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ORIGINAL ARTICLE

Doxorubicin-Induced Renal Inflammation in Rats: Protective Role of 3-Hydrazinoquinoxaline-2-Thiol

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ABSTRACT

Background: **Doxorubicin (DOX) is a well-recognized, potent, and commonly used clinical drug that belongs to the anthracycline family and is used as an antineoplastic agent to manage different types of solid tumors. However, it's therapeutic application has been compromised by its significant multiple organ toxicities, including nephrotoxicity. Over the past few years, various approaches, including bioactive and chemically synthesized molecules, have been discovered that offer protective effects against these drug-related problems. 3-Hydrazinoquinoxaline-2-thiol (3-HQT) belongs to the quinoxaline family and possesses pharmacological properties, such as antioxidant, anti-inflammatory, and antimicrobial activities. The present study was designed to assess the shielding effects of 3-HQT against DOX-induced renal inflammation in an *in vivo* rat model.**

Methods: **Renal inflammation was induced by a single DOX dose (15 mg/kg, i.p. on the 6th day), and the test molecule 3-HQT (30 mg/kg low and 60 mg/kg high dose) was administered orally as a co-treatment daily for 7 days with DOX. DOX-treated rodents displayed an upsurge in renal function parameters (urea, uric acid, and creatinine) as well as a significant decrease in antioxidants and an upsurge in lipid peroxidation in renal cells.**

Results: **DOX treatment also increases the expression of proinflammatory cytokines, namely, tumor necrosis factor-α, interleukin-6 (IL-6), and IL-1β, and inflammatory mediators such as inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), interferon-γ (IFN-γ), and triggered nuclear factor kappa-B (NF-κβ) in kidney cells, causing injury, infiltration, and cell death. These metabolic and immunological changes were further supported by histopathological findings, which confirmed structural changes in renal cells. Nevertheless, treatment with 3-HQT restored renal function parameters and antioxidant status, mitigated lipid peroxidation, and repressed elevated levels of proinflammatory cytokines and inflammatory mediators in DOX-induced rodents. Finally, it can**

be summarized that 3-HQT can reduce DOX-induced renal inflammation by inhibiting oxidative stress and the expression of pro-inflammatory cytokines and mediators in rats.
Conclusions: **This study suggests that 3-HQT could be beneficial in diminishing the adverse effects of DOX and may be a probable candidate for renal treatment.**
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KEYWORDS

doxorubicin, quinoxaline, renal inflammation, kidney damage

LIST OF ABBREVIATIONS

3-HQT - 3-hydrazinoquinoxaline-2-thiol
DOX - Doxorubicin
AKI - Acute kidney injury
CKD - Chronic kidney disease
ANOVA - Analysis of variance
CAT - Catalase
COX-2 - Cyclooxygenase-2
ELISA - Enzyme-linked immunosorbent assay
GSH - Reduced glutathione
GAPDH - Glyceraldehyde 3-phosphate dehydrogenase
H&E staining - Hematoxylin and eosin staining
IFN-γ - Interferon-gamma
IL-1β - Interleukin-1 beta
IL-6 - Interleukin-6
iNOS - Inducible nitric oxide synthase
MDA - Malondialdehyde
NF-κβ - Nuclear factor kappa B
PBS - Phosphate-buffered saline
PGE_2 - Prostaglandin E_2
ROS - Reactive oxygen species
SOD - Superoxide dismutase
TNF-α - Tumor necrosis factor-alpha
p38α MAPK - p38α mitogen-activated protein kinase

INTRODUCTION

Renal inflammatory diseases, caused by different conditions, including acute kidney injury (AKI), chronic kidney disease (CKD), diabetic nephropathy, glomerulonephritis, and lupus nephritis, have a noteworthy global effect due to their prevalence and severity. These diseases are major community health concerns, disturbing roughly 10% or more of the worldwide residents and notably increasing in recent years [1]. The severity of these diseases varies significantly; for example, AKI predominantly affects critically-ill patients and is associated with high morbidity and mortality, whereas CKD is a globally prevalent condition that affects approximately half of individuals aged over 70 years. CKD is characterized by chronic inflammation and fibrosis, leading to progressive and largely irreversibly damage to renal tissues and function [2]. Renal inflammation generates systemic complications and elevates the risk of cerebrovascular diseases, stroke, hypertension, type 2 diabetes mellitus, and vascular and metabolic disturbances due to kidney failure [3,4]. Renal inflammation is characterized by complex immune dysregulation and metabolic imbalance, in which immune cells, cytokines, and inflammatory signaling pathways contribute to renal injury and fibrosis. Novel forms of inflammatory cell death, such as pyroptosis, have been involved in the pathogenesis of various renal disorders, including AKI and CKD, making them potential therapeutic targets [5]. The interplay between immune pathways, such as cytokine signaling, and metabolic dysfunction, exacerbates disease severity by promoting ongoing inflammation and tissue damage [6,7]. Perioperative acute kidney injury driven by inflammation, ischemia, and endothelial dysfunction is linked to increased hospitalization duration, premature onset of CKD, and elevated mortality rates, showing the broad settings in which renal inflammation severity manifests [8].

Doxorubicin (DOX) is a widely-used chemotherapeutic drug, effective against various solid tumors and hematological cancers. However, its clinical utility is substantially restricted due to its dose-dependent toxic effect across multiple organs, including the liver, kidney, heart, reproductive organs, adipose tissue, nervous system, and intestines. The primary mechanism behind doxorubicin toxicity is the production of free radicals, leading to oxidative stress, which causes inflammation and apoptosis [9-11]. In cases of renal inflammation, doxorubicin induces oxidative stress, activation of inflammatory pathways, fibrosis, and cell death. Specifically, doxorubicin promotes excessive reactive oxygen species (ROS) production in kidney tissues, which leads to oxidative damage of cellular components and triggers a cascade of inflammatory responses [12,13]. This oxidative stress activates key inflammatory mediators, such as NF-κβ, COX-2, and the NLRP3 inflammasome, elevating pro-inflammatory cytokines, including IL-1β and TNF-α, which exacerbate renal tissue injury [13,14]. In addition to inflammation, doxorubicin stimulates tissue remodeling and fibrosis through transforming growth factor-beta signaling pathways, resulting in extracellular matrix accumulation and chronic kidney damage [12]. Apoptosis pathways, such as pyroptosis, are also induced, involving molecules like caspase-1 and gasdermin D, which contribute to the loss of functional renal cells [13]. Inflammation involves the infiltration of immune cells, such as macrophages and T cells, into renal tissue, thereby amplifying inflammatory damage and fibrosis [15]. These pathological events manifest as structural kidney damage, weakened renal function (demonstrated by raised serum creatinine and urea levels), and progressive renal inflammation and fibrosis. Suppression of NF-κβ and inflammasome activation pathways is a common therapeutic target to reduce renal inflammation caused by doxorubicin [12]. The application of protective agents having significant antioxidant potential could serve as a probable key to treat DOX-induced nephrotoxicity.

3-Hydrazinoquinoxaline-2-thiol (3-HQT) represents a valuable pharmacophore that combines the bioactivities of quinoxaline, hydrazino, and thiol groups, with potential therapeutic applications in inflammation, infection, oxidative stress, and other pharmacological conditions [16,17]. Quinoxaline and hydrazinoquinoxaline derivatives are known for their broad spectrum of pharmacological and biological properties, such as antimicrobial, anticancer, antioxidant, anti-inflammatory, antitubercular, and antiviral activities. The structural versatility of hydrazinoquinoxaline makes it a valuable scaffold in medicinal chemistry for designing new therapeutic agents with enhanced biological activity [18,19]. These compounds and derivatives have been documented for their antioxidant ability to neutralize ROS and other free radicals, thereby reducing oxidative stress in cells. They also have the ability to inhibit key inflammatory pathways and mediators, thereby controlling inflammation associated with various diseases [20]. These compounds often inhibit key enzymes, such as cyclooxygenase-2 (COX-2), nuclear factor kappa B (NF-κβ), and p38α mitogen-activated protein kinase (p38α MAPK), as well as inducible nitric oxide synthase (iNOS), which play pivotal roles in the inflammatory response. By selectively inhibiting COX-2, these derivatives reduce the synthesis of proinflammatory prostaglandins, thereby alleviating inflammation. Additionally, the deactivation of pro-inflammatory cytokines, like tumor necrosis factor-alpha (TNF-α) and interleukins, may also contribute. The combined antioxidant and enzyme inhibition effects can lead to significant anti-inflammatory outcomes [19].

This study explores the protective properties of 3-hydrazinoquinoxaline-2-thiol in the *in vivo* rat model system against doxorubicin-induced renal inflammation. The protective properties of 3-HQT were analyzed based on the estimation of renal-specific function markers, oxidative stress markers, inflammatory cytokines, renal stress markers, and the expression of tumor markers. The histopathology study of kidney tissue also confirms the protective role of 3-HQT treatment against the DOX-induced kidney inflammation.

MATERIALS AND METHODS

Reagents

3-Hydrazinoquinoxaline-2-thiol ($C_8H_8N_4S$; 3-HQT) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification. Doxorubicin was obtained from Sigma-Aldrich (St. Louis, MO, USA). In this study, ELISA kits were used, including Rat IL-1β (cat. no. MBS265868; detection range 15.6 - 1,000 pg/mL; sensitivity usually < 5 pg/mL; intra-assay CV ≤ 8%; inter-assay CV ≤ 12%), Rat IL-6 (cat. no. MBS269892; detection range 15.6 - 1,000 pg/mL; sensitivity up to 5 pg/mL; intra-assay CV ≤ 8%; inter-assay CV ≤ 12%), Rat TNF-α (cat. no. MBS282960; detection range 6.25 - 400 pg/mL; sensitivity typically < 3.1 pg/mL; intra-assay CV ≤ 8%; inter-assay CV ≤ 12%), Rat IFN-γ (cat. no. MBS2019134; detection range 15.6 - 1,000 pg/mL; sensitivity typically ≤ 6.1 pg/mL; intra-assay CV ≤ 10%; inter-assay CV ≤ 12%), Rat iNOS (inducible nitric oxide synthase; cat. no. MBS2505942; detection range 0.31 - 20 ng/mL; sensitivity typically ≤ 0.19 ng/mL; intra-assay CV ≤ 4.94%; inter-assay CV ≤ 5.19%), Rat NF-κβ (MBS287521, detection range 0.31 - 20 ng/mL; sensitivity typically ≤ 0.15 ng/mL; intra-assay CV ≤ 8%; inter-assay CV ≤ 12%), Rat Cyclooxygenase-2 (COX-2; cat. no. MBS266603; detection range 0.156 - 10 ng/mL; sensitivity typically up to 0.05 ng/mL; intra-assay CV ≤ 8%; inter-assay CV ≤ 12%), and Rat PGE_2 (cat. no. MBS262150; detection range 15.6 - 1,000 pg/mL; sensitivity typically up to 5 pg/mL; intra-assay CV ≤ 8%; inter-assay CV ≤ 12%) were procured from Sigma-Aldrich in St. Louis, MO, USA. All other chemicals that are used in the present research were of the highest purity grade.

Experimental animals

An in vivo experimental study was conducted using Wistar rats (160 - 220 g, approximately 2 months old). Prior to the experiment, all animals were acclimatized for one week in a controlled and sanitized animal facility maintained at 22 ± 0.5°C with a 12 hours light/12 hours dark cycle and relative humidity of 40 - 45%. Rats had free access to standard laboratory chow and drinking water throughout the study. Animals were monitored daily for general health, behavior, and any signs of distress. Humane endpoints were predefined and included severe lethargy, inability to access food or water, or marked body weight loss. If any of these conditions had occurred, the affected animal would have been humanely euthanized and removed from the study. However, none of the animals met these criteria during the experimental period. All procedures were reviewed and approved by the Institutional Ethics Committee of Umm Al-Qura University and were conducted in accordance with ARRIVE guidelines and applicable national regulations for the care and use of laboratory animals (approval number: HAP0-02-K-012-2025-10-2956). All animals included in the study were analyzed, and no animals or samples were excluded from the final dataset. Exclusion would only have occurred in the event of a clear technical or procedural error during sample collection, processing, or analysis; however, no such events occurred in this study. After adaptation, 24 healthy Wistar rats were randomly assigned to four test groups using a simple randomization method, with six rats per group (n = 6):

Group I (Control): Rats were not treated with any drug or solvent.

Group II (DOX): Single dose of DOX (15 mg/kg; i.p.) on the 6th day after the start of the test [13].

Group III (DOX + 3-HQT-30): Rats receive a low dose of 3-HQT (30 mg/kg/day) by oral administration once a day for 7 consecutive days and doxorubicin (15 mg/kg, i.p.) in a single dose on the 6th day [21].

Group IV (DEN + 3-HQT-60): Rats receive a high dose of 3-HQT (60 mg/kg/day) by oral administration once a day for 7 consecutive days and doxorubicin (15 mg/kg, i.p.) in a single dose on the 6th day [21].

To evaluate the protective efficacy of 3-HQT, a prophylactic/co-treatment design was adopted. Animals received 3-HQT orally for seven consecutive days. DOX was administered on day 6 of the experimental schedule. This timing allowed for five days of pretreatment prior to DOX exposure, followed by continued administration on the day of DOX injection and one additional day thereafter.

Collection of blood samples and renal tissues

All of the tested rodents were fasted overnight and first anesthetized using sodium pentobarbital (45 mg/kg IP) to attain surgical plane anesthesia, confirmed by the absence of pedal withdrawal reflex and corneal reflex. Under maintained anesthesia retro-orbital blood collection was performed once per animal (maximum 100 - 200 µL, < 10% total blood volume) using heparinized capillary tubes. Anesthetic depth (loss of righting reflex, absent pedal withdrawal, and palpebral reflex) was confirmed, followed immediately by cervical dislocation as the primary physical euthanasia method, ensuring rapid unconsciousness and death. All procedures were conducted by trained personnel in a designated laboratory space during daylight hours, with rationale for anesthetic selection based on rapid onset (sodium pentobarbital), stable maintenance, and humane endpoint confirmation prior to cervical dislocation to minimize distress. Post-procedure, death was confirmed by the absence of heartbeat and respiration [22]. After authorization, the kidneys were carefully removed and cleaned of debris with ice-cold phosphate-buffered saline (PBS; pH - 7.4; 1M), and the tissue was macerated in ice-cold PBS (1M; 1 g of tissue/9 mL PBS). After proper maceration, the obtained mixture was centrifuged at low rotation (5,000 rpm) for 20 minutes. The clear supernatant was cautiously divided and used for different biochemical tests. Renal function tests were performed on the collected blood serum samples, which were extracted by centrifuging whole blood at 10,000 x g at 4°C for 15 minutes.

Excised tissues were rinsed in ice-cold phosphate-buffered saline (PBS, pH 7.4) to remove residual blood, gently blotted dry, and weighed. Each kidney sample was homogenized on ice in ice-cold PBS (1 g tissue in 9 mL PBS) using a glass homogenizer. The homogenates were centrifuged at 5,000 rpm for 20 minutes at 4°C. The clear supernatant was carefully collected and divided into aliquots for biochemical and cytokine analyses. All steps were carried out on ice to preserve protein stability.

Aliquoted samples were stored at -80°C until analysis. Each sample was thawed only once, and repeated freeze-thaw cycles were avoided. Total protein concentration in the tissue homogenates was measured by a commercial kit using the biuret method, and cytokine levels were expressed relative to total protein content (pg/mL) to ensure normalization across samples.

For serum analysis, whole blood was collected at sacrifice and allowed to clot at room temperature. Samples were centrifuged at 10,000 × g for 15 minutes at 4°C to obtain serum. The serum was separated promptly, aliquoted, and stored at -80°C until renal function parameters were measured.

Quantification of renal functions

Renal function test markers, such as serum urea, uric acid, and creatinine levels, were quantified to assess DOX-induced nephrotoxicity in each control and test group rat. The quantification of serum urea, uric acid, and creatinine concentrations was determined by a spectrophotometric method using commercially available kits.

Quantification of oxidative stress markers

Lipid peroxidation was estimated as malondialdehyde (MDA) concentration using a spectrophotometric method according to Atia et al. [22]. In this method, MDA present in the kidney tissue homogenate reacts with thiobarbituric acid at 95°C in an acidic medium and produced pink color end product, which was calculated at 532 nm and was represented as nmol/mg of protein. SOD activity was calculated using a spectrophotometric method adopted from Shahid Nadeem et al. [23] and was represented as unit/mg, where one unit of SOD activity was calculated as the protein amount required to inhibit 50% of NBT reduction.

In the serum supernatant, CAT activity was measured using a method in which it was prepared in phosphate buffer, reacted with 50 nM hydrogen peroxide, and kept for half an hour. The absorbance was measured every 15 seconds at 510 nm, and CAT activity was expressed as μM per minute/g of serum supernatant [24].

The GSH level was measured by mixing the clear serum supernatant with an equal volume of trichloroacetic acid. The resultant solution was then mixed with a DTNB solution prepared in phosphate buffer. The GSH level was quantified using a standard curve after recording the absorbance at 412 nm, and the results were presented as units per U/mg of GSH [25].

Quantification of the renal expression of pro-inflammatory cytokines and mediators

Tissue dissected from the kidney was homogenized in ice-cooled PBS (pH 7.4, 1M), and the clear supernatant was isolated by centrifugation at 6,000 rpm for 10 minutes under cooling conditions. The clear upper layer was used as the test material for quantification of inflammatory cytokines, such as IL-1β, IL-6, and other markers such as TNF-α and NF-κβ expression levels were measured using a market-purchased ELISA kit. The method was applied as per the directions given by the manufacturer. The levels of these markers were presented as ng/mL or pg/mL.

Quantification of the other inflammatory mediators

To identify renal stress and kidney function at the serum level, markers such as iNOS, GAPDH (glyceraldehyde 3-phosphate dehydrogenase), and marker PGE_2 were analyzed to assess the immune response produced in renal stress condition. The serum levels of these markers were quantified by market-purchased ELISA kits according to the provided manufacturer's protocol and their quantity was represented in ng/mL or pg/mL.

Quantification of pro-inflammatory (IFN-γ) and inflammatory response (COX-2) markers

To assess the tumor markers IFN-γ and rat COX-2 levels, the ELISA method was applied using commercially available kits. These kits were based on the sandwich ELISA antibody-based capture method to precisely measure the expression levels of these tumor markers, and the results were represented as picograms per milliliter (pg/mL).

Renal histopathology

The histopathological study of kidney tissue was performed to measure DOX-induced inflammatory damage and recovery status after 3-HQT treatment. For this, the separated kidney tissue was immediately treated with ice-cooled 10% buffered formalin. Subsequently, to prepare thin sections, the samples were embedded in paraffin wax. About 5-mm thin vertical section was prepared and stained with hematoxylin and eosin (H&E). Representative photomicrographs were captured at a scale of 50 μm (200 × magnification), and scale bars were included in all images. Leucocytes infiltration and epidermal thickness (indicators of histological changes) were analyzed microscopically. At least six dissimilar regions of stained kidney sections from each group were examined, and the results were carefully recorded. For injury scoring, two parameters were graded on a scale from 0 to 4, each corresponding to normal histology (0), mild injury (< 25%), moderate injury (25 - 50%), severe injury (50 - 75%), and extensive injury (> 75%). Each animal was scored to obtain a final histopathological score, which was the mean of scores from several random microscopic fields. For each kidney, at least 8 non-overlapping fields from the kidney section were randomly selected and examined to ensure representative evaluation. The final score for each animal was calculated as the mean of the observed fields. Histological assessment was performed by an experienced pathologist who was unaware of the treatment allocation. To ensure consistency, a subset of sections was independently reviewed by a second evaluator. Any discrepancies in scoring were discussed and resolved by consensus.

Statistical analysis

All statistical analyses were performed with GraphPad Prism version 8.0 (GraphPad Software, San Diego, USA). Data normality was assessed using the Shapiro-Wilk test, and homogeneity of variance was assessed using Levene's test before performing ANOVA. The results are shown as mean ± SEM (n = 6 animals per group). When assumptions of normality and equal variance were satisfied, comparisons among groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. For pairwise comparisons, mean differences with 95% confidence intervals were calculated to estimate the magnitude and precision of treatment effects. Data are presented as mean ± SEM. Statistical significance was set at $p < 0.05$. No data points were excluded unless a clear procedural error was identified. No such exclusions occurred in the present study.

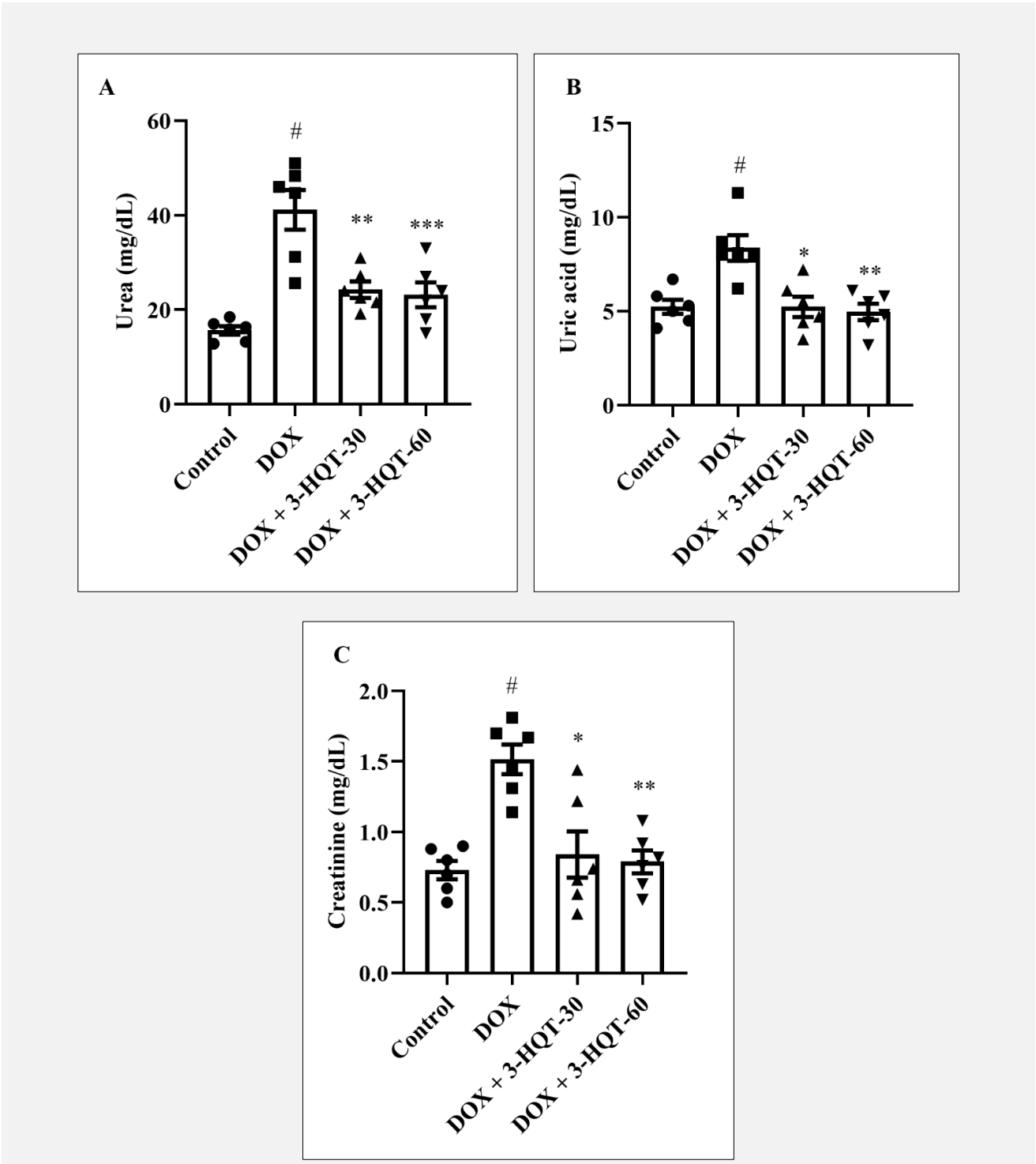


Figure 1. Therapeutic effects of 3-HQT treatment on blood urea, uric acid, and creatinine in DOX-administered rats.

A: Urea, B: Uric acid, C: Creatinine. Data are presented as mean ± S.E.M. (n = 6). Treatment groups: Control, DOX - doxorubicin, DOX + 3-HQT-30 - DOX with 30 mg/kg/day 3-HQT, DOX + 3-HQT-60 - DOX with 60 mg/kg/day 3-HQT. One-way ANOVA followed by Tukey's post hoc test, $^{\#}p < 0.001$ DOX vs. control, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. DOX.

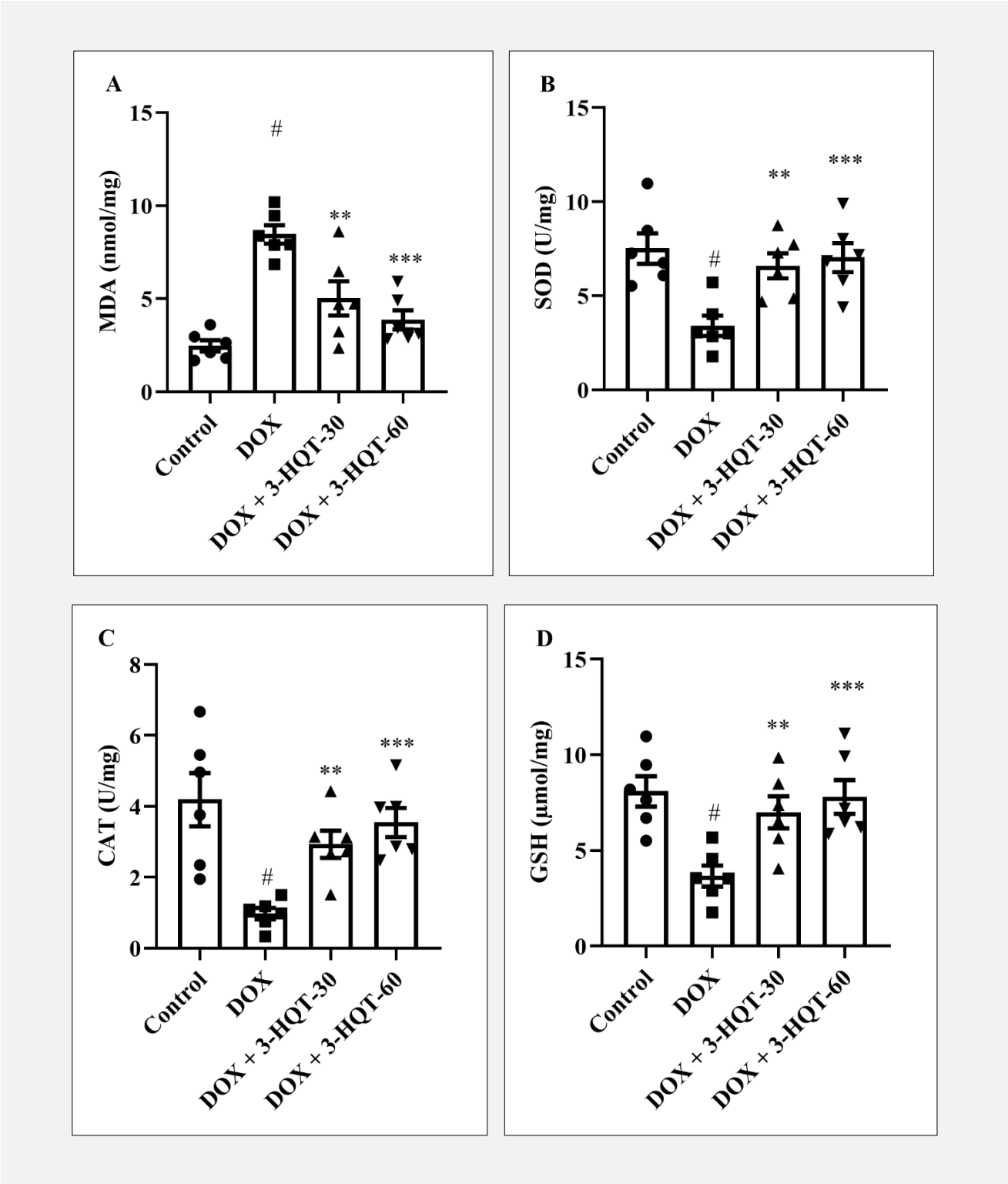


Figure 2. Therapeutic effect of 3-HQT treatment on the expression of renal oxidative markers in DOX-administered rats.

A: MDA, B: SOD, C: CAT, and D: GSH. Data are presented as mean ± S.E.M. (n = 6). Treatment groups: Control; DOX - doxorubicin; DOX + 3-HQT-30 - DOX with 30 mg/kg/day 3-HQT; DOX + 3-HQT-60 - DOX with 60 mg/kg/day 3-HQT. One-way ANOVA followed by Tukey's post hoc test, $^{\#}p < 0.001$ DOX vs. control, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. DOX.

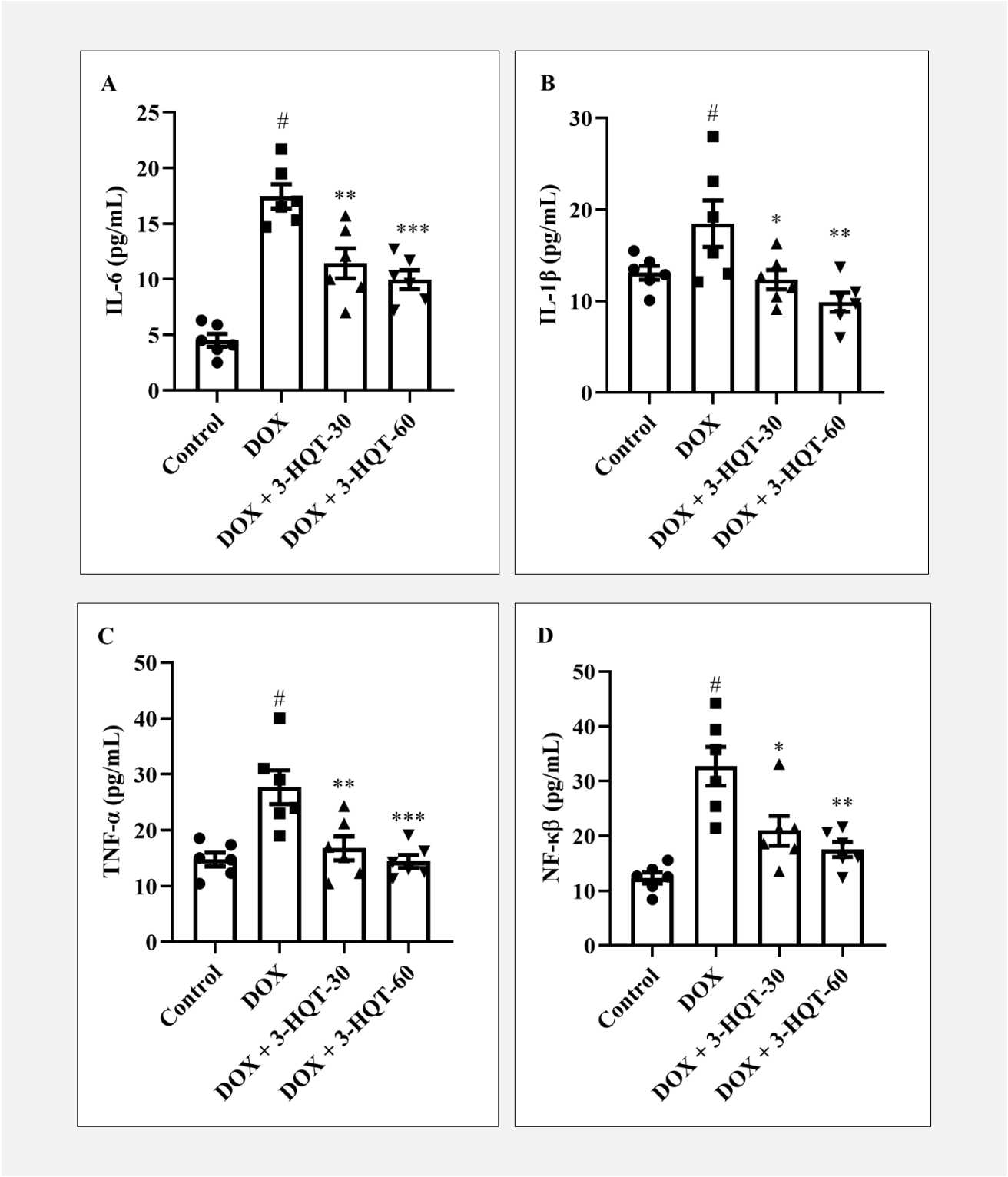


Figure 3. Therapeutic effect of 3-HQT treatment on the expression of cytokines and pro-inflammatory mediators in DOX-administered rats.

A: IL-6, B: IL-1β, C: TNF-α, D: NF-κβ. Data are presented as mean ± S.E.M. (n = 6). IL-6 - interleukin-6; IL-1β - interleukin-1β; TNF-α - tumor necrosis factor-α; NF-κβ - nuclear factor-κβ. Treatment groups: Control; DOX - doxorubicin; DOX + 3-HQT-30 - DOX with 30 mg/kg/day 3-HQT; DOX + 3-HQT-60 - DOX with 60 mg/kg/day 3-HQT. One-way ANOVA followed by Tukey's post hoc test, $^{\#}p < 0.001$ DOX vs. control, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. DOX.

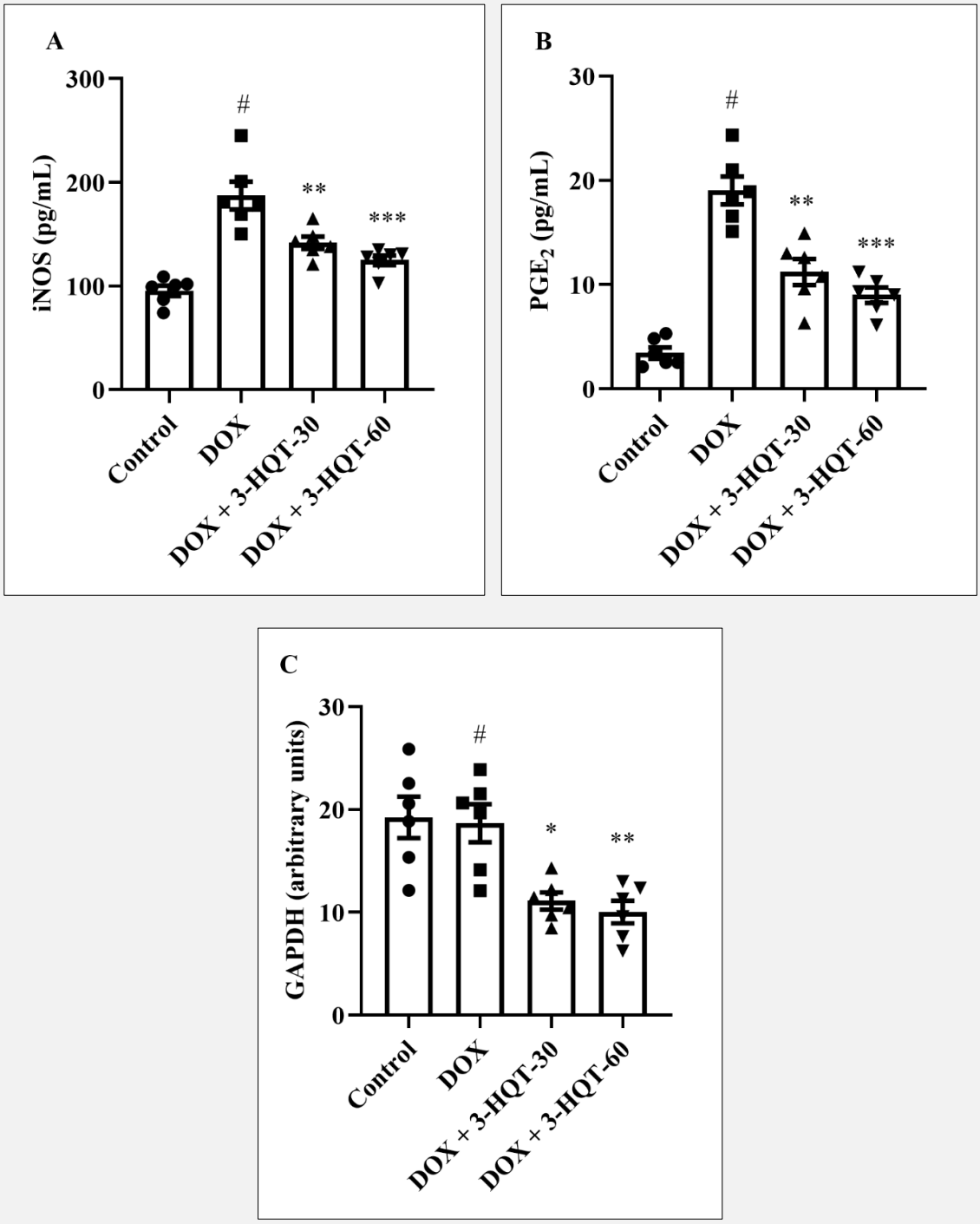


Figure 4. Therapeutic effect of 3-HQT treatment on renal stress markers in DOX-administered rats.

A: iNOS, B: PGE_2, and C: GAPDH. Data are presented as mean ± S.E.M. (n = 6). iNOS - nitric oxide synthase; GAPDH - glyceraldehyde 3-phosphate dehydrogenase; and PGE_2 - prostaglandin E_2. Treatment groups: Control; DOX - doxorubicin; DOX + 3-HQT-30 - DOX with 30 mg/kg/day 3-HQT; DOX + 3-HQT-60 - DOX with 60 mg/kg/day 3-HQT. One-way ANOVA followed by Tukey's post hoc test, $^{\#}p < 0.001$ DOX vs. control, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. DOX.

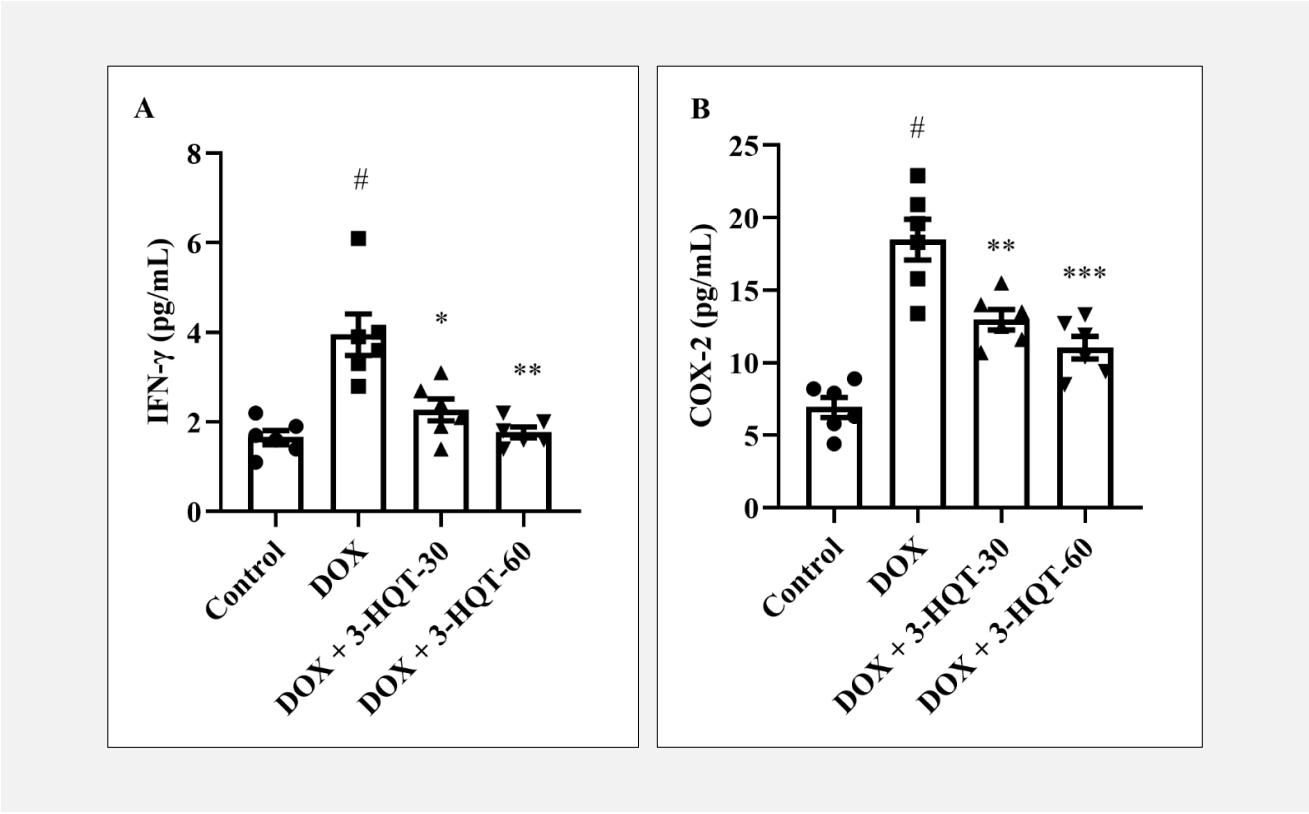


Figure 5. Therapeutic effect of 3-HQT treatment on pro-inflammatory mediators in DOX-administered rats.

A: IFN-γ and B: COX-2. Data are presented as mean ± S.E.M. (n = 6). IFN-γ - interferon - gamma; and COX-2 - cyclooxygenase-2; treatment groups: control; DOX - doxorubicin; DOX + 3-HQT-30 - DOX with 30 mg/kg/day 3-HQT; DOX + 3-HQT-60 - DOX with 60 mg/kg/day 3-HQT. One-way ANOVA followed by Tukey's post hoc test, # $p < 0.001$ DOX vs. control, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. DOX.

RESULTS

Effect of 3-HQT on renal function parameters

In this study, indicators of renal function, specifically urea, uric acid, and creatinine amount in the serum, were quantified. The results showed a considerable upsurge in renal function parameters in the DOX-treated rats, including serum urea ($p < 0.001$, Figure 1A), uric acid ($p < 0.001$, Figure 1B), and serum creatinine ($p < 0.001$, Figure 1C) levels, compared with the control. In contrast, the rats that received 3-HQT at 30 mg/kg/day significantly recovered from the upsurge in serum urea (95% CI: 6.391 to 27.39, $p < 0.01$), uric acid (95% CI: 1.070 to 5.196, $p < 0.05$), and creatinine (95% CI: 0.2383 to 1.112, $p < 0.05$) levels. However, in group IV treated rats, which received a high dose (60 mg/kg/day) of 3-HQT, serum urea (95% CI: 7.476 to 28.48, $p < 0.001$), uric acid (95% CI: 1.337 to 5.463, $p < 0.01$), and creatinine (95% CI: 0.2899 to 1.163, $p < 0.01$) levels were almost similar to those of control rats, indicating a dose-dependent recovery effect.

Effect of 3-HQT on the levels of antioxidant markers in blood

In respect to control rats, group II rats treated with DOX displayed an unusual upsurge in the MDA level ($p < 0.001$), while SOD, catalase, and GSH activities were decreased ($p < 0.001$). 3-HQT treatment significantly recovered the necessary antioxidant level in all treated rats. Compared to the DOX-control group rats, the 3-HQT (at low dose, i.e., 30 mg/kg/day) significantly downgraded the MDA level (95% CI: 1.053 to 5.814, $p < 0.01$) and upsurged the renal SOD (95% CI: -5.966 to -0.4102, $p < 0.01$), CAT (95% CI: -3.848 to -0.07175, $p < 0.01$), and GSH (95% CI: -6.416 to -0.2375, $p < 0.01$) activity (Figure 2 A - D). Compared to group IV rats, there was a noteworthy improvement in the activity levels in rats treated with a high dose (60 mg/kg/day) of 3-HQT and in the activity levels of SOD (95% CI: -6.405 to -0.8486, $p < 0.001$), CAT (95% CI: -4.462 to -0.6851, $p < 0.001$), and GSH (95% CI: -7.221 to -1.042, $p < 0.001$), while the MDA (95% CI: 2.204 to 6.966, $p < 0.001$) activity showed a slight reduction compared to group III rats, representing the dose-dependent effect of 3-HQT.

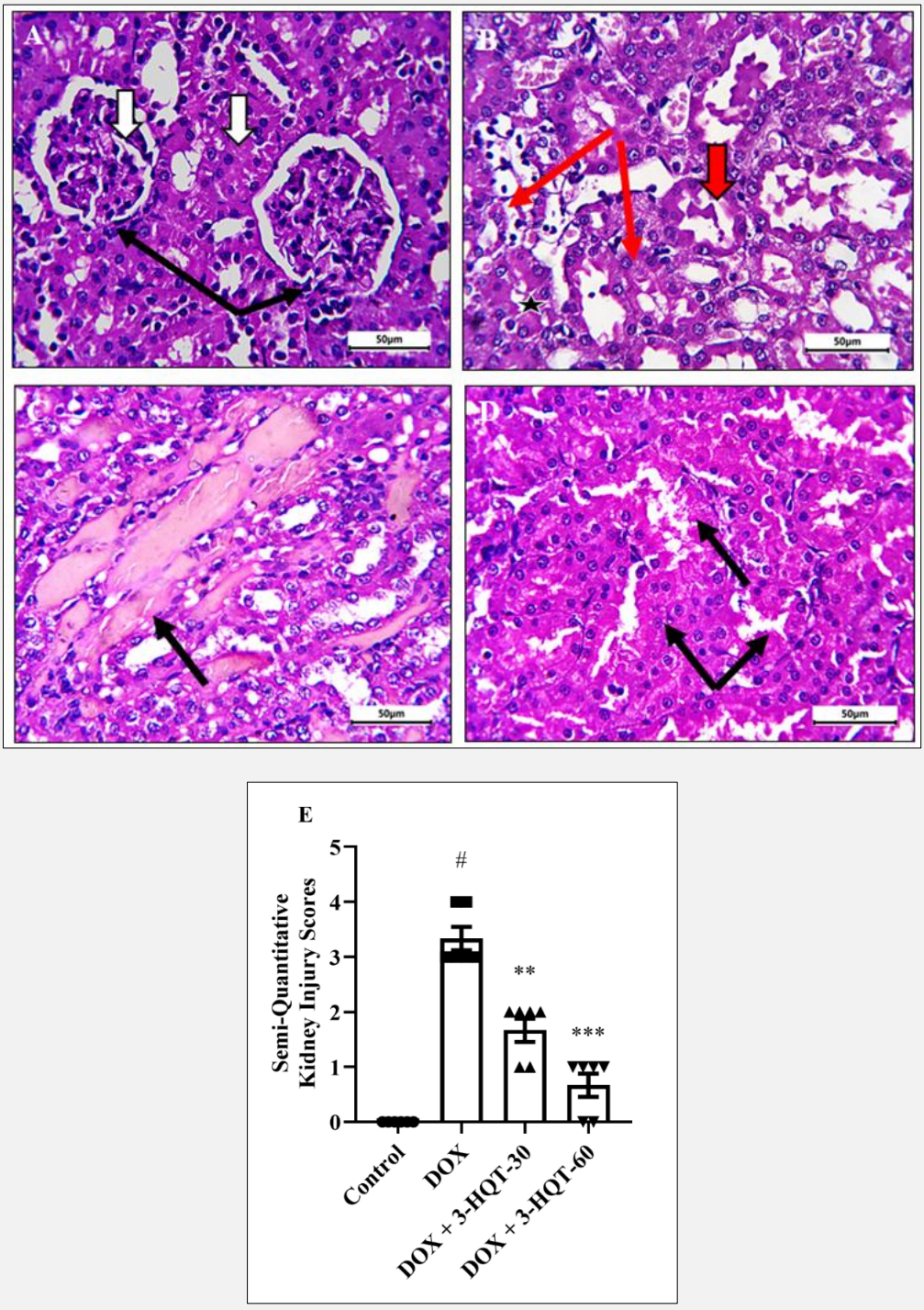


Figure 6. Renal histological alterations of rat kidneys were observed using H&E staining (scale 50 µm).

A: Control rats, B: DOX treated - sporadic marked atrophy of renal corpuscle glomeruli (thick arrow), dilation of many tubules with deformed shapes and epithelial lining atrophy (thin arrow), and dilated tubules contain epithelial and hyaline casts, dilated vessels show hemolyzed blood (star) by DOX administration, C: DOX + 3-HQT-30 - DOX with 30 mg/kg/day 3-HQT, pre-treatment with 3-HQT reverted with these alterations, with mild dilation of many tubules with deformed shape and epithelial lining atrophy (thin black arrow); D) DOX + 3-HQT-60 - DOX with 60 mg/kg/day 3-HQT pretreatment showing improvement of atrophy of renal corpuscle glomeruli and dilation of many tubules (black arrow), E: quantitative analysis of diabetic kidney injury was determined by semi-quantitative kidney injury scoring (0 - 4), bar graphs show mean ± S.E.M. = ne-way ANOVA followed by Tukey's post hoc test, $^{\#}$ p < 0.001 DOX vs. control, ** p < 0.01, *** p < 0.001 vs. DOX.

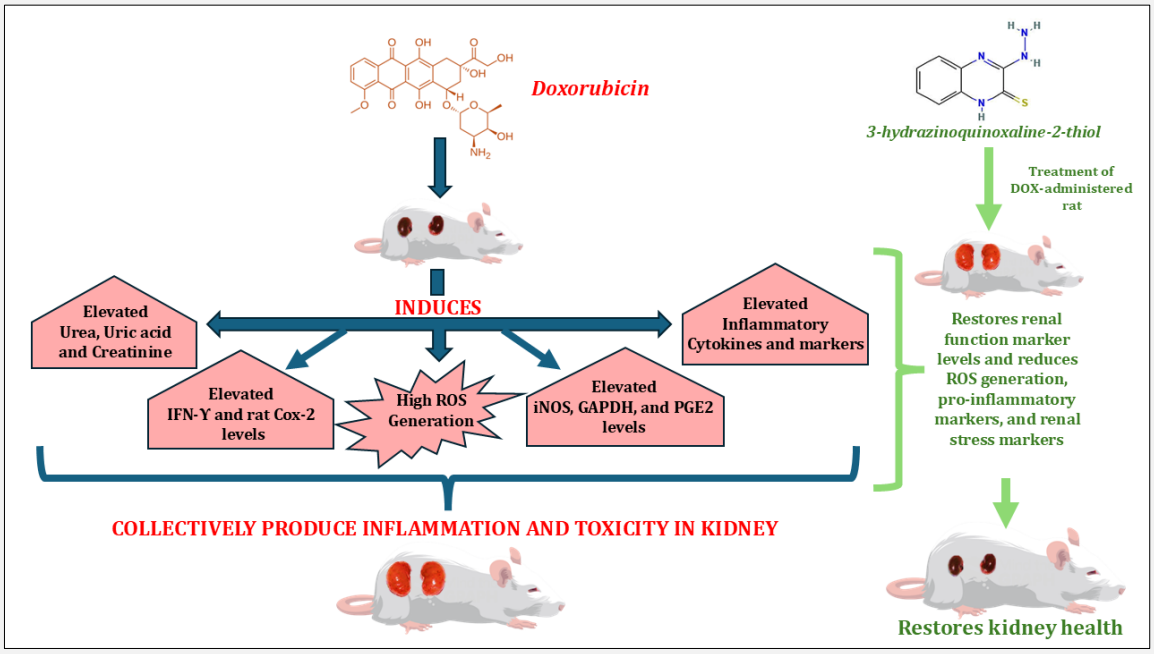


Figure 7. Graphical summary.

Effect of 3-HQT on pro-inflammatory cytokines in the kidney

The activity levels of pro-inflammatory cytokines such as IL-6, IL-1β, TNF-α, and NF-κβ in renal tissue were unusually high ($p < 0.001$) in DOX-treated rats compared with control rats. Rats that received 30 mg/kg/day 3-HQT (group III rats) possibly reversed the DOX-generated surge in the activity levels of proinflammatory cytokines IL-6 (95% CI: 2.063 to 10.00, $p < 0.01$), IL-1β (95% CI: 0.09001 to 12.11, $p < 0.05$), TNF-α (95% CI: 2.849 to 19.02, $p < 0.01$), and NF-κβ (95% CI: 2.309 to 21.20, $p < 0.01$) efficiently, compared to the DOX-treated rats (Figure 3 A-D). Rats that received low and high doses (60 mg/kg/day) of 3-HQT displayed a greater reduction in DOX-induced effects with respect to pro-inflammatory markers, IL-6 (95% CI: 3.529 to 11.47, $p < 0.001$), IL-1β (95% CI: 2.557 to 14.58, $p < 0.01$), TNF-α (95% CI: 5.194 to 21.37, $p < 0.001$), and NF-κβ (95% CI: 5.699 to 24.59, $p < 0.01$).

Effect of 3-DTQ on inflammatory mediators in the kidney

In DOX-treated rats, renal stress markers iNOS ($p < 0.001$) and PGE2 ($p < 0.001$) were unusually high, whereas GAPDH levels ($p < 0.001$) were low compared with control rats. The 3-HQT treatment in group IV (high dose) rats remarkably reduced the upsurged iNOS ($p < 0.001$) and PGE_2 ($p < 0.01$) levels and recovered GAPDH ($p < 0.01$) in comparison with the DOX-received group (Figure 4 A-C). Group III rats, which received a low dose (30 mg/kg/day) of 3-HQT, showed less recovery of renal stress marker [iNOS (95% CI: 13.54 to 77.79, $p < 0.01$), PGE_2 (95% CI: 3.795 to 11.87, $p < 0.01$), and GAPDH (95% CI: 1.478 to 13.64, $p < 0.05$)] levels than group IV (high-dose, i.e. 60 mg/kg/day) [iNOS (95% CI: 30.37 to 94.63, $p < 0.001$), PGE_2 (95% CI: 6.028 to 14.10, $p < 0.001$), and GAPDH (95% CI: 2.554 to 14.72, $p < 0.01$)] rats.

Effect of 3-HQT on the pro-inflammatory and inflammatory response markers in DOX-induced nephrotoxicity rats

The expression levels of renal tumor protein IFN-γ ($p < 0.001$) and COX-2 ($p < 0.001$) were remarkably upsurged in the rats treated with DOX compared to those in control rats (Figure 5 A - B). Treatment with 3-HQT (low dose, i.e. 30 mg/kg/day) resulted in a remarkable drop in the overexpression of tumor protein IFN-γ (95% CI: 0.5710 to 2.796, $p < 0.05$) and COX-2 (95% CI: 1.773 to 9.260, $p < 0.01$). Nevertheless, rats that received a high dose (60 mg/kg/day) of 3-HQT exhibited a remarkable decline in expression of tumor markers (IFN-γ (95% CI: 1.071 to 3.296, $p < 0.01$) and COX-2 (95% CI: 3.707 to 11.19, $p < 0.001$), compared to rats treated with a low dose of 3-HQT.

Effect of 3-HQT treatment on renal histology in DOX-induced nephrotoxicity

The renal histological deviations induced by DOX and the effect of 3-HQT treatment are shown in Figure 6 (A - D) and were evaluated by neutrophil infiltration. DOX treatment resulted in marked irregular atrophy of the renal corpuscle glomeruli, along with deformed tubules with dilation observed in many renal sections. Epithelial lining atrophy and dilated tubules containing epithelial and hyaline casts were also noted. In the dilated vessels, hemolysis was observed, and histological injury in renal cells was found in DOX-treated rats compared with the kidney section of control rats. 3-HQT treatment at low and high doses displayed a sporadic recovery in the atrophy of the renal corpuscle glomeruli and a decrease in the dilation of many deformed-shape tubules. 3-HQT treatment also recovered epithelial lining atrophy and contracted neutrophil seep-out conditions. These findings were supported by a significant increase in the renal injury score compared to the control group, in relation to DOX ($p < 0.001$) (Figure 6E).

Rats given both doses of 3-HQT showed fewer morphological changes, indicated by a notable decrease in the renal injury score compared to the DOX group (30 mg/kg/day, 95% CI: 0.9440 to 2.389, $p < 0.01$; 60 mg/kg/day, 95% CI: 1.944 to 3.389, $p < 0.001$).

DISCUSSION

Renal inflammatory diseases affect a significant portion of the global population, contribute to high morbidity and mortality, and pose a substantial economic burden on healthcare systems worldwide. Their severity is exacerbated by immune dysregulation, metabolic abnormalities, infectious triggers, and comorbid conditions such as diabetes, emphasizing the need for improved early detection, preventive strategies, and targeted therapies to mitigate their impact [26]. Doxorubicin-induced renal inflammation is a significant cause of nephrotoxicity, characterized by oxidative stress, inflammation, apoptosis, and impaired renal function. Several studies have shown that treatment with compounds having antioxidant properties can effectively prevent inflammation-induced renal inflammation and toxicity [27-29]. Hence, this study aimed to assess the probable defensive properties of 3-HQT against DOX-induced renal inflammation.

The experimental evidence in rat models showed that DOX-treated rats displayed elevated serum urea, uric acid, and creatinine levels. All these elevated parameters found in doxorubicin-treated animals cause nephrotoxicity due to doxorubicin-induced oxidative stress, inflammation, and apoptosis, which disrupt renal tubular cell architecture, decrease the glomerular filtration rate, and damage the excretory functions of urea, uric acid, and creatinine. Increased concentrations of serum urea and creatinine are standard markers of renal dysfunction, which may be due to compromised glomerular filtration rate and reduced tubular elimination functions caused by doxorubicin-induced renal injury [30,31]. Elevated uric acid levels may also cause hyperuricemia, which further contributes to kidney damage by activating inflammatory pathways and promoting renal fibrosis [32]. The variations in these serum functional markers were parallel to the grade of renal damage induced by DOX. Treatment with 3-HQT can help reverse the effects and restore kidney function.

Doxorubicin persuades renal oxidative stress as the main mechanism of renal inflammation and nephrotoxicity. Furthermore, our result data showed that DOX treatment generated a noteworthy drop in CAT and SOD activity and GSH renal content with a high level of MDA. DOX treatment amplified lipid peroxidation products such as MDA, while diminishing endogenous antioxidant defenses, such as SOD, CAT, and GSH, which disturb the cellular redox equilibrium and increase the production of ROS. This disturbance in oxidative status leads to renal tissue damage, fibrosis, and apoptosis, as confirmed by previous findings [12,13]. Treatment with 3-HQT reduced the oxidative stress levels in the kidney cells and helped restore kidney health. Some quinoxaline derivatives have ability to modulate cellular redox status, mimic antioxidant enzymes such as glutathione peroxidase, and upregulate endogenous antioxidant defense systems [33].

Cytokines are major players in the initiation and development of renal inflammation through facilitating immune cell recruitment, stimulation, and continuous inflammatory responses. Key pro-inflammatory cytokines such as TNF-α, IFN-γ, IL-6, and IL-1β support local immune stimulation and subsidize to kidney tissue injury [34]. In this study, DOX-treated rat models showed that DOX increases renal pro-inflammatory cytokines like TNF-α, IL-1β, and IL-18, which enhance renal inflammation and contribute to its nephrotoxic effects, similar to the result of a previous study [12,13,35]. At the transcriptional level, NF-κβ controls the expression of iNOS, COX-2, and other proinflammatory cytokines. In conjunction with this, DOX also boosts the expression and activity of inflammatory mediators with NF-κβ and COX-2, which amplify oxidative injury and inflammatory responses in the kidney [36]. Treatment with 3-HQT reduced the expression of these pro-inflammatory markers and renal inflammation.

As GAPDH is the principal glycolytic enzymes routinely employed as a housekeeping reference gene, DOX-induced oxidative stress and associated metabolic instabilities may potentially modulate its expression and functional activity. PGE_2 levels and COX-2 expression were amplified in the kidneys after DOX treatment. This surge imitates inflammatory responses, as COX-2 is the enzyme that catalyzes PGE_2 synthesis, which is involved in mediating inflammation and oxidative injury. DOX upregulates COX-2 gene expression and protein levels in the kidney, thereby increasing PGE_2 levels and promoting inflammation [35,36]. DOX administration also markedly increased iNOS mRNA and

protein levels and activity in the kidney tissue. High iNOS contributes to the amplified generation of nitric oxide, which, in excess, can generate reactive nitrogen species that cause oxidative and nitrosative stress, aggravating renal inflammation and injury. These upsurges in COX-2/PGE_2 and iNOS relate to stimulation of inflammatory signaling pathways like NF-κβ and deteriorate oxidative stress induced by DOX [36,37]. IFN-γ is another critical immune cytokine that is responsible for inflammation, immune regulation, and tumor suppression. IFN-γ signaling influences different cellular pathways including NF-κβ and MAP kinase cascades, which are frequently involved in inflammatory responses induced or modulated by DOX treatment [38]. Treatment with 3-HQT reversed the elevated levels of renal stress markers, thereby protecting against DOX-induced nephrotoxicity.

DOX treatment significantly promoted histopathological changes in the kidney identified as tissue damage, inflammation, and cellular apoptosis. DOX administration is associated with increased expression of proinflammatory mediators, enhanced apoptosis, and reduced endogenous antioxidant levels. Experimental studies in rat models have displayed that DOX leads to structural injury, tubular necrosis, glomerular basement membrane thickening, interstitial inflammation, and fibrosis [27]. Parallel findings have been reported in DOX-induced acute kidney damage models, where cytokines and inflammation markers (NF-κβ, COX-2, iNOS) are overexpressed in kidney tissue, contributing to the observed histopathological damage [39].

DOX-induced renal toxicity arises due to oxidative stress, inflammation, apoptosis, and impaired renal homeostasis in the kidneys, and treatment with 3-HQT offsets these effects and helps to restore kidney health. Through this study, we propose the probable defensive mechanisms of 3-HQT against DOX-induced renal inflammation in a rat model system, where 3-HQT reduces the elevated urea, uric acid, and creatinine levels and supports the regaining of renal health. 3-HQT also successfully decreased the production of ROS, nitric oxide synthase, and PGE_2 levels, which might result in a rise in antioxidant enzymes such as SOD and CAT and a decrease in GSH and MDA levels, resulting in a reduction in lipid peroxidation and cellular damage. Further, 3-HQT decreases inflammation by lowering the inflammatory cytokines expression, such as TNF-α, IL-6, and IL-1β, which ultimately diminishes the activation of NF-κβ and MAPK signaling pathways. 3-HQT also proficiently regulated the increased production of IFN-γ and COX-2 to counter DOX-induced renal inflammation. 3-HQT may also suppress DOX-induced renal fibrosis by reducing the production of fibrosis-related proteins, ultimately recovering enlarged capsular space and renal tubule damage-associated structural alterations in kidney tissues. Thus, 3-HQT enhances renal function markers and improves histopathological outcomes in a model of doxorubicin-induced nephrotoxicity (Figure 7). While our study provides valuable insights into 3-HQT treatment for DOX-induced nephrotoxicity, several limitations should be acknowledged. First, findings from the rodent model may not fully translate to human physiology due to differences in metal metabolism and renal handling between species. Second, the relatively short 14-day exposure window captures acute injury patterns but does not reflect chronic occupational exposures typical in humans. Third, we focused on serum and tissue biomarkers, without assessing functional urine output or emerging renal markers such as albuminuria, KIM-1, or NGAL, which would strengthen nephrotoxicity assessment. Fourth, while randomization was performed by cage, we recognize the need for more explicit blinding details during analysis to fully address potential bias. Fifth, comprehensive pharmacokinetics and safety profiling of 3-HQT remains preliminary, warranting further toxicological evaluation. Finally, doxorubicin's systemic effects, including weight loss and reduced feed intake, may confound DOX-induced specific renal outcomes, though control groups help mitigate this. Future studies addressing these gaps will better bridge preclinical findings to occupational health applications.

CONCLUSION

DOX, widely used as an anthracycline antibiotic for treating different types of cancer due to its cytotoxic properties, also generates a wide array of side effects, including renal inflammation and renal toxicity. The present study revealed that 3-HQT has nephroprotective activity, as it decreased the doxorubicin-induced renal inflammation and renal toxicity in a rat model system. 3-HQT has potential to serve as an antioxidant by suppressing the overproduction of reactive oxygen species generation, and it also conquers the doxorubicin-induced upsurge in cytokine and pro-inflammatory markers levels. 3-HQT ameliorated renal injury and restored kidney architecture and inflammation induced by doxorubicin. Further research is needed to evaluate other possible mechanisms behind the nephroprotective effects of 3-HQT and to examine the safety of DOX.

Ethical Approval Statement:
This research was reviewed and approved by the institutional Ethics Committee of Umm Al-Qura University, in accordance with the ARRIVE guidelines for research, and the experimental protocols were strictly followed (approval number: HAP0-02-K-012-2025-10-2956).

Data Availability Statement:
All data supporting the conclusion of this study have been included in the manuscript and the supplemental file.

Declaration of Generative AI in Scientific Writing:
During the peroration of this manuscript, the authors used Grammarly to improve language and readability. After its use, the authors thoroughly reviewed, verified, and revised all AI-assisted content to ensure accuracy and originality. The authors take full responsibility for the integrity and final content of the published article.

Declaration of Interest:
The authors declare that they have no conflicts of interest.

References:

1. Volpon LC, Sugo EK, Consulin JC, Tavares TL, Aragon DC, Carlotti AP. Epidemiology and outcome of acute kidney injury according to pediatric risk, injury, failure, loss, end-stage renal disease and KDIGO criteria in critically ill children: a prospective study. Pediatr Crit Care Med 2016;17:e229-38. (PMID: 26890198)

2. Sato Y, Yanagita M. Functional heterogeneity of resident fibroblasts in the kidney. Proc Jpn Acad Ser B Phys Biol Sci 2019;95: 468-78. (PMID: 31611502)

3. Kelly DM, Ademi Z, Doehner W, et al. Chronic kidney disease and cerebrovascular disease: consensus and guidance from a KDIGO controversies conference. Stroke 2021;52:e328-46. (PMID: 34078109)

4. Thomas MC, Cooper ME, Zimmet P. Changing epidemiology of type 2 diabetes mellitus and associated chronic kidney disease. Nat Rev Nephrol 2016;12:73-81. (PMID: 26553517)

5. Hu B, Ma K, Wang W, et al. Research progress of pyroptosis in renal diseases. Curr Med Chem 2024;31:6656-71. (PMID: 37861024)

6. Wei W, Zhao Y, Zhang Y, Jin H, Shou S. The role of IL-10 in kidney disease. Int Immunopharmacol 2022;108:108917. (PMID: 35729842)

7. Chen WY, Li LC, Yang JL. Emerging roles of IL-33/ST2 axis in renal diseases. Int J Mol Sci 2017;18(4):783. (PMID: 28387719)

8. Ostermann M, Cennamo A, Meersch M, Kunst G. A narrative review of the impact of surgery and anaesthesia on acute kidney injury. Anaesthesia 2020;75(Suppl 1):e121-33. (PMID: 31903567)

9. Renu K, Pureti LP, Vellingiri B, Valsala Gopalakrishnan A. Toxic effects and molecular mechanism of doxorubicin on different organs-an update. Toxicology Reports 2022;41:650-74. https://www.tandfonline.com/doi/full/10.1080/15569543.2021.1912099?utm_source=researchgate.net&utm_medium=article

10. Aziz AUR, Geng C, Li W, et al. Doxorubicin induces ER calcium release via Src in rat ovarian follicles. Toxicol Sci 2019;168:171-8. (PMID: 30476324)

11. van der Zanden SY, Qiao X, Neefjes J. New insights into the activities and toxicities of the old anticancer drug doxorubicin. FEBS J 2021;288:6095-111. (PMID: 33022843)

12. Altinoz E, Cetinavci D, Abdulkareem Aljumaily SA, Elbe H, Cengil O, Bicer Y. Crocin restores doxorubicin-induced renal dysfunction, oxidative stress, inflammation, and TGF-β signaling pathways. Nat Prod Res 2025;39:4609-22. (PMID: 38662441)

13. Abd-Ellatif RN, Nasef NA, El-Horany HE, et al. Adrenomedullin mitigates doxorubicin-induced nephrotoxicity in rats: role of oxidative stress, inflammation, apoptosis, and pyroptosis. Int J Mol Sci 2022;23:14570. (PMID: 36498902)

14. Su Z, Ye J, Qin Z, Ding X. Protective effects of madecassoside against doxorubicin-induced nephrotoxicity *in vivo* and *in vitro*. Sci Rep 2015;5:18314. (PMID: 26658818)

15. Ma Z, Jin X, He L, Wang Y. CXCL16 regulates renal injury and fibrosis in experimental renal artery stenosis. Am J Physiol Heart Circ Physiol 2016;311:H815-21. (PMID: 27496882)

16. Thakur S, Kumar D, Jaiswal S, et al. Medicinal chemistry-based perspectives on thiophene derivatives: exploring structural insights to discover plausible druggable leads. RSC Med Chem 2025;16:481-510. (PMID: 39601022)

17. Bhat M, Lagamappa Belagali S. Structure–activity relationship of benzothiazole derivatives in medicinal chemistry: A Comprehensive Review. Mini Rev Org Chem 2020;17:323-50. https://www.benthamdirect.com/content/journals/mroc/10.2174/1570193X16666190204111502

18. Dorababu A. Recent update on antibacterial and antifungal activity of quinoline scaffolds. Arch Pharm (Weinheim) 2021;354: e2000232. (PMID: 33210348)

19. Anjali, Kamboj P, Amir M. Synthetic methods of quinoxaline derivatives and their anti-inflammatory properties. Mini Rev Med Chem 2025;25:138-62. (PMID: 38910487)

20. Farghaly TA, Alqurashi RM, Masaret GS, Abdulwahab HG. Recent methods for synthesis of quinoxaline derivatives and biological activities. Mini Rev Med Chem 2024;24:920-82. (PMID: 37885112)

21. Hussam D, Yousef A, Taghreed S, et al. Possible Gastroprotective Impact of 3-hydrazinoquinoxaline-2-thiol on Gastric Ulcers in Rats. Bahrain Med Bull 2025;47:2413. https://openurl.ebsco.com/EPDB%3Agcd%3A8%3A28962584/detailv2?sid=ebsco%3Aplink%3Ascholar&id=ebsco%3Agcd%3A189176778&crl=c&link_origin=www.google.com

22. Atia HA, Elariny HA, Abdallah MH, et al. Repositioning perindopril for mitigation of methotrexate-induced hepatotoxicity in rats. Pharmaceuticals 2025;18:358. (PMID: 40143135)

23. Shahid Nadeem M, Khan JA, Al-Abbasi FA, et al. Protective effect of hirsutidin against rotenone-induced parkinsonism via Inhibition of Caspase-3/Interleukins-6 and 1β. ACS Omega 2023;8: 13016-25. (PMID: 37065035)

24. El-Moselhy MA, El-Sheikh AAK. Protective mechanisms of atorvastatin against doxorubicin-induced hepato-renal toxicity. Biomed Pharmacother 2014;68:101-10. (PMID: 24268999)

25. Khadrawy YA, Salem AM, El-Shamy KA, et al. Neuroprotective effect of caffeine in rotenone-induced Parkinson's disease induced by Rotenone. J Diet Suppl 2017;14:553-72. (PMID: 28301304)

26. Qu L, Jiao B. Interplay between immune and metabolic pathways in kidney disease. Cells 2023;12:1584. (PMID: 37371054)

27. El-Sayed EM, Mansour AM, El-Sawy WS. Alpha-lipoic acid prevents doxorubicin-induced nephrotoxicity in rats. J Biochem Mol Toxicol 2017;31:e21965. (PMID: 28598563)

28. Ayla S, Seckin I, Tanriverdi G, et al. Doxorubicin-induced nephrotoxicity: protective effect of nicotinamide. Int J Cell Biol 2011; 2011:390238. (PMID: 21789041)

29. Arozal W, Eziefule OM, Wanandi SI, et al. Protective role of Andrographis paniculata against doxorubicin-induced nephrotoxicity. Front Pharmacol 2025;16:1585965. (PMID: 40894204)

30. Griffin BR, Faubel S, Edelstein CL. Biomarkers of drug-induced kidney toxicity. Ther Drug Monit 2019;41(2):213-26. (PMID: 30883514)

31. Zhao X, Wang J, Tang L, et al. Withaferin A protects against hyperuricemia-induced kidney injury and its possible mechanisms. Bioengineered 2021;12:589-600. (PMID: 33517833)

32. Braga TT, Foresto-Neto O, Camara NOS. The role of uric acid in inflammasome-mediated kidney injury. Curr Opin Nephrol Hypertens 2020;29:423-31. (PMID: 32452918)

33. Cheng G, Sa W, Cao C, et al. Quinoxaline 1,4-di-N-oxides: biological activities and mechanisms of actions. Front Pharmacol 2016;7:64. (PMID: 27047380)

34. Cantero-Navarro E, Rayego-Mateos S, Orejudo M, et al. Role of macrophages and cytokines in kidney disease. Front Med (Lausanne) 2021;8:688060. (PMID: 34307414)

35. Atta AH, Atta SA, Khattab MS, et al. Ceratonia siliqua extract alleviates doxorubicin-induced nephrotoxicity via antioxidant, anti-inflammatory and anti-apoptotic pathways in rats. Environ Sci Pollut Res 2023;30:83421-38. (PMID: 37341944)

36. Hussain MA, Abogresha NM, AbdelKader G, Hassan R, Abdelaziz EZ, Greish SM. Antioxidant and Anti-Inflammatory Effects of Crocin Ameliorate Doxorubicin-Induced Nephrotoxicity in Rats. Oxid Med Cell Longev 2021:8841726. (PMID: 33628387)

37. Molehin OR, Adeyanju AA, Adefegha SA, Oyeyemi AO, Idowu KA. Protective mechanisms of protocatechuic acid against doxorubicin-induced nephrotoxicity in rat model. J Basic Clin Physiol Pharmacol 2019;30(4). (PMID: 31280244)

38. Bhat MY, Solanki HS, Advani J, et al. Comprehensive network map of interferon gamma signaling. J Cell Commun Signal 2018; 12:745-51. (PMID: 30191398)

39. Kim YS, Sung SH, Tang Y, et al. Protective Effect of Taurine on Mice with Doxorubicin-induced Acute Kidney Injury. Adv Exp Med Biol 2017:975 Pt 2:1191-201. (PMID: 28849533)

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