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Nephroprotective effects of the soluble guanylyl cyclase stimulator, riociguat in doxorubicin-induced acute kidney injury in rats

Raya Al-Maskari [a], Aly M. Abdelrahman [a], Haytham Ali [b], Priyadarsini Manoj [a], Yousuf Al Suleimani [a,*]

[a] Department of Pharmacology and Clinical Pharmacy, College of Medicine and Health Sciences, Sultan Qaboos University, P.O. Box 35, Al Khod 123, Oman
[b] Department of Animal and Veterinary Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University, Muscat 123, Oman

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ABSTRACT

This study aimed to investigate the potential protective effects of riociguat, a soluble guanylyl cyclase (sGC) stimulator, on kidney function and structure in rats with acute kidney injury (AKI) induced by the chemotherapeutic drug doxorubicin (DX). Rats were subjected to a single intraperitoneal injection of DX (13.5 mg/kg) on the 5th day, either alone or in combination with low-dose riociguat (3 mg/kg/day), or high-dose riociguat (10 mg/kg/day) for 8 consecutive days. Various markers related to kidney function, oxidative stress, and inflammation were measured in plasma and urine. Kidney tissues were examined histopathologically. DX-induced nephrotoxicity was characterized by increased plasma urea, creatinine, uric acid and neutrophil gelatinase-associated lipocalin (NGAL). DX also decreased creatinine clearance and albumin levels and increased urinary N-acetyl-β-D-glucosaminidase (NAG) activity. Furthermore, DX increased the inflammatory markers interleukin 1 beta (IL-1 β), interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α). DX further induced oxidative stress injury evidenced by decreased glutathione reductase (GR) activity, total antioxidant capacity (TAC), superoxide dismutase (SOD) and catalase levels and increased malondialdehyde (MDA) levels. Concomitant treatment with riociguat ameliorated these DX-induced changes with parallel histopathological improvements but the effects were more favorable with high-dose riociguat. The observed renoprotective effects of riociguat can be partly attributed to the anti-inflammatory and anti-oxidant properties of this drug.

1. Introduction

Cyclic guanosine monophosphate (cGMP) is a ubiquitous second messenger which activates key effector proteins such as cGMP-protein kinases, phosphodiesterases and cyclic nucleotide-gated ion channels [1,2]. cGMP is synthesized from guanosine triphosphate (GTP) through two primary pathways: one involves nitric oxide (NO) stimulation of soluble guanylyl cyclase (sGC) while the other involves natriuretic peptides stimulation of particulate guanylyl cyclase (pGC) [1,3]. The NO-sGC-cGMP signaling pathway regulates a wide range of cellular and physiological processes essential for cardiovascular, central nervous system, metabolic, lungs, hepatic and renal function [2,3]. Disruptions in this signaling pathway are linked to the pathogenesis of heart failure, hypertension, pulmonary hypertension, several neurological conditions and hepatic and kidney disease [2,4]. Consequently, restoring this signaling pathway pharmacologically has been the focus of ongoing research in recent years. sGC stimulators have emerged as a novel class of drugs that modulate the action of sGC [5].

Riociguat was the first sGC stimulator to be approved for clinical use and is currently indicated for pulmonary artery hypertension and chronic thromboembolic pulmonary hypertension [5]. Riociguat acts by stabilizing the structure of the sGC enzyme in its active catalytic state while simultaneously increasing its responsiveness to circulating NO, thereby increasing cGMP levels [6,7]. This dual action allows sGC stimulators to increase cGMP even when NO production is impaired or absent [6,7]. Given the diverse functions of the NO-sGC-cGMP signaling pathway across multiple tissues and organs, the therapeutic potential of riociguat and other sGC stimulators warrants investigation for a broader range of diseases. sGC is highly expressed in both the vascular and interstitial regions of the kidneys, underscoring the importance of this pathway in kidney function and dysfunction [8]. Experimental studies in animal models of hypertension, diabetes and kidney disease demonstrated that riociguat provides significant protection against renal damage, improving renal hemodynamics and ameliorating

* Corresponding author.
E-mail address: yousufm@squ.edu.om (Y. Al Suleimani).

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glomerulosclerosis and interstitial renal fibrosis [9–11]. Other sGC modulators have similarly shown kidney protective effects in various experimental models [12,13].

Recent *in vitro* experiments show that riociguat protects podocytes against doxorubicin (DX)-induced injury [14]. DX is a widely used chemotherapeutic drug for treating various solid and hematological cancers [15]. However, treatment with DX is complicated by severe side effects, resulting in increased morbidity, longer hospitalizations and treatment interruptions [15,16]. A large proportion of patients on DX therapy develop acute kidney injury (AKI) with impaired filtration, reabsorption and excretion [15]. A combination of oxidative stress, inflammation and apoptosis are believed to be the main processes driving DX-induced nephrotoxicity [17]. There are currently no approved pharmacological agents to prevent or minimize kidney toxicity in patients receiving DX, and treatment relies primarily on supportive care. Therefore, there is a pressing need to develop new therapeutic strategies to protect against DX-induced renal damage.

Given the emerging role of sGC stimulators in renoprotection, we sought to investigate the renal effects and the potential renoprotective mechanisms of two doses of riociguat (3 mg/kg and 10 mg/kg) in an experimental rat model of DX-induced AKI.

2. Materials and methods

Male Wistar rats, weighing between 200 and 300 g, were obtained from the small animal facility at Sultan Qaboos University. They were housed under controlled environmental conditions, with a temperature maintained at 22 ± 2 °C, relative humidity at approximately 60 %, and a 12-hour light-dark cycle. The animals had access to a standard diet and tap water freely. The study involving the use of these rats received approval from the Animal Ethics Committee of Sultan Qaboos University (Approval Code: SQU/EC-AUR/2021–2022–13). All animal procedures and care adhered to both national and international regulations and ethical guidelines.

2.1. Experimental design

In this study, 24 male male Wistar rats were randomly assigned into four equal groups and treated for 8 days as follow:

Group 1: Control; maintained on a normal diet for 8 days, given oral 0.5 % carboxymethylcellulose (CMC) for 8 days, and received a saline injection on day 5.

Group 2: Doxorubicin (DX); treated similarly to the control group and administered intraperitoneal (i.p.) injection of DX at a dose of 13.5 mg/kg on day 5.

Group 3: Doxorubicin + Riociguat (DX+ R3); Maintained on a normal diet for 8 days, given riociguat (suspended in 0.5 % CMC) orally at a dose of 3 mg/kg/day for 8 days, and injected with DX on day 5, as in Group 2.

Group 4: Doxorubicin + Riociguat (DX+ R10); treated the same as group 3 but with riociguat at a dose of 10 mg/kg/day.

The doses of DX and riociguat was selected based on previous studies [9,18,19].

Urine was collected from each rat using a metabolic cage over a 24-hour period, one day prior to the conclusion of the treatment. The total urine volume was recorded. At the end of the treatment phase, rats were anesthetized with an intraperitoneal injection of ketamine (75 mg/kg) and xylazine (5 mg/kg). Blood samples were drawn from the abdominal aorta into heparinized tubes, followed by centrifugation at 900 x g for 15 minutes at 4°C to isolate plasma, which was subsequently frozen at −80°C for biochemical analyses to be conducted within 10 days. The rats were then euthanized by administering an overdose of the anesthetics. Kidneys were harvested, rinsed in ice-cold saline, blotted dry, and weighed, with the kidney-to-body weight ratio calculated. A small section of the right kidney was preserved in 10 % buffered formalin for histological examination, while the remaining portions of both kidneys were individually wrapped in aluminum foil, flash-frozen in liquid nitrogen, and stored at −80°C for analysis within 10 days. Creatinine clearance was determined using the following formula:

Urinary creatinine (μmol/L) × 24-hour urine volume (mL) / Serum creatinine (μmol/L) × 1440.

Table 1
Effect of riociguat (3 mg/kg, R3 and 10 mg/kg, R10) treatment on some physiological parameters in rats with doxorubicin (DX) - induced acute kidney injury (AKI).

Parameters/ Treatment	Control	DX	DX + R3	DX + R10
Base line body weight (g)	325.67 ± 13.98	325.00 ± 12.32	325.67 ± 15.32	325.33 ± 18.69
Final body weight (g)	334.67 ± 12.95	311.33 ± 13.82	315.83 ± 15.24	315.33 ± 17.93
Body weight change (%)	2.89 ± 1.03	−4.34 ± 0.98[a]	−3.05 ± 0.32[a]	−3.03 ± 0.66[a]
Relative kidney weight (%)	0.53 ± 0.006	0.57 ± 0.027	0.55 ± 0.013	0.55 ± 0.011
Water intake (mL)	19.33 ± 2.5	17.75 ± 4.87	12.50 ± 1.98	11.75 ± 2.74
Urine output (mL/ 24 hr)	6.38 ± 1.05	10.67 ±2.02	5.50 ± 1.06[b]	5.58 ± 0.71[b]
Urine osmolality (mOsmol/kg)	1903.17 ± 87.4	781.86 ± 91.82[a]	1291.5 ± 221.7[b]	1630.2 ± 221.5[b]

Values in the table are means ± SEM (n = 6).
Differences between the groups were assessed by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test, Where $P < 0.05$.
[a]denotes significant from control group[b] denotes significant from DX group

2.2. Drugs and chemicals

Doxorubicin was sourced from Sultan Qaboos University pharmacy, while riociguat was purchased from ZHI Shang Chemical, China. Urea, creatinine, uric acid, and albumin were measured using a Mindray BS-120 chemistry analyzer (Shenzhen Mindray Bio-Medical Electronics Co., China). Superoxide dismutase (SOD) and glutathione reductase (GR) were measured using Biovision colorimetric kits (Milpitas, CA, USA). Total antioxidant capacity (TAC) and malondialdehyde (MDA) kits were obtained from MyBioSource, Inc. (San Diego, CA, USA). ELISA kits for interleukin-1β (IL-1β) and N-acetyl-β-D-glucosaminidase (NAG) were from Cusabio Biotech Co. Ltd. (Wuhan, Hubei, China). Neutrophil gelatinase-associated lipocalin (NGAL), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), and catalase kits were purchased from Thermo Fisher Scientific, Inc. (Waltham, MA, USA). Osmolality was measured using the Osmomat 3000 osmometer (Gonotec GmbH, Berlin, Germany).

2.3. Histopathological analysis

Kidneys were fixed in 10 % neutral buffered formalin and processed for histopathology. Sections of 4 μm were stained with Hematoxylin and Eosin (H & E) and Picro-Sirius red (ab150681, Abcam). Renal tubular necrosis was evaluated using a semi-quantitative scoring method [18] on a scale of 0–4: 0 = normal, no necrosis; 1 = <10 %; 2 = 10–25 %; 3 = 26–75 %; 4 = >75 %. Three 40X microscopic fields were examined per kidney section per animal, and the score was calculated as the mean percentage. The fibrosis index was determined using Picro-Sirius red staining, which colors collagen red.

2.4. Statistical analysis

The normality of the data was assessed using the Shapiro-Wilk test, and statistical analyses were conducted using one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test for multiple comparisons. Results are expressed as the mean ± standard error of the mean (SEM), and all analyses were performed using GraphPad Prism

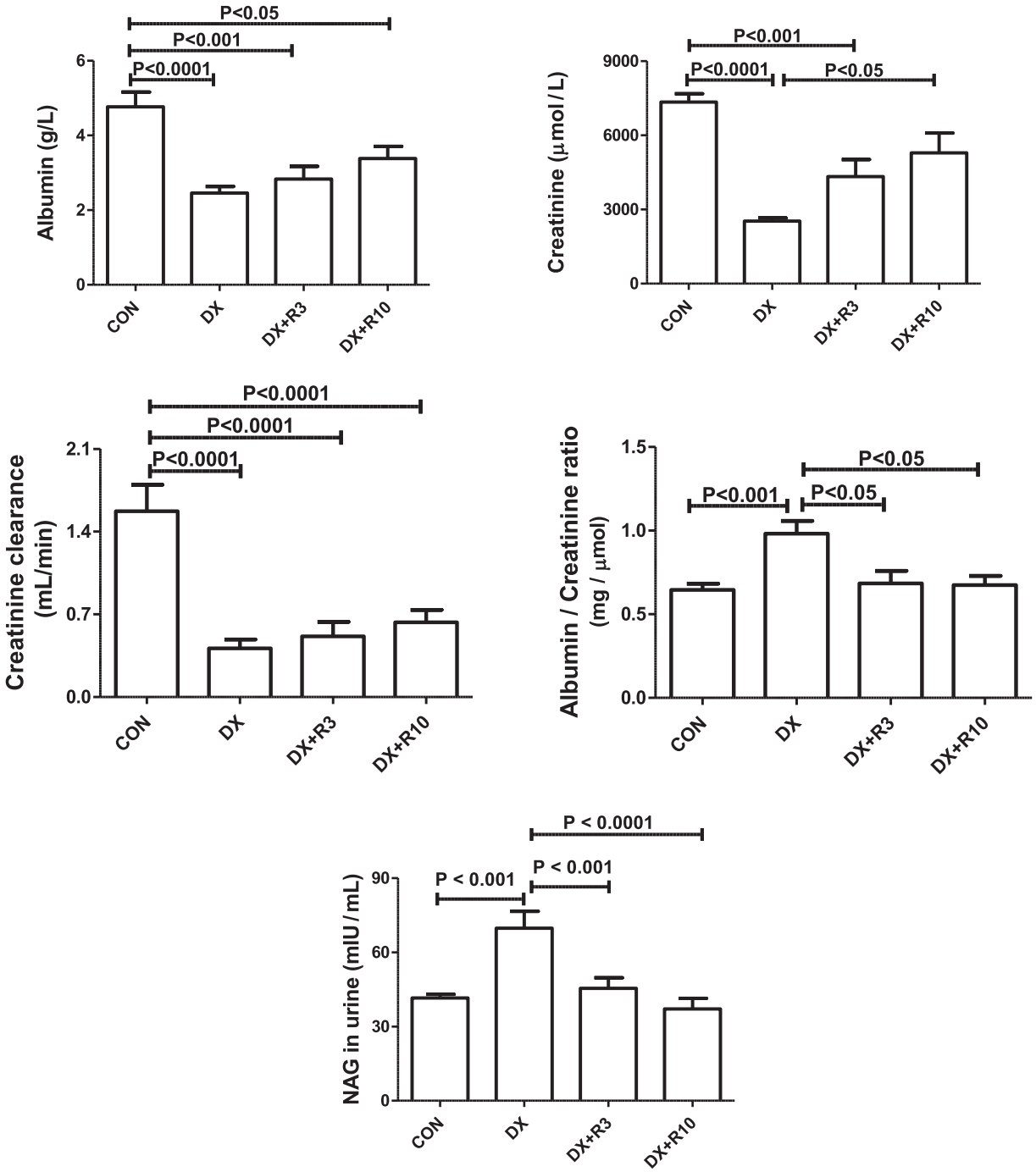

Fig. 1. The urinary levels of albumin over 24 hours, creatinine, urine albumin-to-creatinine ratio (UACR), creatinine clearance, and N-acetyl-β-D-glucosaminidase (NAG) in control rats and those treated with doxorubicin (DX) alone or in combination with two doses of riociguat (3 mg/kg, R3 and 10 mg/kg, R10). Each bar represents the mean ± SEM (n = 6). Differences between groups were assessed using one-way ANOVA followed by Bonferroni's multiple comparison test, with significance set at $P < 0.05$.

software, version 5.03 (San Diego, CA, USA). A P-value of less than 0.05 was considered to indicate statistical significance.

3. Results

3.1. Physiological parameters

DX significantly reduced body weight but did not cause any significant changes in the relative kidney weight (Table 1). DX also significantly reduced urine osmolality with no effect on water intake and increased urine output. Cotreatment with the two doses of riociguat significantly reversed the decrease in urine osmolality caused by DX.

3.2. Renal function and injury markers

DX significantly reduced urinary albumin, creatinine and creatinine clearance and significantly increased albumin/creatinine ratio and NAG (Fig. 1). Both doses of riociguat significantly reduced the DX-induced increase in albumin/creatinine ratio and urinary NAG. However, only the higher dose of riociguat (R10) attenuated the DX-induced changes in creatinine. Furthermore, DX significantly increased plasma urea, creatinine, uric acid and NGAL (Table 2). Both doses of riociguat significantly

Table 2
Effect of riociguat (3 mg/kg, R3 and 10 mg/kg, R10) treatment on plasma parameters in rats with doxorubicin (DX) - induced acute kidney injury (AKI).

Parameters/ Treatment	Control	DX	DX + R3	DX + R10
Urea (mmol/L)	4.80 ± 0.28	14.35 ± 1.47[a]	9.51 ± 0.91[a, b]	7.79 ± 0.63[b]
Creatinine (μmol/L)	21.87 ± 0.87	45.82 ± 2.88[a]	34.57 ± 2.43[b]	32.45 ± 2.69[b]
Uric acid (μmol/L)	17.38 ± 0.80	41.99 ± 1.87[a]	33.53 ± 1.89[a, b]	26.27 ± 2.96[a, b]
NGAL (ng/mL)	34.32 ± 3.26	61.18 ± 2.35[a]	49.2 ± 4.91[a, b]	44.7 ± 3.36[a, b]

Values in the table are means ± SEM (n = 6).
NGAL: Neutrophil gelatinase-associated lipocalin.
Differences between the groups were assessed by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test, Where $P < 0.05$.
[a] denotes significant from control group [b] denotes significant from DX group

ameliorated the changes in all parameters.

3.3. Inflammatory and oxidative stress markers

DX significantly increased IL-1 β, IL-6 and TNF-α (Fig. 2). Both doses of riociguat significantly reversed the DX-induced changes in TNF-α while only the higher dose of riociguat (R10) reduced IL-1 β and IL-6 significantly.

On the other hand, DX significantly decreased GR activity, TAC, SOD and catalase levels and increased MDA levels (Fig. 3). Both doses of riociguat significantly reversed the DX-induced changes in SOD, catalase and MDA while only the higher dose (R10) reversed the changes in GR activity and TAC significantly.

3.4. Histopathology

Microscopic examination of the renal tissues of the treated rats showed a normal renal histological structures and architecture with intact glomeruli and renal tubules (lesion score 0) in the control group (Fig. 4A). Examined renal tissues of DX-treated rats revealed tubular cystic dilatation, cellular casts and sclerotic glomeruli with marked basophilia of the renal tubules and mononuclear cells infiltrations (Score 4) (Fig. 4C). Tissues from rats treated with DX+R3 exhibited mild tubular basophilia with intact glomeruli (Score 2) (Fig. 4E). Renal tissues of rats treated with DX+R10 showed marked tubular dilatation with intact glomerular tufts (Score 2) (Fig. 4G). The distribution of collagen fibers stained in red and the non-collagen structures stained in yellow was demonstrated using Picro-sirus red stain in all four groups (Figs. 4B, 4D, 4F, 4H) and the fibrosis index percentage was highest in DX-treated rats (Table 3).

4. Discussion

The NO-sGC-cGMP signaling pathway plays an important role in renal homeostasis and physiological function. Thus, restoring cGMP as a therapeutic target for kidney dysfunction has been suggested previously [14,20]. Here, we investigated for the first time the effect of riociguat, a novel sGC stimulator currently indicated for pulmonary hypertension, on an animal model of AKI induced by the chemotherapeutic drug DX. The pathophysiology of DX-mediated AKI typically involves proteinuria, renal fibrosis, oxidative stress and inflammation [17]. These features were successfully replicated in our experimental animal model using a single intraperitoneal injection of DX at a dose of 13.5 mg/kg. DX-treated animals showed several indicators of nephrotoxicity including significant proteinuria, impaired clearance and elevated markers of tubular and glomerular injury. Histologically, renal damage was characterized by glomerulosclerosis, tubular dilatation and

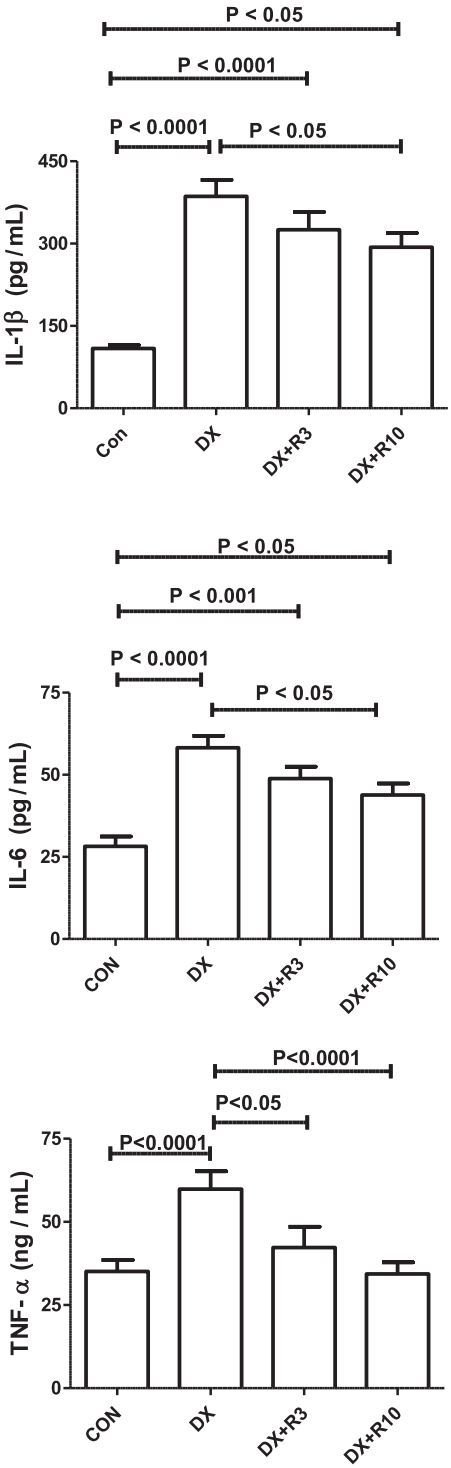

Fig. 2. The plasma levels of tumor necrosis factor-alpha (TNF-α), Interleukin 1-beta (IL-1β), and Interleukin-6 (IL-6) in control rats and those treated with doxorubicin (DX) alone or in combination with two doses of riociguat (3 mg/kg, R3 and 10 mg/kg, R10). Each bar represents the mean ± SEM (n = 6). Differences between groups were assessed using one-way ANOVA followed by Bonferroni's multiple comparison test, with significance set at $P < 0.05$.

necrosis, severe fibrosis and proinflammatory cell infiltration. In addition, DX-treated animals had reduced antioxidant capacity, increased markers of oxidative damage and lipid peroxidation along with increased proinflammatory cytokines.

Treatment with both doses of riociguat improved renal function with parallel improvements in tubular and glomerular injury, tubular

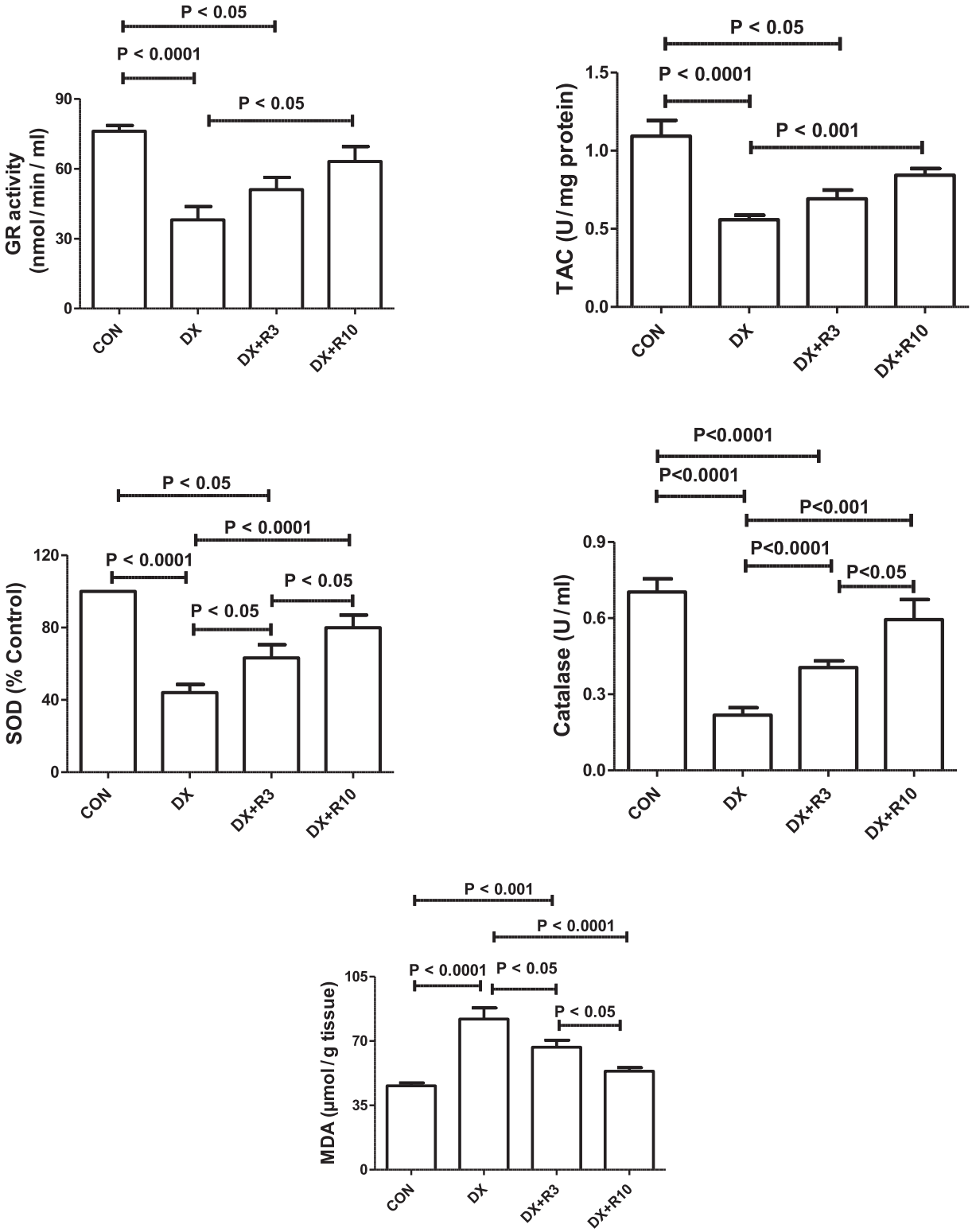

Fig. 3. The renal levels or activity of superoxide dismutase (SOD), catalase (CAT), total antioxidant capacity (TAC), glutathione reductase (GR), and malondialdehyde (MDA) in control rats and those treated with doxorubicin (DX) alone or in combination with two doses of riociguat (3 mg/kg, R3 and 10 mg/kg, R10). Each bar represents the mean ± SEM (n = 6). Differences between groups were assessed using one-way ANOVA followed by Bonferroni's multiple comparison test, with significance set at $P < 0.05$.

necrosis and renal tissue fibrosis. Treatment with riociguat also showed dose-dependent improvements in antioxidant and inflammatory indices. The results of the present study are consistent with earlier studies where riociguat showed significant renoprotective and antifibrotic effects in diverse animal models including hypertensive Dahl salt sensitive, low-renin and high-renin rat models as well as in diabetic endothelial nitric oxide synthase (eNOS) knockout mice [9–11]. More recently, Sravani et al. [19] demonstrated the antifibrotic activity of riociguat in the kidneys with doses as low as 1 mg/kg in animals with unilateral ureteral obstruction– an experimental model that yields progressive renal

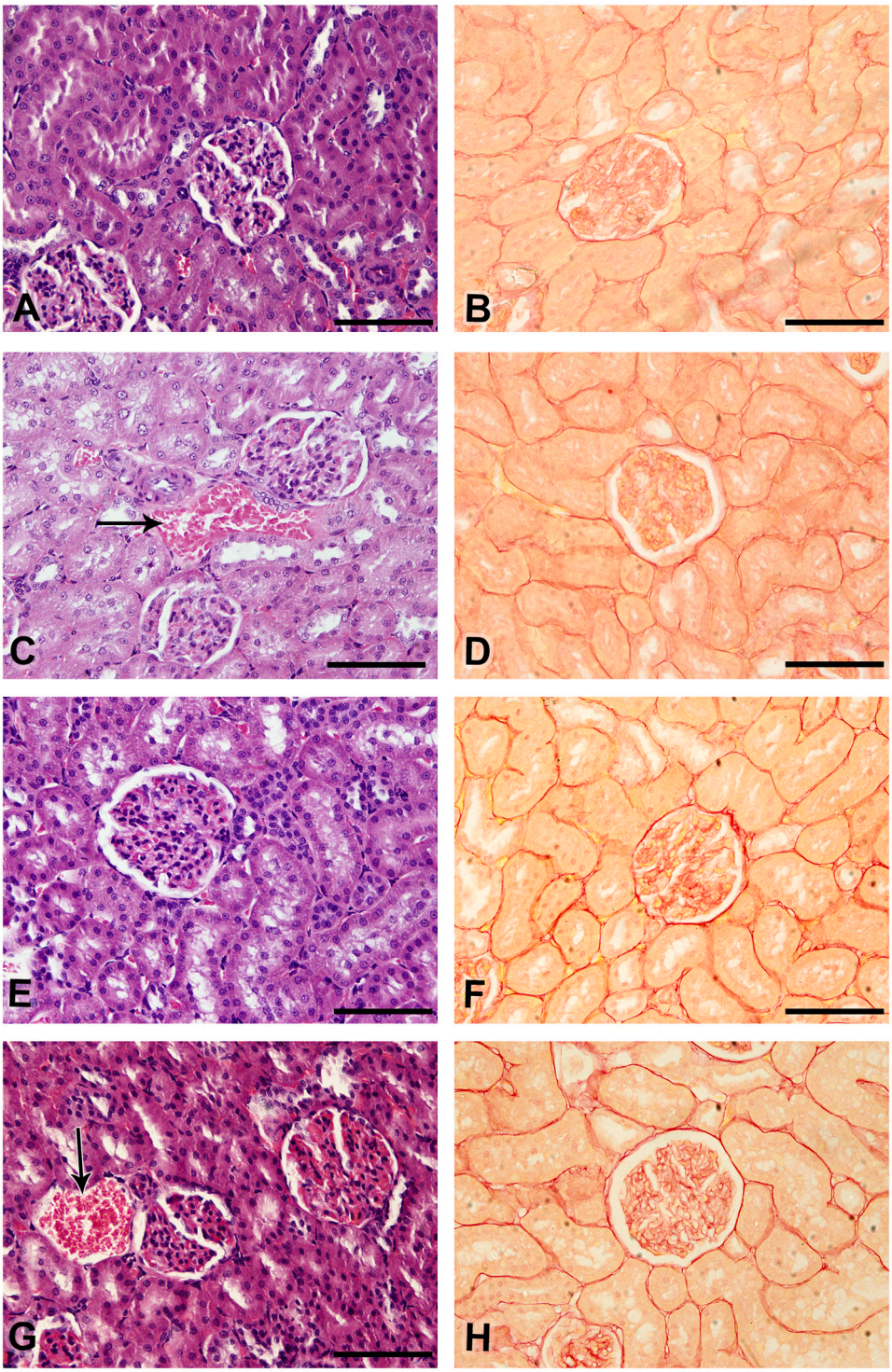

Fig. 4. Photomicrographs of the renal cortex (Bar= 100 μm; A, C, E, G: H&E; B, D, F, G: Picro-Sirius red). (A) The control group displays normal renal histology with intact glomeruli and renal tubules (Score 0). (C) The doxorubicin (DX)-treated group exhibits marked congestion of a blood vessel (arrow), basophilia of the renal tubules, (Score 4). (E) The DX + riociguat (3 mg/kg))-treated group shows mild tubular basophilia with intact glomeruli (Score 2). (G) The DX + riociguat (10 mg/kg)-treated group reveals mild congestion of blood vessel (arrow) with intact glomerular tufts (Score 2). B, D, F, and G highlight the red-stained collagen fibers and yellow-stained non-collagen structures across all four groups.

fibrosis. In fact, the antifibrotic effects of riociguat and other sGC modulators has consistently been shown across multiple tissues and organs including the skin, liver, heart and lungs [21–24]. The main mechanism underlying this effect is largely attributed to the pleiotropic effects resulting from cGMP's inhibition of transforming growth factor (TGF)-β signaling [25,26]. Indeed, TGF-β1 is considered to be the most potent fibrogenic cytokine across all organ systems [27]. Accumulating evidence indicate that upregulating cGMP prevents TGF-β1 mediated extracellular matrix production, cell proliferation and the phenotypic conversion of fibroblasts into myofibroblasts [25]. However, this represents a simple interpretation of what is otherwise a complex pathological process involving several intersecting molecular pathways. For

Table 3
Lesion score and fibrosis index of rats isolated renal cortical tissues.

Treatment	Lesion score (Tubular necrosis)	Fibrosis index %
Control	0	5.4 ± 0.2
Doxorubicin (DX)	4	28.3 ± 1.1[a]
DX+ riociguat (3 mg/kg)	2	11.3 ± 0.3[b]
DX+ riociguat (10 mg/kg)	2	8.4 ± 1.1[b]

Values are means ± SEM (n = 6).
[a] Significant from control
[b] Significant from DX

example, cGMP-dependent protein kinase 1 (PKG) regulates the phosphorylation of numerous substrates that are also implicated in renal fibrosis [28]. The anti-fibrotic actions may further be explained in light of the reciprocal relationship between tumor growth factor (TGF)-β and the redox cycle. That is, while TGF-β1 stimulates the production of reactive oxygen species (ROS) and dampens antioxidant enzymes, it is conversely upregulated by ROS and oxidative stress [27]. In the present study, riociguat counteracted the redox imbalance caused by DX, as evidenced by improvements in total antioxidant capacity, SOD, glutathione reductase activity and catalase as well as a reduction in MDA levels. Thus, restoration of the antioxidant defenses may have contributed to the downregulation of TGF-β and dampening of its profibrogenic impact in the riociguat treatment group. In addition, oxidative stress has been shown to have an inhibitory effect on cGMP signaling, either through direct interaction of ROS with NO or by impairing the ability of cGMP to interact with downstream effector molecules such as cGMP-PKG [19,29]. Hence, the impact of riociguat in reducing oxidative stress may have further contributed to the preservation of renal function against DX-induced injury by correcting these perturbations in the NO-cGMP signaling axis.

Immune cell infiltration and activation of proinflammatory cytokines is a well-recognized response to renal injury [30,31]. This process accelerates kidney damage, drives renal cell apoptosis and exacerbates fibrogenesis [31]. In line with our previous findings and those of other studies, DX-treated animals in this study had increased levels of the proinflammatory cytokines IL-1β, IL-6 and TNF-α [15,18]. Treatment with riociguat significantly reduced the levels of these cytokines in a dose-dependent manner. These results further support the anti-inflammatory effects of riociguat and other sGC modulators in the specific context of renal impairment [19,32,33]. The specific renal anti-inflammatory mechanism of sGC modulators is not fully understood, but it is hypothesized to be partly driven by the blockade of TGF-β signaling [34]. In addition to the well-known role of TGF-β in systemic inflammation, impaired TGF-β1 signaling specifically enhances renal expression of proinflammatory cytokines [35]. However, a recent study examining the effects of the sGC stimulator praliciguat on renal proximal tubular cells suggests that the anti-inflammatory mechanisms may also involve the nuclear factor kappa (NF-κ)B pathway [33]. The authors show that monocyte chemoattractant protein-1, a cytokine that is transcriptionally regulated through the NF-κB signaling pathway, is suppressed in cells treated with praliciguat [33]. Consistent with this observation, praliciguat was found to induce PKG-mediated reduction of NF-κB activity in Kupffer cells, supporting the role of this pathway in the anti-inflammatory actions of sGC stimulators [36]. Given that IL-1β, IL-6 and TNF-α are regulated by both the TGF-β and the NF-κB signaling pathways [37,38], it is difficult to discern from the current study which of these pathways represent the major anti-inflammatory driver of riociguat. It is possible that both pathways, or a compensatory interaction between them, contribute to the observed anti-inflammatory effects of riociguat and other sGC stimulators in kidney disease.

Finally, while the improvements in kidney function may be explained by the antifibrotic, antioxidant and anti-inflammatory properties of riociguat, additional mechanisms possibly contribute to the overall renoprotective effect observed in this study. One possibility is that the vasodilatory property of riociguat [39] enhances the hemodynamic profile of the kidneys, thereby improving renal function. There is also evidence that riociguat inhibits DX-induced overexpression of specific calcium channels which are crucial for the adaptation of podocyte cytoskeleton to changes in glomerular blood pressure [14]. Future research should focus on providing further mechanistic insight into the renoprotective actions of riociguat.

Taken together, these findings support the therapeutic potential of riociguat in the management of renal dysfunction. It is noteworthy that DX has been shown to specifically reduce sGC activity in the heart [40], suggesting that a similar toxicological process contributes to the pathological features of DX toxicity in the kidneys. This further underscores riociguat as a promising candidate for the treatment or prevention of DX-induced AKI.

5. Conclusion

Riociguat significantly mitigated DX-induced AKI in a dose-dependent manner, where the effects were more pronounced with the higher dose. Both low-dose and high-dose riociguat improved renal function as evidenced by reduced serum creatinine and urea and urinary albumin creatinine ratio. In addition, riociguat attenuated DX-induced tubular necrosis and renal fibrosis. Riociguat attenuated oxidative stress and reduced inflammatory markers. Our findings suggest that the renoprotective effects are driven by the anti-inflammatory and antioxidant properties of riociguat, although other mechanisms may also be involved. Future studies should therefore aim to identify these specific mechanisms.

Ethics

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Sultan Qaboos University Medical Research Ethics Committee (SQU/EC-AUR/2021–2022–13).

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Author statement

All the author declare that the work described has not been published previously, that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

CRediT authorship contribution statement

Priyadarsini Manoj: Software, Investigation, Formal analysis. **Haytham Ali:** Writing – review & editing, Software, Methodology, Investigation, Formal analysis. **Aly M Abdelrahman:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Data curation, Conceptualization. **Raya Al-Maskari:** Writing – review & editing, Writing – original draft, Validation, Software, Investigation, Formal analysis. **Yousuf Al Suleimani:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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