Chinese Herbal Medicines 15 (2023) 102–109

Contents lists available at ScienceDirect

Chinese Herbal Medicines

journal homepage: www.elsevier.com/locate/chmed

Original Article

Protective effect of *Cistanche deserticola* on gentamicin-induced nephrotoxicity in rats

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ARTICLE INFO

Article history:
Received 17 January 2022
Revised 15 April 2022
Accepted 24 June 2022
Available online 16 December 2022

Keywords:
apoptosis
Cistanche deserticola Y. C. Ma
gentamicin
inflammation
nephrotoxicity
Orobanchaceae
oxidative stress

ABSTRACT

Objective: Gentamicin (GM) is a commonly used aminoglycoside antibiotic, however, renal toxicity has limited its usage. The present study was designed to evaluate the ameliorative effect of *Cistanche deserticola* on GM-induced nephrotoxicity in rats.

Methods: The nephrotoxicity in rats was induced by intraperitoneal administration of GM (100 mg/kg) for 10 consecutive days. Glomerular filtration rate, blood urea nitrogen, creatinine and kidney histopathology were detected to assess the GM-induced nephrotoxicity. The oxidative stress (catalase, superoxide dismutase, glutathione and malondialdehyde) was assessed. The inflammatory response (tumor necrosis factor-α, interleukin-6, myeloperoxidase and nuclear factor-kappa B) and apoptotic marker (Bax and Bcl-2) were also evaluated.

Results: The results showed that water and 75% ethanol extracts of *C. deserticola* (named CDW and CDE, respectively) (100, 200 and 400 mg/kg) in combination with GM could recover the reduction of glomerular filtration rate and enhance the renal endogenous antioxidant capability induced by GM. The increase in the expression of renal inflammatory cytokines (tumor necrosis factor-α and interleukin-6), nuclear protein of nuclear factor-kappa B (p65) and the activity of myeloperoxidase induced by GM was significantly decreased upon CDW or CDE treatment. In addition, CDW or CDE treatment could decrease the Bax protein expression and increase the Bcl-2 protein expression in GM-induced nephrotoxicity in rats significantly.

Conclusion: The study demonstrated that *C. deserticola* treatment could attenuate kidney dysfunction and structural damage in rats induced by GM through the reduction of inflammation, oxidative stress and apoptosis.

1. Introduction

Gentamicin (GM) is one of the aminoglycoside antibiotics and used for the treatment of gram-negative bacterial infection (Nabavi et al., 2012). However, it's reported that about 10%–30% of patients receiving GM treatment will develop nephrotoxicity (Rodrigues et al., 2014). It's widely recognized that oxidative stress occurred in the GM-induced nephrotoxicity, which appears as the increase of lipid peroxidation level and decrease of antioxidant enzyme activities (Lee et al., 2012). Studies have also found that renal inflammation is involved in this process, which is characterized by release of pro-inflammatory cytokines, infiltration of inflammatory cells and activation of nuclear factor-kappa B (NF-κB) signal pathway (Ansari, Raish, Ahmad, Fayaz, et al., 2016). Furthermore, GM can induce the apoptosis of renal tubular epithelial cells as well (El Gamal et al., 2014).

Cistanche deserticola Y. C. Ma (Roucongrong in Chinese), generally referred to as "Ginseng of the deserts", belongs to the Orobanchaceae family (Yang et al., 2019). *C. deserticola* is widely used in China to treat cure renal disorders and infertility. The phenylethanoid glycosides and polysaccharides are major active components isolated from *C. deserticola* (Yuan et al., 2018; Zhi et al, 2021). Recent studies have shown that *C. deserticola* have potential antioxidant (Liu et al., 2018), anti-inflammatory (Jia et al., 2014), anti-osteoporotic (Zhang et al., 2019), hypoglycemia (Xiong et al., 2013), anti-tumor (Yuan et al., 2018) and immunomodulatory (Zhang et al., 2018) effects. However, to the best of our knowledge, the protective effect of *C. deserticola* against GM-induced nephrotoxicity has not been studied. Therefore, in this study, the protec-

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https://doi.org/10.1016/j.chmed.2022.03.008
1674-6384/

tive effects of *C. deserticola* extracts on GM-induced nephrotoxicity in rats are assessed by glomerular filtration rate (GFR), blood urea nitrogen (BUN) and creatinine. The possible mechanisms are explored through oxidative stress, inflammation levels and renal tubular cell apoptosis.

2. Materials and methods

2.1. Plants and materials

Dried *Cistanche deserticola* was collected from Qingtuhu, Minqin County (Gansu Province, China). A voucher specimen was identified by Xicang Yang, Department of Pharmacy, Affiliated Hospital of Gansu University of Chinese Medicine. A voucher specimen (accession number LZU-Y-CD-20190426) of the retained material was preserved at the herbarium of Chinese Medicine and Natural Medicine Research Institute, School of Pharmacy, Lanzhou University.

GM sulfate was obtained from Beijing Solarbio Science & Technology Co., ltd. (Beijing, China); Blood urea nitrogen (BUN), creatinine, superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), malondialdehyde (MDA), myeloperoxidase (MPO) assay kits were purchased from Nanjing Jiancheng Biotechnology Co., ltd. (Nanjing, China); Antibodies against Bax, α-tublin and HRP conjugated secondary antibody were purchased from ImmunoWay Biotechnology Company (Plano, TX, USA). Antibodies against Bcl-2, nuclear factor-kappa B (NF-κB) and β-actin were purchased from GENETEX, Inc (CA, USA). All other reagents used were analytical grade.

2.2. Extracts preparation

The dried succulent stem of *C. deserticola* was crushed into powder and sieved through 850 μm mesh. *C. deserticola* powder (500 g) was extracted thrice by 5 L 75% ethanol and concentrated to obtain ethanol extract, named as CDE. *C. deserticola* powder (500 g) was extracted thrice by 5 L distilled water and concentrated to obtain water extract, named as CDW. Then the extracts were frozen at −80 ℃ and lyophilized. The yield of CDW and CDE were 66.29% and 58.91%, respectively.

2.3. Chemical characterization analysis

The carbohydrates content of CDW or CDE was measured using the phenol–sulfuric acid method using D-glucose as the standard (Bai et al., 2018). The contents of echinacoside and verbascoside in CDW or CDE were determined by high performance liquid chromatography (HPLC). The HPLC systems (Agilent 1260, Santa clara, CA, USA) were connected with an autosampler and a diode array detector. Agilent ZORBAX C_{18} column (250 mm × 4.6 mm, 5 μm) was used to perform analysis with following chromatographic conditions: A mobile phase consisting of 0.2% phosphoric acid solution (A) and 50% methanol–acetonitrile (B) in the ration of 75:25 (volume percentage) was run at a flow rate of 1 mL/min for 20 min. The injection volume was 10 μL. Detection was performed at a wavelength of 330 nm at 25 °C.

2.4. Experimental animals

Adult male Wistar rats weighing between 180 and 250 g were obtained from Experimental Animal Center in Lanzhou University, China (the license number SCXK (Gan) 2018–0002). Animals were housed under controlled environmental conditions at room temperature [(25 ± 2) °C] with a 12 h light–dark cycle and humidity of 55%−60%, and maintained on a basal diet and water ad libitum. All animal experiments were carried out ethically approved by the Ethics Committee of School of Pharmacy, Lanzhou University (LZU-Y-CD-20190426), and conformed to the ethical standards for the use of animals in research.

2.5. Experimental design

After one-week adaptive feeding, 80 rats were randomly divided into 10 groups (A − J) of eight rats for each:

Group A, normal group (NC): Rats were intraperitoneally (i.p.) injected with 0.9% sodium chloride saline from the 6th d and lasted for 10 consecutive days.
Group B, model group (GM): Rats were treated with GM 100 mg/kg.bw (i.p.) from the 6th d and lasted for 10 consecutive days.
Groups C and D, CDW or CDE groups (Negative): Rats were treated orally with 200 mg/kg.bw CDW or CDE for 16 consecutive days.
Groups E, F and G, CDW + GM groups (GCDW 100, 200, 400): Rats were treated orally with CDW 100, 200 and 400 mg/kg.bw for 16 d and treated with GM 100 mg/kg.bw (i.p.) from the 6th d and lasted for 10 consecutive days.
Groups H, I and J, CDE + GM groups (GCDE 100, 200, 400): Rats were treated orally with CDE 100, 200 and 400 mg/kg.bw for 16 d and treated with GM 100 mg/kg.bw (i.p.) from the 6th d and lasted for 10 consecutive days.

The dose of *Cistanche* extracts were selected according to literature reports (Zhang et al., 2019). At the end of the study, whole blood samples were collected using abdominal aortic blood collection to obtain serum for identification of serum-specific renal function parameters (BUN and creatinine). The right kidney tissue was harvested for histological studies. Another kidney was frozen in liquid nitrogen and stored at −80 ℃ for other studies.

2.6. Glomerular filtration rate (GFR) measurement

In this study, a novel device was used for the transcutaneous measurement of the elimination kinetics of the fluorescent-labeled exogenous GFR marker fluorescein isothiocyanate (FITC)-sinistrin in freely moving rats (Schock-Kusch et al., 2011; Ellery et al., 2015; Cunningham et al., 2018). Briefly, after intraperitoneal injection of GM on the 16th d, rats were anesthetized and all of the hair on the upper back below the ears were shaved and removed to reduce interference or auto-fluorescence of sinistrin. The determination of GFR was performed on a miniaturized device (NIC-Kidney, Mannheim Pharma & Diagnostics, Mannheim, Germany) composed of 2-light-emmiting diodes that can transcutaneously excite and measure the clearance of FITC-sinistrin. The baseline fluorescence was collected for 2–3 min, followed by a bolus injection of FITC-sinistrin (5 mg/100 g body weight in 0.2 mL of 0.9% irrigation saline). Continuous fluorescence was measured for 2 h and clearance curves were analyzed using the MPD Lab Ver 1.0RC3 software. The half-life ($t_{1/2}$) for the clearance of FITC-sinistrin was determined after the injection of 120 min using one-compartment model. The $t_{1/2}$ value was converted to GFR (mL/min/100 g body weight) using the following semi-empirical equation developed and validated by the manufacturer: GFR = 31.26 [mL/100 g body weight]/$t_{1/2}$ [min] (Cunningham et al., 2018; Weber et al., 2018; Pastor-Arroyo et al., 2018).

2.7. Histological analysis

Renal tissues were fixed with 4% paraformaldehyde. They were then dehydrated with 75% alcohol and embedded in paraffin. The blocks were cut into 4-μm-thick sections, stained with hema-

toxylin and eosin (H&E), and observed under a light microscope (×200).

2.8. Measurement of oxidative stress markers

Kidneys were homogenized in phosphate-buffered saline using tissue homogenizer (Shanghai Onebio Technology Co., Ltd., Shanghai, China) and then centrifuged at 10 000 rpm for 10 min. The precipitate was discarded and protein content was measured in the supernatant using a Coomassie Brilliant Blue assay kit (Nanjing Jiancheng Biotechnology, Nanjing, China). Supernatant aliquots were used to determine oxidative markers, including glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT). The measurements were performed according to the instruction of each commercial kit attached.

2.9. Myeloperoxidase (MPO) levels

MPO is an enzyme expressed in neutrophils that usually is used as an indirect indicator of lipid peroxidation in tissue samples (Ansari, Raish, Ahmad, Fayaz, et al., 2016). MPO concentrations in kidney tissue samples were estimated using commercial kit (Nanjing Jiancheng Biotechnology, Nanjing, China).

2.10. Determination of inflammatory cytokines gene expression

The total RNA was extracted from spleen using the Trizol reagent. The resulting RNA was reverse transcribed using a Hifair™ II 1st Strand cDNA Synthesis SuperMix Kit (Yeasen, China). The RT reaction was performed at 42 °C for 15 min and 85 °C for 2 min to prepare cDNA which was then stored at −80 °C for use. Real-time PCR was carried out using an LightCycle 480 real-time PCR system (Roche, Munich, Germany) and a real-time PCR master mix (SYBR Green) reagent kit (Yeasen, China). Custom-made primers included the following:

tumor necrosis factor-α (TNF-α), 5′-CAGCAGATGGGCTG TACCTT-3′ (forward) and 5′-AAATGGCAAATCGGCTGACG'(reverse);
interleukin-6 (IL-6), 5′-CTCTCCGCAAGAGACTTCCA-3′ (forward) and 5′-TGGTCTGTTGTGGGTGGTATC-3′ (reverse);
GAPDH, 5′-AGTGCCAGCCTCGTCTCATA-3′ (forward) and 5′-GAGAAGGCAGCCCTGGTAAC −3′ (reverse).

The conditions for real-time PCR were 95 °C for 5 min, then 40 cycles at 95 °C for 10 s, 60 °C for 30 s, and 95 °C for 15 s, 60 °C for 1 min, 95 °C for 15 s. Relative expression was calculated by the $2^{-\triangle\triangle CT}$ method (Livak & Schmittgen, 2001).

2.11. Western blot assay

The kidney from each group frozen at − 80 °C were homogenized in an ice bath with lysis buffer for 5 min and centrifuged (12 000 *g* for 30 min). The concentration of protein was determined by BCA method. The denatured proteins were loaded onto 12% SDS-PAGE by electrophoresis, transferred to PVDF membranes and probed with primary antibody against Bax (1:1000), Bcl-2 (1:000), NF-κB (p65) (1:1000) according to the manufacturer's instructions. 5% BSA (prepared in TBST containing 0.1% Tween 20) was used to block the membranes at 4 °C with gentle shaking, overnight. After washing with TBST, the membranes were incubated at room temperature for 1 h with secondary antibody (1:5000). The signals were analyzed by ECL Western Blotting Substrate and quantified by Tanon 5200 Chemiluminescence imaging system (Tanon Science & Technology Co., Ltd., Shanghai, China).

2.12. Statistical analysis

Results were expressed as means ± SD. All data were analyzed by one-way ANOVA with Tukey post-test. Significance was defined as $P < 0.05$.

3. Results

3.1. Chemical characterization of CDW and CDE

Total carbohydrates contents of CDW and CDE were 72.13% and 62.00%, respectively. The contents of echinacoside and verbascoside in CDW were 3.80 mg/g and 0.17 mg/g, respectively. The contents of echinacoside and verbascoside in CDE were 3.90 mg/g and 0.78 mg/g, respectively. The HPLC chromatogram of CDW and CDE were shown in Fig. 1.

3.2. Effect of C. deserticola on GM-induced renal dysfunction

Compared to normal rats, GM induced a significant reduction ($P < 0.01$) in GFR (Fig. 2A). Compared to GM group rats, rats treated with CDW or CDE at 100, 200, 400 mg/kg doses along with GM significantly increased GFR (CDW: 100, 200, 400 mg/kg, $P < 0.01$; CDE: 100 mg/kg, $P < 0.05$; 200, 400 mg/kg, $P < 0.01$). In addition, the levels of serum specific renal function parameters such as BUN (Fig. 2B) and creatinine (Fig. 2C) were significantly ($P < 0.01$) increased in GM group rats than normal rats. Compared with GM administered rats, co-administration of CDW or CDE at all doses (100, 200 and 400 mg/kg) with GM have significantly decreased serum levels of BUN (CDW: 100 mg/kg, $P < 0.05$; 200, 400 mg/kg, $P < 0.01$; CDE: 200, 400 mg/kg, $P < 0.05$) and creatinine (CDW: 200, 400 mg/kg, $P < 0.01$; CDE: 200, 400 mg/kg, $P < 0.01$). CDW showed better potent protective effects against GM-induced nephrotoxicity than CDE (GFR: 100, 400 mg/kg, $P < 0.01$; BUN: 200 mg/kg, $P < 0.01$; 400 mg/kg, $P < 0.05$; creatinine: 200 mg/kg, $P < 0.05$). The differences in GFR and serum BUN and creatinine levels in negative groups (CDW and CDE groups) were insignificant ($P > 0.05$) when compared to normal rats.

3.3. Histopathological examination

As shown in Table 1 and Fig. 3, light microscopic examination of kidney tissue in NC group (Fig. 3A) showed normal tubular and glomerular structures. GM-intoxicated rats displayed many structural changes (Fig. 3B). Renal tubules showed necrosis and vacuolation with occasional desquamation appeared on epithelial cells of the proximal convoluted tubules. The interstitium showed mild inflammatory cell infiltration, edema, and extravasated red blood corpuscle. Kidney sections of mice given CDW (Fig. 3C–E) or CDE (Fig. 3F–H) simultaneous with GM showed alleviation of GM-induced changes as indicated by the reduction of epithelial vacuolization, tubular necrosis and extravasated red blood corpuscles.

3.4. Effect of C. DESERTICOLA on GM-induced catalase (CAT) activity

Compared to normal rats, GM administration caused a significant decrease of 17.10% on catalase levels [(20.58 ± 1.03) to (17.06 ± 1.07) U/mg port, $P < 0.05$)], which was shown in Fig. 4A. Compared to GM alone group, addition of CDW at the dose of 200 mg/kg body weight to GM significantly restored the levels of catalase with an increase of 16.45% ($P < 0.05$). However, addition of CDE treatment at doses of 100, 200 and 400 mg/kg body weight to GM all had no significant change of the activity of catalase ($P > 0.05$).

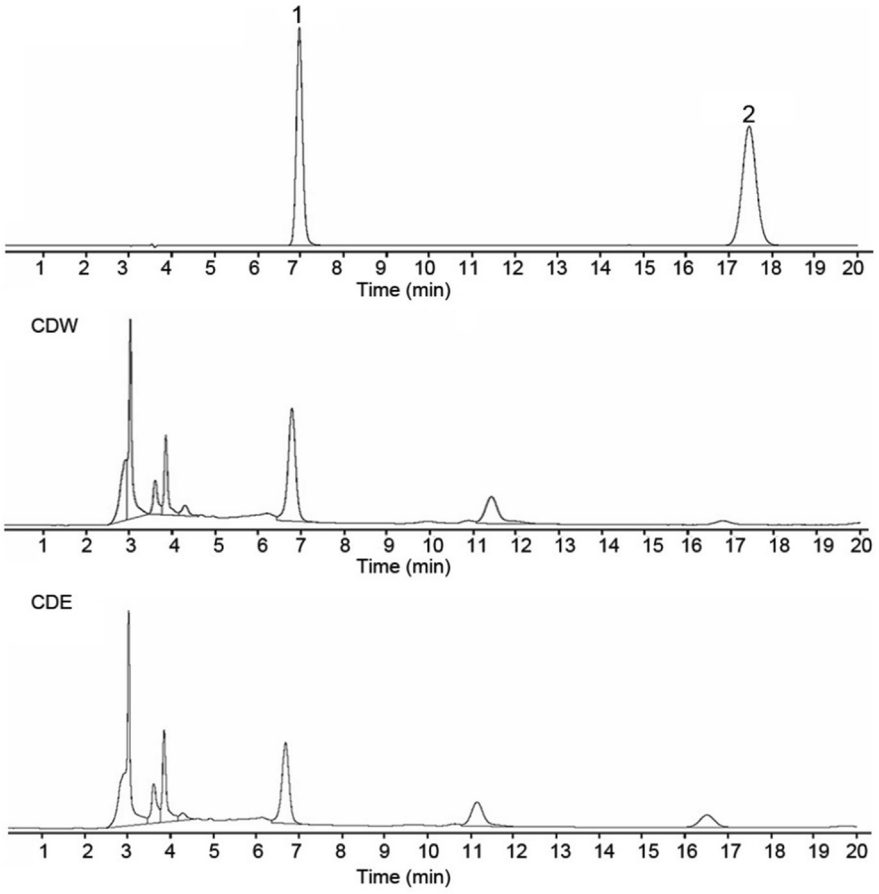

Fig. 1. HPLC chromatographic profile of water and 75% ethanol extracts of *C. deserticola* (CDW and CDE). peak1, echinacoside; peak2, verbascoside.

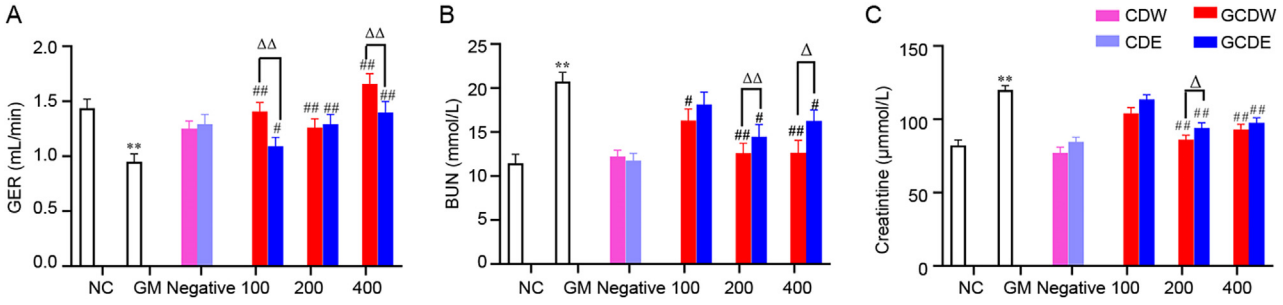

Fig. 2. Effect of *C. deserticola* on gentamicin (GM)-induced changes in glomerular filtration rate (GFR, A) and serum specific renal function parameters of blood urea nitrogen (BUN, B) and creatinine (C). Values are the means ± SD (n = 8). NC, group of rats treated with normal saline daily; GM, group of rats treated with GM (100 mg/kg, i.p.); Negative, group of animals treated with CDW (water extract of *C. deserticola*) or CDE (ethanol extract of *C. deserticola*) (200 mg/kg, p.o.) and normal saline (i.p.); 100, 200, 400, group of animals treated with both CDW or CDE (100, 200, 400 mg/kg, p.o.) and gentamicin (100 mg/kg, i.p.); $^{**}P < 0.01$ *vs* NC group, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ *vs* GM control group, $^{\Delta}P < 0.05$ and $^{\Delta\Delta}P < 0.01$ *vs* GCDE group.

3.5. Effect of C. deserticola on GM-induced superoxide dismutase (SOD) activity

Compared to normal rats, GM administration caused a significant reduction of 26.77% in SOD levels [(75.49 ± 2.51) to (55.28 ± 2.24) U/mg port, $P < 0.05$], which was shown in Fig. 4B. Compared with the GM group, when the oral administration of CDW to rats with GM-induced nephrotoxicity was 100, 200, 400 mg/kg, it could significantly increase the SOD activity of GM-induced rats by 20.34%, 36.00% and 25.67%, respectively (100, 200 mg/kg, $P < 0.01$, 400 mg/kg, $P < 0.05$). Compared to GM group rats, CDE (100, 200 and 400 mg/kg) pretreatment of GM-induced rats could significantly increase the SOD level of GM-induced rats by 20.34%, 36.00% and 25.67% (200 mg/kg, $P < 0.01$, 100, 400 mg/kg, $P < 0.05$). Furthermore, CDW presented significantly stronger effect than CDE at a dose of 200 mg/kg ($P < 0.05$).

3.6. Effect of C. deserticola on GM-induced malondialdehyde (MDA) content

The magnitude of lipid peroxidation as measured by the formation of MDA was shown in Fig. 4C. Compared with the normal rats,

Table 1
Effect of *C. deserticola* on kidney histology scores in all experimental groups (Mean ± SD, n = 3).

Experimental groups	Histopathological rating (mean ± SD)
NC	1.27 ± 0.12
GM	4.32 ± 0.93$^{\#\#}$
GCDW100	3.42 ± 0.28*
GCDW200	2.15 ± 0.37*
GCDW400	2.96 ± 0.45*
GCDE100	3.64 ± 0.62*
GCDE200	2.27 ± 0.42*
GCDE400	2.43 ± 0.29*

Note: $^{\#\#}P < 0.01$*vs* NC group, $^{*}P < 0.05$ *vs* GM group, 1.0, normal; 1.5, very mild, no remarkable lesions noted; 2.0, mild tubular degeneration on the cortex and medulla; 2.5, mild tubular degeneration on the cortex and medulla with very mild fibroblasts proliferation; 3.0, moderate degeneration on the cortex and medulla with mild fibroblasts proliferation; 3.5, moderate to severe tubular degeneration on the cortex and medulla; 4, severe tubular degeneration on the cortex and medulla.

the MDA content of GM-induced nephrotoxicity rats increased significantly by 18.55% [(0.27 ± 0.02) to (0.32 ± 0.02) nmol/mg port, $P < 0.05$], resulting in oxidative stress. Compared with GM group rats, CDW pretreatment at 100, 200 and 400 mg/kg doses resulted in significant reduction of 35.39%, 32.83% and 19.82% respectively in GM-induced oxidative stress in terms of MDA levels (100, 200 mg/kg, $P < 0.01$, 400 mg/kg, $P < 0.05$); CDE pretreatment at 200 and 400 mg/kg resulted in significant reduction of 29.50% and 23.15% respectively in GM-induced oxidative stress in terms of MDA levels (200 mg/kg, $P < 0.01$, 400 mg/kg, $P < 0.05$). In addition, CDW presented significantly stronger effect than CDE at a dose of 100 mg/kg ($P < 0.05$).

3.7. Effect of C. deserticola on GM-induced glutathione (GSH) content

As shown in Fig. 4D, GM-treated rats showed significant decrease in GSH content of 17.65% [(0.51 ± 0.04) to (0.42 ± 0.03)

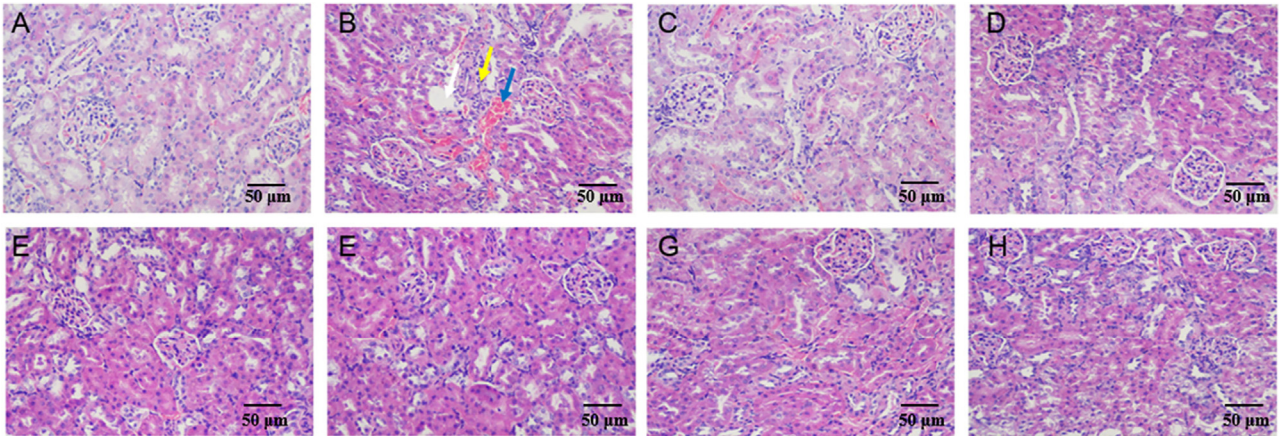

Fig. 3. Representative microscopic images of Hematoxylin & Eosin-stained kidney tissue sections. Light microscopic examination of kidney tissue in (A) NC, (B) GM, (C) GCDW100, (D) GCDW200, (E) GCDW400, (F) GCDE100, (G) GCDE200, (H) GCDE400. White arrow: tubular degeneration; Yellow arrow: massive necrosis and foci of inflammation containing infiltration of inflammatory cells; Blue arrow: the tubular lumen filled with pink color fluid accumulation. Magnification, ×200.

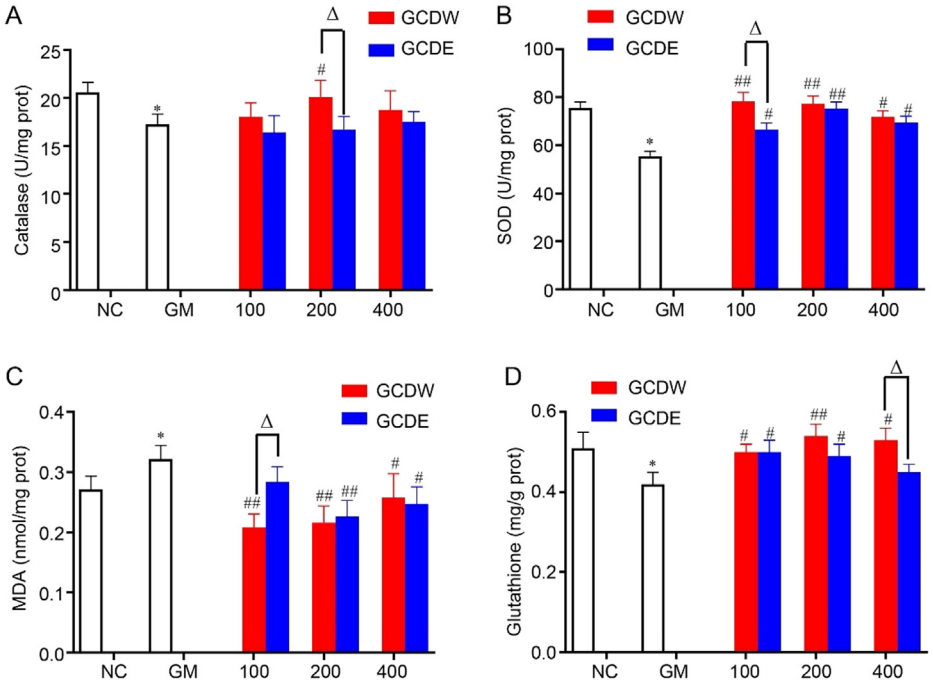

Fig. 4. Effect of *C. deserticola* on GM-induced nephrotoxicity with respect to kidney lipid peroxidation (means ± SD, n = 8). (A) catalase (CAT) activity, (B) superoxide dismutase (SOD) activity, (C) malondialdehyde (MDA) content, and (D) glutathione (GSH) content. $^{*}P < 0.05$ *vs* NC group, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ *vs* GM control group, $^{\Delta}P < 0.05$ *vs* GCDE group.

mg/g port, $P < 0.05$] in comparison with normal rats. In comparison with GM group rats, CDW (100, 200, 400 mg/kg) pretreatment of GM-induced rats could significantly increase GSH content by approximately 18.51%, 28.35% and 25.82% (200 mg/kg, $P < 0.01$, 100, 400 mg/kg, $P < 0.05$); CDE (100, 200 mg/kg) pretreatment of GM-induced rats could significantly increase GSH content by approximately 19.06% and 16.16% (100, 200 mg/kg, $P < 0.05$). In addition, CDW presented significantly stronger effect than CDE at a dose of 400 mg/kg ($P < 0.05$).

3.8. Effect of C. deserticola on GM-induced myeloperoxidase (MPO) levels

As depicted in Fig. 5A, MPO levels increased significantly by 30.99% [(0.71 ± 0.08) to (0.93 ± 0.09) U/g, $P < 0.05$] in the kidneys of GM group rats compared to normal rats. Compared with the GM group rats, CDW (100, 200, 400 mg/kg) pretreatment of GM-induced rats significantly prevented the elevations in MPO levels by approximately 35.63%, 44.58% and 48.06%, respectively (100 mg/kg, $P < 0.05$, 200, 400 mg/100 g, $P < 0.01$); CDE (100, 200, 400 mg/kg) pretreatment of GM-induced rats significantly prevented the elevations in MPO levels by approximately 26.27%, 43.63% and 46.41%, respectively (100 mg/kg, $P < 0.05$, 200, 400 mg/kg, $P < 0.01$). These results indicated that CDE and CDW had anti-inflammatory activity.

3.9. Effect of C. deserticola on GM-induced renal inflammation

As shown in Fig. 5B and C, GM administration significantly upregulate the mRNA levels of TNF-α and IL-6 ($P < 0.01$) when compared to normal rats. CDW or CDE treatment (100, 200, 400 mg/kg) along with GM significantly decreased the TNF-α mRNA (CDW: 100, 200, 400 mg/kg, $P < 0.01$; CDE: 100 mg/kg, $P < 0.05$, 200, 400 mg/kg, $P < 0.01$) and IL-6 mRNA (CDW: 100 mg/kg, $P < 0.05$, 200, 400 mg/kg, $P < 0.01$; CDE: 100, 200 mg/kg, $P < 0.05$, 400 mg/kg, $P < 0.01$) levels in comparison with GM group rats. CDW presented significantly stronger effects than CDE at the dose of 400 mg/kg ($P < 0.05$).

In addition, GM significantly increased expression of the nuclear protein NF-κB (p65) (Fig. 5D and E) which compared to normal rats ($P < 0.01$). Compared to GM group rats, CDW or CDE treatment significantly reduced nuclear NF-κB (p65) protein expression (CDW: 100 mg/kg, $P < 0.05$, 200, 400 mg/kg, $P < 0.01$; CDE: 100, 200, 400 mg/kg, $P < 0.01$). CDW presented significantly stronger effects than CDE (100, 200, 400 mg/kg, $P < 0.05$).

3.10. Effect of C. deserticola on GM-induced renal apoptosis

As shown in Fig. 6, GM administration significantly increased the renal protein expression of Bax ($P < 0.01$) and decreased Bcl-2 ($P < 0.01$) in comparison with normal rats. Compared to GM group rats, addition of CDW or CDE (100, 200, 400 mg/kg) treatment along with GM effectively reduced the GM-induced renal expression of Bax (CDW: 200, 400 mg/kg, $P < 0.01$; CDE: 100, 200, 400 mg/kg, $P < 0.01$) and increased Bcl-2 (CDW: 100, 200, 400 mg/kg, $P < 0.01$; CDE: 100, 200, 400 mg/kg, $P < 0.01$) expression.

4. Discussion

The serious nephrotoxicity limits the use of GM (Nafiu et al., 2019). Thus, it is of great clinical value to seek a therapeutic approach to reverse or protect GM-induced kidney injury. In recent years, many natural plants have been proved to have protective effects against GM-induced nephrotoxicity (Boozari & Hosseinzadeh, 2017). For instance, the gingerol fraction from *Zingiber officinale* had been proved to promoted the protective effect on GM-mediated nephropathy by inflammatory processes, oxidative stress and renal dysfunction (Rodrigues et al., 2014). Red ginseng extract treatment significantly attenuated tubular damage, renal dysfunction and cell apoptosis and decreased ROS production in GM-induced rats (Ansari et al., 2016). *C. deserticola* is an important traditional Chinese herbal for renal dysfunction treatment (Chao et al., 2019). Decoction or medicinal liquor of this plant have been widely used. However, the protective effect of *C. deserticola* on kidney injury caused by GM has not been studied yet. In this study, we aimed to study the renal protective effects of CDW and CDE on GM-induced nephrotoxicity in rats.

GFR is an important index in detecting kidney function (Endre et al., 2011). To determine whether kidney function decline in

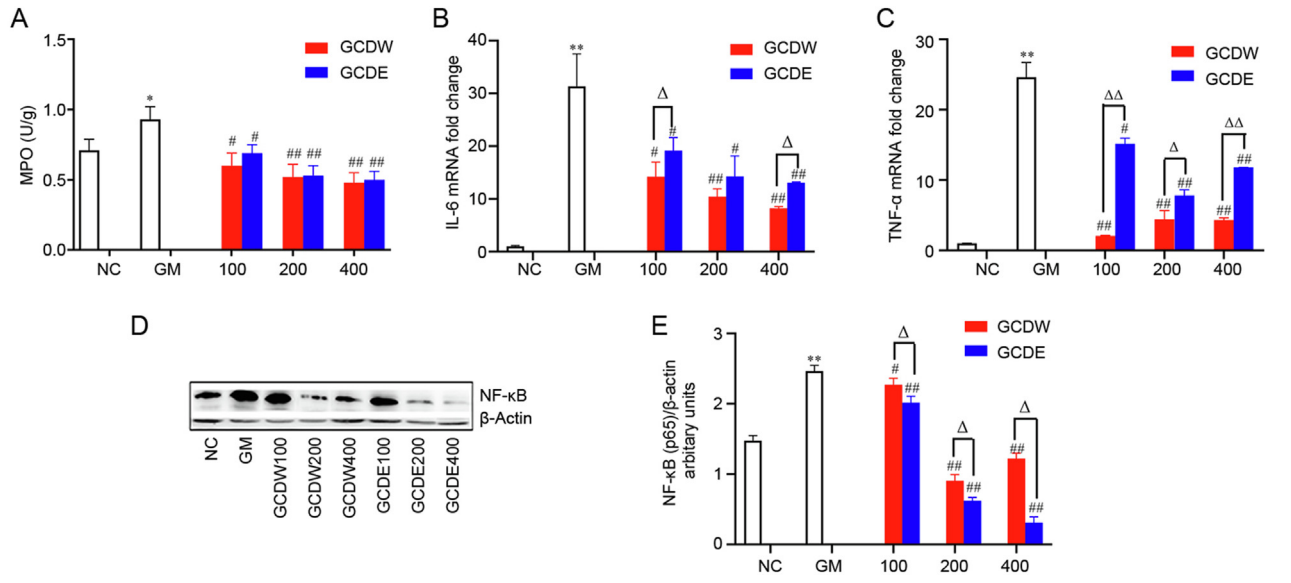

Fig. 5. Effect of *C. deserticola* on GM-induced changes in inflammatory markers in kidney tissues of rats. (A) Myeloperoxidase (MPO) activity, (B) relative mRNA expression of tumor necrosis factor-α (TNF-α) and (C) relative mRNA expression of interleukin-6 (IL-6) (means ± SD, $n = 8$) (D) and (E) Western blot analysis of nuclear nuclear factor-kappa B (NF-κB) (p65) expression in the kidney tissue (means ± SD, $n = 3$). $^{*}P < 0.05$ and $^{**}P < 0.01$ *vs* NC group, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ *vs* GM control group, $^{\Delta}P < 0.05$ and $^{\Delta\Delta}P < 0.01$ *vs* GCDE group.

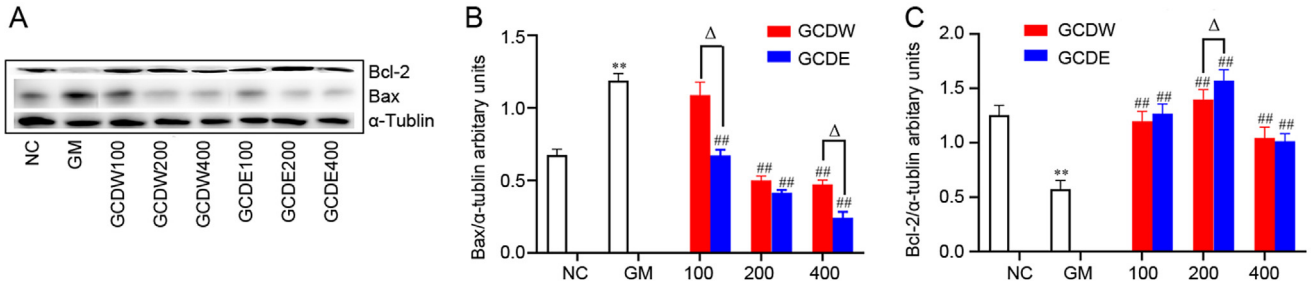

Fig. 6. Effect of *C. deserticola* on GM-induced changes in apoptotic markers in kidney tissues of rats (means ± SD, $n = 3$). (A) Western blot analysis of Bax and Bcl-2 protein expression in the kidney tissue of rats, α-tublin expression was used as a loading control. (B) and (C) representative bar diagram showing quantitative results for relative levels of Bax and Bcl-2 proteins. $^{**}P < 0.01$ *vs* NC group, $^{\#\#}P < 0.01$ *vs* GM control group, $^{\Delta}P < 0.05$ *vs* GCDE group.

GM-treated rats, we injected FITC-sinistrin into the femoral vein of anesthetized rats and connected a detector to transcutaneously measure the efficiency of FITC-sinistrin filtered by the kidney (Eriguchi et al., 2018). GM administration at the dose of 100 mg/kg (i.p.) for 10 consecutive days produced a significant reduction of GFR, which indicated that GM reduced kidney function as reported (Lee et al., 2019). At the same time, the levels of BUN and creatinine in the blood of rats were increased in GM-treated rats, and renal tubules were degenerated with a large amount of necrosis, pink fluid accumulation and infiltration of inflammatory cells, which further proved that GM has serious nephrotoxicity. Compared with the GM-alone treated rats, addition of CDW or CDE (100, 200, 400 mg/kg) treatment to GM significantly reduced the BUN and creatinine levels, increased GFR and produced apparently normal histoarchitecture with slight inflammatory infiltration and tubular degeneration. Because there were no significant differences in GFR, serum levels of BUN and creatinine and kidney histomorphology between normal and negative (CDE and CDW) groups of rats, we excluded negative groups (CDW and CDE) for further biochemical analysis.

Oxidative stress plays a crucial role in GM-induced nephrotoxicity (Veljković et al., 2017). MDA is the marker of oxidative stress and the final product of lipid peroxidation (Rodrigues et al., 2014). In this study, GM-induced renal injury rats showed a significant elevation of MDA content indicating the increase of lipid peroxidation. Pretreatment with CDW or CDE at doses of 100, 200 and 400 mg/kg greatly reduced the GM-induced MDA level. Reactive oxygen species generated during normal cellular processes are instantly detoxified by endogenous antioxidant enzymes, such as catalase and SOD and non-enzymatic antioxidant glutathione (Wang et al., 2011). However, in the GM-induced renal injury model, the content of reactive oxygen species were increased, such as hydroxyl free radicals and superoxide anion free radicals, leading to excessive consumption of catalase, SOD and glutathione, which exacerbated the deterioration of renal structure (Cao et al., 2019). In the present study, addition of CDW or CDE treatment (100, 200, 400 mg/kg) along with GM significantly recovered the GM-induced decrease in SOD and glutathione levels in comparison with GM treated alone rats. However, only addition of CDW at a dose of 200 mg/kg body weight along with GM could increase the catalase activity in comparison with GM treated alone rats. Our results confirmed the results of earlier studies that the enhancement of oxidative stress played a major role in the nephrotoxicity induced by GM (Randjelović et al., 2017).

Earlier studies reported that renal damage caused by GM could stimulate inflammatory events and enhance the migration of monocytes (Lopez-Novoa et al., 2011). By regulating the secretion of pro-inflammatory cytokines, the nuclear translocation of activated NF-κB in oxidative stress responses is a key process in the progress of renal inflammation (Quiros et al., 2011). In the present investigation, there were significant increases of mRNA level of renal cytokines (TNF-α and IL-6) and nuclear protein expression of NF-κB in GM-induced rats compared with normal rats. Pretreatment of CDW or CDE to GM-treated rats significantly reduced the expression of renal nuclear protein of NF-κB and mRNA levels of pro-inflammatory cytokines (TNF-α and IL-6) compared to GM-treated rats. MPO activity in is an indicator of neutrophil accumulation and an increase in MPO activity reveals the inflammatory infiltration of tissue injury sites (Abd-Elhamid et al., 2018). This study showed that MPO activity in rat kidney tissue increased after GM action, suggesting neutrophil infiltration and inflammatory response occurred. CDW and CDE (100, 200, 400 mg/kg) combined with GM can inhibit the activity of MPO in kidney tissues of rats and improve the histological characteristics of kidneys. The above findings indicated that *C. deserticola* could improve GM-induced nephrotoxicity through its anti-inflammatory effect.

Apoptosis plays an important role not only in the physiological processes of the kidney, but also in nephrotoxicity caused by various kidney diseases and drugs (El Gamal et al., 2014). Prolonged GM treatment may lead to renal injury with acute tubular apoptosis. It is well known that during apoptosis, Bax acts as a proapoptotic protein, whereas Bcl-2 acts as an anti-apoptotic protein (Chen & Huang, 2017). In this study, GM administration upregulated the protein expression of Bax and down regulated Bcl-2 expression. The CDW or CDE (100, 200, 400 mg/kg) treatment to GM treated rats significantly prevented renal tubular apoptosis compared with GM treated alone rats. Previous reports suggested that NF-κB activation promoted GM-induced renal tubular apoptosis in rats (Gong et al., 2012). NF-κB may directly activate apoptotic proteins such as Bax or down-regulate of anti-apoptotic proteins such as Bcl-2 (Z. Yuan et al., 2017) (Fan et al., 2017). Our results indicated that the combination of CDW and CDE with GM significantly reduced the expression of NF-κB in the kidney, thereby inhibiting renal tubular apoptosis/necrosis due to GM. These findings were also in agreement with previously reported literatures (Ansari, Raish, Ahmad, Fayaz, et al., 2016). However, further study of the biochemical basis of its therapeutic potentials (particularly at a molecular level) is expedient in order to properly elucidate the extract's mechanism of action. Also, further studies are needed to identify and characterize the phytoconstituents from *C. deserticola* and explore the exact mechanism to act as nephroprotective, before being established it in clinical setting.

5. Conclusion

In summary, this study indicated that the water and 75% ethanol extracts of *C. deserticola* had a protective effect on kidney, and its protective effect on GM-induced nephrotoxicity could be attributed to its anti-apoptotic, anti-inflammatory and antioxidant prop-

erties. Thus, *C. deserticola* might be a promising drug for the treatment of nephrotoxicity caused by GM.

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.

Acknowledgements

This work was supported by the National Key Research and Development Program of China (2018YFC1706300, 2018YFC17063003), the Key Research and Development Project of GanSu Province (20YF2FA019), the People's Livelihood Special Project from Technical Innovation Guide Plan of Gansu Province (20CX4FK014), the Key Talent Project of Organization Department of Gansu Provincial Party Committee ([2022] Gan-71), the Talent Innovation and Entrepreneurship Project of Lanzhou (2017-RC115, 2020-RC-41).

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