase-3 expression was associated with thymoquinone dosage. Pairwise comparison revealed significant differences between the groups in Bax and caspase-3 protein expression. Bcl-2 expression differed (although nonsignificantly) between groups A and G and between groups C and D [Fig. 3a, b].

3.3. Effects of thymoquinone on NGAL and KIM-1 expression in rat kidney tissue

Relative to the normal control, NGAL and KIM-1 protein expressions were significantly elevated following cisplatin treatment. Relative to cisplatin treatment, NGAL and KIM-1 expression significantly reduced following thymoquinone and cisplatin treatment in a dose-dependent manner [Fig. 4a, b].

3.4. Effects of thymoquinone on kidney histopathology

In the normal control group, the kidney tissue was well structured and normal, with no pathology (Fig. 5a). In the cisplatin group, the renal tissue was severely damaged, with many inflammatory cells and vacuolar degeneration. Renal tubule brush edges were absent, although protein-like tubules were present (Fig. 5b). In the cisplatin group with thymoquinone at 1 mg/kg, a few inflammatory cells were present, parts of the tubular epithelial cells were exuded and necrotic, and the brush border was absent (Fig. 5c). With increasing thymoquinone dosage (3 and 5 mg/kg; Fig. 5d and e, respectively), renal pathology was progressively ameliorated; at 10 mg/kg (Figs. 5f) and 20 mg/kg (Fig. 5g), the renal morphology appeared to be normal. The statistical results of paller score were shown in Fig. 6. Paller score was significantly elevated in the cisplatin-treated group relative to the normal control. However, thymoquinone reduced the high paller score caused by cisplatin.

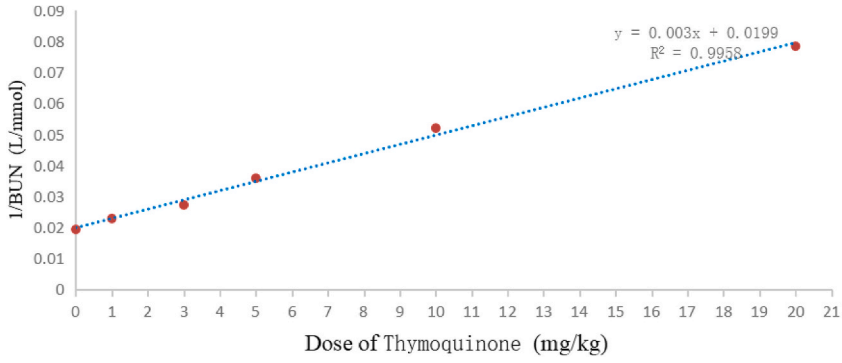


Fig. 2. Relationship between serum BUN concentration and thymoquinone dose in rats.

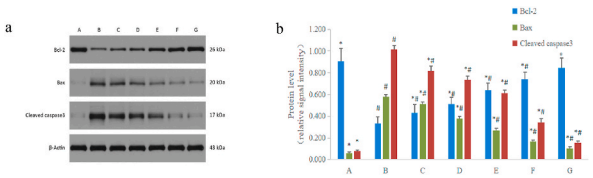


Fig. 3. Effects of thymoquinone on Bcl-2, Bax, and caspase-3 expression in rat kidney in cisplatin induced nephrotoxicity in rats (n = 3). a: Western blot of Bcl-2, Bax and caspase-3 expression in rat kidney in cisplatin induced nephrotoxicity in rats b: Statistical analysis plot of the effect of thymoquinone on Bcl-2, Bax and caspase-3 expression in rat kidney in cisplatin induced nephrotoxicity in rats *: Significantly different from group B; #: Significantly different from group A
Group A: normal control group, Group B: in rats treated with cisplatin only, Group C: in rats treated with cisplatin and 1 mg/kg thymoquinone, Group D: in rats treated with cisplatin and 3 mg/kg thymoquinone, Group E: in rats treated with cisplatin and 5 mg/kg thymoquinone, Group F: in rats treated with cisplatin and 10 mg/kg thymoquinone, Group G: in rats treated with cisplatin and 20 mg/kg thymoquinone.

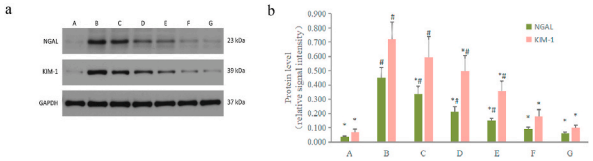


Fig. 4. Effects of thymoquinone on NGAL and KIM-1 expression in rat kidney in cisplatin induced nephrotoxicity in rats (n = 3). a: Western blot of NGAL and KIM-1 expression in rat kidney in cisplatin induced nephrotoxicity in rats
b: Statistical analysis plot of the effect of thymoquinone on NGAL and KIM-1 expression in rat kidney in cisplatin induced nephrotoxicity in rats *: Significantly different from group B; #: Significantly different from group A.Group A: normal control group, Group B: in rats treated with cisplatin only, Group C: in rats treated with cisplatin and 1 mg/kg thymoquinone, Group D: in rats treated with cisplatin and 3 mg/kg thymoquinone, Group E: in rats treated with cisplatin and 5 mg/kg thymoquinone, Group F: in rats treated with cisplatin and 10 mg/kg thymoquinone, Group G: in rats treated with cisplatin and 20 mg/kg thymoquinone.

4. Discussion

Although cisplatin is the first-line drug for treating various solid tumors, its renal toxicity can limit its clinical use, even causing treatment to be stopped in cases of severe toxicity. There is therefore an urgent clinical need to for strategies to ameliorate its nephrotoxic effects. In this study, we examined the ameliorative effects of thymoquinone on kidney cell apoptosis in a cisplatin-induced rat model of renal damage. Building on earlier studies, we tested the effects of five dosages (1, 3, 5, 10, and 20 mg/kg).

Apoptosis occurs via endogenous pathways mediated by mitochondria as well as exogenous pathways mediated by death receptors. Endoplasmic reticulum stress can lead to apoptosis. Few studies have addressed the anti-apoptotic effects of thymoquinone in treating drug-induced kidney injury. In a gentamicin-induced renal cell apoptosis rat model, gentamicin significantly reduced renal Bcl-2 expression, while thymoquinone significantly improved it, thereby reducing cell apoptosis [7]. El-Sheikh et al. [10] examined the effects of thymoquinone on methotrexate renal toxicity: caspase-3 expression was significantly upregulated in the methotrexate-treated group, especially in the renal tubule superior cortex, and administering thymoquinone significantly reversed this effect. In a rat study on reducing the renal toxicity of diclofenac, diclofenac significantly reduced renal mitochondrial activity and increased the levels of DNA fragmentation, caspase-3 activity, and cytochrome C; administering thymoquinone rescued these effects [13]. Kaymak et al. [9] examined the effects of doxorubicin on kidney cell apoptosis and caspase-3, IL-17, GRP78, and TNF-α expression: in a doxorubicin-induced renal injury model, thymoquinone reduced the expression of these proteins, thus ameliorating renal damage by inhibiting apoptosis. Thymoquinone protects against silver nanoparticle-induced cytotoxicity via an anti-apoptotic effect: when administered along with silver nanoparticles, thymoquinone reduced Bax and increased Bcl-2 levels [20]. These studies suggest that thymoquinone has renoprotective effects, inhibiting apoptosis, when administered with nephrotoxic drugs.

Only one study has included Bcl-2 and Bax expression as outcomes [20]. Bcl-2 and Bax, which have opposing effects, form an important regulatory protein pair in the mitochondria-mediated endogenous apoptosis pathway [23]. Together with caspase-3, a key executive protease in cell apoptosis, these proteins play central roles in the mitochondria-mediated apoptosis pathway. Based on this, we selected these proteins as study targets.

Bcl-2 expression was significantly reduced following cisplatin treatment, while that of Bax and caspase-3 were significantly elevated. Thymoquinone treatment significantly and dose-dependently reversed these effects. Bcl-2 expression was higher (by 18.56 %, although nonsignificantly) at 3 mg/kg thymoquinone than at 1 mg/kg, while Bax and caspase-3 expression were significantly lower at 3 mg/kg than at 1 mg/kg, suggesting that the inhibitory effect of thymoquinone on apoptosis increases dose-dependently. This is consistent with our biochemical and histological findings. These findings indicate that thymoquinone protects renal function by inhibiting mitochondria-mediated apoptosis.

The anti-apoptotic effect of thymoquinone exhibited here appears to contradict its pro-apoptotic effects in anticancer, and we suspect that this depends on the dosage. However, comparing the dosages used for renoprotection with those used to fight cancer [24],

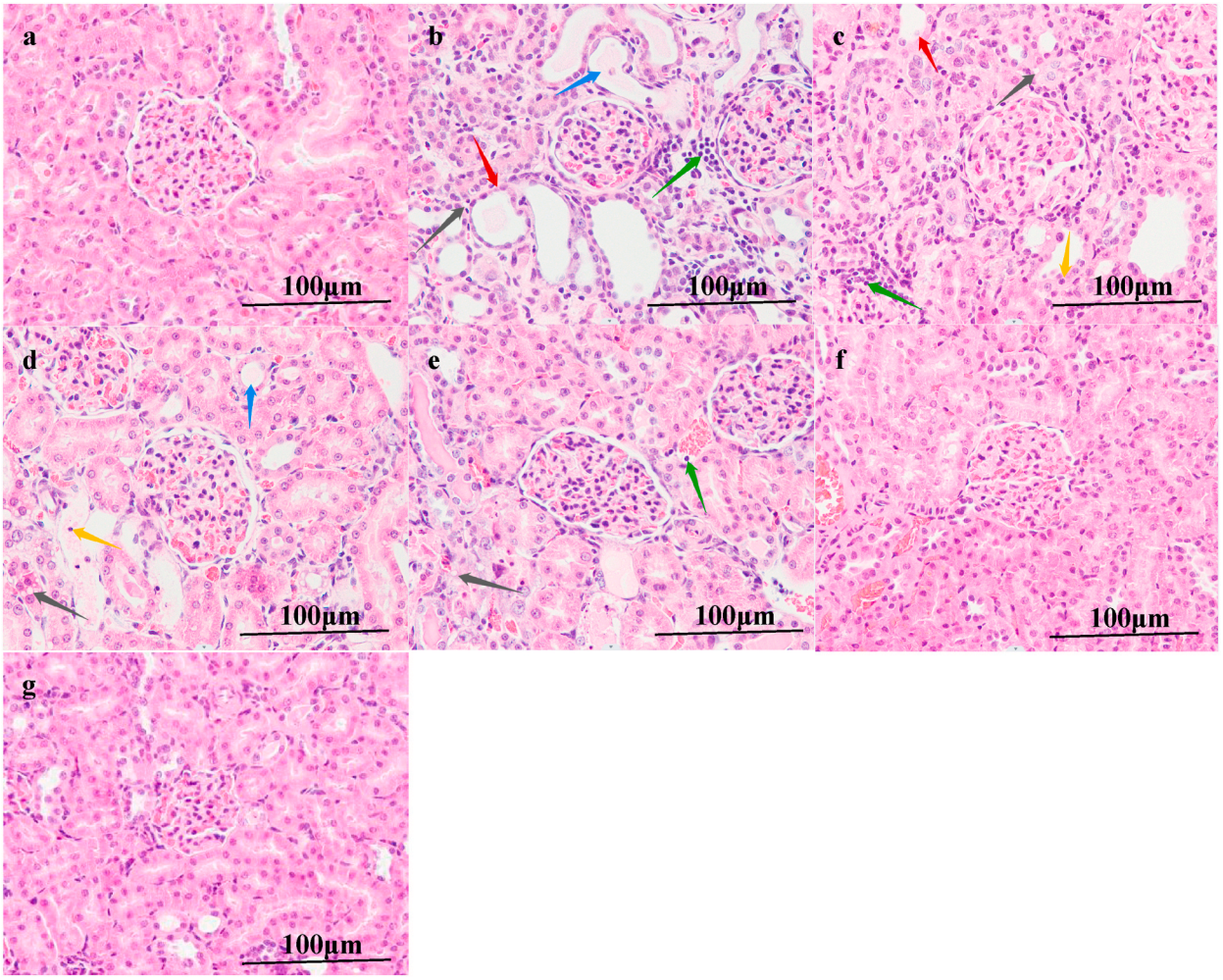


Fig. 5. Effect of thymoquinone on histopathology of kidney tissue in cisplatin induced nephrotoxicity in rats (HE × 400) (n = 3)
a: normal control group; b: in rats treated with cisplatin only; c: in rats treated with cisplatin and 1 mg/kg thymoquinone; d: in rats treated with cisplatin and 3 mg/kg thymoquinone; e: in rats treated with cisplatin and 5 mg/kg thymoquinone; f: in rats treated with cisplatin and 10 mg/kg thymoquinone; g: in rats treated with cisplatin and 20 mg/kg thymoquinone. The renal tissue was well structured and normal in morphology (a).A large number of inflammatory cells are accumulated (green arrow), the brush edges of the renal tubules are absent (red arrow), vacuolar degeneration can be observed (black arrow), and protein-like tubules were present (blue arrow)(b). A small number of inflammatory cells are gathered, parts of the tubular epithelial cells are exuded and necrotic (yellow arrow), and the brush border is absent (c). The micrographs in groups (d–g) showed kidney damage gradually reduced with the increase of thymoquinone dose. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

there is a large degree of overlap.

Thymoquinone at 1–20 mg/kg significantly and dose-dependently reduced Cr and BUN, consistent with an earlier finding that it dose-dependently ameliorated gentamicin toxicity [25]. However, it seems to contradict the results of a previous study [17]. Considering the different design of the experiment, we suspect that the low stability of the thymoquinone aqueous solution may affect the experimental results [26]. In our research, Cr declined more slowly above 10 mg/kg, returning to a normal level (although still elevated by 36.13 % relative to the normal control) at 20 mg/kg. With a statistical point of view, this result is consistent with a previous research published in 1997 [18]. However, while considering the protective effects of thymoquinone on the kidney from other indicators, the dose of 20 mg/kg is still superior to 10 mg/kg. In addition, researchers [27]. used thymoquinone (a dosage of 50 mg/kg) combined with curcumin to protect cisplatin-induced kidney injury in rats, suggesting that the therapeutic dose of thymoquinone may continue to increase above the level that we tested. However, the thymoquinone dosage cannot be increased endlessly, and the benefits seem to be limited beyond a saturation point. There was a strong linear association between the reciprocal of BUN concentration and thymoquinone dosage. Serum BUN is therefore potentially useful for determining individualized therapeutic thymoquinone dosages.

In order to improve the early diagnosis rate of acute kidney injury (AKI),a number of new biomarkers were added to the study, including NGAL and KIM-1. NGAL is a 25-kDa protein of the lipocalin family. It is conjugated to gelatinase and specific to neutrophils. KIM-1 is a 38.7-kDa type I transmembrane glycoprotein with an extracellular immunoglobulin-like domain topping a long mucin-like

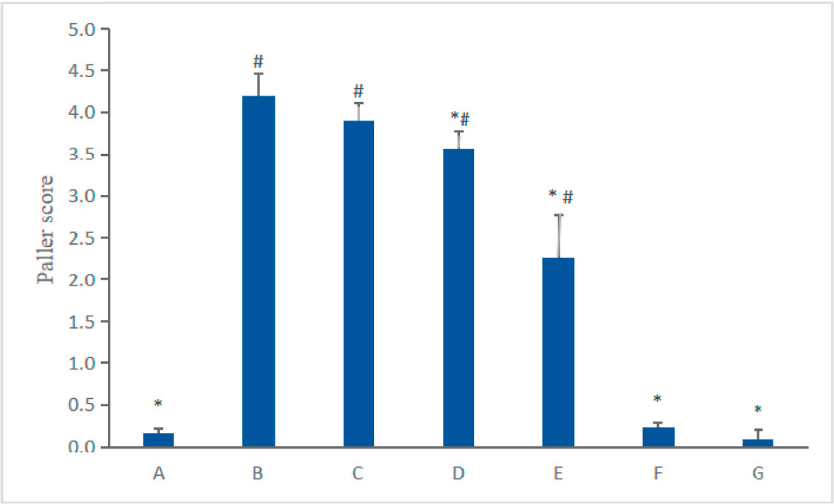


Fig. 6. Comparison of Paller score of renal injury in each group in cisplatin induced nephrotoxicity in rats (n = 3) *: Significantly different from group B; # : Significantly different from group A.Group A: normal control group, Group B: in rats treated with cisplatin only, Group C: in rats treated with cisplatin and 1 mg/kg thymoquinone, Group D: in rats treated with cisplatin and 3 mg/kg thymoquinone, Group E: in rats treated with cisplatin and 5 mg/kg thymoquinone, Group F: in rats treated with cisplatin and 10 mg/kg thymoquinone, Group G: in rats treated with cisplatin and 20 mg/kg thymoquinone.

domain [28]. Under normal circumstances, NGAL and KIM-1 are expressed at very low levels. However, they are highly upregulated at mRNA and protein level in drug-induced AKI rodents. Vikash Sinha [29] confirmed that the abnormal increase of NGAL and KIM was earlier than Cr in rats treated with cisplatin. KIM-1 levels remained elevated for 10 days, but NGAL only maintained for 3 days. However, a high level of NGAL was still observed on 5th day after cisplatin injection [30]. The duration of elevated NGAL might be related to the severity of renal damage [28]. In our study, NGAL and KIM-1 expression levels were significantly elevated in the cisplatin group. Thymoquinone improved these changes in a dose-dependent manner. While the thymoquinone dose reached 10 mg/kg, NGAL and KIM-1 levels became statistically non-different from normal control group. Although NGAL and KIM-1 levels in the group of thymoquinone 20 mg/kg decreased by 31.11 % and 44.51 %, respectively, comparing with thymoquinone 10 mg/kg group, the differences were not statistically significant.

Owing to financial constraints, the thymoquinone dosage used here did not exceed 20 mg/kg. Based on the 50 mg/kg dosage mentioned earlier [27], further research using higher dosages is warranted to determine the optimal thymoquinone dosage for improving renal function. However, thymoquinone LD50 should be considered. Apoptosis-related protein and RNA expression are now often examined concurrently, and this approach may be more effective in designing experimental studies to examine molecular mechanisms. However, considering that protein expression occurs downstream of RNA regulation, we were able to achieve our objectives even though our observations were limited to changes of apoptosis-related protein expression.

Drugs to treat or prevent cisplatin-induced kidney damage would be invaluable in clinical practice. In our rat model, thymoquinone at 1–20 mg/kg effectively improved cisplatin-induced renal dysfunction, which is characterized by reduced serum Cr and BUN. Renal tissue morphology returned to normal, with a reduction in inflammatory cell count, renal tubule edema, and vacuolar degeneration. This protective effect is related to the inhibition of mitochondria-mediated apoptosis. These findings provide an accurate reference for future determination of the therapeutic dosage of thymoquinone.

Declarations

Ethical Approval

This study was performed in line with the regulations on the Administration of Laboratory Animals of the State Science and Technology Commission of the People's Republic of China (Revised in 2017). Approval was granted by the Ethics Committee of the Qinghai University Affiliated Hospital (P-SL-2021150).

Funding

This work was supported by the Young and middle-aged scientific research fund project of Qinghai University Affiliated Hospital (No. ASRF-2021-YB-19) and Qinghai Association for Science and Technology young and middle-aged talents lifting project 2023.

CRediT authorship contribution statement

Shuai Li: Writing – original draft, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Zhanxue Zhao:** Writing – review & editing, Supervision, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24840.

References

[1] Y. Qiu, Y.F. Yang, Z.G. Huang, J.L. Zhou, X.Y. Liang, Li Huang, CAI ZW, protection effect of panax notoginseng saponins in rats with cisplatin-induced nephrotoxicity, Chin. J. Exp. Tradit. Med. Formulae 22 (2016) 104–109, https://doi.org/10.13422/j.cnki.syfjx.2016210104.

[2] C. Tang, M.J. Livingston, R. Safirstein, Z. Dong, Cisplatin nephrotoxicity: new insights and therapeutic implications, Nat. Rev. Nephrol. 19 (2023) 53–72, https://doi.org/10.1038/s41581-022-00631-7.

[3] M.M. Farag, A.A. Khalifa, W.F. Elhadidy, R.M. Rashad, Thymoquinone dose-dependently attenuates myocardial injury induced by isoproterenol in rats via integrated modulations of oxidative stress, inflammation, apoptosis, autophagy, and fibrosis, Naunyn-Schmiedeberg's Arch. Pharmacol. 394 (2021) 1787–1801, https://doi.org/10.1007/s00210-021-02087-1.

[4] S. Li, Z.X. Zhao, Research progress of mechanism of Nigella sativa seed oil to ameliorate drug induced kidney injury, Chin Hosp Pharm J 37 (2017) 1997–2000, https://doi.org/10.13286/j.cnki.chinhosppharmacyj.2017.19.27.

[5] E.M. Yimer, K.B. Tuem, A. Karim, N. Ur-Rehman, F. Anwar, Nigella sativa L. (black cumin): a promising natural remedy for wide range of illnesses, Evid Based Complement Alternat Med (2019) 1528635, https://doi.org/10.1155/2019/1528635, 2019.

[6] Z. Zhao, L. Liu, S. Li, X. Hou, J. Yang, Advances in research on the relationship between thymoquinone and pancreatic cancer, Front. Oncol. 12 (2022) 1092020, https://doi.org/10.3389/fonc.2022.1092020.

[7] A.M. Mahmoud, O.M. Ahmed, S.R. Galaly, Thymoquinone and curcumin attenuate gentamicin-induced renal oxidative stress, inflammation and apoptosis in rats, EXCLI J 13 (2014) 98–110.

[8] F. Basarslan, N. Yilmaz, S. Ates, T. Ozgur, M. Tutanc, V.K. Motor, V. Arica, C. Yilmaz, M. Inci, S. Buyukbas, Protective effects of thymoquinone on vancomycin-induced nephrotoxicity in rats, Hum. Exp. Toxicol. 31 (2012) 726–733, https://doi.org/10.1177/0960327111433185.

[9] E. Kaymak, E. Öztürk, A.T. Akİn, D. Karabulut, B. Yakan, Thymoquinone alleviates doxorubicin induced acute kidney injury by decreasing endoplasmic reticulum stress, inflammation and apoptosis, Biotech. Histochem. 97 (2022) 622–634, https://doi.org/10.1080/10520295.2022.2111465.

[10] A.A.K. El-Sheikh, M.A. Morsy, A.M. Abdalla, A.H. Hamouda, I.A. Alhaider, Mechanisms of thymoquinone hepatorenal protection in methotrexate-induced toxicity in rats, Mediat. Inflamm. (2015) 859383, https://doi.org/10.1155/2015/859383, 2015.

[11] R. Ulu, A. Dogukan, M. Tuzcu, H. Gencoglu, M. Ulas, N. Ilhan, I. Muqbil, R.M. Mohammad, O. Kucuk, K. Sahin, Regulation of renal organic anion and cation transporters by thymoquinone in cisplatin induced kidney injury, Food Chem. Toxicol. 50 (2012) 1675–1679, https://doi.org/10.1016/j.fct.2012.02.082.

[12] İ.Ö. Aycan, O. Tokgöz, A. Tüfek, U. Alabalık, O. Evliyaoğlu, H. Turgut, F. Çelik, A. Güzel, The use of thymoquinone in nephrotoxicity related to acetaminophen, Int. J. Surg. 13 (2015) 33–37, https://doi.org/10.1016/j.ijsu.2014.11.020.

[13] K.S. Hashem, A.Z. Abdelazem, M.A. Mohammed, A.M. Nagi, B.E. Aboulhoda, E.T. Mohammed, M.M. Abdel-Daim, Thymoquinone alleviates mitochondrial viability and apoptosis in diclofenac-induced acute kidney injury (AKI) via regulating Mfn2 and miR-34a mRNA expressions, Environ. Sci. Pollut. Res. Int. 28 (2021) 10100–10113, https://doi.org/10.1007/s11356-020-11313-x.

[14] U. Sener, R. Uygur, C. Aktas, E. Uygur, M. Erboga, G. Balkas, V. Caglar, B. Kumral, A. Gurel, H. Erdogan, Protective effects of thymoquinone against apoptosis and oxidative stress by arsenic in rat kidney, Ren. Fail. 38 (2016) 117–123, https://doi.org/10.3109/0886022X.2015.1103601.

[15] A. Mabrouk, H.B. Cheikh, Thymoquinone ameliorates lead-induced suppression of the antioxidant system in rat kidneys, Libyan J. Med. 11 (2016) 31018, https://doi.org/10.3402/ljm.v11.31018.

[16] M. Erboga, M. Kanter, C. Aktas, U. Sener, Z. Fidanol Erboga, Y. Bozdemir Donmez, A. Gurel, Thymoquinone ameliorates cadmium-induced nephrotoxicity, apoptosis, and oxidative stress in rats is based on its anti-apoptotic and anti-oxidant properties, Biol. Trace Elem. Res. 170 (2016) 165–172, https://doi.org/10.1007/s12011-015-0453-x.

[17] Z. Farooqui, F. Shahid, S. Abidi, I. Parwez, F. Khan, Oral thymoquinone administration ameliorates: the effect of cisplatin on brush border membrane enzymes, energy metabolism, and redox status in rat kidney, Naunyn-Schmiedeberg's Arch. Pharmacol. 390 (2017) 1271–1284, https://doi.org/10.1007/s00210-017-1428-6.

[18] O.A. Badary, M.N. Nagi, O.A. al-Shabanah, H.A. al-Sawaf, M.O. al-Sohaibani, A.M. al-Bekairi, Thymoquinone ameliorates the nephrotoxicity induced by cisplatin in rodents and potentiates its antitumor activity, Can. J. Physiol. Pharmacol. 75 (1997) 1356–1361.

[19] B.H. Ali, M. Al Za'abi, A. Shalaby, P. Manoj, M.I. Waly, J. Yasin, M. Fahim, A. Nemmar, The effect of thymoquinone treatment on the combined renal and pulmonary toxicity of cisplatin and diesel exhaust particles, Exp. Biol. Med. 240 (2015) 1698–1707, https://doi.org/10.1177/1535370215579013.

[20] B. Salama, K.J. Alzahrani, K.S. Alghamdi, O. Al-Amer, K.E. Hassan, M.A. Elhefny, A.J.A. Albarakati, F. Alharthi, H.A. Althagafi, H. Al Sberi, H.K. Amin, M. S. Lokman, K.F. Alsharif, A. Albrakati, A.E. Abdel Moneim, R.B. Kassab, A.S. Fathalla, Silver nanoparticles enhance oxidative stress, inflammation, and apoptosis in liver and kidney tissues: potential protective role of thymoquinone, Biol. Trace Elem. Res. 201 (2023) 2942–2954, https://doi.org/10.1007/s12011-022-03399-w.

[21] M.A. Hannan, M.S. Zahan, P.P. Sarker, A. Moni, H. Ha, M.J. Uddin, Protective effects of black cumin (Nigella sativa) and its bioactive constituent, thymoquinone against kidney injury: an aspect on pharmacological insights, Int. J. Mol. Sci. 22 (2021) 9078, https://doi.org/10.3390/ijms22169078.

[22] F. Li, J.M. Cao, P.F. Zhai, J.S. Wang, D. Hong, Resveratrol improves renal inflammatory injury in exhaustive exercise rats by regulating NOD-like receptor protein 3 inflammasome, Journal of Army Medical University 44 (2022) 1229–1236, https://doi.org/10.16016/j.2097-0927.202110114.

[23] R. Singh, A. Letai, K. Sarosiek, Regulation of apoptosis in health and disease: the balancing act of BCL-2 family proteins, Nat. Rev. Mol. Cell Biol. 20 (2019) 175–193, https://doi.org/10.1038/s41580-018-0089-8.

[24] J. Li, J.J. Fu, Research advances in the antitumor activity of thymoquinone and its mechanism of action, Journal of Southwest Medical University 42 (2019) 378–382, https://doi.org/10.3969/j.issn.2096-3351.2019.04.019.

[25] S. Samarghandian, M. Azimi-Nezhad, H. Mehrad-Majd, S.R. Mirhafez, Thymoquinone ameliorates acute renal failure in gentamicin-treated adult male rats, Pharmacology 96 (2015) 112–117, https://doi.org/10.1159/000436975.

[26] J.M.M. Salmani, S. Asghar, H. Lv, J. Zhou, Aqueous solubility and degradation kinetics of the phytochemical anticancer thymoquinone; probing the effects of solvents, pH and light, Molecules 19 (2014) 5925–5939, https://doi.org/10.3390/molecules19055925.

[27] M. Al Fayi, H. Otifi, M. Alshyarba, A.A. Dera, P. Rajagopalan, Thymoquinone and curcumin combination protects cisplatin-induced kidney injury, nephrotoxicity by attenuating NFκB, KIM-1 and ameliorating Nrf 2/HO-1 signalling, J. Drug Target. 28 (2020) 913–922, https://doi.org/10.1080/1061186X.2020.1722136.

[28] E.V. Schrezenmeier, J. Barasch, K. Budde, T. Westhoff, K.M. Schmidt-Ott, Biomarkers in acute kidney injury - pathophysiological basis and clinical performance, Acta Physiol. 219 (2017) 554–572, https://doi.org/10.1111/apha.12764.

[29] V. Sinha, L.M. Vence, A.K. Salahudeen, Urinary tubular protein-based biomarkers in the rodent model of cisplatin nephrotoxicity: a comparative analysis of serum creatinine, renal histology, and urinary KIM-1, NGAL, and NAG in the initiation, maintenance, and recovery phases of acute kidney injury, J. Invest. Med. 61 (2013) 564–568, https://doi.org/10.2310/JIM.0b013e31828233a8.

[30] A.J. Won, S. Kim, Y.G. Kim, K.-B. Kim, W.S. Choi, S. Kacew, K.S. Kim, J.H. Jung, B.M. Lee, S. Kim, H.S. Kim, Discovery of urinary metabolomic biomarkers for early detection of acute kidney injury, Mol. Biosyst. 12 (2016) 133–144, https://doi.org/10.1039/c5mb00492f.