The Journal of Basic and Applied Zoology

RESEARCH

Open Access

Protective effect of curcumin on the kidney of diclofenac sodium-challenged mice: apoptotic, redox potential and histopathological outcomes

Sohair M. M. Ragab[1], Mahmoud Abd-Elkareem[2], Nasser S. Abou Khalil[3*] and Mona M. Atia[4]

Abstract

Background: The renal burden imposed by diclofenac sodium (DS) remedy is a significant concern and limits the extension in its clinical application. Curcumin (Cur) can be used as a promising natural phytochemical in rescuing chemotherapy-associated renal dysfunction owing to its redox stabilizing and cytoprotective nature. Thus, the current experiment aims to highlight the possible ameliorative impact of Cur on DS-induced renal damage and its mediating mechanisms in adult male mice.

Methods: A total number of eighteen healthy adult mice of the male sex were classified into 3 groups for 21 days. The first group served as a control, whereas the second one received DS at 10 mg/kg body weight by intraperitoneal route of administration daily during the last 14 days of the experiment. The third group was supplemented with Cur at 100 mg/kg body weight during the entire duration of the intervention in conjunction with the DS burden. At the end of the experimental protocol, kidney functions, redox parameters, histopathological investigation and TUNEL assay were performed.

Results: Cur succeeded in restoring the typical histomorphometric features and reducing the apoptosis in the kidney. The redox disturbances in the kidney of DS-challenged mice rebalanced were manifested by normalizing the level of renal reduced glutathione and immunostaining of glutathione reductase and superoxide dismutase 2. No marked alteration in plasma urea level in the DS group could be noticed compared to the control. Nevertheless, an obvious reduction in plasma urea level was observed in the DS+Cur group relative to the control and DS groups. The comparison between all experimental groups revealed the absence of significant difference in plasma creatinine and renal lipid peroxide levels.

Conclusions: Cur might exert its renoprotective action through its cytoprotective, anti-apoptotic and antioxidant characteristics. The findings of this study shed light on using natural phytochemicals to alleviate the adverse influences of chemotherapies.

Keywords: Nonsteroidal anti-inflammatory drugs, Curcumin, Anti-apoptotic, Redox balance, Renoprotection

Background

Diclofenac sodium (DS) is the most recommended worldwide drug in relieving painful and inflammatory complaints (Ledakowicz et al., 2019). However, its destructive effect on the renal system is a fundamental obstacle restraining its practical utilization (Huo et al.,

*Correspondence: nasser82@aun.edu.eg

[3] Department of Medical Physiology, Faculty of Medicine, Assiut University, Assiut, Egypt
Full list of author information is available at the end of the article

2020) as the kidney is highly susceptible to the harmful impacts of drug metabolites during their disposal (Hosohata, 2016). Following hepatic biotransformation of DS to 4-hydroxydiclofenac and other hydroxylated derivatives, they are excreted mainly in the urine (Kumar et al., 2002). The excretion and accumulation of these conjugates are strongly linked to renal dysfunction and end-stage renal failure (Ahmed et al., 2017).

DS is strongly incriminated in triggering renal cell death by increasing the pro-apoptotic effectors Bax, caspase-3 and cytochrome C, and decreasing in the anti-apoptotic effector Bcl2 (Abdou et al., 2021; Hashem et al., 2020). It also impedes the pathway of oxidative phosphorylation from ATP generation in the renal cells (Bao et al., 2012). The benzoquinone imine intermediates are the end products of the biotransformation of DS. They share in redirecting redox homeostasis toward the oxidant side, resulting in renal destruction (Peter & Prince, 2018). DS causes a marked increase in hydrogen peroxide, malondialdehyde, superoxide dismutase and catalase (Alabi & Akomolafe, 2020). It stimulates the production of reactive oxygen and nitrogen species and depresses the total antioxidant capacity (Aycan et al., 2018). These outcomes are emerged from the ability of DS to modulate the transcript level of nuclear factor erythroid 2-related factor 2; an essential regulator of cellular resistance to oxidants protein levels (Elbaz et al., 2022). DS reduced glomerular filtration and plasma flow with subsequent renal tubular necrosis (Nethathe et al., 2021). DS-intoxicated renal tissues are characterized by several other histopathological affections including shrinkage of the glomerulus, widening of the Bowman's space, congestion of the blood vessels and interstitial edema and mononuclear cellular infiltration (Abdou et al., 2021; Hashem et al., 2020). Serological and histomorphometrical studies revealed a marked decrease in mean volume, numerical density and total number of glomeruli following exposure to DS (Khoshvakhti et al., 2015). These abnormalities are reflected on the kidney functional potency in raising circulating urea and creatinine levels (Ahmed et al., 2017; Aycan et al., 2018). These facts drive toward using phytochemical substances to prevent chemotherapy-induced nephrotoxicity due to their efficacy, safety and attainability.

In folk medicine, curcumin (Cur) is a well-known therapeutic approach for numerous respiratory and gastrointestinal ailments (Araujo & Leon, 2001). According to Oriental beliefs, it has habitually been used in fighting abnormalities linked to inflammatory and peroxidative injury (Trujillo et al., 2013). Most of the biological actions of Curcuma longa L. are attributed to Cur, which is suggested to be a top-ranked alternative in protecting against chemotherapy-associated disturbances as it reduces the production of apoptotic mediators and free radicals. The anti-apoptotic ability of Cur is mediated by activating the DNA repair process (Chen et al., 2017), down-regulating the transcript levels of pro-apoptotic mediators, up-regulating those of anti-apoptotic mediators and inducing cytoprotective proteins (Ben Yehuda Dai et al., 2016; Greenwald et al., 2017). Neutralization of reactive oxygen species, up-regulation of enzymatic antioxidants expression and stimulation of the production of non-enzymatic ones (Barzegar & Moosavi-Movahedi, 2011; Lavoie et al., 2009) reflect the dual functional antioxidant role of Cur.

The above-mentioned multifaceted properties of Cur offer a solid intellectuality for interfering with the different toxicological targets of DS. However, the extent to which Cur can guard against DS-associated renal impairments remains open to debate in the light of its biphasic dose-related effects, rapid degradation, low oral bioavailability and presence of conflict data regarding its effectiveness in combating chemotherapy-induced nephrotoxic injury (Antunes et al., 2001; Moghaddam et al., 2019; Tsuda, 2018). To our knowledge, a single scholarly article indicated the ameliorating impact of Cur on DS-induced nephrotoxicity in Albino rats (Ahmed et al., 2017). The short-term administration of both DS and Cur and complete ignorance of the potential anti-apoptotic and antioxidant expression modulatory effects of Cur add fuel to our study to continue exploring this issue.

Identifying the roles of natural antioxidant agents in offsetting the harms induced by DS is valuable in presenting helpful nutritional methods in combination with chemotherapy to diminish its unwanted effects on vital organs such as the kidney. Thus, this study highlights the possible protective effects of Cur on DS-associated renal impairment in adult mice and its essential mechanistic tools.

Methods

Diclofenac sodium

Diclofenac sodium ampoules (Voltaren® 75 mg/3 ml Novartis Pharma S.A.E. Cairo, Egypt) was obtained from a local registered medical store.

Curcumin

Curcumin from *Curcuma longa* (Turmeric), powder (diferulylmethane, (E,E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, diferuloylmethane, CAS # 458-37-7) was purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA).

Experimental animals

Healthy adult male mice (average body weight 30–40 g, average age 5–6 weeks) were used in the current study.

Animals were obtained from the Animal House, Faculty of Medicine, Assuit University, Assiut, Egypt. They were supplied with water and standard pelleted diets and exposed to typical diurnal photoperiods. The therapeutic intervention began after a 7-day adaptation period.

Experimental groups

Animals were equally allocated into three groups (Fig. 1). The intervention continued for 21 days using a single animal as an experimental unite. One group served as a control which received no treatment except water and

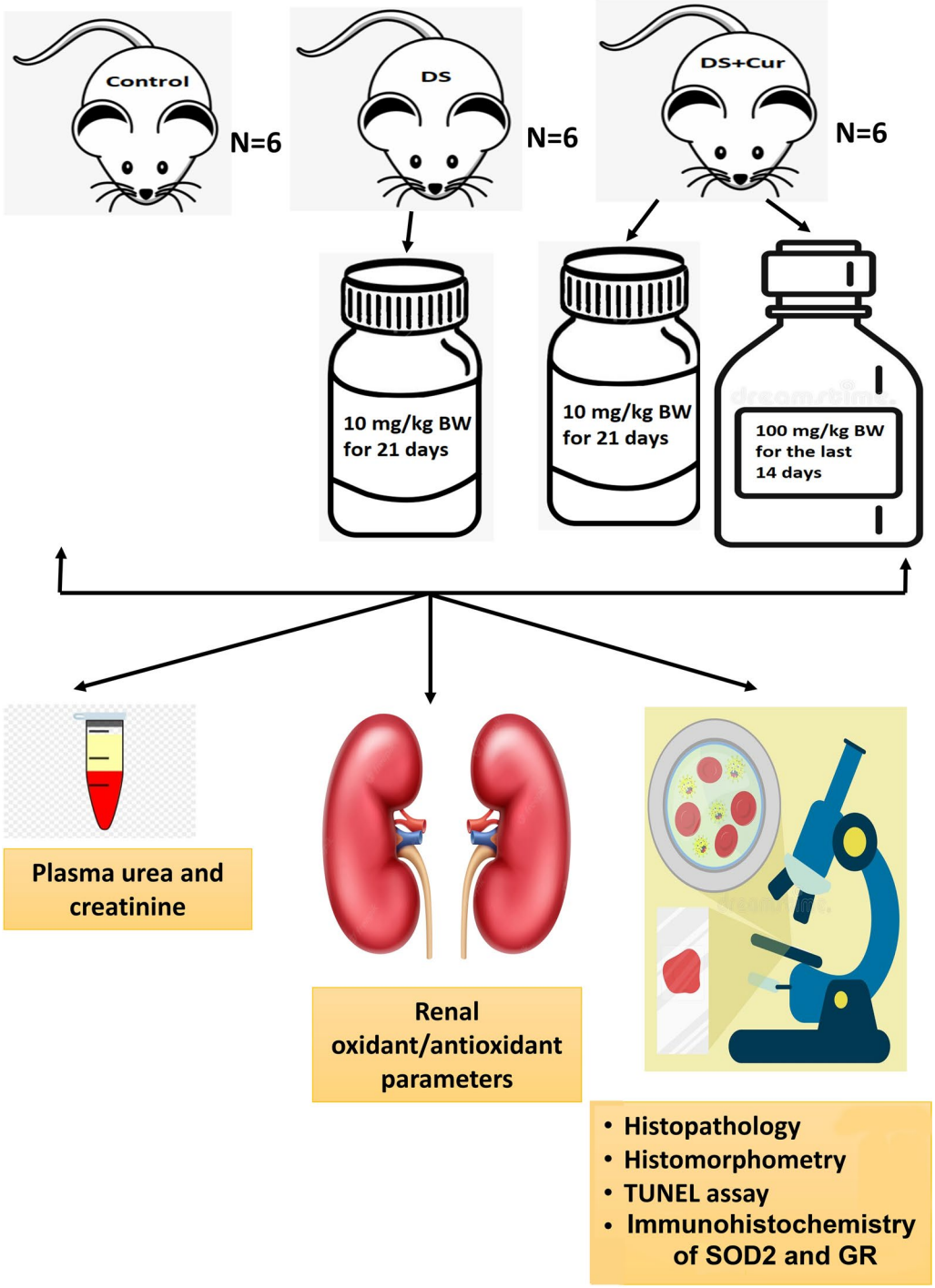

Fig. 1 Chart clarifies the experimental design

food ad libitum, while the second one (DS) was subjected to DS challenge every day by injection of a single dose of 10 mg/kg body weight through intraperitoneal route of injection (Waly et al., 2022) during the last 14 days of the experiment. The third group (DS + Cur) was supplemented with Cur (CAS number 458-37-7, Sigma-Aldrich, St. Lous, MO, USA) daily orally by stomach tube at a dose of 100 mg/kg body weight liquefied in 0.5% carboxymethyl cellulose (Li et al., 2019) along the entire duration of the intervention concurrently with the exposure to DS. The primary outcome measure in this investigation was the changes in renal functional biomarkers following the treatments.

Sampling

At the end of experimental interventions, blood and testicular tissue samples were collected from eighteen healthy adult male mice (6 mice/group) to be subjected to biochemical measurements, histopathological and morphometrical examination and TUNNEL assay.

Collection and preparation of plasma and kidney

Twenty-one days after the start of the experiment, the blood samples were obtained following overnight fasting from a jugular vein in EDTA-containing tubes. The withdrawn samples were centrifuged at 3000 rpm for 10 min to get plasma which was kept at − 20 °C to estimate urea and creatinine later. Mice were euthanized by cervical dislocation, kidneys were harvested and cleaned with normal saline 0.9%. 10% neutral buffered formalin was used for fixation of one kidney for the histological studies. The other kidney was preserved at − 20 °C to evaluate reduced glutathione (GSH) and lipid peroxides (LPO). The supernatant obtained from kidney homogenate were kept at − 20 °C for assessment of redox status. To ensure blinding during the experiment, animal care technician was uninformed of distribution of groups.

Histological and histomorphometrical assessments

Formalin-fixed samples were dehydrated in ascending grades of ethanol, cleared in methyl benzoate and embedded in paraffin wax. Paraffin sections of 5 µm in thickness were cut and stained with the following histological stains: hematoxylin and eosin (H&E) for general histological evaluation, periodic acid Schiff (PAS) technique for the histochemical demonstration of glycogen (Bancroft & Gamble, 2008) and Masson's trichrome method to stain collagen fibers (Crossmon, 1937).

Immunohistochemistry of superoxide dismutase 2 and glutathione reductase

Polyclonal anti-glutathione reductase, anti-superoxide dismutase 2 antibodies (Chongqing Biospes Co., Ltd, China) were utilized for the immunohistochemical detection of glutathione reductase (GR) and superoxide dismutase 2 (SOD2) in the tissue samples. The procedure of immunohistochemistry was following the instructions in Power-Stain™ 1.0 Poly horseradish peroxidase (HRP) DAB Kit (Genemed Biotechnologies, Inc, 458 Carlton Ct. South San Francisco, CA 94,080, USA).

TUNEL assay

Evaluation of apoptosis was performed using In Situ Cell Death Detection Kit, Fluorescein (Sigma-Aldrich) according to a previously published protocol (Abd-Elkareem et al., 2021).

An Olympus BX51 microscope was used to examine all staining preparations, and the photographs were captured by an Olympus DP72 camera linked to the microscope.

Kidney functional and redox parameters

Plasma urea, creatinine and total antioxidant capacity (TAC) were measured colorimetrically using commercial kits (Egyptian Company for Biotechnology, Egypt, catalog # 318,001, 234,001 and TA2513, respectively). Renal total protein level was estimated using Folin–Ciocalteu solution reagent (Lowry et al., 1951), and the concentration was calculated from standard curve. Renal GSH content was assessed using 5,5 dithiobis (2-nitrobenzoic acid) (Beutler, 1963). Tetramethoxypropane was used as external reagent for determination of renal LPO (Ohkawa et al., 1979). Catalase (CAT) activity was measured according to the method of Lück (1963), based on its ability to decompose hydrogen peroxide. All assessed measures were normalized with the total protein concentration in the kidney tissue to avoid the effects of dilution/concentration difference.

Statistical analysis

The data were expressed as a mean ± standard error. One-way analysis of variance was used to determine statistical significance, followed by a Duncan post hoc test. The significance was considered at p values < 0.05. The results were analyzed using SPSS software 16.

Results

Effect of curcumin on histo-architecture and histomorphometry of kidney in diclofenac sodium-challenged mice

Histologically, the kidney of the control group illustrated typical architecture of renal corpuscles, proximal tubules and distal tubules. The renal corpuscle was formed of a normal-sized glomerulus and Bowman's capsule. The glomerulus was a cluster of blood capillaries surrounded by the Bowman's capsule. Bowman's capsule was formed

of a parietal and visceral layer separated by a Bowman's space. The continuous healthy parietal layer of Bowman's capsule was formed of single layer of simple squamous epithelium resting on the basement membrane, while the visceral layer was formed of podocytes. The proximal tubules included large cuboidal cells with well-developed brush borders and rounded centrally located nuclei. Macula densa cells of juxtaglomerular apparatus were modified epithelial cells of the distal convoluted tubules close to the vascular pole of its glomerulus. These cells were numerous tall columnar cells with deeply stained nuclei (Fig. 2A, D and G). The present study revealed that the kidney of DS group showed degenerated renal corpuscles, degenerated proximal tubules, small-sized glomerulus, wide Bowman's space, destructed parietal layer of Bowman's capsule and detached macula densa cells of juxtaglomerular apparatus (Fig. 2B, E and H). The kidney of DS group also showed renal tubules with mildly edematous epithelial lining, intra-tubular cellular debris and intra-tubular hyaline casts (Fig. 3B). The kidney of DS + Cur group showed restoration of the typical architecture of renal corpuscles, proximal tubules, normal size of the glomerulus and Bowman's space, continuous healthy parietal layer of Bowman's capsule and normal macula densa cells of juxtaglomerular apparatus (Fig. 2C, F and I). Also, the kidney of the DS + Cur group showed normal renal tubules with few or no intra-tubular cellular debris and intra-tubular hyaline casts (Figs. 3C).

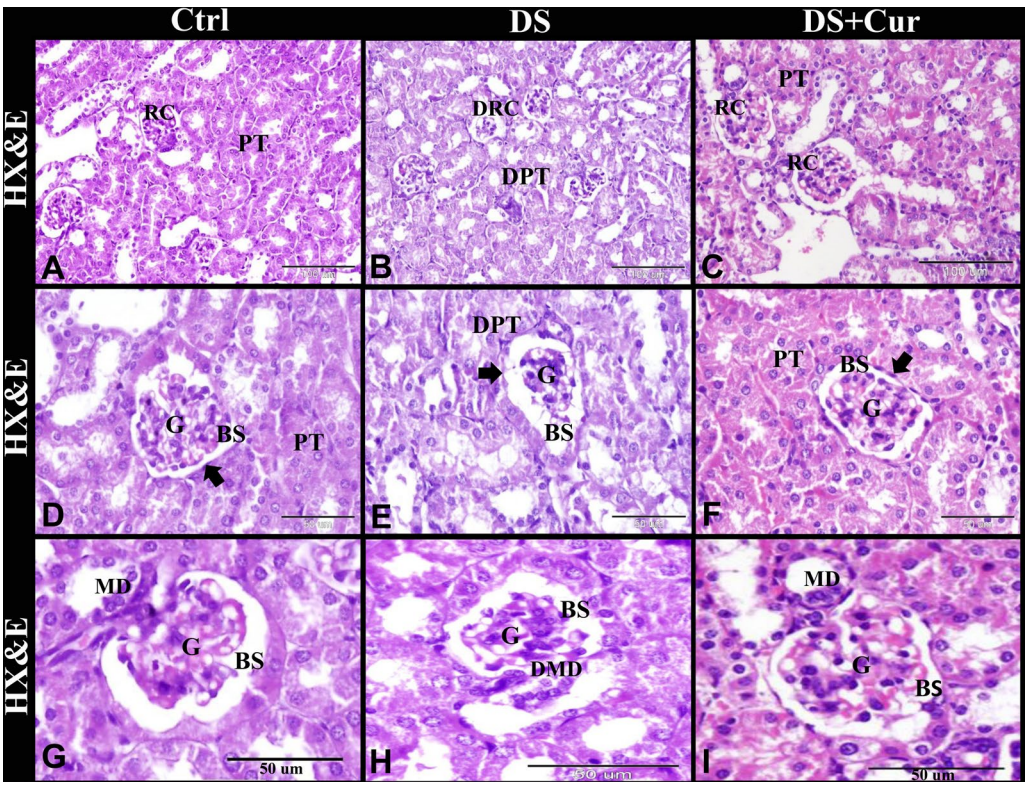

Fig. 2 Photomicrographs of paraffin sections stained with Hematoxylin and Eosin (HX&E) illustrated the protective effect of curcumin on diclofenac sodium induced renal damage in rat. **A** The kidney of control (Ctrl) group showed normal architecture of renal corpuscles (RC) and proximal tubules (PT). **B** The kidney of diclofenac sodium (DS) group showed degenerated renal corpuscles (DRC) and degenerated proximal tubules (DPC). **C** The kidney of diclofenac sodium + curcumin (DS + Cur) group showed restoration of the normal architecture of renal corpuscles (RC) and proximal tubules (PC). **D** The kidney of Ctrl group showed normal-sized glomerulus (G) and Bowman's space (BS), along with continuous healthy parietal layer of Bowman's capsule (arrow) and normal proximal tubules (PT). **E** The kidney of DS group showed small-sized glomerulus (G), wide Bowman's space (BS), destructed parietal layer of Bowman's capsule (arrow) and degenerated proximal tubules (DPC). **F** The kidney of DS + Cur group showed the restoration of the normal size of the glomerulus (G) and Bowman's space (BS), along with continuous healthy parietal layer of Bowman's capsule (arrow) and normal proximal tubules (PT). **G** The kidney of Ctrl group showed normal-sized glomerulus (G) and Bowman's space (BS) and normal macula densa cells of juxtaglomerular apparatus (MD). **H** The kidney of DS group showed small-sized glomerulus (G), wide Bowman's space (BS) and detached macula densa cells of juxtaglomerular apparatus (DMD). **I** The kidney of DS + Cur group showed restoration of the normal size of the glomerulus (G) and Bowman's space (BS) and normal macula densa cells of juxtaglomerular apparatus (MD). Original magnification (A–C) X200, scale bar = 100 µm, (D–I) X400, scale bar = 50 µm

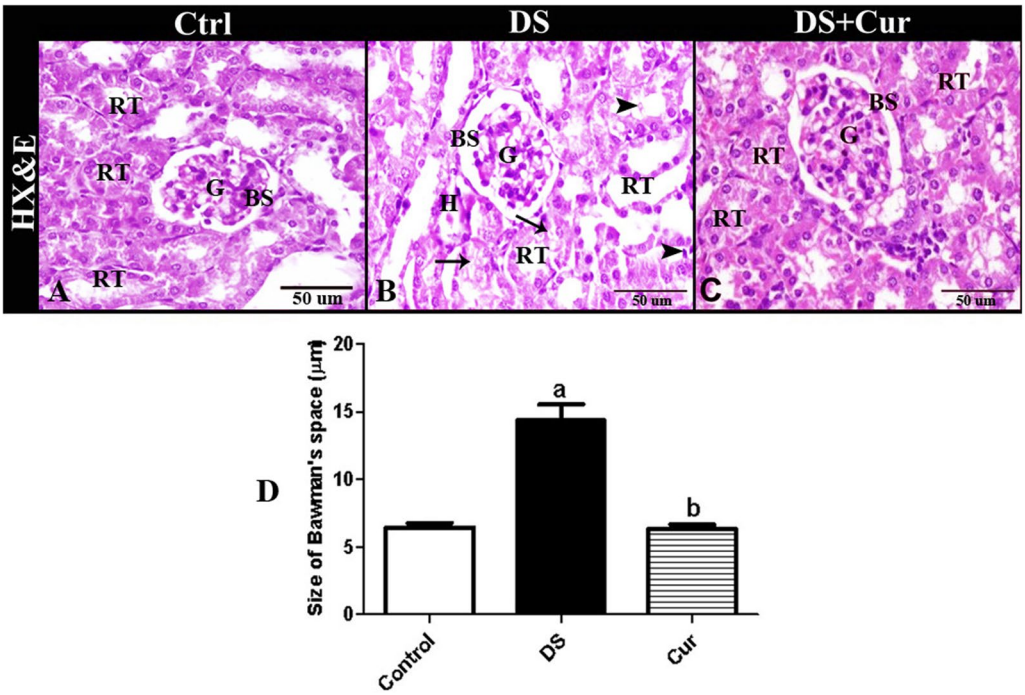

Fig. 3 Photomicrographs of paraffin sections stained with Hematoxylin and Eosin (HX&E) illustrated the protective effect of curcumin (Cur) on diclofenac sodium (DS) induced renal damage in rat. **A** The kidney of control (Ctrl) group showed normal-sized glomerulus (G), Bowman's space (BS) and renal tubules (RT). **B** The kidney of DS group showed small-sized glomerulus (G) and wide Bowman's space (BS) and renal tubules with mildly edematous epithelial lining (arrow), intra-tubular cellular debris (arrowhead) and intra-tubular hyaline casts (H). **C** The kidney of diclofenac sodium + curcumin (DS + Cur) group showed restoration of the normal size of the glomerulus (G), Bowman's space (BS) and normal renal tubules (RT). Original magnification X400, scale bar = 50 µm. **D** Histogram showed that the size of Bowman's space significantly increased in DS group compared to the control group, and oral supplementation of Cur restored the normal size of the Bowman's space. Results are expressed as mean ± SEM (6 mice/group). Data are analyzed by one-way ANOVA followed by Duncan posttest. [a]indicates significant difference between control and DS groups at $p < 0.05$; [b]indicates significant difference between DS and DS + Cur groups at $p < 0.05$

The morphometrical measurements revealed that the number of the renal corpuscles per unit area and glomerular podocytes significantly decreased (Fig. 4B and C, respectively). In contrast, the size of Bowman's space and glomerular vessels increased dramatically in the DS group compared to the control one. Cur supplementation restored these morphometric features to normal (Fig. 3D and 5D, respectively). A significant decrease in the renal corpuscles and glomerulus size was found in the DS group versus the control one. At the same time, the administration of Cur returned these outcome measures to the baseline levels (Fig. 5E and F, respectively). Alternatively, there were insignificant changes in the thickness of the epithelium and brush borders of the proximal tubules in the different groups (Fig. 6G and H, respectively). Nevertheless, there was a significant increase in the diameter of the lumen of the proximal tubules in the DS group in comparison with the control one, and Cur restored it near to normal (Fig. 6I).

Typical collagen contents in glomeruli was found in the kidney of the control group. It also exhibited a normal basement membrane of the renal tubules and of the parietal layer of the Bowman's capsule (Fig. 5A). The kidney of the DS group showed fewer collagen contents in glomeruli. It also displayed a thin, disrupted basement membrane of the renal tubules and of the parietal layer of the Bowman's capsule (Fig. 5B). In contrast, the supplementation with Cur rescued these changes (Fig. 5C).

The kidney of the control group showed average-sized glomerular capillaries and normal proximal tubules with intense PAS-positive brush borders, narrow lumen and continuous PAS-positive renal tubular basement membrane (Fig. 6A and D). However, the kidney of the DS group showed large-sized (widened) glomerular capillaries and proximal tubules with decreased PAS-positive brush borders, wide lumen and disrupted renal tubular basement membrane (Fig. 6B and E). In contrast, the kidney of the DS + Cur group showed restoration of the average-sized glomerular capillaries and normal proximal tubules with intense PAS-positive brush borders, narrow lumen and continuous healthy

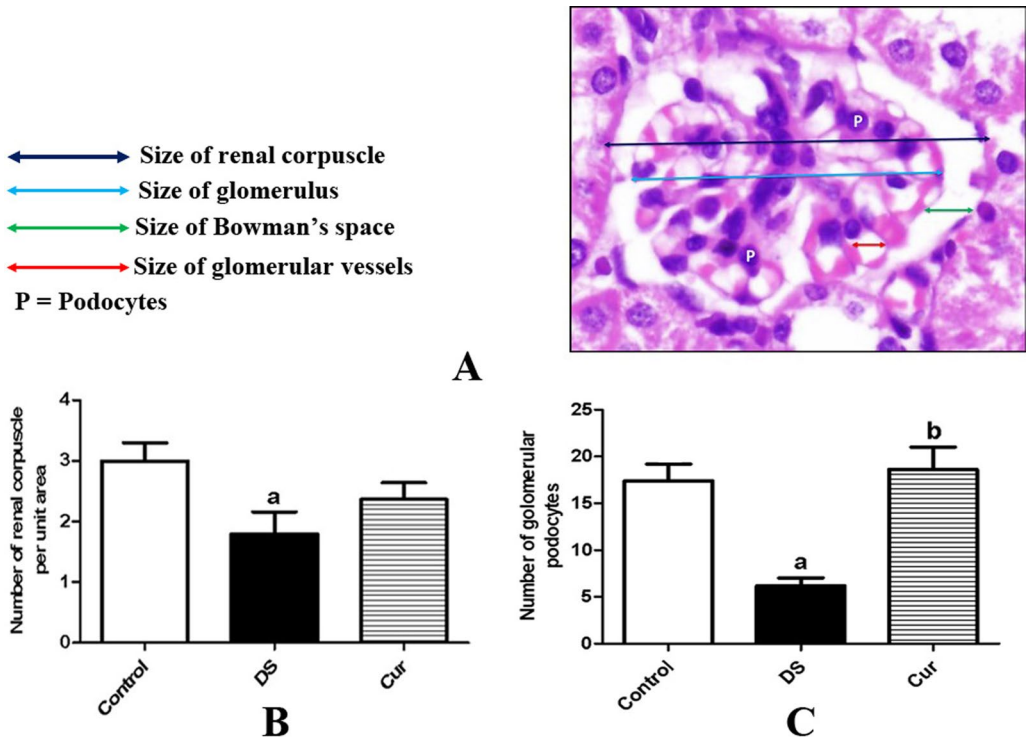

Fig. 4 **A** Photograph showed the measured morphometric parameters in the renal corpuscles. **B** Histogram showed that the number of the renal corpuscles per unit area significantly decreased in diclofenac sodium (DS) group compared to the control (Ctrl) group, and curcumin (Cur) restored the number of renal corpuscles to normal. 3C: Histogram showed that the number of glomerular podocytes significantly decreased in DS group while oral supplementation of Cur prevented this decrease and kept the normal number of glomerular podocytes. Results are expressed as mean ± SEM (6 mice/group). Data are analyzed by one-way ANOVA followed by Duncan posttest. [a]indicates significant difference between control and DS groups at $p < 0.05$; [b]indicates significant difference between DS and DS + Cur groups at $p < 0.05$

PAS-positive renal tubular basement membrane (Fig. 6C and F).

Effect of curcumin on immunostaining of superoxide dismutase 2 and glutathione reductase of kidney in diclofenac sodium-challenged mice

To examine the anti-oxidative effect of Cur, we stained paraffin sections with SOD2 and GR immunostaining. We found that the kidney of the control group exhibited a slight SOD2 and GR immunostaining in the renal corpuscles and tubules (Fig. 7A and D, respectively). While, the kidney of DS group showed mild SOD2 and GR immunoexpression in the renal corpuscles and tubules (Fig. 7B and E, respectively). On the other hand, the kidney of DS + Cur group displayed a minor SOD2 and GR immunostaining in the renal corpuscles and tubules (Fig. 7C and F, respectively).

Effect of curcumin on apoptosis

The kidney of the control group exhibited fewer apoptotic cells in the renal corpuscles and tubules (Fig. 8A and D) than those observed in the DS group (Fig. 8B and E). In contrast, the kidney of the DS + Cur group exhibited few apoptotic cells in the renal corpuscles and tubules (Fig. 8C and F).

All the renal damages induced by DS were mild to moderate, and there were renal compensatory changes to overcome these damages.

Effect of curcumin on kidney functional and redox parameters in diclofenac sodium-challenged mice

Table 1 shows the changes in kidney function and the parameters of redox homeostasis in the DS-challenged mice. The plasma urea level was insignificantly changed in the DS group in comparison with the control one. However, a significant decrease in plasma urea level was observed in the DS + Cur group relative to the control and DS groups. The comparison between all the experimental groups revealed the absence of significant differences in plasma creatinine and renal LPO levels. The disturbance in reductive/oxidative balance in the kidney of DS-exposed mice was evident by a significant elevation in GSH level and reduction in CAT activity in

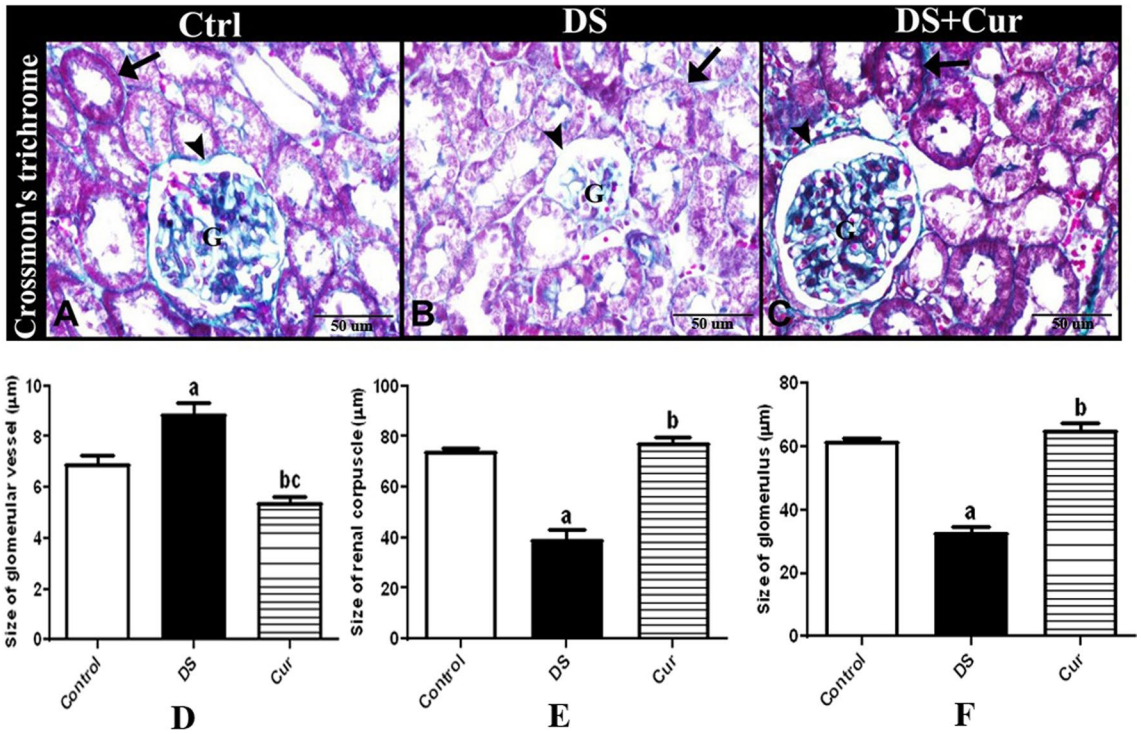

Fig. 5 Photomicrographs of paraffin sections stained with Crossmon's trichrome technique illustrated the protective effect of curcumin (Cur) on diclofenac sodium (DS) induced renal damage in rat. **A** The kidney of control (Ctrl) group showed normal collagen contents in glomeruli (G) in the continuous renal tubular basement membrane (arrow) and in the continuous basement membrane of the parietal layer of the Bowman's capsule (arrowhead). **B** The kidney of DS group showed a less collagen contents in glomeruli (G) in the thin disrupted renal tubular basement membrane (arrow) and in the thin disrupted basement membrane of the parietal layer of the Bowman's capsule (arrowhead). **C** The kidney of diclofenac sodium + curcumin (DS + Cur) group showed restoration of the normal collagen contents in glomeruli (G) in the continuous renal tubular basement membrane (arrow) and in the continuous basement membrane of the parietal layer of the Bowman's capsule (arrowhead). Original magnification X400, scale bar = 50 µm. **D** Histogram showed that the size of glomerular vessels significantly increased in DS group compared to the control group, and oral supplementation of Cur restored the normal size of the glomerular vessels. **E** Histogram showed that the size of the renal corpuscles significantly decreased in DS group compared to the control group, and oral supplementation of Cur restored the normal size of the renal corpuscles. **F** Histogram showed that the size of the glomerulus significantly decreased in DS group compared to the control group, and oral supplementation of Cur restored the normal size of the glomerulus. Results are expressed as mean ± SEM (6 mice/group). Data are analyzed by one-way ANOVA followed by Duncan posttest. [a]indicates significant difference between control and DS groups at $p < 0.05$; [b]indicates significant difference between DS and DS + Cur groups at $p < 0.05$; [c]indicates significant difference between control and DS + Cur groups at $p < 0.05$

comparison with the control group. Oral supplementation with Cur protected against renal redox imbalance as manifested by restoring the renal GSH toward the control level. However, the renal CAT activity of the DS + Cur group was significantly lower than that of the control and DS groups. The TAC of the DS and DS + Cur groups was significantly higher than that of the control group, without a significant difference between DS and DS + Cur groups.

Discussion

The key outcome of this study is the renoprotection of Cur against renal deteriorations driven by DS in mice by restoring the normal histo-architecture and redox homeostasis and suppressing apoptotic cascade. These outcomes open new opportunities in the front for further research investigating the potential protective effects of this natural supplement on other DS-related health hazards.

The histopathological lesions in the kidney of DS-intoxicated mice are consistent with Peter and Prince (2018) and Shafeek et al. (2019). Taken into account the plenty of polyunsaturated fatty acids in renal tissues, DS specifically invades the renal microenvironment to induce oxidative and nitrosative stress compromising the renal cytological patterns, blood flow in addition to glomerular capillary ultrafiltration coefficient (Aycan et al., 2018; Garcia-Cohen et al., 2000; Kubo et al., 1997). The renal microvasculature is constricted and perfusion is impaired owing to the ability of DS to suppress generation of

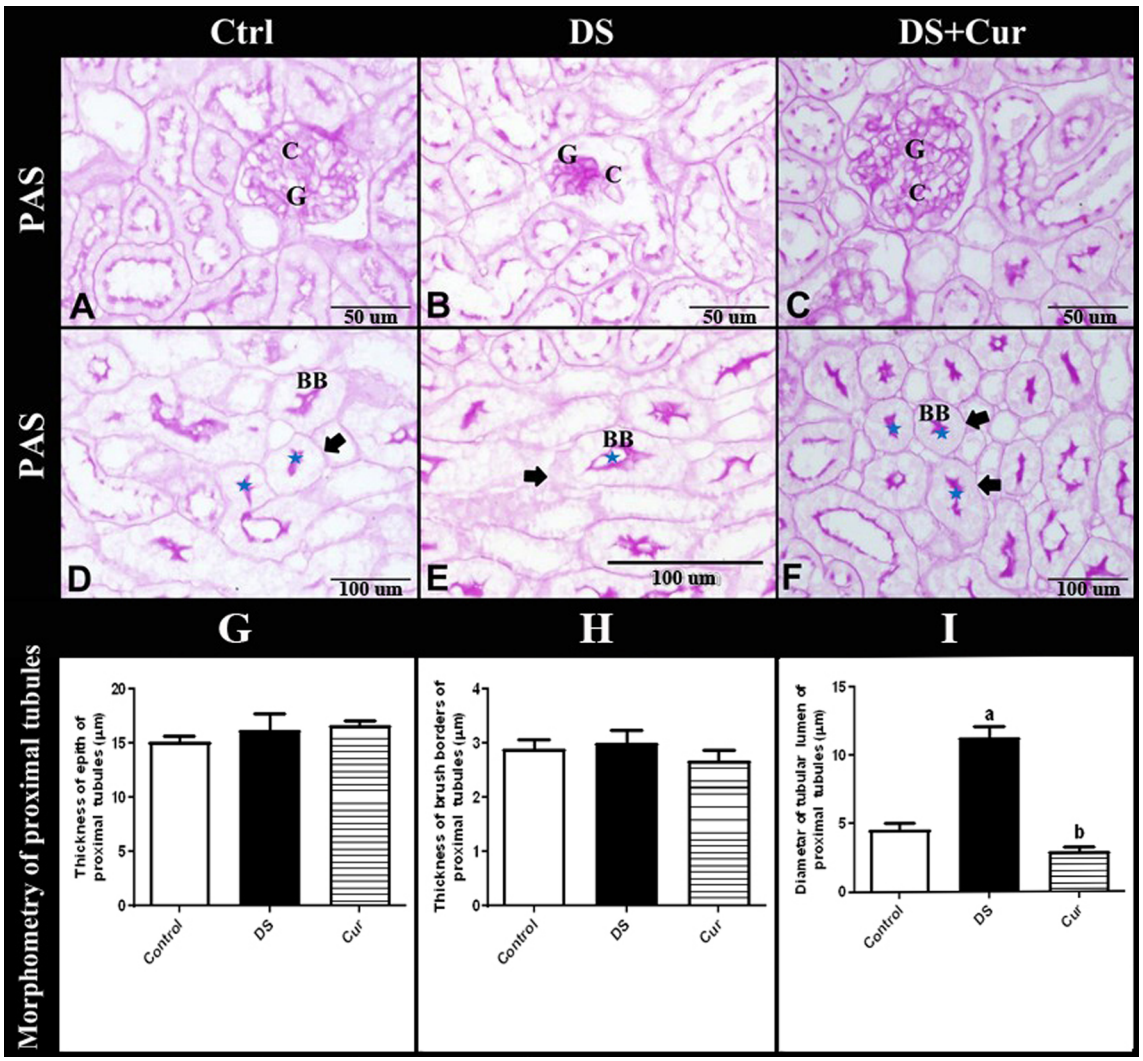

Fig. 6 Photomicrographs of paraffin sections stained with periodic acid Schiff (PAS) illustrated the protective effect of curcumin (Cur) on diclofenac sodium (DS) induced renal damage in rat. **A** The kidney of control (Ctrl) group showed normal-sized glomerulus (G) and glomerular capillaries (C). **B** The kidney of DS group showed small-sized glomerulus (G) and large-sized (widened) glomerular capillaries (C). **C** The kidney of diclofenac sodium + curcumin (DS + Cur) group showed restoration of the normal-sized glomerulus (G) and glomerular capillaries (C). **D** The kidney of control (Ctrl) group showed normal proximal tubules with intense PAS-positive brush borders (BB), narrow lumen (blue star) and continuous healthy PAS-positive renal tubular basement membrane (arrow). **E** The kidney of DS group showed proximal tubules with decreased PAS-positive brush borders (BB), wide lumen (blue star) and disrupted renal tubular basement membrane (arrow). **F** The kidney of DS + Cur group showed restoration of the normal proximal tubules with intense PAS-positive brush borders (BB), narrow lumen (blue star) and continuous healthy PAS-positive renal tubular basement membrane (arrow). Original magnification X400, scale bar = 50 µm. **G** and **H**: Histograms showed that there were no significant changes in the thickness of epithelium and brush borders (respectively) of the proximal tubules in the different groups. **I**: histogram showed that there was a significant increase in the diameter of the lumen of the proximal tubules in DS group compared to the control group, and oral supplementation of Cur restored the diameter of the lumen of the proximal tubules near to normal. Results are expressed as mean ± SEM (6 mice/group). Data are analyzed by one-way ANOVA followed by Duncan posttest. [a]indicates significant difference between control and DS groups at $p < 0.05$; [b]indicates significant difference between DS and DS + Cur groups at $p < 0.05$

vasodilator prostaglandins in the kidneys (Mizuno et al., 2012). This pathological condition promotes renal tubular damage and impairs regenerative ability, which is the leading cause of renal interstitial fibrosis (Wang et al., 2012). Depletion of intracellular ATP synthesis in renal tubular epithelial cells adds another dimension to the nephrotoxic nature of DS (Bao et al., 2012). From a different perspective, excretion and accumulation of the detoxification products of DS have been closely implicated in renal dysfunction and might have direct harmful

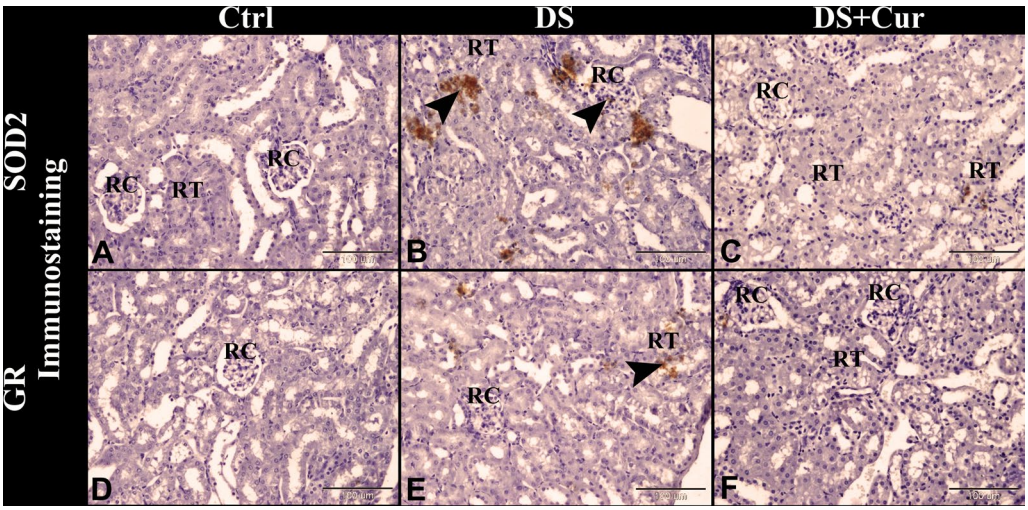

Fig. 7 Photomicrograph of superoxide dismutase 2 (SOD2) (A–C) and glutathione reductase (GR) (D–F) immunostaining in the kidney. **A** The kidney of control (Ctrl) group showed slight SOD2 immunostaining in the renal corpuscles (RC) and tubules (PT). **B** The kidney of diclofenac sodium (DS) group showed mild SOD2 immunoexpression (arrowheads) in the renal corpuscles (RC) and tubules (PT). **C** The kidney of diclofenac sodium + curcumin (DS + Cur) group showed slight SOD2 immunostaining in the renal corpuscles (RC) and tubules (PT). **D** The kidney of Ctrl group showed slight GR immunostaining in the renal corpuscles (RC) and tubules (PT). **E** The kidney of DS group showed mild GR immunoexpression (arrowheads) in the renal corpuscles (RC) and tubules (PT). **F** The kidney of DS + Cur group showed slight GR immunostaining in the renal corpuscles (RC) and tubules (PT). Original magnification, X200, scale bar = 100 µm

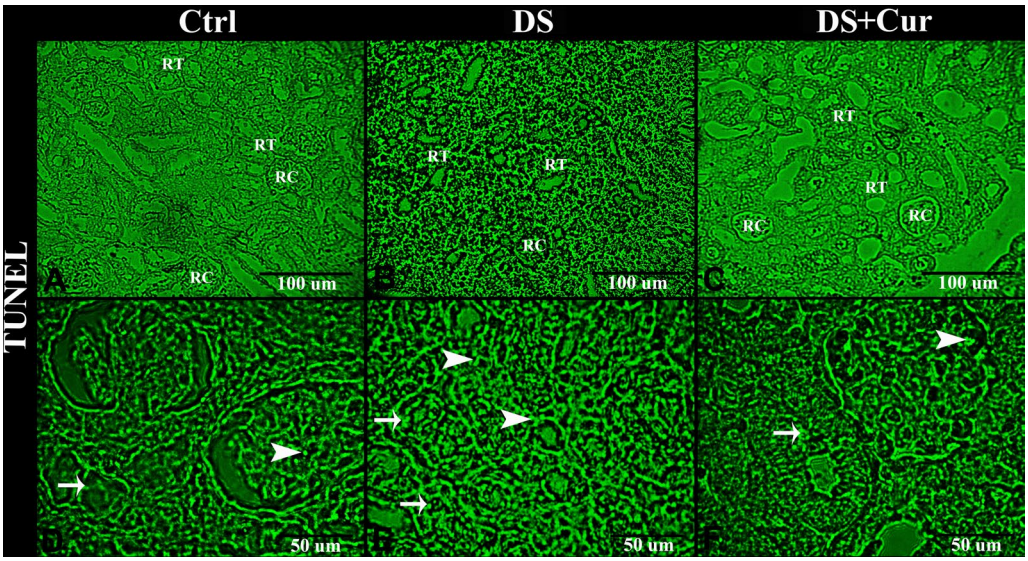

Fig. 8 Fluorescent photomicrograph of TUNEL assay in the paraffin sections of kidney. **A** (low magnification) & **D** (high magnification): The kidney of control (Ctrl) group showed few numbers of apoptotic cells in the renal corpuscles (RC, arrowhead) and tubules (RT, arrow). **B** (low magnification) & **E** (high magnification): The kidney of diclofenac sodium (DS) group showed high number of apoptotic cells in the renal corpuscles (RC, arrowheads) and tubules (RT, arrow). **C** (low magnification) & **F** (high magnification): The kidney of diclofenac sodium + curcumin (DS + Cur) group showed few numbers of apoptotic cells in the renal corpuscles (RC, arrowheads) and tubules (RT, arrow). Original magnification in A–C, X200, scale bar = 100 µm, in D–F X400, scal bar = 50um

effects (Ahmed et al., 2017; Rankin et al., 2008). In addition, CAT deficiency in the DS group rendered the renal tissues more susceptible to oxidative injury and renal fibrosis (Kobayashi et al., 2005).

Table 1 Effects of curcumin on kidney function parameters and oxidant/antioxidant balance of diclofenac sodium-challenged mice

Parameter	Group			
	Control	DS	DS + Cur	*p* value
Plasma urea level (mg/dl)	53.761 ± 3.556	65.000 ± 6.380	33.561 ± 3.049[bc]	0.001
Plasma creatinine level (mg/dl)	0.407 ± 0.024	0.422 ± 0.014	0.486 ± 0.031	0.098
Plasma TAC level (mM/l)	0.043 ± 0.005	0.068 ± 0.005[a]	0.062 ± 0.007[c]	0.022
Renal LPO level (nmol/mg protein)	2.193 ± 0.306	1.864 ± 0.167	1.673 ± 0.187	0.302
Renal CAT activity (U/mg protein)	3.843 ± 0.193	2.655 ± 0.383[a]	0.935 ± 0.162[bc]	0.000
Renal GSH level (µg/mg protein)	34.494 ± 1.604	61.039 ± 6.927[a]	33.165 ± 3.670[b]	0.002

Results are expressed as mean ± SEM (6 mice/group)

Data are analyzed by one-way ANOVA followed by Duncan posttest

DS Diclofenac sodium; *Cur* curcumin; *TAC* total antioxidant capacity; *LPO* lipid peroxides; *CAT* catalase; *GSH* reduced glutathione

[a] indicates significant difference between control and DS groups at $p < 0.05$

[b] indicates significant difference between DS and DS + Cur groups at $p < 0.05$

[c] indicates significant difference between control and DS + Cur groups at $p < 0.05$

The essential structural constituent in the glomerular basement membrane is collagen, which is responsible for ultrafiltration barrier integrity. Loss of this integrity results in a defect in one of the essential functional criteria of glomerular membranes, such as selective permeability (Miner, 2012). The renal tubular basement membrane is responsible for conserving cellular attachment, growth, proliferation and angiogenesis and assists as a growth factor storehouse (van Genderen et al., 2018). Structural and compositional basement membrane defects have been linked to kidney dysfunction and adverse renal outcomes (Jones et al., 2016). Collagen fibers represent the basement membrane's main constituent, forming an extensively cross-linked network to provide a mechanical scaffold (Bhave et al., 2017). Depletion of collagen content in the basal lamina of glomerular membrane and tubular epithelial cells indicates glomerular hyperpermeability and disturbance in the processes of renal handling (Abd-Elkareem et al., 2020; Kotb et al., 2018; Zayed et al., 2018). DS has a suppressive impact on the cellular protein synthesizing machinery by blocking DNA synthesis and mRNA translation, evoking lysosomal damage to release proteolytic enzymes and irreversible protein modification (Barcelos et al., 2017; Dastidar et al., 2000; Nouri & Heidarian, 2019; Pourahmad et al., 2011).

The significant expansion in Bowman's space following DS exposure could result from glomerular atrophy. The presence of glomerular edema may explain the increase in capsular area. Glomerular edema results from obstruction in the renal tubules (Zayed et al., 2018), evident by the presence of edematous epithelial lining, intra-tubular cellular debris and intra-tubular hyaline casts in the renal tubules. Redox disturbance is a main causative factor in renal obstruction by hindering fluid outflow (Jin et al., 2018).

As evident by our findings, dilatation of the glomerular tuft is involved in increased vascular permeability, allowing translocation of fluid from the glomerular space to the renal tubules (Cara-Fuentes et al., 2016). DS causes dilation of glomerular blood vessels corresponding to a previous histological ultrastructural study (Taib et al., 2004). This outcome could antagonize the effect of vasoconstrictor agents through a decline in the levels of prostanoid-derived agents (Annuk et al., 2002). Increased intra-glomerular hydrostatic pressure, as implied by the dilation of glomerular blood vessels, could underlie the separation of viable and non-viable podocytes from the glomerular basal lamina via induction of apoptosis and structural modifications in adhesion proteins (Hamad et al., 2017).

According to our findings concerning the immunoexpression of enzymatic antioxidants, induction of oxidative stress in the renal tissues could be responsible for the decline in glomerular podocyte count by suppressing essential podocyte-related proteins and up-regulating chemokine receptors, which further aggravates podocyte injury through the vicious circle of activation of NADPH oxidase and production of reactive oxidants (Mo et al., 2017). Podocyte depletion leads to instability of the glomerular filtration apparatus, culminating in proteinuria and glomerulosclerosis (Nagata, 2016).

The apparent enhancement in the renal histo-architecture and morphometrical aspects of DS-exposed mice following Cur supplementation is in line with other investigators (Ahmed et al., 2017). Quenching the free oxidants, vasodilation of the renal microvasculature and blocking the apoptotic pathways by Cur (Shao et al., 2019; Trujillo et al., 2016; Wu et al., 2017) could normalize the

renal histopathological changes. The cytoprotective influence of Cur could explain the absence of intra-tubular cast and debris and the restoration of normal histological features of glomerular corpuscles and renal tubule. Reduction in cell detachment and cast development may arise from attenuation in tubular blockage and consequent enhancement of renal functionality. The role of Cur in DNA repair, cell energy preservation and conservation of tissue integrity (Ahmed-Farid et al., 2017) could be implicated in this outcome.

DS increased the protein expression of renal SOD2 and GR and the level of renal GSH in our experimental model. SOD2 is a crucial mitochondrial enzymatic antioxidant that is indispensable for mitigating DS-induced apoptosis (Cecere et al., 2010). GSH is a central participant in the cellular redox network by scavenging reactive oxygen and nitrogen species (Couto et al., 2016). It is binding under the catalytic effect of glutathione-S-transferase allows xenobiotic harmful complexes to convert to a highly soluble harmless ones predisposing to its elimination (Dasari et al., 2018). A compensatory activation and up-regulation in gamma-glutamylcysteine synthetase and cystine/glutamate transporter (Kim et al., 2001; Ray et al., 2002) could be contributory factors in increasing renal GSH levels. DS triggers intracellular reactive oxygen and nitrogen species over generation leading to oxidative stress (Aycan et al., 2018). As stimulation of antioxidant defensive mechanisms was an essential prerequisite to counteract the harmful effects of peroxidative damage, peroxidative insult induces up-regulation in antioxidant defenses by stimulation of redox-dependent transcription mediators (Done & Traustadóttir, 2016). For instance, DS up-regulates the transcript abundance of nuclear factor erythroid 2-related factor 2 (Bao et al., 2017), which controls the expression of a network of genes initiating acclimatization to oxidative stress. In light of our observations, it seems that redox imbalance following DS dosage prompts redox stabilizers such as GSH, GR and SOD2 to afford the cells with a firewall against the harmful influences of reactive oxidants (El-Shafei & Saleh, 2016). Although the insignificant changes in renal LPO levels in the DS group in comparison with the control one contradicts previous observations (Borghi et al., 2018; El-Shafei & Saleh, 2016), peroxidative interactions on the other biological molecules, including protein and DNA cannot be omitted.

Down-regulation of GR following Cur administration is in harmony with that observed previously (Manal, 2016). In the current study, reduced immunostaining of SOD2 and GR in the renal tissue after Cur supplementation contrasts the up-regulation in gene expression of hepatic antioxidants in aflatoxin B1 intoxicated rats (El-Bahr, 2015). This conflicting finding might be owing to alterations in experimental design. The minor immunostaining of SOD2 and GR in the DS + Cur group reflects the inhibition of free radical overgeneration by preserving mitochondrial function and down-regulating some NADPH subunits (Trujillo et al., 2013). From another prospective, the reduction in the immunoexpression of GR explain the drop in GSH level taken into consideration that GR is responsible for maintaining the normal supply of GSH (Couto et al., 2016).

The apoptotic impact of DS on renal tissues goes hand to hand with a previous report (Shafeek et al., 2019). From another perspective, the reduction in the immunoexpression of GR explains the drop in GSH level, considering that GR is responsible for maintaining the normal supply of GSH (Couto et al., 2016). This outcome is mediated by mitochondrial damage, loss of lysosomal membrane integrity and escape of hydrolyzing proteases which result in the emission of cytochrome C from the mitochondrial compartment motivating downstream apoptotic effectors (Pourahmad et al., 2011). Overproduction of free radicals, depletion of ATP content, induction of nuclear fragmentation, overexpression of death ligand tumor necrosis factor-alpha and activation of c-Jun NH2-terminal protein kinase (Ng et al., 2006; Shafeek et al., 2019; Singh et al., 2011) might be also responsible for the apoptotic nature of DS. Conversely, Cur markedly decreased the TUNEL-positive cells similar to that observed in a glycol-induced nephrotoxic rat model by activation of PI3K/Akt pathway resulting in attenuation in release of pro-apoptotic caspases, inhibition of tumor necrosis factor-gamma signaling pathway and reduction of Bax/Bcl-2 ratio (Awad & El-Sharif, 2011; Huang et al., 2018; Wu et al., 2017). A close inspection of our findings revealed that the marked elevation in CAT activity following Cur supplementation could play a role in counteracting apoptosis by maintaining low levels of reactive oxygen species and controlling chromosomal stability (Kang et al., 2013).

In contradiction of a broad spectrum of scientific articles (Borghi et al., 2018; Mousa et al., 2020; Peter & Prince, 2018), the absence of significant modulation in plasma urea and creatinine levels in the DS group represents a surprise. Nevertheless, the dramatic increase in the size of Bowman's space and glomerular vessels in parallel with a significant increase in the diameter of the lumen of the proximal tubules could be part of compensatory mechanisms that stabilize urea and creatinine in the bloodstream. According to our histopathological examination, the injurious modifications in the renal tissues of mice inflicted with DS were of a reasonable degree. Based on our findings, the compensatory up-regulation in SOD2 and GR and a pronounced increase in TAC could play a pivotal role in minimizing the adverse

changes of renal damage biomarkers in the face of DS exposure. El-Shafei and Saleh (2016) found an insignificant change in serum creatinine level of DS-challenged rats. It seems that DS needs an extended period to reach threshold of end-stage renal failure. For example, as an index of kidney injury, the glomerular filtration rate must be reduced to about one-half of its standard value before this become enough to alter plasma urea and creatinine concentrations (Baum et al., 1975). Adaptive mechanisms, including hypertrophy, decrease in vascular resistance and tubular reabsorption in remaining nephrons, allow maintenance of internal homeostasis, even when the kidney mass is reduced to 20–25% of normal (Hall & Hall, 2020). Urea is a principal nitrogenous waste product of protein metabolism. The ability of Cur to decrease plasma urea levels is similar to prior findings (Ahmed et al., 2017) due to increased renal urea clearance (Tirkey et al., 2005). The significant drop in plasma urea in the DS + Cur group contributes in limiting reactive oxidant generation and apoptosis in the kidneys (Vanholder et al., 2018).

Conclusions

The adverse renal consequences of DS were ameliorated by oral intake of Cur in mice by rebalancing redox potential, blocking programmed cell death and providing cytoprotection. These outcomes are of utmost significance in opening windows toward incorporating Cur in fighting the DS-related defects and touching a new ground for exploring its usefulness in combating the other dangerous effects of DS.

Abbreviations

CAT: Catalase; Cur: Curcumin; DS: Diclofenac sodium; GR: Glutathione reductase; GSH: Reduced glutathione; LPO: Lipid peroxides; NSAID: Nonsteroidal anti-inflammatory drug; SOD2: Superoxide dismutase 2; TAC: Total antioxidant capacity.

Acknowledgements

Not applicable.

Author contributions

SMMR and NSA designed the experiment and carried out the biochemical assay. MA and MMA carried out the histological and immunohistochemical examination and TUNEL assay. NSA carried out the statistical analysis and was the main contributor in writing the manuscript. All authors read and approved the final manuscript.

Funding

Not applicable.

Availability of data and materials

All data are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

All experimental protocols were approved by the ethical committee of Faculty of Medicine, Assiut University, Assiut, Egypt, Approval No. IRB17300552, and were performed according to ARRIVE guidelines of animal experiments.

Consent for publication

Not applicable.

Competing interests

The authors declare that there is no competing interests.

Author details

[1]Laboratory of Physiology, Department of Zoology, Faculty of Science, Assiut University, Assiut, Egypt. [2]Department of Cell and Tissues, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt. [3]Department of Medical Physiology, Faculty of Medicine, Assiut University, Assiut, Egypt. [4]Laboratory of Molecular Cell Biology, Department of Zoology, Faculty of Science, Assiut University, Assiut 71516, Egypt.

Received: 9 September 2022 Accepted: 16 November 2022
Published online: 02 December 2022

References

Abd-Elkareem, M., Abou Khalil, N. S., & Sayed, A. E. D. H. (2020). Cytoprotective effect of Nigella sativa seed on 4-nonylphenol-induced renal damage in the African catfish (*Clarias gariepinus*). *Chemosphere, 259*, 127379. https://doi.org/10.1016/j.chemosphere.2020.127379

Abd-Elkareem, M., El-Rahman, A., Mokhless, A., Khalil, N. S. A., & Amer, A. S. (2021). Antioxidant and cytoprotective effects of *Nigella sativa* L. seeds on the testis of monosodium glutamate challenged rats. *Scientific Reports, 11*, 1–16. https://doi.org/10.1038/s41598-021-92977-4

Abdou, R. M., El-Maadawy, W. H., Hassan, M., El-Dine, R. S., Aboushousha, T., El-Tanbouly, N. D., & El-Sayed, A. M. (2021). Nephroprotective activity of Aframomum melegueta seeds extract against diclofenac-induced acute kidney injury: A mechanistic study. *Journal of Ethnopharmacology, 273*, 113939. https://doi.org/10.1016/j.jep.2021.113939

Ahmed, A. Y., Gad, A. M., & El-Raouf, O. M. (2017). Curcumin ameliorates diclofenac sodium-induced nephrotoxicity in male albino rats. *Journal of Biochemical and Molecular Toxicology, 31*(10), e21951. https://doi.org/10.1002/jbt.21951

Ahmed-Farid, O. A. H., Nasr, M., Ahmed, R. F., & Bakeer, R. M. (2017). Beneficial effects of curcumin nano-emulsion on spermatogenesis and reproductive performance in male rats under protein deficient diet model: Enhancement of sperm motility, conservancy of testicular tissue integrity, cell energy and seminal plasma amino acids content. *Journal of Biomedical Science, 24*(1), 66. https://doi.org/10.1186/s12929-017-0373-5

Alabi, Q. K., & Akomolafe, R. O. (2020). Kolaviron diminishes diclofenac-induced liver and kidney toxicity in wistar rats via suppressing inflammatory events, upregulating antioxidant defenses, and improving hematological indices. *Dose-Response, 18*(1), 1559325819899256. https://doi.org/10.1177/1559325819899256

Annuk, M., Fellström, B., & Lind, L. (2002). Cyclooxygenase inhibition improves endothelium-dependent vasodilatation in patients with chronic renal failure. *Nephrology Dialysis Transplantation, 17*(12), 2159–2163. https://doi.org/10.1093/ndt/17.12.2159

Antunes, L. M. G., Darin, J. D. C., & Maria de Lourdes, P. B. (2001). Effects of the antioxidants curcumin or selenium on cisplatin-induced nephrotoxicity and lipid peroxidation in rats. *Pharmacological Research, 43*(2), 145–150. https://doi.org/10.1006/phrs.2000.0724

Araujo, C. A. C., & Leon, L. L. (2001). Biological activities of *Curcuma longa* L. *Memórias Do Instituto Oswaldo Cruz, 96*(5), 723–728. https://doi.org/10.1590/S0074-02762001000500026

Awad, A. S., & El-Sharif, A. A. (2011). Curcumin immune-mediated and anti-apoptotic mechanisms protect against renal ischemia/reperfusion and distant organ induced injuries. *International Immunopharmacology, 11*(8), 992–996. https://doi.org/10.1016/j.intimp.2011.02.015

Aycan, İÖ., Elpek, Ö., Akkaya, B., Kıraç, E., Tuzcu, H., Kaya, S., Coşkunfırat, N., & Aslan, M. (2018). Diclofenac induced gastrointestinal and renal toxicity is alleviated by thymoquinone treatment. *Food and Chemical Toxicology, 118*, 795–804. https://doi.org/10.1016/j.fct.2018.06.038

Bancroft, J. D., & Gamble, M. (2008). *Theory and practice of histological techniques*. Churchill Livingstone.

Bao, H., Ge, Y., Zhuang, S., Dworkin, L. D., Liu, Z., & Gong, R. (2012). Inhibition of glycogen synthase kinase-3β prevents NSAID-induced acute kidney injury. *Kidney International, 81*(7), 662–673. https://doi.org/10.1038/ki.2011.443

Bao, S., Nie, X., Ou, R., Wang, C., Ku, P., & Li, K. (2017). Effects of diclofenac on the expression of Nrf2 and its downstream target genes in mosquito fish (Gambusia affinis). *Aquatic Toxicology, 188*, 43–53. https://doi.org/10.1016/j.aquatox.2017.04.008

Barcelos, R. P., Bresciani, G., Cuevas, M. J., Martínez-Flórez, S., Soares, F. A. A., & González-Gallego, J. (2017). Diclofenac pretreatment modulates exercise-induced inflammation in skeletal muscle of rats through the TLR4/NF-κB pathway. *Applied Physiology, Nutrition, and Metabolism, 42*(7), 757–764. https://doi.org/10.1139/apnm-2016-0593

Barzegar, A., & Moosavi-Movahedi, A. A. (2011). Intracellular ROS protection efficiency and free radical-scavenging activity of curcumin. *PLoS ONE, 6*(10), e26012. https://doi.org/10.1371/journal.pone.0026012

Baum, N., Dichoso, C. C., & Carlton, C. E., Jr. (1975). Blood urea nitrogen and serum creatinine: physiology and interpretations. *Urology, 5*(5), 583–588. https://doi.org/10.1016/0090-4295(75)90105-3

Ben Yehuda Greenwald, M., Frušić-Zlotkin, M., Soroka, Y., Ben Sasson, S., Bitton, R., Bianco-Peled, H., & Kohen, R. (2017). Curcumin protects skin against UVB-induced cytotoxicity via the Keap1-Nrf2 pathway: the use of a microemulsion delivery system. *Oxidative Medicine and Cellular Longevity, 2017*, 5205471. https://doi.org/10.1155/2017/5205471

Beutler, E. (1963). Improved method for the determination of blood glutathione. *Journal of Laboratory and Clinical Medicine, 61*, 882–888.

Bhave, G., Colon, S., & Ferrell, N. (2017). The sulfilimine cross-link of collagen IV contributes to kidney tubular basement membrane stiffness. *American Journal of Physiology-Renal Physiology, 313*(3), F596–F602. https://doi.org/10.1152/ajprenal.00096.2017

Borghi, S. M., Fattori, V., Ruiz-Miyazawa, K. W., Bertozzi, M. M., Lourenco-Gonzalez, Y., Takakihara, R. I., Bussmann, A. J. C., Mazzuco, T. L., Casagrande, R., & Verri, W. A., Jr. (2018). Pyrrolidine dithiocarbamate inhibits mouse acute kidney injury induced by diclofenac by targeting oxidative damage, cytokines and NF-κB activity. *Life Sciences, 208*, 221–231. https://doi.org/10.1016/j.lfs.2018.07.038

Cara-Fuentes, G., Clapp, W. L., Johnson, R. J., & Garin, E. H. (2016). Pathogenesis of proteinuria in idiopathic minimal change disease: molecular mechanisms. *Pediatric Nephrology, 31*(12), 2179–2189. https://doi.org/10.1007/s00467-016-3379-4

Cecere, F., Iuliano, A., Albano, F., Zappelli, C., Castellano, I., Grimaldi, P., Masullo, M., De Vendittis, E., & Ruocco, M. R. (2010). Diclofenac-induced apoptosis in the neuroblastoma cell line SH-SY5Y: possible involvement of the mitochondrial superoxide dismutase. *BioMed Research International, 2010*, 801726. https://doi.org/10.1155/2010/801726

Chen, X., Wang, J., Fu, Z., Zhu, B., Wang, J., Guan, S., & Hua, Z. (2017). Curcumin activates DNA repair pathway in bone marrow to improve carboplatin-induced myelosuppression. *Scientific Reports, 7*(1), 1–11. https://doi.org/10.1038/s41598-017-16436-9

Couto, N., Wood, J., & Barber, J. (2016). The role of glutathione reductase and related enzymes on cellular redox homoeostasis network. *Free Radical Biology and Medicine, 95*, 27–42. https://doi.org/10.1016/j.freeradbiomed.2016.02.028

Crossmon, G. (1937). A modification of Mallorus's connective tissue stain with discussion of the principle involved. *Anatomical Record, 69*, 33–38. https://doi.org/10.1002/ar.1090690105

Dai, C., Li, D., Gong, L., Xiao, X., & Tang, S. (2016). Curcumin ameliorates furazolidone-induced DNA damage and apoptosis in human hepatocyte L02 cells by inhibiting ROS production and mitochondrial pathway. *Molecules, 21*(8), 1061. https://doi.org/10.3390/molecules21081061

Dasari, S., Ganjayi, M. S., Yellanurkonda, P., Basha, S., & Meriga, B. (2018). Role of glutathione S-transferases in detoxification of a polycyclic aromatic hydrocarbon, methylcholanthrene. *Chemico-Biological Interactions, 294*, 81–90. https://doi.org/10.1016/j.cbi.2018.08.023

Dastidar, S. G., Ganguly, K., Chaudhuri, K., & Chakrabarty, A. N. (2000). The anti-bacterial action of diclofenac shown by inhibition of DNA synthesis. *International Journal of Antimicrobial Agents, 14*(3), 249–251. https://doi.org/10.1016/s0924-8579(99)00159-4

Done, A. J., & Traustadóttir, T. (2016). Nrf2 mediates redox adaptations to exercise. *Redox Biology, 10*, 191–199. https://doi.org/10.1016/j.redox.2016.10.003

El-Bahr, S. M. (2015). Effect of curcumin on hepatic antioxidant enzymes activities and gene expressions in rats intoxicated with aflatoxin B1. *Phytotherapy Research, 29*(1), 134–140. https://doi.org/10.1002/ptr.5239

Elbaz, E. M., Ahmed, K. A., & Abdelmonem, M. (2022). Resveratrol mitigates diclofenac-induced hepatorenal toxicity in rats via modulation of miR-144/Nrf2/GSH axis. *Journal of Biochemical and Molecular Toxicology, 36*(9), e23129. https://doi.org/10.1002/jbt.23129

El-Shafei, R. A., & Saleh, R. M. (2016). Pharmacological effects of vitamin C & E on diclofenac sodium intoxicated rats. *Biomedicine & Pharmacotherapy, 84*, 314–322. https://doi.org/10.1016/j.biopha.2016.09.005

Garcia-Cohen, E., Marin, J., Diez-Picazo, L. D., Baena, A. B., Salaices, M., & Rodriguez-Martinez, M. A. (2000). Oxidative stress induced by tert-butyl hydroperoxide causes vasoconstriction in the aorta from hypertensive and aged rats: Role of cyclooxygenase-2 isoform. *Journal of Pharmacology and Experimental Therapeutics, 293*(1), 75–81.

Hall, J. E., & Hall, M. E. (2020). *Textbook of medical physiology* (14th ed.). Elsevier.

Hamad, R. A., Berman, S., Hachmo, Y., Stark, M., Hasan, F., Doenyas-Barak, K., & Efrati, S. (2017). Response of renal podocytes to excessive hydrostatic pressure: a pathophysiologic cascade in a malignant hypertension model. *Kidney and Blood Pressure Research, 42*(6), 1104–1118. https://doi.org/10.1159/000485774

Hashem, K. S., Abdelazem, A. Z., Mohammed, M. A., Nagi, A. M., Aboulhoda, B. E., Mohammed, E. T., & Abdel-Daim, M. M. (2021). Thymoquinone alleviates mitochondrial viability and apoptosis in diclofenac-induced acute kidney injury (AKI) via regulating Mfn2 and miR-34a mRNA expressions. *Environmental Science and Pollution Research, 28*(8), 10100–10113. https://doi.org/10.1007/s11356-020-11313-x

Hosohata, K. (2016). Role of oxidative stress in drug-induced kidney injury. *International Journal of Molecular Sciences, 17*(11), 1826. https://doi.org/10.3390/ijms17111826

Huang, J., Yao, X., Weng, G., Qi, H., & Ye, X. (2018). Protective effect of curcumin against cyclosporine A-induced rat nephrotoxicity. *Molecular Medicine Reports, 17*(4), 6038–6044. https://doi.org/10.3892/mmr.2018.8591

Huo, X., Meng, Q., Wang, C., Wu, J., Wang, C., Zhu, Y., Ma, X., Sun, H., & Liu, K. (2020). Protective effect of cilastatin against diclofenac-induced nephrotoxicity through interaction with diclofenac acyl glucuronide via organic anion transporters. *British Journal of Pharmacology, 177*(9), 1933–1948. https://doi.org/10.1111/bph.14957

Jin, H., Wang, Y., Wang, D., & Zhang, L. (2018). Effects of qingshen granules on the oxidative stress-NF/kB signal pathway in unilateral ureteral obstruction rats. *Evidence-Based Complementary and Alternative Medicine, 2018*, 4761925. https://doi.org/10.1155/2018/4761925

Jones, F. E., Bailey, M. A., Murray, L. S., Lu, Y., McNeilly, S., Schlötzer-Schrehardt, U., Lennon, R., Sado, Y., Brownstein, D. G., & Mullins, J. J. (2016). ER stress and basement membrane defects combine to cause glomerular and tubular renal disease resulting from Col4a1 mutations in mice. *Disease Models & Mechanisms, 9*(2), 165–176. https://doi.org/10.1242/dmm.021741

Kang, M. Y., Kim, H. B., Piao, C., Lee, K. H., Hyun, J. W., Chang, I. Y., & You, H. J. (2013). The critical role of catalase in prooxidant and antioxidant function of p53. *Cell Death & Differentiation, 20*(1), 117–129. https://doi.org/10.1038/cdd.2012.102

Khoshvakhti, H., Yurt, K. K., Altunkaynak, B. Z., Türkmen, A. P., Elibol, E., Aydın, I., Elfide, G. K., Emin, M., & Ö., Kaplan, S. (2015). Effects of melatonin on diclofenac sodium treated rat kidney: a stereological and histopathological study. *Renal Failure, 37*(8), 1379–1383. https://doi.org/10.3109/0886022X.2015.1073556

Kim, J. Y., Kanai, Y., Chairoungdua, A., Cha, S. H., Matsuo, H., Kim, D. K., Inatomi, J., Sawa, H., Ida, Y., & Endou, H. (2001). Human cystine/glutamate transporter: cDNA cloning and upregulation by oxidative stress in glioma cells. *Biochimica Et Biophysica Acta-Biomembranes, 1512*(2), 335–344. https://doi.org/10.1016/s0005-2736(01)00338-8

Kobayashi, M., Sugiyama, H., Wang, D. H., Toda, N., Maeshima, Y., Yamasaki, Y., Masuoka, N., Yamada, M., Kira, S., & Makino, H. (2005). Catalase deficiency renders remnant kidneys more susceptible to oxidant tissue injury and

renal fibrosis in mice. *Kidney International, 68*(3), 1018–1031. https://doi.org/10.1111/j.1523-1755.2005.00494.x

Kotb, A. M., Abd-Elkareem, M., Abou Khalil, N. S., & Sayed, A. E. D. H. (2018). Protective effect of Nigella sativa on 4-nonylphenol-induced nephrotoxicity in *Clarias gariepinus* (Burchell, 1822). *Science of the Total Environment, 619*, 692–699. https://doi.org/10.1016/j.scitotenv.2017.11.131

Kubo, K., Saito, M., Tadokoro, T., & Maekawa, A. (1997). Changes in susceptibility of tissues to lipid peroxidation after ingestion of various levels of docosahexaenoic acid and vitamin E. *British Journal of Nutrition, 78*(4), 655–669. https://doi.org/10.1079/bjn19970181

Kumar, S., Samuel, K., Subramanian, R., Matthew, P. B., Ralph, A. S., Shuet-Hing, L. C., David, C. E., & Thomas, A. B. (2002). Extrapolation of diclofenac clearance from in vitro microsomal metabolism data: role of acyl glucuronidation and sequential oxidative metabolism of the acyl glucuronide. *Journal of Pharmacology and Experimental Therapeutics, 303*(3), 969. https://doi.org/10.1124/jpet.102.038992

Lavoie, S., Chen, Y., Dalton, T. P., Gysin, R., Cuénod, M., Steullet, P., & Do, K. Q. (2009). Curcumin, quercetin, and tBHQ modulate glutathione levels in astrocytes and neurons: importance of the glutamate cysteine ligase modifier subunit. *Journal of Neurochemistry, 108*(6), 1410–1422. https://doi.org/10.1111/j.1471-4159.2009.05908.x

Ledakowicz, S., Drozdek, E., Boruta, T., Foszpańczyk, M., Olak-Kucharczyk, M., Żyłła, R., & Gmurek, M. (2019). Impact of hydrogen peroxide on the UVC photolysis of diclofenac and toxicity of the phototransformation products. *International Journal of Photoenergy, 2019*, 1–11. https://doi.org/10.1155/2019/1086704

Li, Y., Zhang, J., Liu, H., Yuan, J., Yin, Y., Wang, T., Cheng, B., Sun, S., & Guo, Z. (2019). Curcumin ameliorates glyoxylate-induced calcium oxalate deposition and renal injuries in mice. *Phytomedicine, 61*, 152861. https://doi.org/10.1016/j.phymed.2019.152861

Lowry, O. L., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein determination with the Folin phenol reaction. *Journal of Biological Chemistry, 93*, 265–273.

Lück, H. (1963). Catalase. In H. U. Bergmeyer (Ed.), *Methods of enzymatic analysis*. Academic Press.

Manal, I. (2016). Detoxification and antioxidant effects of garlic and curcumin in *Oreochromis niloticus* injected with aflatoxin B 1 with reference to gene expression of glutathione peroxidase (GPx) by RT-PCR. *Fish Physiology and Biochemistry, 42*(2), 617–629. https://doi.org/10.1007/s10695-015-0164-4

Miner, J. H. (2012). The glomerular basement membrane. *Experimental Cell Research, 318*(9), 973–978. https://doi.org/10.1016/j.yexcr.2012.02.031

Mizuno, T., Ito, K., Miyagawa, Y., Ishikawa, K., Suzuki, Y., Mizuno, M., Ito, Y., Funahashi, Y., Hattori, R., & Gotoh, M. (2012). Short-term administration of diclofenac sodium affects renal function after laparoscopic radical nephrectomy in elderly patients. *Japanese Journal of Clinical Oncology, 42*(11), 1073–1078. https://doi.org/10.1093/jjco/hys145

Mo, H., Wu, Q., Miao, J., Luo, C., Hong, X., Wang, Y., Tang, L., Hou, F. F., Liu, Y., & Zhou, L. (2017). CXC chemokine receptor type 4 plays a crucial role in mediating oxidative stress-induced podocyte injury. *Antioxidants & Redox Signaling, 27*(6), 345–362. https://doi.org/10.1089/ars.2016.6758

Moghaddam, N. S. A., Oskouie, M. N., Butler, A. E., Petit, P. X., Barreto, G. E., & Sahebkar, A. (2019). Hormetic effects of curcumin: What is the evidence? *Journal of Cellular Physiology, 234*(7), 10060–10071. https://doi.org/10.1002/jcp.27880

Mousa, A. A., Elweza, A. E., Elbaz, H. T., Tahoun, E. A. E., Shoghy, K. M., & Elsayed, I. (2020). Eucalyptus Globulus protects against diclofenac sodium induced hepatorenal and testicular toxicity in male rats. *Journal of Traditional and Complementary Medicine, 10*(6), 521–528. https://doi.org/10.1016/j.jtcme.2019.11.002

Nagata, M. (2016). Podocyte injury and its consequences. *Kidney International, 89*(6), 1221–1230.

Nethathe, B., Chipangura, J., Hassan, I. Z., Duncan, N., Adawaren, E. O., Havenga, L., & Naidoo, V. (2021). Diclofenac toxicity in susceptible bird species results from a combination of reduced glomerular filtration and plasma flow with subsequent renal tubular necrosis. *PeerJ, 9*, e12002. https://doi.org/10.7717/peerj.12002

Ng, L. E., Vincent, A. S., Halliwell, B., & Wong, K. P. (2006). Action of diclofenac on kidney mitochondria and cells. *Biochemical and Biophysical Research Communications, 348*(2), 494–500. https://doi.org/10.1016/j.kint.2016.01.012

Nouri, A., & Heidarian, E. (2019). Ameliorative effects of N-acetyl cysteine on diclofenac-induced renal injury in male rats based on serum biochemical parameters, oxidative biomarkers, and histopathological study. *Journal of Food Biochemistry, 43*(8), e12950. https://doi.org/10.1111/jfbc.12950

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry, 95*(2), 351–358. https://doi.org/10.1016/0003-2697(79)90738-3

Peter, J., & Prince, S. (2018). Diclofenac-induced renal toxicity in female Wistar albino rats is protected by the pre-treatment of aqueous leaves extract of Madhuca longifolia through suppression of inflammation, oxidative stress and cytokine formation. *Biomedicine & Pharmacotherapy, 98*, 45–51. https://doi.org/10.1016/j.biopha.2017.12.028

Pourahmad, J., Mortada, Y., Eskandari, M. R., & Shahraki, J. (2011). Involvement of lysosomal labilisation and lysosomal/mitochondrial cross-talk in diclofenac induced hepatotoxicity. *Iranian Journal of Pharmaceutical Research, 10*(4), 877–887.

Rankin, G. O., Hong, S., Anestis, D. K., Ball, J. G., & Valentovic, M. A. (2008). Mechanistic aspects of 4-amino-2, 6-dichlorophenol-induced in vitro nephrotoxicity. *Toxicology, 245*(1–2), 123–129. https://doi.org/10.1016/j.tox.2007.12.014

Ray, S., Watkins, D. N., Misso, N. L. A., & Thompson, P. J. (2002). Oxidant stress induces gamma-glutamylcysteine synthetase and glutathione synthesis in human bronchial epithelial NCI-H292 cells. *Clinical & Experimental Allergy, 32*(4), 571–577. https://doi.org/10.1046/j.0954-7894.2002.01294.x

Shafeek, F., Abu-Elsaad, N., El-Karef, A., & Ibrahim, T. (2019). Gum Acacia mitigates diclofenac nephrotoxicity by targeting monocyte chemoattractant protein-1, complement receptor-1 and pro-apoptotic pathways. *Food and Chemical Toxicology, 129*, 162–168. https://doi.org/10.1016/j.fct.2019.04.050

Shao, J., Han, J., Zhu, Y., Mao, A., Wang, Z., Zhang, K., Zhang, X., Zhang, Y., Tang, C., & Ma, X. (2019). Curcumin induces endothelium-dependent relaxation by activating endothelial TRPV4 channels. *Journal of Cardiovascular Translational Research, 12*(6), 600–607. https://doi.org/10.1007/s12265-019-09928-8

Singh, R., Cadeddu, R., Fröbel, J., Wilk, C. M., Bruns, I., Zerbini, L. F., Prenzel, T., Hartwig, S., Brünnert, D., & Schroeder, T. (2011). The non-steroidal anti-inflammatory drugs sulindac sulfide and diclofenac induce apoptosis and differentiation in human acute myeloid leukemia cells through an AP-1 dependent pathway. *Apoptosis, 16*(9), 889. https://doi.org/10.1007/s10495-011-0624-y

Taib, N. T., Jarrar, B. M., & Mubarak, M. M. (2004). Ultrastructural alterations in renal tissues of rabbits induced by diclofenac sodium (Voltaren). *Saudi Medical Journal, 25*(10), 1360–1365.

Tirkey, N., Kaur, G., Vij, G., & Chopra, K. (2005). Curcumin, a diferuloylmethane, attenuates cyclosporine-induced renal dysfunction and oxidative stress in rat kidneys. *BMC Pharmacology, 5*(1), 15. https://doi.org/10.1186/1471-2210-5-15

Trujillo, J., Chirino, Y. I., Molina-Jijón, E., Andérica-Romero, A. C., Tapia, E., & Pedraza-Chaverrí, J. (2013). Renoprotective effect of the antioxidant curcumin: Recent findings. *Redox Biology, 1*(1), 448–456. https://doi.org/10.1016/j.redox.2013.09.003

Trujillo, J., Molina-Jijón, E., Medina-Campos, O. N., Rodríguez-Muñoz, R., Reyes, J. L., Loredo, M. L., Barrera-Oviedo, D., Pinzón, E., Rodríguez-Rangel, D. S., & Pedraza-Chaverri, J. (2016). Curcumin prevents cisplatin-induced decrease in the tight and adherens junctions: Relation to oxidative stress. *Food & Function, 7*(1), 279–293. https://doi.org/10.1039/c5fo00624d

Tsuda, T. (2018). Curcumin as a functional food-derived factor: degradation products, metabolites, bioactivity, and future perspectives. *Food & Function, 9*(2), 705–714. https://doi.org/10.1039/c7fo01242j

van Genderen, A. M., Jansen, J., Cheng, C., Vermonden, T., & Masereeuw, R. (2018). Renal tubular-and vascular basement membranes and their mimicry in engineering vascularized kidney tubules. *Advanced Healthcare Materials, 7*(19), 1800529. https://doi.org/10.1002/adhm.201800529

Vanholder, R., Gryp, T., & Glorieux, G. (2018). Urea and chronic kidney disease: The comeback of the century?(In uraemia research). *Nephrology Dialysis Transplantation, 33*(1), 4–12. https://doi.org/10.1093/ndt/gfx039

Waly, H., Abd-Elkareem, M., Raheem, S. A., & Abou Khalil, N. S. (2022). Berberine protects against diclofenac sodium-induced testicular impairment in mice by its anti-oxidant and anti-apoptotic activities. *Iranian Journal of Basic Medical Sciences, 25*(6), 767. https://doi.org/10.22038/IJBMS.2022.62811.13895

Wang, Y., Fu, W., Wang, H., Liang, Y., Wang, Y., Yao, W., Chen, W., Li, Q., Ying, P. H., & Shi, X. (2012). Renal microvascular injury in chronic aristolochic acid

nephropathy and protective effects of Cozaar. *Renal Failure, 34*(1), 60–67. https://doi.org/10.3109/0886022X.2011.623561

Wu, J., Pan, X., Fu, H., Zheng, Y., Dai, Y., Yin, Y., Chen, Q., Hao, Q., Bao, D., & Hou, D. (2017). Effect of curcumin on glycerol-induced acute kidney injury in rats. *Scientific Reports, 7*(1), 1–11. https://doi.org/10.1038/s41598-017-10693-4

Zayed, A. E., Saleh, A., Gomaa, A., Abd-Elkareem, M., Anwar, M. M., Hassanein, K., Elsherbiny, M. M., & Kotb, A. M. (2018). Protective effect of Ginkgo biloba and magnetized water on nephropathy in induced type 2 diabetes in rat. *Oxidative Medicine and Cellular Longevity, 2018*, 1785614. https://doi.org/10.1155/2018/1785614

Publisher's Note