International Journal of Pharmacology

*Int. J. Pharmacol.* **2026**; 22(3): 48537
https://doi.org/10.31083/IJP48537

*Research Article*

# Protective Role of 2,3-Dimethylquinoxaline Against Doxorubicin-Induced Renal Inflammation in Rats via Modulation of the iNOS/COX-2/PGE2/TNF-$\alpha$/NF-$\kappa$B Signaling Pathway

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Academic Editor: Mehmet Ozaslan
Submitted: 25 November 2025 Revised: 4 February 2026 Accepted: 24 February 2026 Published: 28 June 2026

**Abstract**

**Background**: A member of the quinoxaline family, 2,3-dimethylquinoxaline (DMQ), has been evaluated for associated nephroprotective properties in an *in vivo* model system to assess renal inflammation induced by the anticancer drug doxorubicin (DOX). **Methods**: Rats were administered DMQ at doses of 30 or 60 mg/kg body weight once daily for 7 consecutive days. Additionally, a single intraperitoneal (i.p.) dose of DOX (15 mg/kg body weight) was administered on the sixth day. DMQ-mediated nephroprotective effects were assessed by measuring renal function markers, oxidative stress markers, lipid peroxidation, renal stress markers, proinflammatory cytokines, and inflammatory mediator levels, as well as by performing histopathological examination of kidney tissues. **Results**: DMQ-treated rats exhibited a marked reduction in kidney toxicity markers and restoration of antioxidant enzyme levels, leading to attenuated oxidative stress. DMQ administration also reduced proinflammatory cytokines, renal stress markers, and inflammatory mediators. Furthermore, DMQ may also attenuate DOX-induced renal structural damage and help restore the dilated capsular space and other histopathological alterations in kidney tissues. **Conclusion**: These findings suggest that DMQ may protect against DOX-induced renal damage by alleviating oxidative stress and inflammation in a rat model, indicating the potential of DMQ as a nephroprotective agent.

**Keywords:** doxorubicin; inflammation; kidney; nephrotoxicity; quinoxaline

## 1. Introduction

Nephrotoxicity is a severe threat to human health because of its potential to induce acute and chronic kidney injury (CKI) [1]. Nephrotoxicity is identified by the rapid degeneration of kidney function due to exposure to toxic substances, including medications, chemicals, and environmental pollutants [2,3]. The consequences of nephrotoxicity on human health are significant, as it often leads to acute kidney injury, and if not promptly diagnosed and managed, this condition may progress to chronic kidney or end-stage renal disease [1]. Nephrotoxicity arises through various mechanisms, along with renal tubular toxicity, renal inflammation, glomerular impairment, crystal nephrosis, and thrombotic microangiopathy [4]. The threat posed by nephrotoxicity extends to the medical management of patients, especially in conditions in which application of nephrotoxic drugs is unescapable, such as treatments in-

volving antibiotics, such as colistin, or chemotherapeutic agents, such as 5-fluorouracil and doxorubicin (DOX) [5,6].

DOX is a potent anthracycline chemotherapeutic drug mainly applied to counter various cancers, including breast cancer, hematologic malignancies, and other solid tumors [7,8]. Despite its efficacy in inducing cancer cell death by embolism with DNA and block topoisomerase II activity [9], its clinical application is restricted by dose-dependent toxicities, including cardio related toxicity, myelosuppression, nephrotoxicity, and hepatotoxicity [10–13]. DOX-induced nephrotoxicity is a major side effect that limits its use as a chemotherapeutic agent in cancer treatment. The main cellular consequences of nephrotoxicity include elevated oxidative stress, inflammation, mitochondrial damage, dysregulation of intracellular calcium and iron levels, and cell death of renal cells. The study also showed that DOX-induced nephrotoxicity results in raised serum creatinine and urea levels [14]. Different studies have indicated that the administration of DOX leads to an increases oxidative stress in the kidneys. This is evidenced by heightened levels of malondialdehyde (MDA) and a reduction in antioxidant defenses, including the activity of glutathione (GSH) and other enzymes, such as superoxide dismutase (SOD) and catalase (CAT) [5,14–17]. This disequilibrium in pro-oxidant and antioxidant defenses results in cellular damage in several organ structures, including the kidneys [18]. Exposure to DOX results in an elevation of inflammation-allied cytokines, including tumor necrosis factor-alpha (TNF-$\alpha$) and interleukin-1beta (IL-1$\beta$). These inflammatory markers contribute to renal damage and exacerbate nephrotoxicity [14,19,20]. DOX-induced pyroptosis, is a programmed cell death type where inflammation of kidney cells and over activity of markers such as caspase-1 and caspase-3 was seen [21].

In recent years, a prominent research challenges have been the identification of clinical therapies to mitigate the renal side effects associated with DOX. Oxidative stress and inflammatory responses are key contributors to DOX-induced kidney damage, indicating that substances with antioxidant and anti-inflammatory properties may offer protection against DOX toxicity [22]. Quinoxaline derivatives are notable for their wide range of therapeutic applications and relatively straightforward chemical synthesis, which makes them highly significant in medicinal chemistry [23]. Quinoxaline derivatives have been extensively studied as potential anticancer agents. These compounds act as protein kinase inhibitors, making them promising candidates for cancer therapy [24,25]. Some quinoxaline derivatives have the potential to inhibit acetylcholinesterase, suggesting their application in neurodegenerative diseases like Alzheimer's disease [26]. These compounds also exhibit significant antibacterial, antifungal, and antiviral activities. They have been evaluated against various plant pathogens and human microbial infections, showing potent antimicrobial effects [27–29]. Some synthetic antimicrobial agents belonging to the quinoxaline-di-N-oxides class, specifically mequindox, olaquindox, and carbadox, which have been reported to have potential genotoxic and carcinogenic effects, have also been identified as toxic to the kidneys in various animal studies [30,31]. This toxicity concern has prompted interest in non-di-N-oxide quinoxaline derivatives with simpler substitution patterns and more favorable safety profiles as potential leads for organ-protective pharmacotherapy [32]. These compounds are also known for their modulation activity of oxidative stress pathways, conquest of pro-inflammatory cytokine production, and regulation of redox-sensitive signaling molecules, such as nuclear factor kappa-B cells (NF-$\kappa$B), which are central to DOX-induced renal injury in various models [33,34]. The pharmacological activities of quinoxaline derivatives are enhanced through structural modifications, making the quinoxaline framework an excellent foundation for developing new pharmacotherapeutic agents [23]. 2,3-Dimethylquinoxaline (DMQ) is a quinoxaline derivative that has been described as non-carcinogenic, non-nephrotoxic, and non-hepatotoxic in both *in vivo* and *in vitro* studies, and also reported for anti-inflammatory properties in an *in vivo* model [23,35]. Alfadil *et al*. [23] performed a 28-day oral toxicity study in mice. DMQ demonstrated an acceptable systemic safety profile, with no overt clinical signs of toxicity or marked impairment of standard serum biochemical indices. However, high doses produced hematological alterations and subtle histological changes, warranting further study. However, the safety and specific biological properties of DMQ remain unknown or limited. Importantly, DMQ lacks the di-N-oxide moiety present in classical quinoxaline growth promoters implicated in renal injury, making it a rational candidate for exploring nephroprotective activity. However, its organ-specific pharmacological actions, including potential antioxidant or anti-inflammatory effects in renal tissue, have not yet been characterized [23]. Therefore, DMQ was selected not only because of its low toxicity but also because its chemical scaffold is associated with biological activities that directly target these pathological mechanisms.

The ability of quinoxaline derivatives to enhance antioxidant defenses and attenuate inflammatory responses provides a rationale for evaluating DMQ as a potential nephroprotective agent. Present study was designed to discover, for the first time, whether a structurally simple, non-di-N-oxide quinoxaline derivative with an emerging acceptable safety profile, DMQ, can attenuate DOX-induced nephrotoxicity in rats. Given the central role of oxidative stress and inflammation in DOX-mediated renal injury, we hypothesized that DMQ may exert functional and histological protection by modulating redox status and inflammatory signaling pathways, despite the absence of prior data specifically addressing its renal effects. Accordingly, we evaluated the impact of DMQ on renal function indices, oxidative stress biomarkers, pro-inflammatory cytokines, and the ex-

pression of key inflammatory mediators in a rat model of DOX-induced nephrotoxicity.

## 2. Materials and Methods

### *2.1 Chemicals and Kits*

DOX, DMQ ($C_{10}H_{10}N_2$; DMQ), and sodium pentobarbital were acquired from Sigma-Aldrich. St. Louis, MO, USA. In this study, testing kits, including urea (Cat. No. MBS168363), uric acid (Cat. No. MBS2540398), creatinine (Cat. No. MBS480387), and enzyme-linked immunosorbent assay (ELISA) kits for IL-1$\beta$ (Cat. No. MBS265868), IL-6 (Cat. No. MBS269892) and TNF-$\alpha$ (Cat. No. MBS282960) and IFN-$\gamma$ (Cat. No. MBS2019134), and nuclear factor kappa-B (NF-$\kappa$B, Cat. No. MBS1603467), glyceraldehyde 3-phosphate dehydrogenase (GAPDH, Cat. No. MBS727873), nitric oxide synthase (iNOS; Cat. No. MBS2505942), cyclooxygenase-2 (COX-2, Cat. No. MBS266603) and prostaglandin E2 (PGE2, Cat. No. MBS262150) were procured from MyBioSource, Inc. (San Diego, CA, USA).

### *2.2 Animals*

Adult male Wistar rats (160–200 g, 8 weeks old) were used in this study. Before experimentation, the rodents were maintained for two weeks to acclimatize to a 12-h light and 12 h dark cycle where they are free to access food and water. All research procedures were done in accordance with institutional guidelines and were approved by the Biomedical Research Ethics Committee of Umm Al-Qura University, with approval number HAPO-02-K-012-2025-10-2956. This study complied with all relevant ethical standards and regulations issued by the committee.

### *2.3 Study Design*

Five groups (n = 6 each) were randomly formed with healthy acclimatized rats as follows:

Control group I (CON): rats did not receive any drug or solvent during the experiment.

Doxorubicin (DOX only) group II: A single dose of test DOX (15 mg/kg, intraperitoneally-i.p.) on day 6 after the experiment started [36].

Doxorubicin and 2,3-Dimethylquinoxaline, low, group III (DOX + DMQ-30): rats received DMQ by oral administration (30 mg/kg body weight [bw]) once a day for seven consecutive days and DOX (15 mg/kg, i.p.) single dose on day 6.

Doxorubicin and 2,3-Dimethylquinoxaline high, group IV (DOX + DMQ-60): rats received DMQ derivatives by oral administration (60 mg/kg bw) once a day for 7 consecutive days and DOX (15 mg/kg, i.p.) single dose on day 6.

2,3-Dimethylquinoxaline, group V (DMQ-60) rats received DMQ by oral administration (60 mg/kg bw) once in a day for seven successive days.

### *2.4 Collection of Blood Samples and Renal Tissues*

Following administration of the final dose of DMQ on day 7, the animals were deeply anesthetized with sodium pentobarbital (50mg/mL, 45 mg/kg, i.p.). Adequate anesthetic depth was confirmed by loss of the righting reflex and absence of pedal withdrawal and palpebral reflexes. While under deep anesthesia, cardiac blood was collected by trained personnel. Immediately thereafter, euthanasia was completed by cervical dislocation to ensure death. Both kidneys were then rapidly excised, rinsed in cold phosphate buffer (PBS) (1 M, pH 7.4), and processed for biochemical analysis. Renal tissues were homogenized in ice-cold PBS (1 g tissue/9 mL), centrifuged at 2500 $\times$g for 20 min under cooled conditions, and the supernatant was used for biochemical assays. Renal function tests were also performed.

### *2.5 Quantification of Renal Functions*

Renal function tests were performed using a commercially available kit to determine serum urea, uric acid, and creatinine levels, thereby assessing DOX-induced nephrotoxicity in rats from the different test groups. For each assay, 100 µL of serum was used according to the manufacturer's instructions, and the concentrations of urea, uric acid, and creatinine were determined accordingly.

### *2.6 Quantification of Oxidative Stress Markers*

For lipid peroxidation estimation, malondialdehyde (MDA) levels were quantified using a spectrophotometric method adopted from Sheikh *et al*. [37] and presented as nmol/mg of protein content. Tetramethoxypropane was used as the internal standard.

Superoxide dismutase (SOD) activity was measured by spectrophotometric method as defined by Shahid Nadeem *et al*. [38]. Briefly, the supernatant was combined with the SOD assay reagent from Sigma-Aldrich Chemie GmbH (Taufkirchen, Bavaria, Germany), 200 µL. Assay reagent contains 40 µL xanthine (0.3 mmol/L), 20 µL ethylenediaminetetraacetic acid (EDTA) solution (0.6 mmol/L), 20 µL of nitro tetrazolium (150 µmol/L), 12 µL of $Na_2CO_3$ (400 mmol/L), and 6 mL of bovine serum albumin (1 g/L), and kept for half-an-hour. After incubation, nitroblue tetrazolium (NBT), 100 µL was added and incubated to form a blue formazan complex, which was assessed by spectrophotometer at 550 nm and presented as U/mg protein. One SOD unit activity is defined as the quantity of protein necessary to inhibit a 50% reduction in the NBT.

Catalase (CAT) activity was measured in the serum supernatant prepared in phosphate buffer, which was mixed with 50 nM hydrogen peroxide and incubated. The optical density was calculated every 15 s at 240 nm. CAT activity was quantified and expressed in units per milligram of protein [39].

The reduced glutathione (GSH) level was measured by adding an equal amount of trichloroacetic acid to the serum supernatant, thoroughly mixing, and incubating for

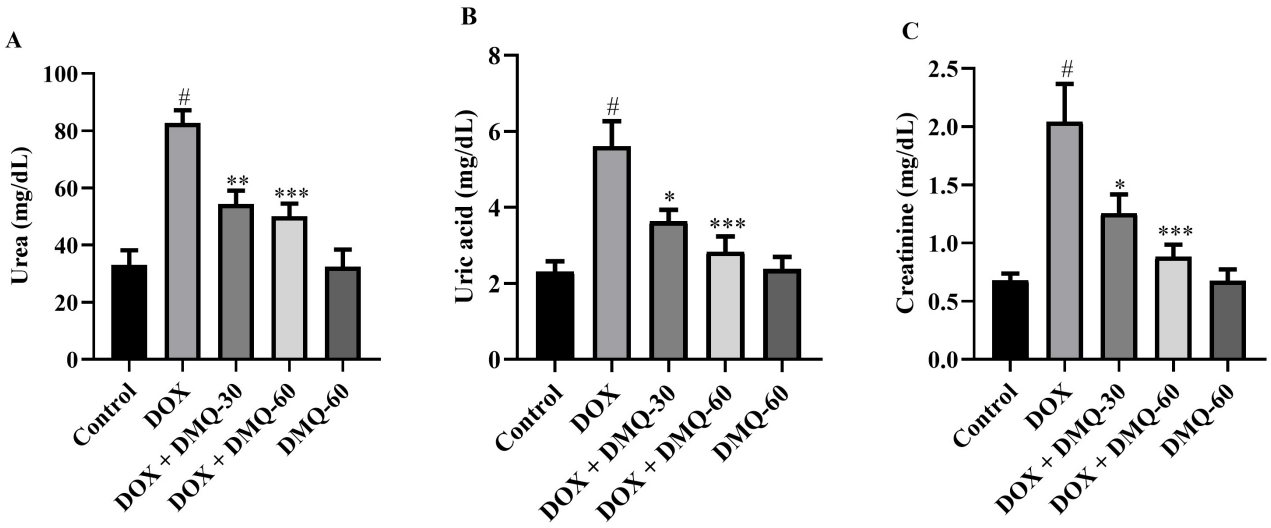


**Fig. 1. Effects of 2,3-DMQ treatment on blood urea, uric acid, and creatinine levels in DOX-treated rats.** (A) Blood urea levels of rats, (B) Uric acid, (C) Creatinine. Data are presented as mean ± SEM ($n$ = 6). Treatment groups: Control, DOX - doxorubicin, DOX + DMQ-30 - DOX with 2,3-DMQ 30 mg/kg bw, DOX + DMQ-60 - DOX with 2,3-DMQ 60 mg/kg bw, DMQ-60 - 2,3-DMQ 60 mg/kg bw. #$p < 0.001$ DOX vs. control; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. DOX. DOX, doxorubicin; DMQ, 2,3-Dimethylquinoxaline.

30 min. The resultant mixture was mixed with DTNB prepared in a phosphate buffer solution (1M; pH 7.4). GSH levels were quantified using a standard curve after measuring the absorbance at 412 nm. The results are presented as µmol GSH/mg protein [40].

### *2.7 Quantification of the Renal Expression of Inflammatory Cytokines and Other Inflammation-Related Markers*

The clear tissue supernatant was used for ELISA. The inflammatory cytokines IL-1$\beta$, IL-6, and TNF-$\alpha$, as well as inflammation-related markers such as NF-$\kappa$B, were measured by market purchased ELISA kits (R&D Systems, Minneapolis, MN, USA). The protocol was followed as described by the manufacturer, and the concentrations were expressed in pg/mL.

### *2.8 Quantification of the Renal Stress*

Tissue levels of iNOS, GAPDH, and PGE2 were analyzed to investigate the immune response, inflammation, and tissue damage. Tissue levels of these markers were evaluated using ELISA kits, following the manufacturer's protocol, and expressed as concentrations in pg/mL (iNOS and PGE2) and as arbitrary units for GAPDH.

### *2.9 Quantification of Inflammatory Mediators*

ELISA kits based on the sandwich ELISA principle were used to assess IFN-$\gamma$ and COX-2 levels. These kits employ an antibody-based capture method within microplates to precisely measure the expression of each inflammatory mediator, and the results are represented as pg/mL of the sample.

### *2.10 Renal Histopathology*

Histopathological analysis of kidney tissue was performed to analyze DOX-induced damage and recovery following DMQ treatment. For this, the dissected kidney was instantly treated with freshly prepared chilled 10% buffered formalin. Subsequently, the sections were fixed in paraffin wax to prepare the sections. A 5-µm thick vertical section was made and hematoxylin and eosin (H&E) staining was done. To analyse the histological changes microscopic examination was done. The diverse regions of the stained kidney sections from each group were examined, and the results were recorded.

### *2.11 Statistical Analysis*

Statistical calculations were done by GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA). The normality of the datasets was verified using the Shapiro-Wilk test ($p > 0.05$), and the results are presented in mean ± SEM, with a sample size of n = 6. For multiple comparisons, Tukey's post hoc test was applied, with significance set at $p < 0.05$.

## 3. Results

### *3.1 Effect of DMQ on Renal Function Parameters*

In the present study, the indicator levels of renal function, primarily urea, uric acid, and creatinine, were evaluated in the serum. Results confirmed that the DOX-induced group II rats showed a noteworthy rise in the level of serum urea ($p < 0.001$, Fig. 1A), uric acid ($p < 0.001$, Fig. 1B), and serum creatinine ($p < 0.001$, Fig. 1C), related to control rats. In group III rats administered DMQ at a dosage of 30

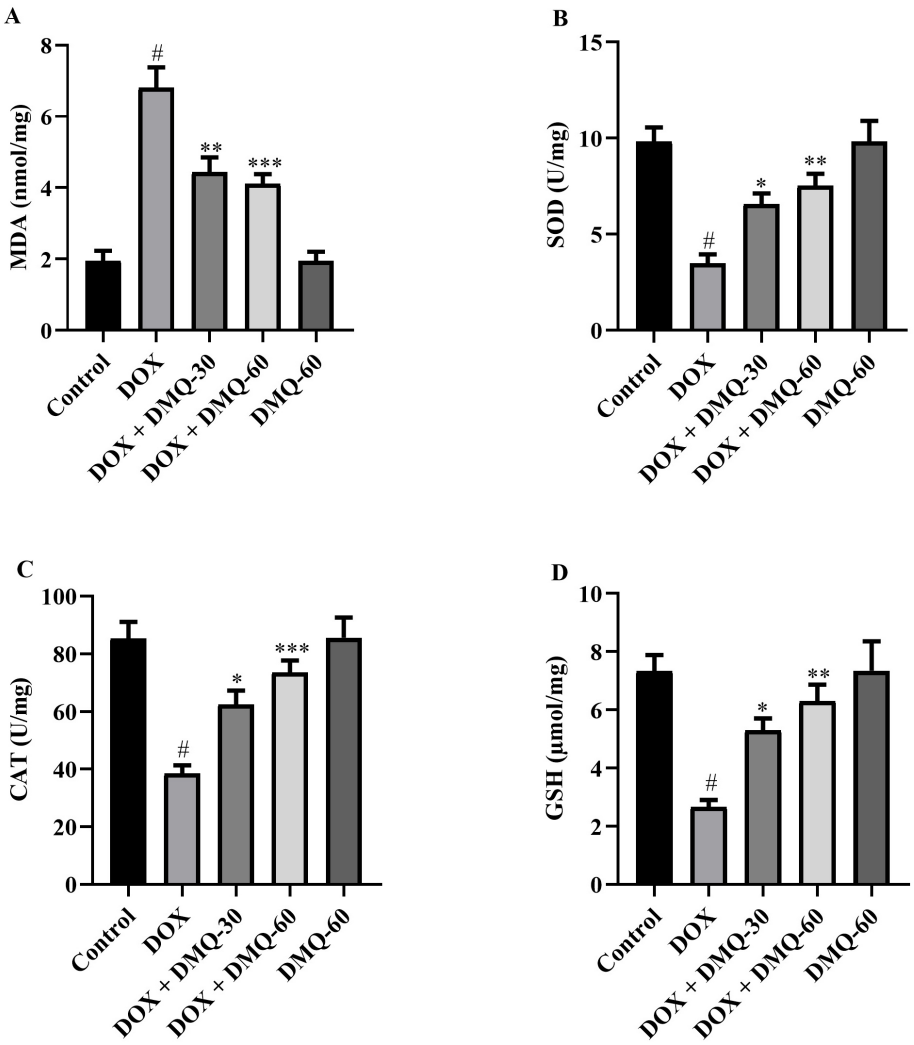


**Fig. 2. Effect of 2,3-DMQ treatment on serum markers in doxorubicin-treated rats.** (A) MDA, (B) SOD, (C) CAT, and (D) GSH levels. Data are presented as mean ± SEM ($n$ = 6). Treatment groups: Control, DOX - doxorubicin, DOX + DMQ-30 - DOX with 2,3-DMQ 30 mg/kg bw, DOX + DMQ-60 - DOX with 2,3-DMQ 60 mg/kg bw, DMQ-60 - 2,3-DMQ 60 mg/kg bw. #$p < 0.001$ DOX vs. control; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. DOX. MDA, Malondialdehyde; SOD, superoxide dismutase; CAT, catalase.

mg/kg bw, there was a remarkably drop in serum urea [F (4, 25) = 16.49; $p < 0.0001$], uric acid [F (4, 25) = 10.93; $p < 0.0001$], and creatinine [F (4, 25) = 10.47; $p < 0.0001$] levels. Nevertheless, the recovery of these indicators in Group IV treated rats (DMQ-60) was comparatively higher than that in Group III treated rats (DMQ-30), indicating a dose-dependent recovery effect. Rats that received only DMQ treatment (Group V) did not show any significant differences in any of the three parameters compared to the control rats (Group I).

### *3.2 Effect of DMQ on the Antioxidant Levels in Blood*

Compared with the control group, rat treated with DOX (Group II) exhibited a noteworthy elevation in MDA levels ($p < 0.001$) along with a marked decrease in the antioxidant markers SOD, CAT, and GSH (all $p < 0.001$) activity. Treatment with DMQ remarkably improved the levels of antioxidant markers, namely, SOD [F (4, 25) = 13.39; $p < 0.0001$], CAT [F (4, 25) = 14.79; $p < 0.0001$], and GSH [F (4, 25) = 10.05; $p < 0.0001$], compared to those in the DOX-induced rats. In Group III rats administered DMQ, there was a remarkably drop in MDA levels ($p < 0.01$), while the activities of SOD, CAT, and GSH were noteworthy raised ($p < 0.01$) in comparison to Group II (Fig. 2A–D). Treatment with DMQ in Group IV showed better recovery in the expression levels of antioxidant markers (SOD, CAT, and GSH) than that in Group III, indicating a dose-dependent effect. Group V rats (DMQ alone) did not show any noteworthy differences related to group I rats.

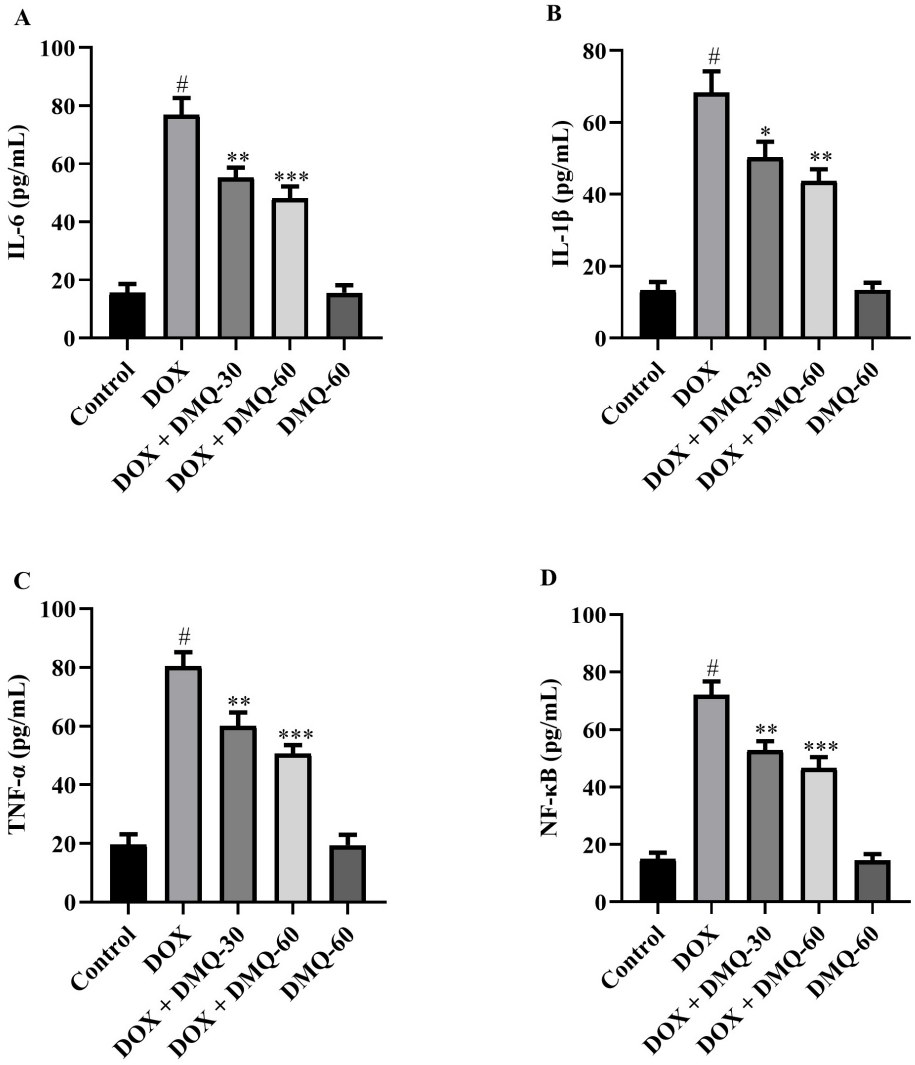


**Fig. 3. Effect of 2,3-DMQ treatment on cytokine levels and inflammatory mediators in doxorubicin-treated rats.** (A) IL-6, (B) IL-1$\beta$, (C) TNF-$\alpha$, and (D) NF-$\kappa$B. Data are presented as mean $\pm$ SEM ($n = 6$). Treatment groups: Control, DOX - doxorubicin, DOX + DMQ-30 - DOX with 2,3-DMQ 30 mg/kg bw, DOX + DMQ-60 - DOX with 2,3-DMQ 60 mg/kg bw, DMQ-60 - 2,3-DMQ 60 mg/kg bw. #$p < 0.001$ DOX vs. control; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. DOX.

### *3.3 Effect of DMQ on Pro-Inflammatory Cytokines in the Kidney*

The activity levels of pro-inflammatory cytokines IL-6, IL-1$\beta$, TNF-$\alpha$, and NF-$\kappa$B in renal tissue were significantly higher ($p < 0.001$) in group II rats in related to control rats. Rats treated with DMQ (groups III and IV) displayed significantly attenuated levels of the renal proinflammatory cytokines IL-6 [F (4, 25) = 46.11; $p < 0.0001$], IL-1$\beta$ [F (4, 25) = 39.27; $p < 0.0001$], TNF-$\alpha$ [F (4, 25) = 45.74; $p < 0.0001$], and NF-$\kappa$B [F (4, 25) = 57.21; $p < 0.0001$] compared to the DOX-treated group II rats (Fig. 3A–D). Rats that received a high dose of DMQ (Group IV) showed a greater reduction in renal proinflammatory cytokine levels. However, no significant difference was observed in renal proinflammatory cytokine activity between Groups I and V.

### *3.4 Effect of DMQ on Renal Stress Markers in the Kidney*

The activity of the renal stress markers iNOS ($p < 0.001$) and PGE2 ($p < 0.001$) were remarkably elevated, whereas the reduced activity level of GAPDH ($p < 0.001$) were found in the DOX-treated rats compared to the control group. DMQ treatment (60 mg/kg bw) in group IV rats remarkably lower the levels of iNOS [F (4, 25) = 38; $p < 0.0001$] and PGE2 [F (4, 25) = 40.60; $p < 0.0001$] and recovered GAPDH [F (4, 25) = 7.082; $p = 0.0006$] compared to the DOX-treated group (Fig. 4A–C). The recovery of renal stress marker levels in group III rats was lower than that in group IV rats. There is no noteworthy variation was detected in the renal stress marker levels between the control (group I) and DMQ alone (group V) groups.

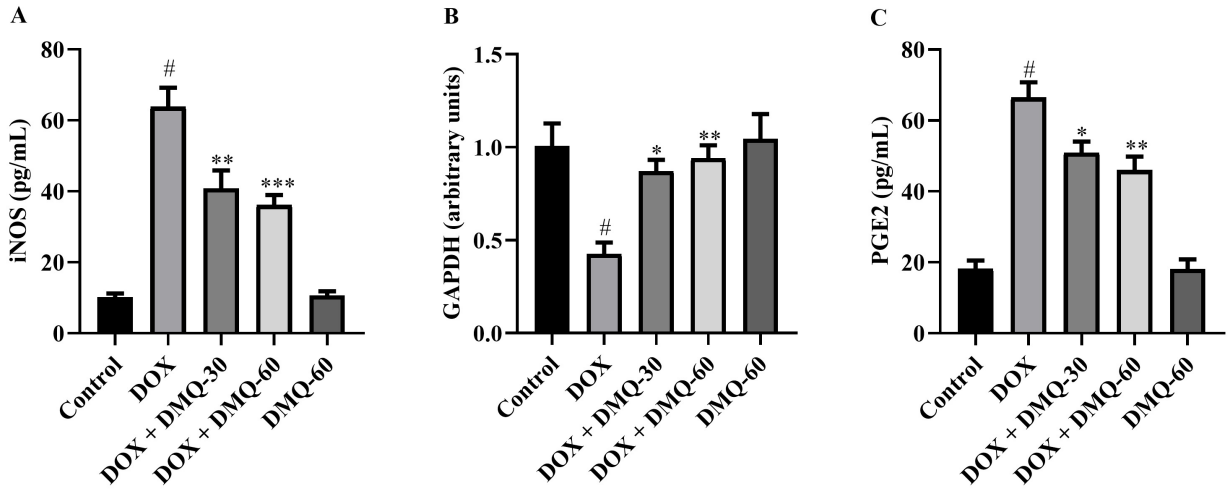


**Fig. 4. Effect of 2,3-DMQ treatment on renal stress in DOX-treated rats.** (A) iNOS, (B) GAPDH, and (C) PGE2. Data are presented as mean ± SEM ($n = 6$). Treatment groups: Control, DOX - doxorubicin, DOX + DMQ-30 - DOX with 2,3-DMQ 30 mg/kg bw, DOX + DMQ-60 - DOX with 2,3-DMQ 60 mg/kg bw, DMQ-60 - 2,3-DMQ 60 mg/kg bw. #$p < 0.001$ DOX vs. control; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. DOX.

### *3.5 Effect of DMQ on the Biomarkers of DOX-Induced Nephrotoxicity*

Renal expression of IFN-$\gamma$ ($p < 0.001$) and COX-2 ($p < 0.001$) was remarkably higher in the DOX-administered rats than in the control group rats (Fig. 5A,B). Treatment with DMQ in groups III and IV showed a significant reduction in IFN-$\gamma$ [F (4, 25) = 40.23; $p < 0.0001$] and COX-2 [F (4, 25) = 39.74; $p < 0.0001$] expression levels. Nevertheless, in group IV (DMQ-60) rats, this reduction in the expression of IFN-$\gamma$ and COX-2 was greater than that observed with the lower dose. There is no any substantial alteration was found in the expression levels of IFN-$\gamma$ and COX-2 among the control and DMQ groups.

### *3.6 Effect of DMQ Treatment on Renal Histology in DOX-Induced Nephrotoxicity*

The effect of DMQ treatment on renal histological deviations caused by DOX treatment is shown in Fig. 6A–D and assessed with respect to neutrophil infiltration. DOX induction resulted in sporadic marked atrophy of the renal corpuscle glomeruli with dilation in many tubules, which deformed their shape. The epithelial lining was atrophied, and the dilated tubules contained epithelial and hyaline casts. Dilated vessels contained hemolyzed blood, and tubular damage and moderate inflammation in renal cells were detected in Group II compared to Group I. Treatment with DMQ (Groups III and IV) showed a reduction in the dilation of many deformed tubules, and a sporadic improvement in the atrophy of the renal corpuscle glomeruli was also observed. Treatment also improved epithelial lining atrophy and reduced neutrophil seep-out. Injury score (Fig. 6E) was calculated for the study and presented as a percentage of the necrotic area [F (4, 25) = 39.16, $p < 0.0001$], indicating a decrease in the treated groups (III and IV).

## 4. Discussion

DOX, a cornerstone anthracycline antibiotic in cancer therapy, exerts potent antitumor effects but carries a significant risk of nephrotoxicity that compromises its clinical use. This damage primarily arises from oxidative stress, inflammatory cascades, and apoptosis, collectively driving acute kidney injury and functional decline [21]. In this pilot study, we examined whether DMQ offers renal protection against DOX-induced inflammation in rats by targeting the iNOS/COX-2/PGE2/TNF-$\alpha$/NF-$\kappa$B signaling pathway. The relatively small sample size tempers the statistical robustness and broader applicability of our results, since the work focused on yielding initial efficacy and safety insights rather than firm mechanistic evidence. We selected only male rats to sidestep hormonal fluctuations and ensure experimental consistency, which is a standard approach in early-stage nephrotoxicity research, although this approach narrows the relevance to female responses or sex-based differences. The short timeframe of the study also limits the views on long-term outcomes, while omitting genetic or transgenic models leaves the gene-specific roles in the pathway unclear. We aimed to explore how DMQ influences the iNOS/COX-2/PGE2/TNF-$\alpha$/NF-$\kappa$B signaling pathways, contributing to its anti-inflammatory benefits in the kidneys. Additionally, we contextualized these findings within the evolving strategies to combat chemotherapy-related kidney damage by managing inflammation and oxidative stress.

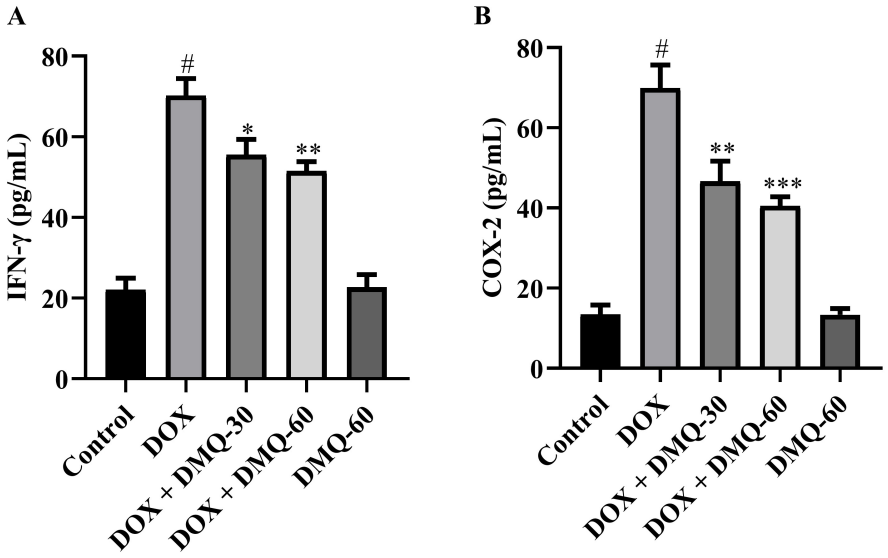


**Fig. 5. Effect of 2,3-DMQ treatment on biomarkers in doxorubicin-treated rats.** (A) IFN-$\gamma$ and (B) COX-2. Data are presented as mean ± SEM (n = 6). Treatment groups: Control, DOX - doxorubicin, DOX + DMQ-30 - DOX with 2,3-DMQ 30 mg/kg bw, DOX + DMQ-60 - DOX with 2,3-DMQ 60 mg/kg bw, DMQ-60 - 2,3-DMQ 60 mg/kg bw. #$p < 0.001$ DOX vs. control; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. DOX.

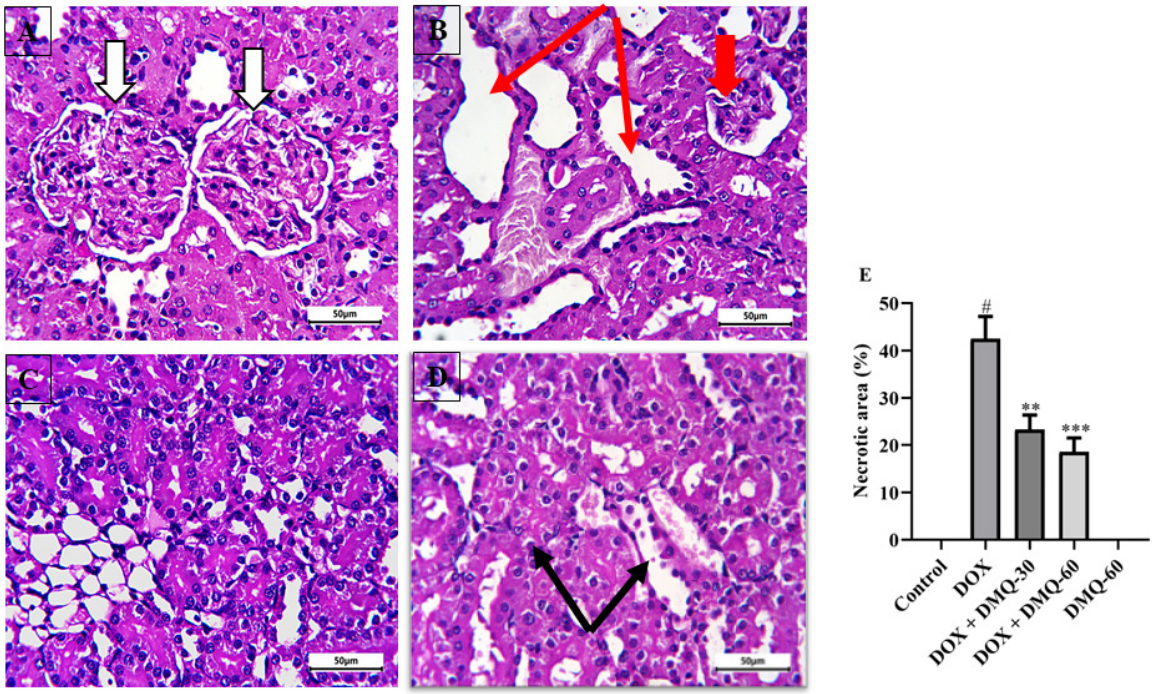


**Fig. 6. Renal histological changes in rat kidneys were detected using H&E staining.** (A) Control rat, Group I. (B) DOX rat, Group II. Sporadic marked atrophy of renal corpuscle glomeruli (thick red arrow), dilation of many tubules with deformed shapes and epithelial lining atrophy (red arrow) by DOX administration were detected. (C) DOX + DMQ-30 - DOX with 2,3-DMQ of 30 mg/kg bw, pre-treatment with 2,3-DMQ reverted with these alterations, with mild dilation of many tubules with deformed shape and epithelial lining atrophy. (D) DOX + DMQ-60: DOX with 2,3-DMQ at 60 mg/kg bw pretreatment showing improvement in atrophy of renal corpuscle glomeruli and dilation of many tubules (black arrow). (E) Injury Score in terms of necrotic area percentage (%). Scale bar = 50 µm. Treatment groups: Control, DOX - doxorubicin, DOX + DMQ-30 - DOX with 2,3-DMQ 30 mg/kg bw, DOX + DMQ-60 - DOX with 2,3-DMQ 60 mg/kg bw, DMQ-60 - 2,3-DMQ 60 mg/kg bw. #$p < 0.001$ DOX vs. control; **$p < 0.01$, ***$p < 0.001$ vs. DOX.

The results of this study highlighted that DOX treatment induces kidney impairment, which is characterized by alterations in serum creatinine, blood urea, and uric acid levels, consistent with previous studies [41,42]. The rise in serum creatinine, blood urea, and uric acid levels is a key clinical indicator of nephrotoxicity, which was reduced by DMQ treatment.

DOX increases serum creatinine and urea levels due to the oxidative stress imposed on renal tissues, leading to the production of oxidative stress markers and a reduction in the activity of antioxidant enzyme [5,21]. The correlation between oxidative stress markers and the severity of DOX-induced renal damage is critical for understanding this relationship. Elevated reactive oxygen species (ROS) levels are a direct indicator of oxidative stress, contributing to extensive cellular damage and dysfunction, and contributing to aging and diseases such as cancer, heart failure, and diabetes [43,44]. Oxidative stress markers, such as MDA, GSH, and the antioxidant enzymes CAT and SOD, play a significant role in this correlation. MDA, a byproduct of lipid peroxidation, is a robust marker of oxidative stress. Elevated MDA levels correlate with the severity of renal damage, as lipid peroxidation reflects the extent of cellular membrane damage caused by ROS [45,46]. Treatment with DMQ significantly reversed the MDA levels. SOD neutralizes superoxide radicals and protects cells from oxidative stress by converting them into oxygen and hydrogen peroxide, which are less harmful [47]. A reduction in SOD activity reflects an increased oxidative burden in the kidneys [14,48,49]. Reduced SOD activity was observed in DMQ-treated rats, which was often paralleled by histological changes in renal tissues, such as tubular necrosis and fibrosis. Glutathione is a crucial antioxidant that detoxifies ROS. Lower GSH levels signal compromised antioxidant defenses and a heightened state of oxidative stress, which correlates with increased tissue damage [47]. Treatment with DMQ in DOX-induced rats was effective in restoring the endogenous antioxidant GSH. CAT detoxifies hydrogen peroxide and prevents oxidative damage. Lower enzyme activity is indicative of an impaired oxidative stress response [50]. DMQ treatment effectively restored CAT levels in rats administered with DOX.

DOX triggers an inflammatory response, as identified by the increased production of interleukins and proinflammatory cytokines. DOX treatment upregulates inflammatory markers such as TNF-$\alpha$, IL-1$\beta$, and IL-6, supporting earlier indications of DOX-induced modifications to these inflammatory markers [36]. These inflammatory mediators exacerbate kidney damage by promoting tissue injury and fibrosis [17,21]. The previously described impact of DOX-induced oxidative stress on the NF-$\kappa$B signaling pathway involves changes in its expression and activation, often leading to higher levels of pro-inflammatory cytokines and survival proteins that help cells respond to stress [51]. In the present study, NF-$\kappa$B protein expression was evaluated as an indicator of inflammation-related signaling in response to doxorubicin (DOX) exposure. However, it should be noted that direct assessment of NF-$\kappa$B activation, such as nuclear translocation or transcriptional activity, was not performed. Therefore, the detected variations in NF-$\kappa$B expression should be interpreted cautiously and viewed in relation to the concurrent alterations in downstream inflammatory mediators rather than as definitive evidence of pathway activation. DMQ treatment remarkably lower the levels of TNF-$\alpha$, IL-1$\beta$, and IL-6, and decreased NF-$\kappa$B protein expression. These findings suggest that the anti-inflammatory effect of DMQ is allied with the attenuation of cytokine-driven renal inflammation. Although this pattern is consistent with reduced inflammatory signaling, the present data support an association rather than direct modulation of the NF-$\kappa$B pathway. Overall, these outcomes indicate that DMQ alleviates DOX-induced renal inflammation primarily by suppressing pro-inflammatory mediators, thereby contributing to improved renal tissue integrity.

The association of iNOS with increased generation of nitric oxide under inflammatory conditions indicates that elevated levels are indicative of both oxidative stress and inflammation [50]. DOX induces the modification of critical cysteine residues in GAPDH, affecting its function, which disrupts its glycolytic role and alters pathways such as the pentose phosphate pathway, which favors antioxidant defense and is essential for cell survival under oxidative stress conditions induced by chemotherapy drugs such as DOX [52,53]. DMQ treatment not only significantly restored GAPDH enzyme levels but also reduced the production of nitric oxide and PGE2, thereby contributing to mitigation of oxidative stress and inflammation in DOX-treated rats.

DOX does not directly stimulate IFN-$\gamma$ production in immune cells, such as macrophages. However, when combined with other inflammatory stimuli, such as lipopolysaccharides and IFN-$\gamma$, it can contribute to an inflammatory environment that may result in elevated IFN-$\gamma$ levels [54]. DOX can also significantly affect COX-2 expression, an enzyme associated with inflammation. This effect is mediated by inflammatory responses that activate COX-2 [55]. The COX-2 and PGE2 pathways are upregulated in response to DOX, facilitating cancer cell survival and proliferation [56]. In this study, DMQ effectively reduced the high expression of IFN-$\gamma$ and COX-2 induced by DOX treatment in rats, demonstrating its potential for the treatment of nephrotoxicity.

While DMQ attenuated DOX-induced elevations in iNOS, IFN-$\gamma$, COX-2, PGE2, IL-1$\beta$, IL-6, TNF-$\alpha$, and NF-$\kappa$B levels, these findings are based on ELISA measurements without corroboration from Western blot (e.g., for NF-$\kappa$B p65 phosphorylation or inhibitor ratios), immunohistochemistry (to localize inflammatory infiltrates in cortical/medullary regions), or gene expression analysis (e.g., RT-qPCR for pathway transcripts). Such validations, which are standard in mechanistic renal inflammation stud-

ies, were beyond the scope of this pilot study due to resource constraints but are essential to confirm signaling cascade engagement beyond mere biomarker suppression. Future studies should prioritize these aspects to dissect DMQ's upstream regulatory effects of DMQ.

Histopathological changes are also evident, with DOX causing renal tubule necrosis [57]. DOX induces structural changes in the kidney, including tubular damage and interstitial inflammation. Histologically, DOX produces typical renal lesions, including tubular degeneration, interstitial inflammatory infiltration, and glomerular alterations, which are well documented in the experimental nephrotoxicity literature [58,59]. DMQ may also downregulate DOX-induced renal damage and recover the dilated capsular space and structural alterations associated with kidney tissues. DMQ treatment mitigated these structural changes, resulting in improved tubular architecture and glomerular morphology, consistent with the biochemical evidence of restored renal function and reduced oxidative and inflammatory damage.

DOX-induced nephrotoxicity is primarily ascribed to oxidative stress, inflammation, apoptosis and impaired renal homeostasis. DMQ treatment counteracted these effects. For present study, we propose the probable defensive mechanisms of DMQ against DOX-induced nephrotoxicity in a rodent model, where DMQ downregulates urea, uric acid, and creatinine levels, which may contribute to the recovery of renal function. DMQ restored the energy production pathway enzyme (GAPDH) level, which supports antioxidant defenses crucial for cell persistence under oxidative stress. DMQ also effectively reduces the production of ROS, nitric oxide synthase, and PGE2 levels, which may increase antioxidant enzymes such as SOD and CAT, and decrease GSH and MDA levels, leading to a reduction in lipid peroxidation and cellular damage. DMQ reduces inflammation by downregulating inflammatory cytokines, such as TNF-$\alpha$, IL-6, and IL-1$\beta$, which may decrease the activation of the NF-$\kappa$B pathway. DMQ also efficiently regulated the high expression of IFN-$\gamma$ and COX-2 to counteract DOX-induced nephrotoxicity. Future research with larger samples, longer timelines, and advanced molecular techniques could improve the robustness of these results. Thus, DMQ enhances renal function markers and improves histopathological outcomes in a DOX-induced nephrotoxicity model.

Taken together, these data support a nephroprotective profile for DMQ in DOX-induced acute kidney injury, mediated by the concurrent modulation of oxidative stress, inflammatory mediators, and GAPDH-linked metabolic resilience in renal tissue.

*Limitations*

There are several limitations to this study that should be acknowledged. The sample size was relatively small, limiting the statistical power and generalizability of the results. Second, the study primarily focused on biochemical and inflammatory markers, rather than pharmacokinetic and pharmacodynamic testing of DMQ, which is important for understanding its systemic behavior and therapeutic profile. The safety profile of DMQ was not further confirmed with comprehensive tissue toxicity assessments and cytotoxicity assays, such as MTT analysis. Moreover, the mechanistic evaluation relied primarily on ELISA-based biomarker measurements without additional confirmation using advanced molecular techniques, such as Western blotting, immunohistochemistry, or gene expression analysis. For validation and strengthening of these results, additional studies with larger samples, extended follow-up periods, pharmacokinetic–pharmacodynamic analyses, as well as detailed toxicity assessments are needed.

## 5. Conclusion

DOX, an anthracycline antibiotic, applied for the treatment of various types of cancers due to its cytotoxic properties; although, nephrotoxicity is a significant side effect of DOX. DOX-induced nephrotoxicity is characterized by a complex interplay between oxidative stress and inflammatory pathways. DOX reduces cellular energy levels and increases oxidative stress by producing ROS, lipid peroxidation, decreasing antioxidant enzyme levels, and activating inflammatory-inducing molecules, leading to cell necrosis. This study demonstrates that DMQ mitigates DOX-induced nephrotoxicity primarily by diminishing oxidative stress and inflammation, resulting in improved renal function and morphology in rats. These results support the use of DMQ as a biologically relevant defensive agent within this experimental framework. Simultaneously, the interpretations were appropriately limited to the measured biochemical, inflammatory, and histological endpoints. Further studies assessing molecular signaling and long-term outcomes are required to define its full mechanistic and therapeutic scope.

## Availability of Data and Materials

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

## Author Contributions

HAbd, NHel, HNiy, and AAzh contributed to the study conception, experimental design, and coordination of the research work. WAlh, KAlk, and OAlh were responsible for data acquisition, laboratory experiments, and preliminary data organization. RAlt, HAls, MAls, and SLab contributed to data analysis, interpretation of results, and statistical evaluation. NAlh, RAlm, KAhm, IAbu, TAsi, and MAlk participated in literature review, methodology refinement, and validation of experimental findings. KIbr contributed through manuscript modification, critical data analysis, and manuscript revision. AAlf supervised the study, contributed to study design, data interpretation, and

critically revised the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

All animal experiments were performed in accordance with the Canadian Council on Animal Care (CCAC) guidelines and received approval from the Biomedical Research Ethics Committee of Umm Al-Qura University, with approval number HAPO-02-K-012-2025-10-2956.


## Acknowledgment

The authors would like to express their sincere gratitude to the Biomedical Research Ethics Committee of Umm Al-Qura University and the Faculty of Medicine, King Abdulaziz University, for their ethical review, guidance, and approval of this study. Their support and oversight were essential in ensuring that the research was conducted in accordance with established ethical standards.

## Funding

This research received no external funding.


## Conflicts of Interest

The authors declare no conflicts of interest.

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