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Protective Effects of 2-Chloro-3-Hydrazinylquinoxaline against Doxorubicin-induced Nephrotoxicity *via* Modulation of Oxidative Stress and Inflammation in Rats

Noha A. Juma[1], Wafaa Alhazmi[2], Noura I. Daffa[1], Bandar H. Saleh[1], Noof R. Helmi[1], Ala A. Azhari[1,3], Hatoon A. Niyazi[1], Mona A. Alqarni[1], Khalil Alkuwaity[2,4], Ahmad M. Alzahrani[5], Mohannad A. Alkasih[6], Turki M. Alharthi[7], Riyadh S. Almalki[8], Ohood Alharbi[9], Abdelbagi Alfadil[1]

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ABSTRACT

Background: Doxorubicin (DOX) is a widely used anthracycline antibiotic effective against multiple human malignancies, but its clinical utility is limited by well-established nephrotoxicity that frequently manifests as acute kidney injury (AKI). In contrast, the potential nephroprotective effects of 2-chloro-3-hydrazinylquinoxaline (CHQ), a quinoxaline derivative with reported antioxidant and anti-inflammatory properties, remain untested. The present study therefore evaluates whether CHQ can mitigate DOX-induced AKI.

Methods: Thirty acclimatized male Wistar rats were randomized distributed into 5 groups: control group; DOX group which received single intraperitoneal (i.p.) injection of DOX (15 mg/kg single) on day 6, DOX + CHQ-30 treated with CHQ (30 mg/kg/day orally) for 7 days and DOX treatment on day 6; DOX + CHQ-60 group receiving the same regime but with CHQ at 60 mg/kg/day orally and CHQ-60 only received CHQ only at 60 mg/kg/day for 7 days. Key parameters assessed included kidney function biomarkers, oxidative stress markers, anti-and pro-inflammatory mediators (IL-1β, IL-6, TNF-α, NF-κB, IFN-γ and COX-2) and additional markers (iNOS, PGE_2 and GAPDH).

Result: The CHQ treatment significantly ameliorated DOX-induced alterations in biochemical parameters and oxidative stress markers, normalized anti-and pro-inflammatory mediator levels and reduced apoptosis-related markers. Histopathological analysis also supports the renoprotective effect. These findings demonstrate that CHQs possess significant antioxidant and anti-inflammatory properties, suggesting their therapeutic potential to attenuate DOX-induced nephrotoxicity.

Key words: Antioxidant, Doxorubicin, Oxidative stress, Quinoxaline.

INTRODUCTION

Doxorubicin (DOX) is an extensively used first-line chemotherapeutic agent for treating various cancers, including breast and metastatic breast cancers. (Lusha and Mei 2021, Bisht *et al*., 2025). DOX-induced nephrotoxicity is a significant adverse effect that limits the clinical utility of this potent chemotherapeutic agent. The nephrotoxic effects of DOX are characterized by acute kidney injury (AKI) and biochemical and histopathological evidence of kidney damage. This toxicity is largely mediated by oxidative stress, inflammation, apoptosis and mitochondrial dysfunction in kidney tissues (Abd-Ellatif *et al*., 2022, Chen *et al*., 2025).

Among quinoxaline analogues, the 3-hydrazinyl substitution pattern has been repeatedly associated with enhanced radical scavenging and metal-chelating potential, while the 2-chloro substituent improves lipophilicity and membrane permeability properties that favor accumulation in renal tissue. Preliminary *in vitro* screening of a small quinoxaline library identified CHQ as exhibiting superior radical scavenging activity and greater suppression of LPS-induced tumor necrotic factor-alpha (TNF-α) and interleukin (IL)-6 release in macrophages relative to other tested analogues, without overt cytotoxicity at pharmacologically relevant concentrations. These structure- activity observations, together with the known nephroprotective mechanisms required to counter DOX toxicity (antioxidant, anti-inflammatory

[1]Department of Clinical Microbiology and Immunology, Faculty of Medicine, King Abdulaziz University, Jeddah 21589, Saudi Arabia. [2]Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah 21589, Saudi Arabia. [3]Vaccines and Immunotherapy Unit, King Fahd Medical Research Center, P.O. Box 80216, King Abdulaziz University, Jeddah, Saudi Arabia. [4]EcoHealth Research Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. [5]Department of Clinical Biochemistry, Faculty of Medicine, King Abdulaziz University, Jeddah 21589, Saudi Arabia. [6]Department of Clinical Anatomy, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia. [7]Department of Clinical Laboratory Sciences, Faculty of Applied Medical Sciences, Umm Al-Qura University, Makkah, Saudi Arabia. [8]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Umm Al-Qura University, Makkah, Saudi Arabia. [9]Department of Microbiology and Parasitology, Faculty of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia.

Corresponding Author: Wafaa Alhazmi, Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah 21589, Saudi Arabia. Email: walhazmi@kau.edu.sa

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and anti-apoptotic effects), provide a strong mechanistic rationale for prioritizing CHQ as the lead candidate for *in vivo* evaluation against DOX-induced nephrotoxicity (Irfan *et al*., 2021, Khan *et al*., 2021).

Current research endeavors are concentrated on the exploration of the anti-nephrotoxic properties of novel quinoxaline derivatives, 2-Chloro-3-hydrazinylquinoxaline ($C_8H_7ClN_4$; MW 194.62; CHQ) and the identification of their potential applications to mitigate the DOX-induced nephrotoxic effects. The protective effects of CHQ were evaluated through the assessment of kidney function markers (urea, uric acid and creatinine), oxidative stress indicators superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH), lipid peroxidation malondialdehyde (MDA), inflammatory cytokines IL-6, IL-1β, TNF-α and interferon-gamma (IFN-γ), inflammatory mediators cyclooxygenase-2 (COX-2) and prostaglandin E_2 (PGE_2) and other markers induced nitric oxide (iNOS) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Furthermore, the histopathological examination of kidney tissue substantiates the protective effect of CHQ treatment against DOX-induced kidney inflammation. The doses of 30 and 60 mg/kg/day CHQ were selected based on dose ranges reported for related quinoxaline derivatives in rodent models of oxidative stress and inflammation and alignment with doses used for other nephroprotective agents in DOX-induced AKI models (*e.g*., curcumin, crocin, alpha-lipoic acid) that have demonstrated efficacy at 20-100 mg/kg/day in similar protocols (Hussain *et al*., 2021; Abd-Ellatif *et al*., 2022).

MATERIALS AND METHODS

Reagents

All chemical details are mentioned in the supplementary material.

Experimental

This study used an *in vivo* animal model of male wistar rats. Details of the randomization procedure and housing/cage structure were provided in the supplementary material. This study received approval from the Institutional Biomedical Ethics Committee, Umm Al-Qura University, Saudi Arabia and adhered strictly to the ARRIVE guidelines (approval number: HAPO-02-K-012-2025-10-2956).

Following the acclimatization period, 30 healthy male Wistar rats were randomly separated into five experimental groups, each comprising six rats (n=6).

1. **Gp I (Control):** In the whole experiment, rats were not treated with any drug. Only vehicle DMSO is provided orally through gavage.
2. **Gp II (DOX only):** Single dose of DOX (15 mg/kg; i.p.) on the 6th day after the start of the test (Abd-Ellatif *et al*., 2022).
3. **Gp III (DOX + CHQ-30):** Rats will receive a 30 mg/kg/day dose of CHQ by oral administration once a day for seven consecutive days and DOX (15 mg/kg, i.p.) in a single dose on the 6th day.
4. **Gp IV (DOX + CHQ-60):** Rats will receive 60 mg/kg/day CHQ by oral administration once a day for seven consecutive days and DOX (15 mg/kg, i.p.) in a single dose on the 6th day.
5. **Gp V (CHQ-only):** Rats will receive 60 mg/kg/day CHQ by oral administration once a day for seven consecutive days.

The detailed dosing procedure is provided in the supplementary material.

Collection of blood samples and renal tissues

The detailed procedure to obtain clear supernatant from kidney tissue and blood serum is explained in the supplementary material.

Assess the effect of CHQ application on renal function parameters

To evaluate nephrotoxicity induced by DOX in all groups of rats, renal function markers, including serum urea, uric acid and creatinine levels, were measured from the serum separated from circulatory blood with a commercially available kit.

Assess the effect of CHQ application on oxidative stress markers

Lipid peroxidation was estimated as MDA concentration using the spectrophotometric method described by Atia *et al*. (2025). The activity of SOD in the supernatant of kidney tissue homogenate was quantified using the nitroblue tetrazolium (NBT) (Shahid *et al*., 2023) and the results were presented in U/mg of protein. Catalase (CAT) activity was assessed in the supernatant of kidney tissue homogenate (Bhangale and Acharya, 2016) and the results were presented in U/mg of protein. The concentration of reduced GSH was quantified in kidney tissue homogenate (Khadrawy *et al*., 2017). The detailed method is explained in the supplementary material.

Assess the effect of CHQ application on the renal inflammatory cytokines and other inflammatory markers

A clear supernatant derived from homogenized rat kidney samples was used quantify IL-1β and IL-6, TNF-α and NF-κB using a commercially available ELISA kit as manufacturer's guidelines. The concentrations of these markers were reported in pg/mL total protein.

Assess the effect of CHQ treatment on iNOS and GAPDH

To assess iNOS levels, GAPDH was measured in the supernatant of kidney tissue homogenate using commercially available ELISA kits and results expressed in pg/mL or arbitrary units.

Assess the effect of CHQ treatment on inflammatory mediator (PGE_2), pro-inflammatory (IFN-γ) and inflammatory response marker (COX-2)

Commercially available ELISA kits were used to assess the levels of the inflammatory mediators (PGE_2 and COX-2)

and the pro-inflammatory cytokine (IFN-γ) in kidney tissue. These kits used a sandwich ELISA antibody-capture method to precisely measure marker levels and results are expressed in pg/mL.

Renal histopathology

To evaluate DOX-induced renal structural and inflammatory injury and the effects of CHQ, kidney tissues were collected after euthanasia, fixed in 10% neutral buffered formalin, routinely processed, embedded in paraffin and sectioned at approximately 5 µm thickness. We stained the sections with hematoxylin and eosin (HandE) and examined them under a light microscope. The Supplementary Material details the parameters and scoring criteria for renal histology. A blinded, experienced pathologist performed the histopathological evaluation and representative photomicrographs were obtained at the stated magnification with scale bars.

Statistical analysis

All results were analyzed using GraphPad Prism (Version 8.0, USA). Before ANOVA, data normality was checked using the Shapiro-Wilk test. Subsequently, for the homogeneity of variance check, Levene's test was performed. The results are presented as mean ± SEM, with six animals per group (n = 6). When the data met the criteria for normality and equal variance, group evaluations were measured by one-way ANOVA, followed by Tukey's post hoc test for multiple comparisons. A Kruskal-Wallis test was used to assess the histological injury scores. The statistical significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Effect of CHQ application on renal function parameters

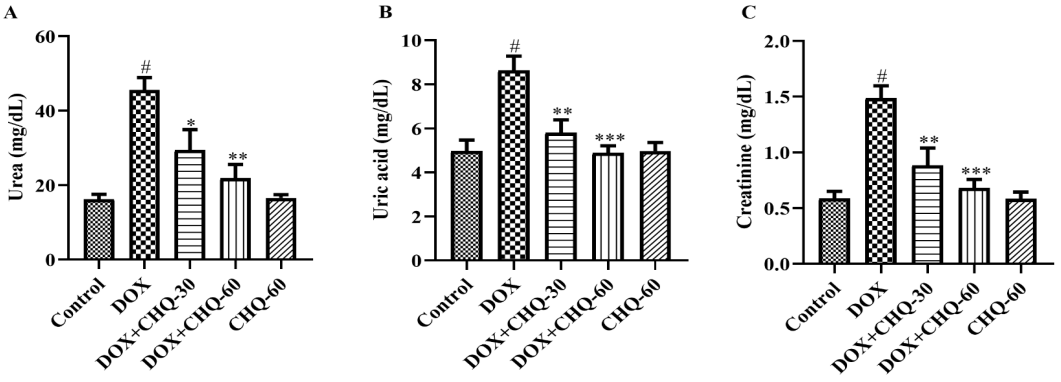
In this study, the levels of urea, uric acid and creatinine were assessed as indicators of renal function. The results demonstrated a significant elevation in these renal function parameters in the DOX-treated rats, with serum urea ($p<0.0001$, Fig 1A), uric acid ($p<0.0002$, Fig 1B) and creatinine ($p<0.0001$, Fig 1C) levels all increased in respect to the control group. In rats, a low dose of CHQ (30 mg/kg/day) was administered, resulting in a notable reduction in elevated levels of urea ($p<0.0196$), uric acid ($p<0.0046$) and creatinine ($p<0.0022$). Furthermore, in Group IV rats, which received a high dose (60 mg/kg/day) of CHQ, the levels of urea ($p<0.0004$), uric acid ($p<0.0002$) and creatinine ($p<0.0001$) were higher than those of the group III rats. Group V rats (CHQ-60 only) showed no significant differences compared with group I (control) rats.

Effect of CHQ treatment on the antioxidant marker levels

Compared with control rats, DOX-treated group II rats showed a significant elevation in MDA levels ($p<0.0001$), while SOD ($p<0.0001$), CAT ($p<0.0007$) and GSH activities ($p<0.0052$) were diminished ($p<0.001$). CHQ treatment effectively restored essential antioxidant levels in all treated rats. Compared with the DOX-control group, the low-dose (30 mg/kg/day) CHQ administration significantly reduced MDA levels ($p<0.0253$) and enhanced the levels of renal SOD ($p<0.0386$), CAT ($p<0.0167$) and GSH ($p<0.0151$) (Fig 2A-D). In contrast to group IV rats, those receiving a high dose (60 mg/kg/day) of CHQ exhibited a noteworthy improvement in the levels of SOD ($p<0.0019$), CAT ($p<0.0014$) and GSH ($p<0.0030$), while MDA activity ($p<0.0004$) showed a high reduction as related to group III rats. Rats that received CHQ-60 only treatment (group V) did not show any significant difference in any of the oxidative stress parameters compared to the control rats (group I).

Effect of CHQ application on pro-inflammatory cytokines and inflammatory markers in the kidney

In the renal tissues of DOX-treated rats, the levels of pro-inflammatory cytokines IL-6 ($p<0.0001$) and IL-1β



(A) Urea, (B) Uric acid and (C) Creatinine levels. Data are presented as mean±S.E.M. (*n* = 6). Treatment groups: Control, DOX- Doxorubicin, DOX+CHQ-30-DOX with 30 mg/kg/day CHQ, DOX+CHQ-60-DOX with 60 mg/kg/day CHQ. A 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 CHQ treated groups vs DOX.

Fig 1 (A-C): Protective effects of CHQ treatment on renal function parameters in DOX-administered rats.

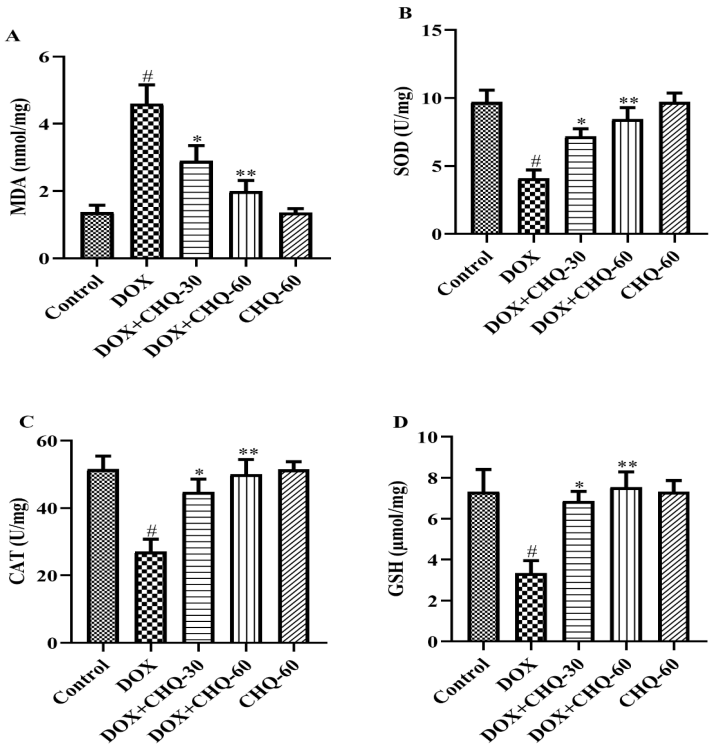
(p<0.0001) and inflammatory markers TNF-α (p<0.0001) and NF-κB (p<0.0001) were significantly elevated compared with the control group (p<0.001 for all; Fig 3A-D). Administration of CHQ at 30 mg/kg/day (DOX + CHQ-30) significantly reduced these elevations relative to the DOX-only group for IL-6 (p=0.0009), IL-1β (p=0.0026), TNF-α (p=0.0016) and NF-κB (p=0.0057). CHQ at 60 mg/kg/day (DOX + CHQ-60) also significantly reduced IL-6 (p<0.0001), IL-1β (p<0.0001), TNF-α (p<0.0001) and NF-κB (p=0.0018) levels compared with the DOX-only group (p<0.001 for all). However, no significant difference was observed in the levels of pro-inflammatory cytokines and inflammatory markers between groups I and V.

Effect of CHQ application on iNOS and GAPDH levels

In rats administered DOX, the activity of the renal inflammatory response marker iNOS was significantly increased (p<0.0001), whereas GAPDH (p<0.0225) levels were markedly decreased (p<0.001) compared to control rats. In group IV, which received a high dose (60 mg/kg bw) of CHQ, there was a noteworthy drop in elevated iNOS levels (p<0.0003) and a recovery in GAPDH levels (p<0.0036) relative to the DOX-treated group (Fig 4 A-B). Rats in group III, which were administered a lower dose of CHQ (30 mg/kg/day), demonstrated less improvement in iNOS (p<0.0037) and GAPDH (p<0.0271) levels than those in group IV, which received a higher dose (60 mg/kg/day). No significant difference was found between the control (group I) and CHQ-60 alone (group V) for iNOS and GAPDH levels.

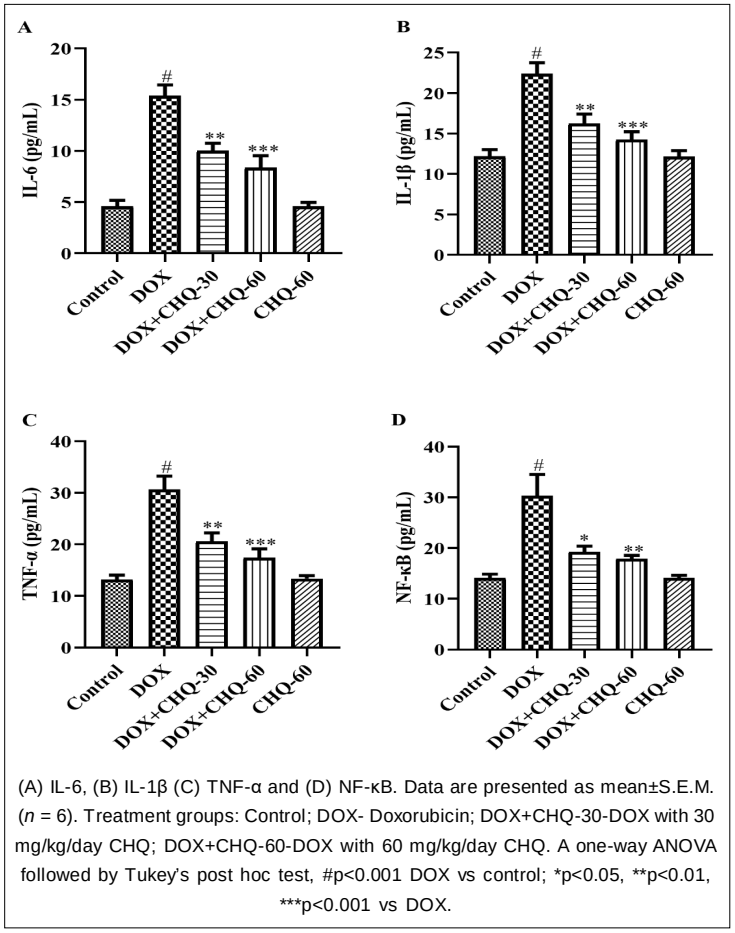
Effect of CHQ application on inflammatory mediator (PGE_2), pro-inflammatory (IFN-γ) and inflammatory response marker (COX-2)

In rats treated with DOX, there was a noteworthy elevation in the levels of the pro-inflammatory cytokine IFN-γ (p<0.0001), inflammatory mediator COX-2 (p<0.0001) and PGE_2 (p<0.0001) related to the control group (Fig 5 A-C). Administration of CHQ at 30 mg/kg/day showed a notable reduction in the level of the pro-inflammatory cytokine IFN-γ (p<0.0023), inflammatory mediator COX-2 (p<0.0029) and PGE_2 (p<0.0168). Conversely, rats receiving a high quantity of CHQ (60 mg/kg/day) exhibited a significantly higher decrease in the level of IFN-γ (p<0.0001), COX-2 (p<0.0001) and PGE_2 (p=0.0009) as compared to those treated with a lower dose (30 mg/kg bw) of CHQ. No significant difference was observed in the expression levels of PEG_2,



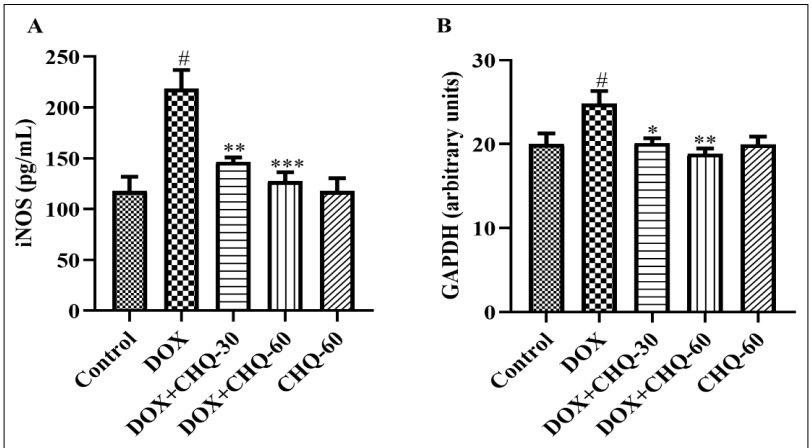
(A) MDA, (B) SOD, (C) CAT and (D) GSH. Data are presented as mean±S.E.M. (*n* = 6). Treatment groups: Control; DOX- Doxorubicin; DOX+CHQ-30-DOX with 30 mg/kg/day CHQ; DOX+CHQ-60-DOX with 60 mg/kg/day CHQ. A one-way ANOVA followed by Tukey's post hoc test, #p<0.001 DOX vs control; *p<0.05, **p<0.01 vs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

Fig 2 (A-D): Protective effect of CHQ treatment on the level of renal-stress markers in DOX-Administered rats.



(A) IL-6, (B) IL-1β (C) TNF-α and (D) NF-κB. Data are presented as mean±S.E.M. (*n* = 6). Treatment groups: Control; DOX- Doxorubicin; DOX+CHQ-30-DOX with 30 mg/kg/day CHQ; DOX+CHQ-60-DOX with 60 mg/kg/day CHQ. A 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.

Fig 3(A-D): Protective effect of CHQ treatment on the level of inflammatory cytokines and pro-inflammatory mediators in DOX-administered rats.



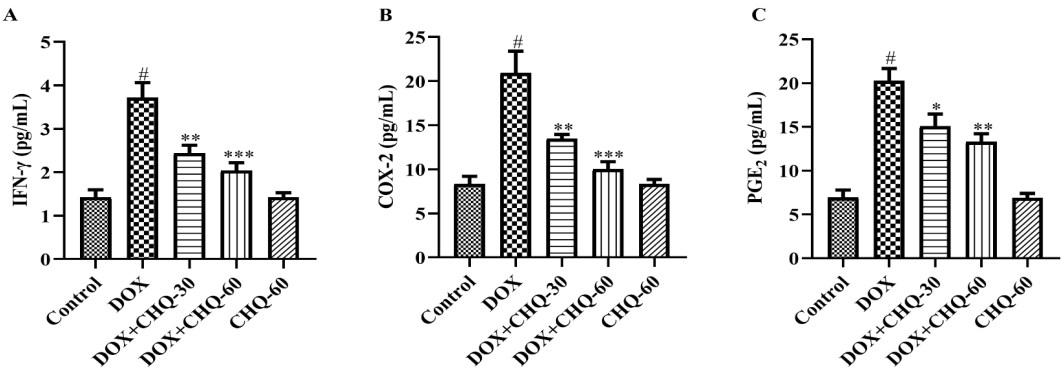
(A) iNOS and (B) GAPDH. Data are presented as mean±S.E.M. (*n* = 6). Treatment groups: Control; DOX- Doxorubicin; DOX+CHQ-30-DOX with 30 mg/kg/day CHQ; DOX+CHQ-60-DOX with 60 mg/kg/day CHQ. A one-way ANOVA followed by Tukey's post hoc test, #p<0.001 DOX vs control; *p<0.05, **p<0.01, ***p<0.001 vs DOX.

Fig 4(A-B): Protective effect of CHQ treatment on nitric oxide-producing enzyme and housekeeping marker (GAPDH) in DOX-administered rats.

IFN-γ and COX-2 between the control and CHQ-60-only group rats.

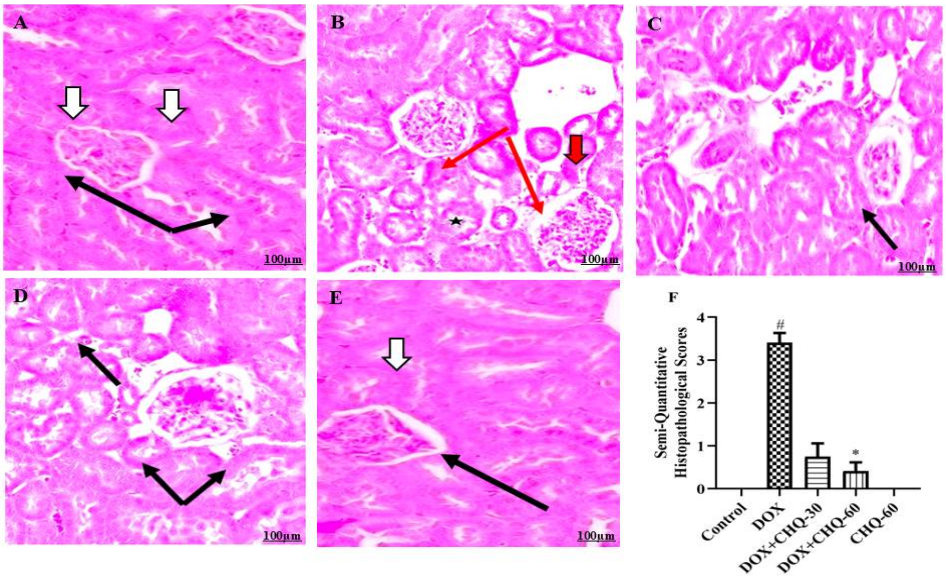
Effect of CHQ application on renal histology in DOX-induced nephrotoxicity

Fig 6(A-D) illustrates renal histopathological changes after DOX administration and the effects of CHQ treatment. Control kidneys showed preserved glomerular and tubular architecture with minimal inflammatory cell infiltration. In contrast, DOX-treated rats exhibited renal lesions, including glomerular architectural distortion, tubular dilation, epithelial degeneration or atrophy, intratubular epithelial and hyaline casts, vascular congestion and interstitial inflammatory cell infiltration, indicating tissue



(A) IFN-γ, (B) COX-2 and (C) PGE_2. Data are presented as mean±S.E.M. (n = 6). Treatment groups: Control; DOX-Doxorubicin; DOX+CHQ-30-DOX with 30 mg/kg/day CHQ; DOX+CHQ-60-DOX with 60 mg/kg/day CHQ. A 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.

Fig 5(A-C): Protective effect of CHQ treatment on inflammatory mediators in DOX-administered rats.



Representative HandE-stained kidney sections from (A) control, (B) DOX, (C) DOX+CHQ low dose and (D) DOX+ CHQ high dose groups. DOX-treated kidneys showed glomerular architectural distortion, tubular dilation, tubular epithelial degeneration/atrophy, intratubular casts, vascular congestion and interstitial inflammatory cell infiltration. CHQ treatment reduced these morphological abnormalities. (E) Quantitative renal injury scores based on animal-level histopathological assessment. Photomicrographs in panels A-D were obtained at magnification 200×, with a scale bar of 100 µm in each panel. Magnification and scale-bar information are provided for each panel. Bar graphs presented show mean±S.E.M. A one-way ANOVA followed by Kruskal-Wallis test, #p<0.001 DOX vs control; *p<0.05 vs DOX.

Fig 6(A-E): Effect of CHQ on renal histopathology in DOX-induced nephrotoxicity.

injury. Inflammatory cells were not further classified on HandE sections because identification was not sufficiently specific without confirmatory immunohistochemical or ancillary staining. CHQ treatment at both doses reduced the severity and extent of these abnormalities. CHQ-treated animals showed preserved glomerular and tubular architecture, reduced tubular dilation and epithelial alterations and less interstitial inflammatory cell infiltration than the DOX-treated group. Quantitative histopathological assessment showed a significant increase in renal injury score after DOX administration versus control ($p<0.0001$; Fig 6E). CHQ at both doses significantly reduced this score, consistent with improved renal function, oxidative stress and inflammatory parameters, supporting its nephroprotective effect.

Nephrotoxicity associated with DOX treatment may cause chronic kidney disease through fibrosis and renal remodeling after acute injury and a decline in renal function, further impairing renal filtration capacity (Altinoz *et al*., 2025). A model study showed that DOX induces nephrotoxicity, affecting kidney function, including the production and clearance of waste products such as urea, uric acid and creatinine, with reduced creatinine clearance indicating diminished glomerular filtration rate and tubular injury (Abdelrahman *et al*., 2020, Zhihua *et al*., 2025). In this study, DOX-induced nephrotoxicity in rats altered these biomarkers, indicating impaired renal function, hyperuricemia and further injury. Hence, the present study aimed to assess the protective effects of CHQ against DOX-induced renal inflammation.

In DOX-induced nephrotoxicity, DOX exposure causes ROS generation in renal tissues, overwhelming antioxidant defenses and cause oxidative damage, inflammation and apoptosis. Specifically, DOX decreases antioxidant enzyme activities such as SOD and CAT and reduces GSH content in the kidney, molecules that neutralize ROS (El-Sayed *et al*., 2017, Altinoz *et al*., 2025). Concurrently, DOX increases oxidative stress markers such as MDA, a lipid peroxidation product that reflect membrane damage, indicating lipid peroxidation in renal cells (Altinoz *et al*., 2025). Interventions that boost antioxidant defenses can protect against DOX-induced nephrotoxicity. The restoration of SOD, CAT and GSH activities, with reduced MDA levels, suggests that CHQ enhances antioxidant defenses in the kidney. These effects align with reports on quinoxaline derivatives that exhibited radical-scavenging capacity and upregulate antioxidant enzymes in injury models. Oxidative injury disrupts kidney function and structure, as evidenced by increased urea, uric acid and creatinine levels and histopathological abnormalities (Wilhelm *et al*., 2017; Hasan, *et al*., 2024). Although quinoxaline derivatives have associated cytoprotective mechanisms including mitochondrial preservation, NADPH oxidase inhibition and metal chelation, the present study did not establish these pathways for CHQ, instead focusing on its antioxidant and anti-inflammatory effects in DOX-induced AKI. Experimental evidence suggests that quinoxaline-based molecules mitigate ROS-induced injury *in vitro* and in animal models, making them potential candidates for treating diseases such as neurodegeneration, cardiovascular conditions and kidney injury (Nithyatharani *et al*., 2024; Singh and Choudhary 2025). Some quinoxaline derivatives chelate metals (*e.g*., iron and copper), inhibiting Fenton reactions that generate hydroxyl radicals from hydrogen peroxide and lipid peroxidation, thereby maintaining membrane integrity and cellular homeostasis under oxidative stress (Abe *et al*., 2022). Antioxidant strategies that restore redox balance and suppress inflammation may protect against DOX-induced kidney damage (Hasan *et al*., 2024; Al-Zharani *et al*., 2025).

Inflammation is intertwined with DOX-induced oxidative stress and involves the upregulation of pro-inflammatory cytokines and inflammatory markers. Specifically, DOX administration elevates IL-6 and IL-1β, key cytokines in the renal inflammatory response and increases TNF-α and NF-κB levels, which contribute to renal inflammation and tissue damage. In the current study, DOX treatment increased renal pro-inflammatory cytokines and inflammatory markers, similar to a previous study (Abd-Ellatif *et al*., 2022, Altinoz *et al*., 2025). CHQ treatment reduced these pro-inflammatory cytokines and renal inflammation markers. DOX also activates the NF-κB and mitogen-activated protein kinase signaling pathways, promoting inflammation and apoptosis in renal tissues. This activation is correlated with oxidative stress, lipid peroxidation and structural kidney damage (Arunachalam *et al*., 2022; Ibtesam, 2026). Histological analysis in a previous study confirmed renal structural injury after DOX treatment (Abd-Ellatif *et al*., 2022).

Furthermore, DOX upregulates the pro-inflammatory cytokine IFN-γ, responsible for inflammation, immune regulation and tumor suppression *in vivo*. This aligns with DOX's promotion of apoptosis by DOX through oxidative and inflammatory kidney signals (Saleh *et al*., 2021, Abd-Ellatif *et al*., 2022). Regarding anti-inflammatory response markers, evidence indicates that DOX treatment alters PGE_2 levels, which is an inflammatory lipid mediator with context-dependent pro- and anti-inflammatory actions. However, in DOX-induced inflammation, PGE_2 levels are generally increased alongside iNOS and COX-2 as part of the inflammatory cascade (Mansouri *et al*., 2015). GAPDH, a "housekeeping" gene sensitive to DOX-induced ROS, plummets, disrupting glycolytic flux, redirecting metabolism toward the pentose phosphate pathway for NADPH production and impairing apoptosis, autophagy and immunomodulation (Butera *et al*., 2019). CHQ treatment attenuated oxidative stress and inflammation markers, suggesting renoprotective effects in this rat model of diabetic nephropathy. The mechanisms by which CHQ modulates autophagy, NF-κB activation, apoptosis and immunomodulatory pathways should be investigated.

DOX-induced nephrotoxicity may primarily involve oxidative stress, characterized by increased ROS production, depletion of antioxidants (SOD, CAT, GSH) and lipid peroxidation. The resultant oxidative damage initiates inflammation and apoptotic pathways, culminating in renal tissue injury and impaired filtration rate and function. DOX treatment also increased the levels of pro-inflammatory cytokines IL-6 and IL-1β, inflammatory enzymes iNOS and COX-2 and PGE_2. This is mediated by the activation of the NF-κB and MAPK pathways. GAPDH inactivation affects apoptosis, autophagy and immunomodulation, whereas IFN-γ may participate in the pro-inflammatory milieu but requires additional stimuli for expression. The study limitations are detailed in the supplementary material.

CONCLUSION

Doxorubicin-induced nephrotoxicity involves oxidative damage and inflammation, causing AKI and impaired function. These mechanisms have guided the search for agents to reduce toxicity and enhance safety. Addressing this challenge needs antioxidants, anti-inflammatory agents and pathway modulation to protect kidneys. CHQ decreases inflammation and oxidative damage *via* antioxidant and immunomodulatory effects, but optimal dosing needs validation.

Ethics approval

This study received approval from the institutional Ethical Committee at the Biomedical Ethics Committee, Umm Al-Qura University, Saudi Arabia (approval number: HAPO-02-K-012-2025-10-2956).

Conflict of interest

On behalf of all authors, the corresponding author declares that there is no conflict of interest.

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