

Protective role of Quinoxaline derivatives (2,3-Dichloro-6-(trifluoromethoxy)) against doxorubicin-induced nephrotoxicity in rats

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ABSTRACT

Drug-induced nephrotoxicity, remains a major global health concern, driven by toxic injury to proximal tubule epithelial cells and by dose-dependent acute tubular necrosis. Doxorubicin (DOX) used as an anticancer antibiotic in human against various cancers and frequently recognized as a causative agent of drug-induced nephrotoxicity, which outcomes in acute renal injury. The current study 2,3 dichloro-6-(trifluoromethoxy) quinoxaline (DTQ), a quinoxaline derivative, aimed to examine the protective effects against DOX-induced nephrotoxicity. About 30 healthy male Wistar rats were randomly distributed into four groups: control group, DOX only group (15 mg/kg single intraperitoneal injection of DOX on 6th day), and DTQ-30/DTQ-60+DOX group (30/60 mg/kg/day orally DTQ 7 consecutive days and DOX on 6th day). Renal injury was assessed based on the parameter such as kidney function parameters, oxidative stress biomarkers, pro-inflammatory cytokines and related mediator levels and renal histology. DTQ treatment enhanced the kidney function biochemical parameters in DOX-induced rats, remarkably downgrade the oxidative damage marker, pro-inflammatory cytokines and mediator levels, and subdue both apoptosis and pyroptosis processes. The histopathological findings established these outcomes, demonstrating that the antioxidant, anti-inflammatory, and anti-apoptotic properties of DTQ could possibly be used to alleviate nephrotoxicity caused by DOX.

Keywords: Antioxidant, Anti-inflammatory, Apoptosis, Doxorubicin, Nephrotoxicity, Quinoxaline.

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INTRODUCTION

Nephrotoxicity, characterized by the rapid decline in kidney function due to the harmful effects of drugs and chemicals, representing a major health concern worldwide. Roughly 13.3% of the global population is affected by acute kidney injury annually, and nephrotoxicity is one of the main contributing factors. Nephrotoxicity can occur through various mechanisms, such as injury to the proximal tubules and acute tubular necrosis, which is frequently dose-dependent. It can also result from tubular blockage began by drug crystals or casts, as well as interstitial nephritis¹. Drug-induced nephrotoxicity accounts for 19–26% of all hospitalized Acute kidney injury(AKI) cases worldwide, representing a critical clinical challenge due to its high morbidity and mortality^{1,2}. Although nephrotoxicity arises from multifactorial causes, drug-induced injury remains predominant contributor. Specific classes of drugs commonly used to treat different diseases include antimicrobials (such as gentamicin, vancomycin, polymyxins, and amphotericin B) and chemotherapeutic agents (such as cisplatin and doxorubicin), which may produce nephrotoxicity²⁻⁴.

Chemotherapeutic drugs, such as doxorubicin (DOX), are also a significant cause of nephrotoxicity worldwide. Doxorubicin-induced nephrotoxicity results from a complex interplay of oxidative stress, inflammatory cascades, and apoptotic/pyroptotic cell death pathways, leading to renal dysfunction and structural damage⁵⁻⁷. At the molecular level, DOX induces the generation of reactive oxygen species (ROS), directing to oxidative damage in renal cells and depletion of antioxidants, such as reduced glutathione and superoxide dismutase activity. This oxidative stress triggers inflammatory responses marked by the upregulation of inflammatory mediators such as nuclear factor kappa-B (NF-κB), Tumour necrosis factor-α (TNF-α), interleukin-1 beta (IL-1β), inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2), which further exacerbate tissue injury^{6,8,9}. Apoptotic processes are also critically involved, as evidenced by increased caspase-3 activity and alterations in pro-apoptotic and anti-apoptotic factors (e.g. Bax and Bcl-2), contributing to the programmed cell death of renal tubular cells⁵. In addition to apoptosis, pyroptosis, an inflammatory form of cell death mediated by inflammasome activation (including NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3) and caspase-1, has been implicated in DOX nephrotoxicity, further amplifying kidney inflammation and damage⁵. Therapeutic strategies that mitigate oxidative stress and inflammation, such as the use of antioxidants (e.g., alpha lipoic acid, crocin, and curcumin)

and anti-inflammatory agents, have shown protective effects against doxorubicin-induced nephrotoxicity in experimental studies^{3,6,7,9}.

Quinoxaline and its derivatives are well-known for their broad spectrum of biological activities, including antimicrobial activity against pathogenic bacteria and fungi, anticancer properties through apoptotic induction and cell cycle arrest^{10,11}, and antiviral, anti-inflammatory, and antioxidant qualities, which make them important in pharmaceutical research and drug development¹². Quinoxaline compounds and their derivatives have demonstrated antioxidant effects that can support their potential nephroprotective role by mitigating oxidative renal damage through scavenging free radicals and reducing inflammatory cytokines¹². Quinoxaline derivatives, which are nitrogen-containing heterocyclic compounds, have been shown to reduce lipid peroxidation and exhibit radical-scavenging activities, even though the exact pathways may diverge based on the specific substituents on the heterocyclic ring¹³. Quinoxaline derivatives also display anti-inflammatory effects, which may involve the downregulation of pro-inflammatory enzymes and mediators. As chronic inflammation is a key driver of kidney damage in various nephropathies, the anti-inflammatory activity of quinoxaline derivatives could contribute to nephroprotection by reducing renal inflammation and fibrosis^{12,14}. Quinoxaline derivatives appear to have less established direct evidence regarding the magnitude and efficacy of their antioxidant effects in renal protection. Quinoxaline derivatives have been shown to inhibit inflammatory mediators, including nitric oxide (NO), (TNF-α), and interleukin-6 (IL-6), which are critical cytokines involved in the inflammatory response. For instance, synthetic quinoxaline-related derivatives demonstrated effective anti-inflammatory potency by blocking Lipopolysaccharide (LPS)-triggered NO release, downregulating COX-2 and iNOS expression, and modulating Mitogen-activated protein kinases (MAPK) signaling pathways very effectively^{15,16}.

2,3-dichloro-6-(trifluoromethoxy) quinoxaline (DTQ), belongs to the class of quinoxaline derivatives and limited studied are reported for its antioxidant and anti-inflammatory properties^{17,18}. Nonetheless, the impact of DTQ on DOX-induced nephrotoxicity has not been explored. Given the pivotal role of oxidative stress and inflammation in DOX-mediated renal injury, the pharmacological activities of DTQ render it a promising candidate for mitigating chemotherapy-associated renal damage. This study aimed to investigate the nephroprotective potential of DTQ through comprehensive biochemical, and histopathological assessments in rats subjected to DOX treatment.

Materials and Methods

Chemicals and kits: DOX, DTQ, and sodium pentobarbital were purchased from Sigma-Aldrich, MO, USA. In this study, kits, including urea (Cat. No. MBS168363), uric acid (Cat. No. MBS2540398), creatinine (Cat. No. MBS480387), and ELISA kits for IL-1 β (Cat. No. MBS265868), IL-6 (Cat. No. MBS269892), TNF- α (Cat. No. MBS282960), IFN- γ (Cat. No. MBS2019134), NF- κ B (Cat. No. MBS1603467), glyceraldehyde 3-phosphate dehydrogenase (GAPDH, Cat. No. MBS727873), iNOS (Cat. No. MBS2505942), COX-2 (Cat. No. MBS266603) and prostaglandin E2 (PGE₂, Cat. No. MBS262150) were procured from MyBioSource, Inc. (San Diego, CA, USA).

Animals: For the *in vivo* study, about 8-week-old male Wistar rats of 150-200 g were used. Before start the experiment, all rats were acclimatizing for two weeks for 12-h light and 12 h dark cycle with free access to food and water. All animal related actions were completed in agreement with ethical standards and regulations permitted by the Biomedical Research Ethics Committee of Umm Al-Qura University, with approval number-HAPO-02-K-012-2025-10-2956.

Study design: Four groups in which about six healthy acclimatized rats (n = 8) were randomly distributed as follows:

1. **Control group I (CON):** rats did not treated with any drug or solvent during the whole experiment.
2. **Group II (DOX only):** On the 6th day after the start of the experiment, a single dose of DOX (15 mg/kg body weight (bw) intraperitoneally i.p.) was administrated¹⁹.
3. **Group III (DOX + DTQ 30mg/kg) :** (Low dose): DTQ (30 mg/kg bw)) treatment was orally administrated once a day, for seven consecutive days and on the 6th day, a single dosage of DOX (15 mg/kg bw i.p) was administrated.
4. **Group IV (DOX + DTQ 60mg/kg) (high dose):** Treatment of DTQ (60 mg/kg bw) was orally administrated as a single dose/day for seven consecutive days. and on the 6th day, a single dose of DOX (15 mg/kg bw i.p) was administrated.

Blood samples and renal tissues collection: After administering the final dose of DTQ on the seventh day, all rats were asleep by the use of sodium pentobarbital (45 mg/kg, i.p.). The cervical displacement was performed by the trained personnel before that anesthetic depth parameter such as loss of righting reflex, absent pedal withdrawal, and palpebral reflex was confirmed. Blood samples were taken from the heart without damage the heart tissues. After scarification, both kidneys were removed and washed by phosphate-buffered (PB; 1M; pH 7.4) and homogenized in sterile PBS (1M; 1 g tissue/9 mL PBS). Clear tissue supernatant was obtained by the centrifugation (at 2500xg for 20 min under cooled conditions) of homogenate mixture and used for different biochemical tests. Serum was also used to perform renal function tests.

Assessment of Renal function parameters: Serum urea, uric acid, and creatinine levels was assessed to analyse the renal function tests by commercially available kit, in the different test groups.

Assessment of oxidative stress markers: MDA is an indirect parameter used for lipid peroxidation levels. MDA level were measured by spectrophotometric method adopted from Sheikh *et al.*²⁰. MDA level was presented as nmol/mg of protein content where tetramethoxypropane was used as the internal standard.

SOD activity was assessed by spectrophotometric method adopted from Nadeem *et al.*²¹. In brief, the clear supernatant was mixed with a reagent which contains 40 μ L xanthine (0.3 mM), 20 μ L EDTA solution

(0.6 mM), 20 μ L of nitro tetrazolium (150 μ M), 12 μ L of Na₂CO₃ (0.4 M), and 6 mL of bovine serum albumin (1 g/L), and incubate for 30 min. Nitroblue tetrazolium (NBT) was added after incubation which form a blue formazan complex. The intensity of this complex was spectrophotometrically assessed at 550 nm and in the form of U/mg protein. One SOD unit activity is defined as the quantity of protein necessary to inhibit a 50% reduction in the NBT.

To measure the CAT activity, serum supernatant diluted in phosphate buffer (1M; pH 7.4), was mixed with 50 nM hydrogen peroxide and incubated in room temperature. The absorbance was taken in every 15 s at 240 nm and expressed in units/mg of protein²².

The GSH activity was assessed in serum supernatant by adding equal quantity of trichloroacetic acid mixed well. After that DTNB was mixed with equal amount and resultant mixture was kept for half-an-hour. GSH activity were spectrophotometrically measured at 412 nm by a standard curve and results are represented as μ mol GSH/mg protein²³.

Assessment of the renal level of inflammatory cytokines and other inflammation-related markers: The inflammatory cytokines IL-1 β , IL-6, TNF- α , and pro-inflammatory markers NF- κ B levels, were measured in clear tissue supernatant by commercially available ELISA kits. The protocol was performed as described by manufacturer, and the cytokines and markers level were presented in pg/mL.

Assessment of the renal stress: Renal tissue stress markers (iNOS, GAPDH, and PGE₂) were measured to detect tissue damage using commercially available ELISA kits. The protocol was done according to manufacturer's instructions, and represented in pg/mL (iNOS and PGE₂) and as arbitrary units for GAPDH.

Assessment of inflammatory mediators: Antibody capture-based sandwich ELISA kits were used to measure inflammatory mediators IFN- γ and COX-2 levels. The procedure was employed to measure the level of inflammatory mediator was done according to manufacturer's instruction and presented as pg/mL of the sample.

Renal histopathology: Histopathological examination of kidney tissue sections was performed to detect damage caused by DOX treatment and the recovery induced by DTQ. The dissected kidneys were immediately immersed in freshly prepared chilled 10% buffered formalin. Subsequently, the tissue was fixed in paraffin wax to create sections. 5 mm-thick sections were cut and stained through H&E staining (hematoxylin and eosin). Microscopic examination was performed to assess histological changes. Various areas of the stained kidney sections from each group were scrutinized, and the findings were recorded.

Statistical Analysis: Statistical studies were conducted by GraphPad Prism version 8.0 (San Diego, USA). The Shapiro-Wilk test (p<0.05) was employed to confirm the normality of the datasets, and the findings are presented as mean $\pm$ SEM, with a sample size of n = 8. Tukey's post hoc test was performed for multiple comparisons, with a significance threshold set at p < 0.05.

RESULTS

Effect of DQT treatment on Renal function parameters

Figure 1 revealed that single dose of DOX (5mg/kg bw) treatment noteworthy (p< 0.001) upsurge the serum urea (216%) creatinine (122%) and uric acid (81%) with related to the control group. Oral treatment of DTQ in a dose of 30 (p< 0.01) and 60 (p< 0.001) mg/kg bw for 6 days before DOX and for 7 days after DOX administration

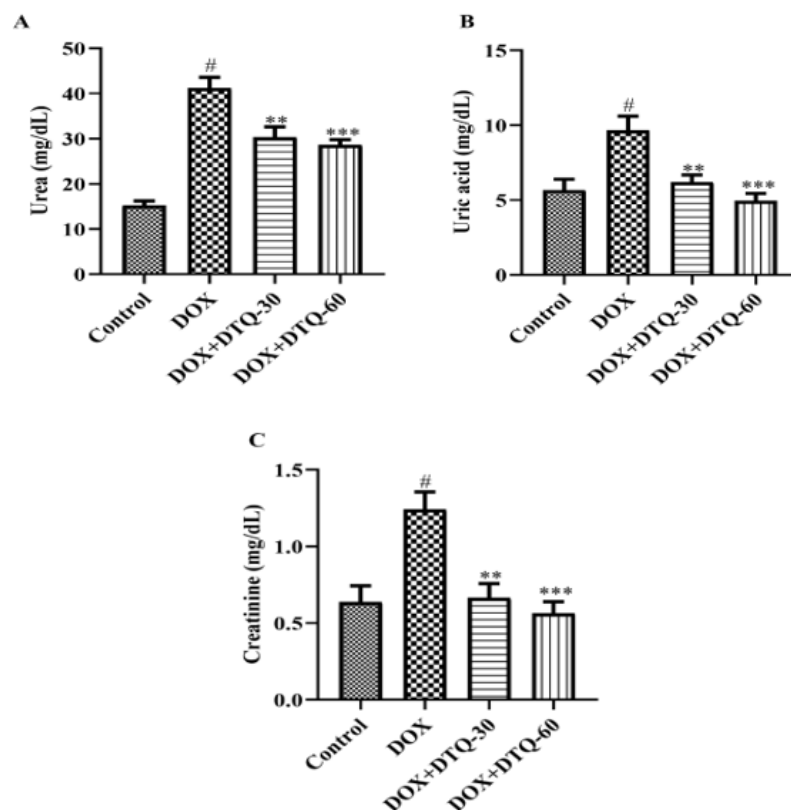


Figure 1. (A-C) Effects of DTQ treatment on renal function parameters (urea, uric acid and creatinine) in DOX treated rats. (A) Urea, (B) Uric acid, (C) Creatinine. Data are presented as mean $\pm$ S.E.M. ($n = 8$). Treatment groups: Control, DOX - doxorubicin, DOX + DTQ-30 - DOX with 30 mg/kg bw DTQ, and DOX + DTQ-60 - DOX with 60 mg/kg bw DTQ. A one-way ANOVA followed by Tukey's post hoc test, # $p < 0.001$ DOX vs control; ** $p < 0.01$, *** $p < 0.001$ vs DOX.

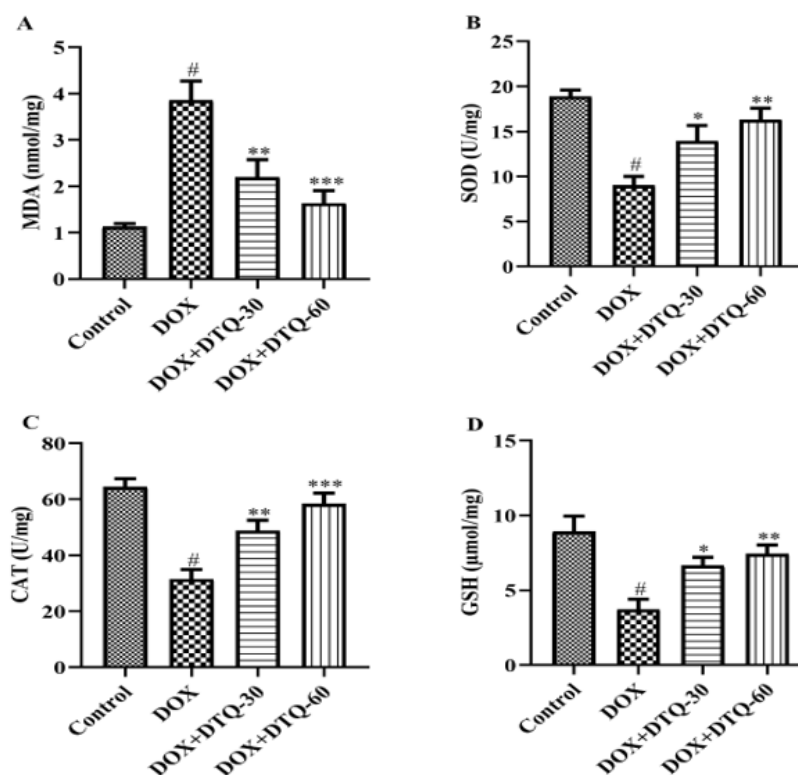


Figure 2. (A-D) Effect of 2,3-DMQ treatment on renal oxidative markers in DOX treated rats. (A) MDA, (B) SOD, (C) CAT and (D) GSH. Data are presented as mean $\pm$ S.E.M. ($n = 8$). Treatment groups: Control, DOX - doxorubicin, DOX + DTQ-30 - DOX with 30 mg/kg bw DTQ, and DOX + DTQ-60 - DOX with 60 mg/kg bw DTQ. 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.

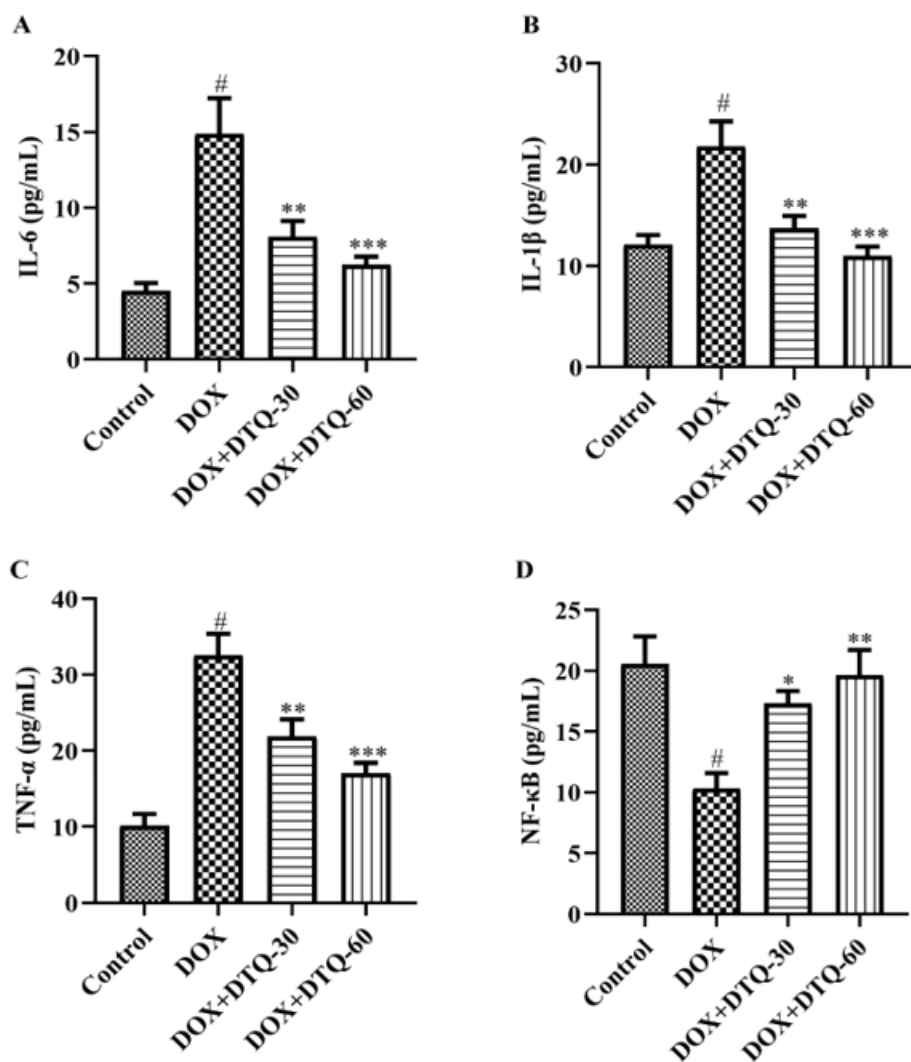


Figure 3(A-D). Effect of 2,3-DMQ treatment on cytokines and inflammatory mediators in DOX treated rats. (A) IL-6, (B) IL-1β (C) TNF-α and (D) NF-κB. Data are presented as mean ± S.E.M. (n = 8). IL-6 - interleukin-6; IL-1β - interleukin-1β; TNF-α - Tumor necrosis factor-α; NF-κB - Nuclear factor-κB. Treatment groups: Control, DOX - doxorubicin, DOX + DTQ-30 - DOX with 30 mg/kg bw DTQ, and DOX + DTQ-60 - DOX with 60 mg/kg bw DTQ. 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.

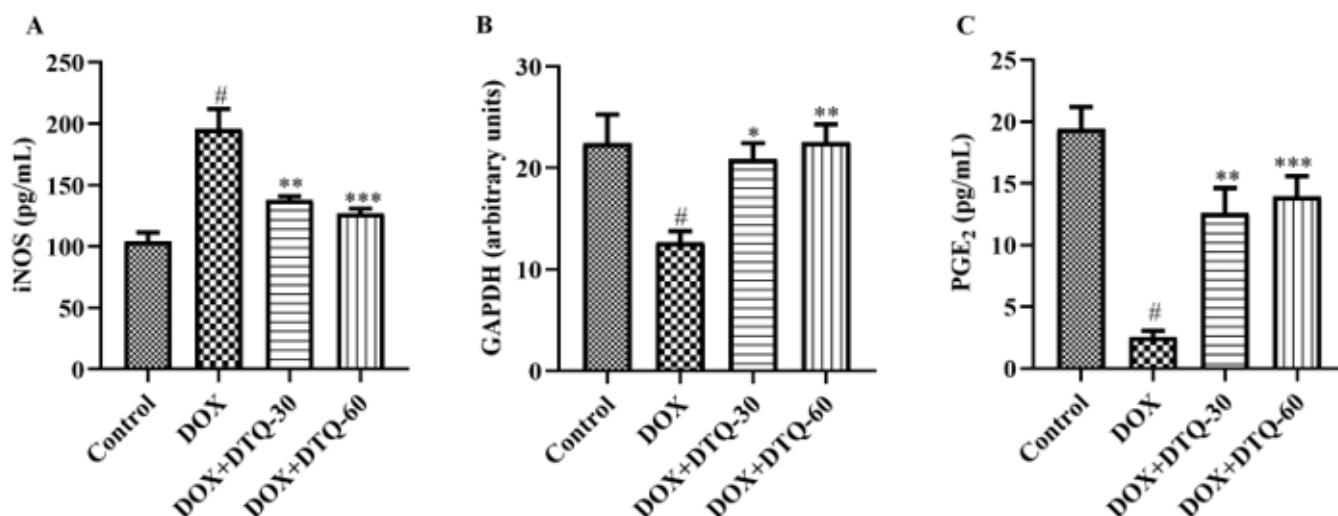


Figure 4(A-C). Effect of 2,3-DMQ treatment on renal stress in DOX treated rats. (A) iNOS, (B) GAPDH, and (C) PGE₂. Data are presented as mean ± S.E.M. (n = 8). iNOS - nitric oxide synthase; GAPDH - Glyceraldehyde 3-phosphate dehydrogenase; and PGE₂ - prostaglandin E₂. Treatment groups: Control, DOX - doxorubicin, DOX + DTQ-30 - DOX with 30 mg/kg bw DTQ, and DOX + DTQ-60 - DOX with 60 mg/kg bw DTQ. 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.

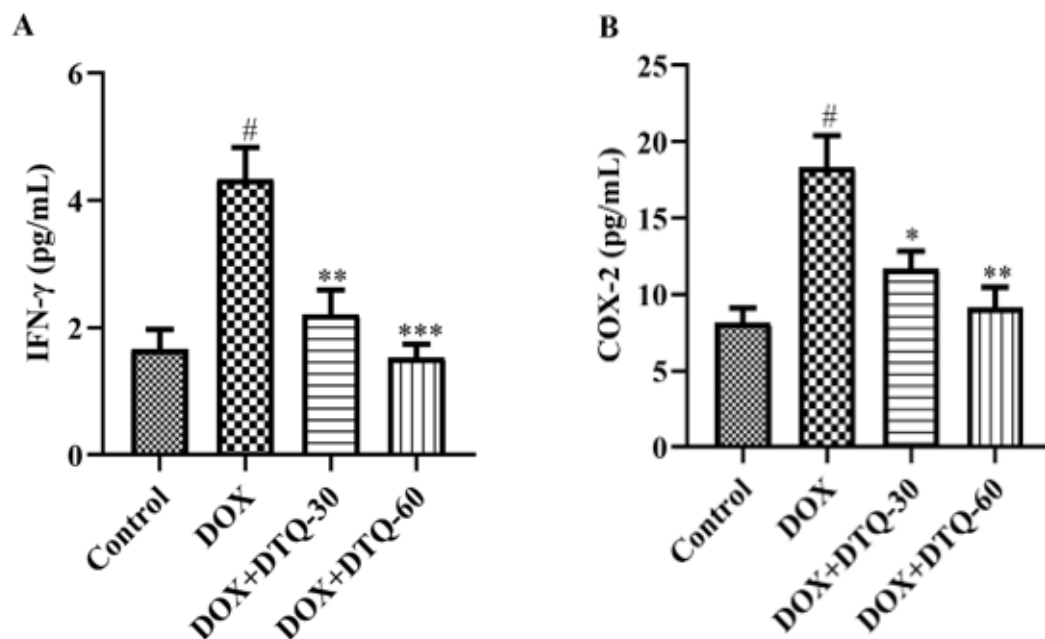


Figure 5(A-B). Effect of 2,3-DMQ treatment on tumour markers in DOX treated rats. (A) IFN- γ and (B) COX-2. Data are presented as mean $\pm$ S.E.M. ($n = 8$). IFN- γ – Interferon - gamma; and COX-2 - Cyclooxygenase-2; Treatment groups: Control, DOX - doxorubicin, DOX + DTQ-30 - DOX with 30 mg/kg bw DTQ, and DOX + DTQ-60 - DOX with 60 mg/kg bw DTQ. 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.

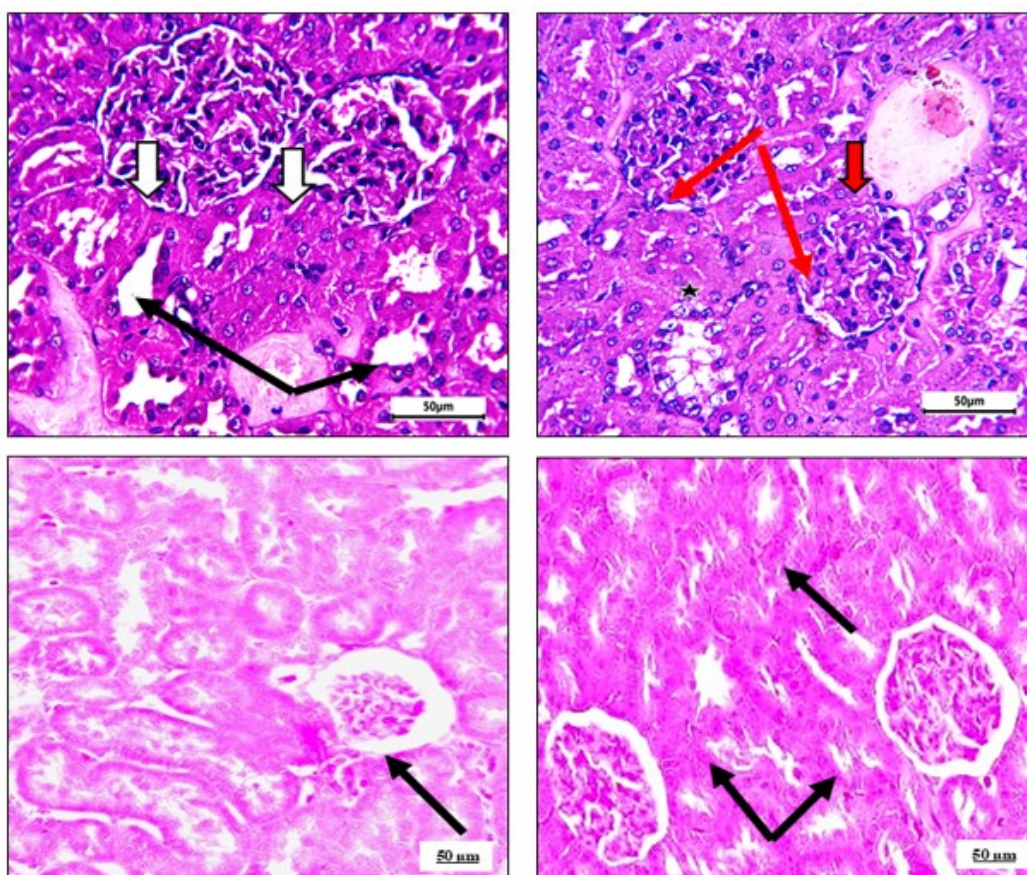


Figure 6(A-D). Renal histological alterations of rat kidneys were observed using H&E staining. (A) Control rat – Group I (B) DOX treated-group II. Sporadic marked atrophy of renal corpuscle glomeruli (thick red arrow), dilation of many tubules with deformed shapes and epithelial lining atrophy (thin red arrow) and dilated tubules contains epithelial and hyaline casts, dilated vessels show haemolysed blood (star) by DOX administration, was detected. (C) DOX + 30DMQ - DOX with 30 mg/kg 2,3-DMQ, pre-treatment with 2,3-DMQ reverted with these alterations, with mild dilation of many tubules with deformed shape and epithelial lining atrophy (thin black arrow). (D) DOX + 60DMQ - DOX with 60 mg/kg 2,3-DMQ pretreatment showing improve of atrophy of renal corpuscle glomeruli and dilation of many tubules

considerably reduced the high levels of urea to 134%, while completely recovered creatinine and uric acid level as control in serum as related to DOX-induced group.

Effect of DQT treatment on oxidative stress markers

Treatment with DOX was linked with noteworthy while significantly decrease in renal levels of SOD ($p < 0.001$, 111%), activity of CAT ($p < 0.001$, 105%) and GSH ($p < 0.001$, 110%) while elevate the renal MDA content ($p < 0.001$, 297%) compared to the control group. Pretreatment with DTQ at 30 and 60 mg/kg bw in DOX-induced rat significantly recover the GSH, CAT, SOD levels of oxidative stress markers while reduce the MDA content (Figure 2). In compared to group IV rats, the MDA ($p < 0.001$) level and activity of GSH ($p < 0.01$), CAT ($p < 0.001$), and SOD ($p < 0.01$) in rats treated with HTQ high dose were significant improved compared to group III rats, shows the dose-dependent effect of HTQ.

Effect of DQT treatment on renal expression of inflammatory cytokines and other inflammation-related markers

In DOX-treated group rats the IL-6 and IL-1 β levels were found markedly raised in related to the control group rats ($p < 0.001$) (Figure 3A-B). DTQ treatment with dose 30, and 60 mg/kg bw pointedly reduce the IL-6 and IL-1 β levels when compared with only DOX-treated group. The DOX treated rats displayed the notable increase in the TNF- α and NF- κ B levels as compare to the normal control group rats ($p < 0.001$). DTQ treatment at a dose of 30 mg/kg bw significantly reduced the TNF- α ($p < 0.01$) and NF- κ B ($p < 0.05$) levels in related with DOX treated rats (group II) (Figure 3C-D). The reduction in IL-6, IL-1 β , TNF- α and NF- κ B level was higher in the group treated high does (60 mg/kg bw) indicating dose-dependent effect.

Effect of DQT treatment on renal stress

In the DOX-treated rats, the activity of renal stress markers iNOS ($p < 0.001$) and PEG₂ ($p < 0.001$) was unusually high, whereas GAPDH levels ($p < 0.001$) were low in respect to the control rats. The DTQ treatment in group IV (high dose) rats remarkably reduced the upsurged iNOS ($p < 0.001$), and PEG₂ ($p < 0.01$) levels and recovered the GAPDH ($p < 0.01$) in comparison with the DOX-received group (Figure 4 A-C). Group III rats, which received a low dose of 3-DTQ, showed less recovery of renal stress marker levels than group IV (high-dose) rats.

Effect of DQT treatment on inflammatory mediators

The level of IFN- γ (110%, $p < 0.001$) and COX-2 (153%, $p < 0.001$) is the renal tissue was noteworthy high in the DOX-treated rats than in the control group rats (Figure 5 A-B). While the treatment with DTQ groups rats (III and IV) found a substantial decrease in IFN- γ ($p < 0.01$ and $p < 0.001$) and COX-2 ($p < 0.01$ and $p < 0.001$) levels. However, in group IV (DTQ-60) rats, this drop in IFN- γ and COX-2 level was higher in compared to the lower dose (group III), represent the dose-dependent effect.

Effect of DQT treatment on Renal histopathology

The histopathological results of the kidney tissues are presented in Figure 6A-D. Kidney tissue sections of control group rats displayed typical histological construction in the glomeruli (g) and tubules (t) in the cortex and corticomedullary portion. However, kidney tissue sections of only DOX-treated rats displayed noteworthy glomerular injury. DOX treatment in rats produces congestion of blood vessels (c), focal haemorrhage (h) in between the tubules, and degeneration in the

tubular lining epithelium with focal inflammatory cell infiltration in the cortical portion. Pretreatment with DTQ in rats for 07 days before and for 7 days after DOX treatment noticeably reduced the DOX-induced histopathological changes. Glomerular damage was significantly decreased in DTQ-treated rats at the 30 and 60 mg/kg bw dosages.

DISCUSSION

DOX is commonly applied as an anthracycline antibiotic with significant applications in the medical field, primarily as a chemotherapeutic drug for numerous cancers, including liver cancer, breast cancer, and hematopoietic malignancies²⁴⁻²⁶. Its antitumor activity stems from several mechanisms, including intercalation in DNA, topoisomerase enzyme inhibition, ROS generation, and chromatin damage, all of which lead to DNA damage and tumor cell death^{25,26}. Despite its broad efficacy, the use of DOX is restricted by several significant side effects, including nephrotoxicity^{26,27}. DOX induces nephrotoxicity primarily through mechanisms involving oxidative stress, inflammation, and apoptosis in the kidney. DOX administration causes significant increases in kidney weight, serum urea, and creatinine levels, demonstrating weakened renal function, alongside increased levels of TNF- α , MDA, NO, COX-2, and caspase-3 in renal tissues, which collectively signify oxidative injury, inflammation, and activation of apoptotic pathways⁷. Quinoxaline derivatives exhibit a range of pharmacological effects, and further focused toxicological studies are necessary to determine their nephrotoxic or nephroprotective characteristics. In this study, the mechanisms underlying the probable nephroprotective effect of DTQ in DOX-induced renal injury were examined by assessing the renal-related biochemical, oxidative stress, inflammatory, and apoptotic markers.

DOX treatment is associated with nephrotoxicity, which affects kidney function and consequently alters the serum levels of urea, creatinine, and potentially uric acid, possibly leading to hyperuricemia. Studies in which DOX-induced nephrotoxicity involved in animal models, the impact on uric acid transport and clearance correspondingly modifies serum urea and creatinine levels, which typically increase, indicating impaired renal function due to kidney damage caused by doxorubicin²⁸⁻³⁰. Increased serum urea and creatinine are classical indicators of reduced glomerular filtration rate and kidney injury, while elevated uric acid levels may also contribute to oxidative stress and inflammation in renal tissue³¹. The elevation of these markers reflects a reduced ability of the kidneys to excrete waste products effectively. In the present study, DTQ treatment reduced doxorubicin-induced renal injury and normalized the elevated levels of urea and creatinine, signifying the nephrotoxic impact of DOX chemotherapy.

DOX induces renal oxidative stress by promoting excessive ROS production, depleting antioxidant defences, and activating inflammatory and apoptotic signalling pathways. These processes culminate in renal tissue damage, inflammation, and impaired kidney function^{7,9,32,33}. In the present study, we examine that DOX-treatment suppress the activity of SOD, CAT and GSH, weaken endogenous antioxidant defence system and increase the ROS level, reflects the similar results of previous studies^{7,32}. DOX induces oxidative stress in the renal system primarily through the ROS overproduction, leading to kidney cell damage and impaired function. DOX undergoes redox cycling within renal cells, producing excessive ROS, such as superoxide anions (O₂⁻) and H₂O₂. These active species overwhelm the endogenous antioxidant defences in the kidneys, including enzymes such as SOD and catalase, ending with oxidative injury³². Elevated ROS levels also the origin of lipid peroxidation of the cellular membranes, compromising the integrity of the renal cells. Lipid peroxidation is often reflected by increased MDA levels (known as a secondary product of lipid peroxidation), a marker

of oxidative damage to the cell membranes which was also found in the present study. This finding is in line with the findings of Abd-Ellatif *et al.*⁵ and El-Sayed *et al.*⁷.

Inflammation is a critical part in the progression of renal damage persuaded by DOX. Oxidative stress activates inflammatory pathways leading to the release of pro-inflammatory cytokines such as TNF- α , IL-1 β , and IL-6. This inflammatory response, primarily mediated by NF- κ B activation, amplifies tissue injury by promoting immune cell infiltration and sustaining oxidative stress, generating a malicious cycle of ongoing damage. This inflammatory response further exacerbates renal injury^{9,33-35}. Our findings showed that a considerable upsurge in TNF- α , IL-1 β , and IL-6 levels in the DOX treated group rats this finding is in synchronization with the previous research findings done by Hussain *et al.*⁹ and Kim *et al.*³⁵.

DOX treatment significantly affected the levels of renal tissue stress markers, including iNOS, GAPDH, and PGE₂. iNOS expression is markedly increased in renal tissue following doxorubicin exposure, contributing to nitrosative stress by elevating the production of nitric oxide (NO). This upregulation is linked to inflammation and oxidative damage triggered by DOX-induced nephrotoxicity in the kidneys. In present study the upsurge in the inflammatory mediators, IFN- γ , NF- κ B and COX-2 was found, this result resemble the previous studies which shows that iNOS and related inflammatory mediators, such as NF- κ B and COX-2, are overexpressed in the kidneys after DOX treatment, exacerbating renal injury^{9,36,37}. DTQ treatments that mitigate DOX-induced nephrotoxicity also reduce inflammatory mediators, IFN- γ , NF- κ B and COX-2 level and iNOS expression, highlighting the role of iNOS in mediating renal oxidative and inflammatory stress responses.

GAPDH is primarily involved in glycolysis, under oxidative and nitrosative stress conditions, such as those induced by DOX, GAPDH can aggregate and contribute to mitochondrial dysfunction and cell death. NO (produced via iNOS) promotes GAPDH aggregation, leading to mitochondrial swelling, depolarization, and apoptosis. This abnormal GAPDH aggregation exacerbates renal cell injury during oxidative stress³⁸. PGE₂, a pro-inflammatory lipid mediator synthesized via COX-2 and microsomal PGE synthase-1 (mPGES-1), is also elevated in inflammation and its production is typically increased following COX-2 and iNOS upregulation in inflamed tissues. Elevated PGE₂ levels promote further inflammation and tissue damage^{9,39}. In this study DTQ downregulate the COX-2 and iNOS levels pretend as anti-inflammatory agents to regulate the COX-2 and iNOS levels and also tend to decrease PGE₂ levels, which can alleviate DOX-induced renal inflammation and injury.

Chronic inflammation promotes fibrosis and scarring, thereby worsening kidney dysfunction over time. The inflammatory milieu activates various immune cells, including macrophages and monocytes, which secrete additional cytokines and chemokines that exacerbate tissue damage and fibrosis⁴⁰. Inflammation in DOX-induced nephrotoxicity is linked to apoptosis of renal cells, which further aggravates structural damage and functional decline³⁵. Histopathological examination confirmed structural kidney damage, characterized by tissue necrosis, inflammatory infiltration, and fibrosis. Notably, sex-related differences in nephrotoxicity severity have been observed in animal models, with male subjects showing more pronounced renal lesions and higher levels of fibrotic and apoptotic markers than females, possibly linked to the differential expression of enzymes such as soluble epoxide hydrolase⁴¹.

Nephrotoxicity induced by DOX is primarily attributed to oxidative stress, inflammation, apoptosis, and disruption of renal homeostasis. This study proposes the potential protective mechanisms of DTQ

against DOX-induced renal damage in a rat model system, indicating that DTQ reduces the levels of serum urea, uric acid, and creatinine, thereby potentially aiding in the restoration of renal health. DTQ enhances the activity of the enzyme GAPDH, which is crucial for energy production and supports antioxidant defences essential for cellular survival under oxidative stress. Furthermore, DTQ significantly reduces the production of ROS, NO synthase, and PEG₂, possibly leading to an increase the level of antioxidant enzymes such as SOD, GSH and CAT, while decreasing levels of MDA. This results in reduced lipid peroxidation and cellular damage. DTQ mitigates inflammation by decreasing the levels of inflammatory cytokines, including TNF- α , IL-6, and IL-1 β , which subsequently reduces the activation of NF- κ B signaling pathways. Additionally, DTQ effectively regulates the elevated expression of IFN- γ and COX-2 to counteract DOX-induced nephrotoxicity. DTQ may also attenuate DOX-induced renal fibrosis by reducing the synthesis of fibrosis-associated proteins, thereby aiding in the restoration of the expanded capsular space and structural alterations associated with renal tubule damage in kidney tissues. Consequently, DTQ enhances renal function indicators and improves histopathological outcomes in models of DOX-induced nephrotoxicity.

CONCLUSION

In conclusion, the global burden of drug-induced nephrotoxicity is gradually increasing, especially due to the high application of antimicrobials and chemotherapeutics. Progress in early biomarkers and potential treatments brings optimism for improved management. Nonetheless, nephrotoxicity continues to be a significant global issue because of its high prevalence, intricate pathophysiology, and effects on morbidity and mortality. In nephrotoxicity, the pathological mechanisms involve tubular injury, inflammation, oxidative stress, and apoptosis. Quinoxaline derivatives exhibit a range of pharmacological effects, and further focused toxicological studies are necessary to clearly determine their nephrotoxic or nephroprotective characteristics. The outcomes of present research establish that the nephroprotective properties of DTQ, when used as an adjuvant therapy, attenuated DOX-induced nephrotoxicity in rats through its antioxidant and anti-inflammatory effects in contrast to the DOX-induced increase in oxidative stress, pro-inflammatory cytokine, and apoptotic marker levels. Additional further research is required to assess other probable mechanisms underlying the nephroprotective effects of DTQ and to address the safety of DTQ/DOX cotreatment in humans.

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