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Chronic administration of troxerutin protects mouse kidney against D-galactose-induced oxidative DNA damage

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ABSTRACT

Troxerutin, a natural bioflavonoid, has been reported to have many benefits and medicinal properties. In this study, we evaluated the protective effect of troxerutin against D-gal-induced oxidative DNA damage in mouse kidney, and explored the potential mechanism of its action. Our data showed that troxerutin significantly decreased levels of urea, uric acid and creatinine in serum and the renal histological injury in D-gal-treated mice. Troxerutin markedly restored Cu/Zn-SOD, CAT and GPx activities in the kidney of D-gal-treated mouse. Furthermore, the increase of 8-hydroxydeoxyguanosine (a marker of oxidative DNA damage) induced by D-gal was effectively suppressed by troxerutin. Internucleosomal DNA ladder fragmentation and the number of terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) nick-end-labeling (TUNEL)-positive cells in D-gal-treated mice were inhibited by troxerutin, which might be attributed to its antioxidant property by decreasing activities of nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase) and levels of reactive oxygen species (ROS). In conclusion, these results suggested that troxerutin could protect the mouse kidney against D-gal-induced injury by improving renal function, attenuating histopathologic changes, reducing ROS production, renewing the activities of antioxidant enzymes and decreasing DNA oxidative damage. This study provided novel insights into the protective mechanisms of troxerutin in D-gal-induced kidney injury.

1. Introduction

D-Galactose (D-gal) is a reducing sugar and can be metabolized at normal concentration. However, at high levels, it can be converted into aldose and hydroperoxide under the catalysis of galactose oxidase, resulting in the generation of a superoxide anion and oxygen-derived free radicals (Wu et al., 2008). D-Gal also reacts readily with the free amines of amino acids in proteins and peptides both in vivo and in vitro to form advanced glycation end products (AGEs). Evidence shows that AGEs could remarkably cause the accumulation of reactive oxygen species (ROS), especially superoxide radicals and hydrogen peroxide release **(Fan et al., 2009; Lu et al., 2007, 2010a,b; Wu et al., 2008; Shan et al., 2009). Mice injected with D-gal have been used as an animal model of oxidative stress (Lu et al., 2007, 2010a,b; Fan et al., 2009; Zhang et al., 2009; Shan et al., 2009). Recently, we found that in this mouse model, oxidative damage was associated with DNA damage.

Troxerutin, a trihydroxyethylated derivative of the natural bioflavonoid rutin, is present in tea, coffee, cereal grains and a variety of fruits and vegetables (Fan et al., 2009). Many reports have demonstrated that troxerutin possesses a variety of biological activities, such as anti-oxidative, anti-inflammatory, anti-neoplastic, anti-erythrocytic, anti-thrombotic, anti-fibrinolytic, and anti-γ-radiation induced DNA damage (Blasig et al., 1987, 1988; Boisseau et al.,1995; Gandhi et al., 2004; Kessler et al., 2002; Wenisch and Biffignandi, 2001; Sarkar et al., 2005; Maurya et al., 2005, 2004a,b; Lu et al., 2007, 2010a,b; Zhang et al., 2009). Moreover, the troxerutin has already been used for years to treat some cardiovascular disease (CVD) (Boisseau et al., 1995; Gohel and Davies, 2009). Recently, reports from our laboratory also confirmed that nephroprotective effect of it through anti-inflammation and anti-oxidation (Fan et al., 2009).

However, to our knowledge, it has not yet been reported that troxerutin could attenuate the renal DNA damage in D-gal-treated

Abbreviations: AGEs, advanced glycation end products; CAT, catalase; D-Gal, D-galactose; GPx, glutathione peroxidase; NADPH oxidases, nicotinamide adenine dinucleotide phosphate oxidases; 8-OHdG, 8-hydroxy-2-deoxyguanosine; ROS, reactive oxygen species; SOD, superoxide dismutase; TUNEL, deoxyribonucleotidyl transferase (TdT)-mediated dUTP-fluorescein isothiocyanate (FITC) nick-end-labeling.

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mice. In the present study, we aimed to evaluate whether troxerutin has protective effect against D-gal-induced kidney DNA damage and to explore the mechanism of its action.

2. Materials and methods

2.1. Animals and treatments

Ten-week-old male Kunming strain mice (29.63 ± 4.88 g) were purchased from the branch of national breeder center of rodents (Shanghai). Prior to experiments mice had free access to food and water and were kept under constant conditions of temperature (23 ± 1 °C) and humidity (60%). Eight mice were housed per cage on a 12-h light/dark schedule (lights on 08:30–20:30). After a week of adaptation, mice were divided randomly into four groups. Group 1 and group 4 served as vehicle control with injection of saline (0.9%), and the other two groups of mice (groups 2 and 3) received daily subcutaneous injection of D-gal (Sigma–Aldrich, MO, USA) at dose of 500 mg/(kg day) for 8 weeks, respectively. At the same time, mice in groups 3 and groups 4 received daily troxerutin (troxerutin; Purity >99%; Baoji Fangsheng Biotechnology Co. Ltd., Baoji, China) of 150 mg/(kg day) in distilled water by oral gavage for 8 weeks, and the mice of groups 1 and 2 were given distilled water orally at the same dose (Lu et al., 2010a). After the experiment termination, mice were sacrificed, the whole blood of mice was collected into heparinized test tubes and centrifuged at 2000g for 15 min at 4 °C to separate serum, and the serum was stored at −70 °C freezer for further analysis. The kidney tissues were immediately collected for experiments or stored at −70 °C for later use. All experiments were performed in compliance with the Chinese legislation on the use and care of laboratory animals and were approved by the respective university committees for animal experiments.

2.2. Histological evaluations

The mice were perfused transcardially with 100 ml of normal saline (0.9%). The kidney tissues were fixed in a fresh solution of 4% paraformaldehyde (pH 7.4) at 4 °C for 24 h, incubated overnight at 4 °C in 100 mM sodium phosphate buffer (pH 7.4) containing 30% sucrose; and embedded in optimal cutting temperature (OCT) compound (Leica, CA, Germany). Cryosections were collected on 3-aminopropyl-trimethoxysilane-coated slides (Sigma–Aldrich). The stains applied to renal biopsy specimens used for light microscopy included haematoxylin–eosin, periodic acid-Schiff (PAS), Masson's trichrome. For each kidney, 100 intersections were examined. Tubulointerstitial damage was performed by the method of Francescato et al. (2007). In brief, tubulointerstitial damage was defined as tubular necrosis, inflammatory cell infiltrate, tubular lumen dilation or tubular atrophy. Damage was graded according to Shih et al. (1988) on a scale of 0–4 (0 = normal; 0.5 = small focal areas; 1 = involvement of less than 10% of the cortices and outer medullae; 2 = 10–25% involvement of the cortices and outer medullae; 3 = 25–75% involvement of the cortices and outer medullae; 4 = extensive damage involving more than 75% of the cortices and outer medullae). We also evaluated the number of tubules with cellular necrosis from the renal cortices and outer medulla by grid fields (0.245 mm^2).

Glomerular damage in the present study was scored by light microscopy according to the scoring system of Wu et al. (2005). Briefly, Glomerular lesions (total score: 0–12) included glomerular hypercellularity, glomerular segmental lesions (crescents, adhesions and segmental sclerosis) and global glomerular sclerosis. The glomerular lesion score of each type was determined as follows: 0, no lesion; 1, lesion in <10% of glomeruli; 2, lesion in >10% but <25% of glomeruli; 3, lesion in >25% and <50% of glomeruli; 4, lesion in >50% of glomeruli.

2.3. Estimation of urea, uric acid, creatinine

The levels of urea, uric acid and creatinine in serum were estimated spectrophotometrically using commercial diagnostic kits (Jiancheng Institute of Biotechnology, Nanjing, China).

2.4. Assay of ROS level

ROS was measured as described previously, based on the oxidation of 2′7′-dichlorodihydrofluorescein diacetate to 2′7′-dichloro-fluorescein (Shinomol and Muralidhara, 2007; Lu et al., 2010a). Briefly, the homogenate was diluted 1:20 times with ice-cold Locke's buffer (154 mM NaCl, 5.6 mM KCl, 3.6 mM $NaHCO_3$, 2.0 mM $CaCl_2$, 10 mM D-glucose, and 5 mM HEPES, pH 7.4) to obtain a concentration of 5 mg tissue/ml. The reaction mixture (1 ml) containing Locke's buffer (pH 7.4), 0.2 ml homogenate and 10 ml of DCFH-DA (5 mM) was incubated for 15 min at room temperature to allow the DCFH-DA to be incorporated into any membrane-bound vesicles and the diacetate group cleaved by esterases. After 30 min of further incubation, the conversion of DCFH-DA to the fluorescent product DCF was measured using a spectrofluorimeter with excitation at 484 nm and emission at 530 nm. Background fluorescence (conversion of DCFH-DA in the absence of homogenate) was corrected by the inclusion of parallel blanks. ROS formation was quantified from a DCF-standard curve and data are expressed as pmol DCF formed/min/mg protein.

2.5. Assay of NADPH oxidase activity

NADPH oxidase activity was assayed spectrophotometrically at 600 nm. The assay mixture contained, in a total volume of 1 ml, 0.05 M potassium phosphate buffer (pH 7.7), 95 mol of dichlorophenol indophenol, 100 mol of NADPH (Sigma–Aldrich, MO, USA), and an appropriate amount of the PMS. The reduction of dichlorophenol indophenol was expressed in terms of nanomoles of dichlorophenol indophenol reduced per minute per milligram of protein using an extinction coefficient of 21 cm^{-1} $millimolar^{-1}$ (Ramesh and Begum, 2008).

2.6. Assay of Cu, Zn-SOD activity

Chemicals used in the assay, including xanthine, xanthine oxidase, cytochrome c, bovine serum albumin (BSA) and SOD, were purchased from Sigma Chemical Company (St. Louis, MO, USA). Cu, Zn-SOD activity was measured using the method of McCord and Fridovich (1969). Solution A was prepared by mixing 100 ml of 50 mM PBS (pH 7.4) containing 0.1 mM EDTA and 2 μmol of cytochrome c with 10 ml of 0.001 N NaOH solutions containing 5 μmol of xanthine. Solution B contained 0.2 U xanthine oxidase/mland 0.1 mM EDTA. Fifty microliters of a tissue supernatant was mixed with 2.9 ml of solution A and the reaction was started by adding 50 μl of solution B. Change in absorbance at 550 nm was monitored in a spectrophotometer (Shimadzu UV-2501PC, Japan). A blank was run by replacing the supernatant with 50 μl of ultra pure water. Cu, Zn-SOD levels were expressed as units per mg protein with reference to the activity of a standard curve of bovine copper-, zinc-SOD under the same conditions.

2.7. Assay of CAT activity

CAT activity was assayed by the method of Aebi (1984). In brief, to a quartzcuvette, 0.65 ml of the phosphate buffer (50 mmol/l; pH 7.0) and 50 μl sample were added, and the reaction was started by addition of 0.3 ml of 30 mM hydrogen peroxide (H_2O_2). The decomposition of H_2O_2 was monitored at 240 nm at 25 °C. CAT activity was calculated as nM H_2O_2 consumed/min/mg of tissue protein.

2.8. Assay of GPx activity

The GPx activity assay was based on the method of Paglia and Valentine (1967). *tert*-Butylhydroperoxide was used as substrate. The assay measures the enzymatic reduction of H_2O_2 by GPx through consumption of reduced glutathione (GSH) that is restored from oxidized glutathione GSSG in a coupled enzymatic reaction by GR. GR reduces GSSG to GSH using NADPH (Sigma–Aldrich, MO, USA) as a reducing agent. The decrease in absorbance at 340 nm due to NADPH consumption was measured in a Molecular Devices M2 plate reader (Molecular Devices, Menlo. Park, CA). GPx activity was computed using the molar extinction coefficient of 6.22 mM^{-1} cm^{-1}. One unit of GPx was defined as the amount of enzyme that catalyzed the oxidation of 1.0 μmol of NADPH to $NADP^+$ per minute at 25 °C.

2.9. Assay of nuclear 8-hydroxy-2-deoxyguanosine (8-OHdG) level

Nuclear 8-OHdG contents in kidney were assayed by the method of Umemura et al. (2009). Briefly, to prevent 8-OHdG formation as a byproduct during DNA isolation, nuclear DNA was extracted with a commercially available DNA extractor kit (Wako Pure Chemical Industries, Ltd.) containing an antioxidant NaI solution to dissolve cellular components. For further prevention of autooxidation in the cell lysis step, deferoxamine mesylate (Sigma Chemical Co.) was added to the lysis buffer (Helbock et al.,1998). The DNA was digested to deoxynucleotides with nuclease P1 and alkaline phosphatase, and levels of 8-OHdG (8-OHdG/10^{-5} deoxyguanosine) was assessed by high-performance liquid chromatography (HPLC) with an electrochemical detection system (Agilent Technologies, NYSE:A, USA).

2.10. Deoxyribonucleotidyl transferase (TdT)-mediated dUTP-fluorescein isothiocyanate (FITC) nick-end-labeling (TUNEL) assay

For the TUNEL staining, the standard protocol for frozen sections was followed (BD ApoAlert™ DNA Fragmentation assay kit, BD Biosciences Clontech, Palo Alto, CA, USA). The sections were immersed in a Coplin jar (VWR International, Aurora, CO, USA) containing fresh 4% formaldehyde/PBS, and incubated at room temperature for 5 min. The sections were washed twice with PBS for 5 min. The liquid was allowed to drain thoroughly, and the slides were placed on a flatsurface. Each section was covered with 100 ml of 20 mg/ml Proteinase K solution (section III) and incubated at 37 °C for 5 min. After two washes of 5 min each with PBS, the sections were transferred into a Coplin jar containing 4% formaldehyde/PBS and then washed in PBS again. The cells were covered in equilibration buffer (from the kit) and equilibrated at room temperature for 5 min. The equilibration buffer was drained, and TdT incubation buffer was added to the tissue sections. To perform

the tailing reaction, the slice was placed in a dark and humidified 37 °C incubator for 1 h. The tailing reaction was terminated by immersing the samples in 2× saline-sodium citrate (SSC) at room temperature for 15 min. Samples were washed three times with PBS for 5 min to remove unincorporated fluorescein-dUTP. Finally, strong, nuclear green fluorescencein apoptotic cells were observed on a fluorescent microscopy equipped with a standard fluorescein filter (520 ± 20 nm). All cells stained with propidium iodide exhibit strong red cytoplasmic fluorescence when viewed at 620 nm. Specimens were analyzed with a Zeiss Axioskop 40™ microscope equipped for light microscopy (Carl Zeiss, Oberkochen, Germany). The images were taken with a CCD camera (CoolSNAP™ Color, Photometrics, Roper Scientific Inc., Trenton, NJ, USA) and processed with Image-Pro® Plus 6.0 software (Media Cybernetics Inc., Newburyport, MA, USA). For analysis; plaque areas were excluded, and the number of stained cells in 0.01 mm^2 was estimated by blind manual counting of seven regions located at a consistent position per section.

2.11. DNA fragmentation assay

Apoptotic DNA fragmentation was assayed using the commercial kits (BioVision Inc. USA) according to the manufacturer's instructions. The extracted DNA dissolved in suspension buffer loading buffer were loaded on a 1.2% (w/v) agarose gel and subjected to electrophoresis at 75 V for 1–2 h. DNA ladders were visualized by UV illumination after staining with ethidium bromide (EB).

2.12. Statistic analysis

All statistical analyzes were performed using the SPSS software, version 11.5. A one-way analysis of variance (ANOVA; $p < 0.05$) was used to determine significant differences between groups and the individual comparisons were obtained by Turkey's HSD post hoc test. Statistical significance was set at $p \leqslant 0.05$.

3. Results

3.1. Effects of troxerutin on serum urea, uric acid and creatinine levels in D-gal-treated mice

The levels of serum urea, uric acid and creatinine were considered to serum biochemical marker of renal damage. In order to determine whether troxerutin can attenuate the kidney damage in the D-gal-treated mouse, we measured the levels of serum urea, uric acid and creatinine (Fig. 1). In D-gal-treated mice, the levels of serum urea, uric acid and creatinine significantly increased by 52%, 101% and 105% as compared with vehicle controls, respectively [$F_{urea}(3,8) = 16.872$, $p < 0.01$; $F_{uric\ acid}(3,8) = 46.925$, $p < 0.01$; $F_{creatinine}(3,8) = 49.323$, $p < 0.01$; D-gal group vs. control group]. However, the levels of serum urea, uric acid and creatinine in the mice cotreated with D-gal and creatinine decreased by 25%, 47% and 43% as compared with the D-gal-treated mice, respectively [$F_{urea}(3,8) = 16.872$, $p < 0.01$; $F_{uric\ acid}(3,8) = 46.925$, $p < 0.01$; $F_{creatinine}(3,8) = 49.323$, $p < 0.01$ vs. D-gal group]. Interestingly, there were no significant difference in levels of serum urea, uric acid and creatinine among the control group, the troxerutin group and the troxerutin + D-gal group (Fig. 1).

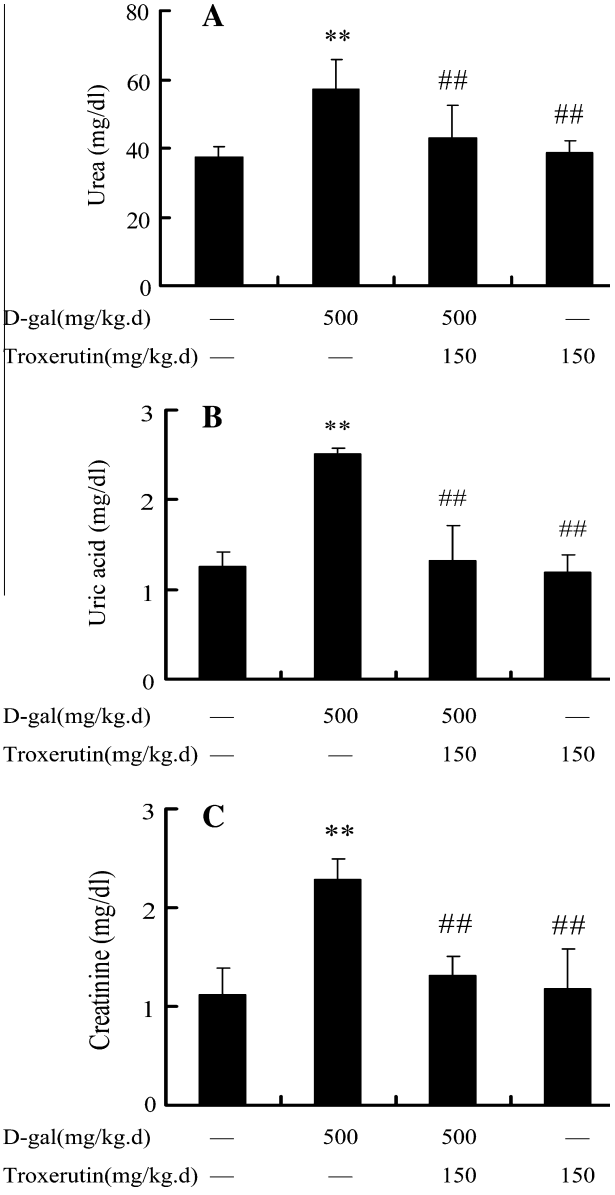

Fig. 1. Effect of troxerutin on D-gal-induced changes in renal functional markers: (A) serum urea, (B) serum uric acid and (C) serum creatinine in mice. All values are expressed as mean ± S.E.M. ($n = 3$). $^{**}p < 0.01$, compared with the control group; $^{\#\#}p < 0.01$, vs. D-gal-treated group.

3.2. Effects of troxerutin on histology changes in D-gal-treated mice kidney

Kidney histological study was used to determine the protective effect of troxerutin on D-gal-induced injury. As shown in Fig. 2, the results of histopathological evaluation showed that troxerutin exhibited protective effect against D-gal-induced kidney injury. D-Gal treatment caused several visible histology changes. In D-gal-treated mice the renal sections showed extensive tubular damage by presence of necrotic epithelial cells. Significant number of nephrons with distended tubular lumen containing copious amounts of proteinaceous glomerular filtrate and characterized by pyknosis and karyolysis of tubular epithelial nuclei were observed. In some areas exfoliation of epithelial cells was observed in the tubular lumen (Fig. 2B). Moreover, the score for tubulointerstitial and glomerular lesions was significantly higher in the D-gal-treated mice as compared with vehicle controls ($p < 0.05$) (Fig. 2E). The number of tubules with cellular necrosis from the renal cortices and outer medulla also increased in the D-gal-treated mice as compared with vehicle controls ($p < 0.01$) (Fig. 2F). Whereas, troxerutin significantly alleviated the kidney damage in D-gal-treated mice. No visible histological changes in the kidney could be observed among the control group, the troxerutin group and the troxerutin + D-gal group (Fig. 2A, C and D).

3.3. Troxerutin decreases ROS in the kidney of D-gal-treated mice

Evidence reveals that D-gal can react with protein non-enzymatically forming Schiff bases and are converted into AGEs, which induces ROS (Cai et al., 2006; Lu et al., 2007, 2010a,b; Shan et al., 2009; Tian et al., 2005; Fan et al., 2009). As shown in Fig. 3A, D-gal administration significantly increased ROS by 68% in the mice kidney [$F(3,8) = 39.086$, $p < 0.01$] as compared to the control group. The data indicated that oxidative stress in vivo was elevated in the

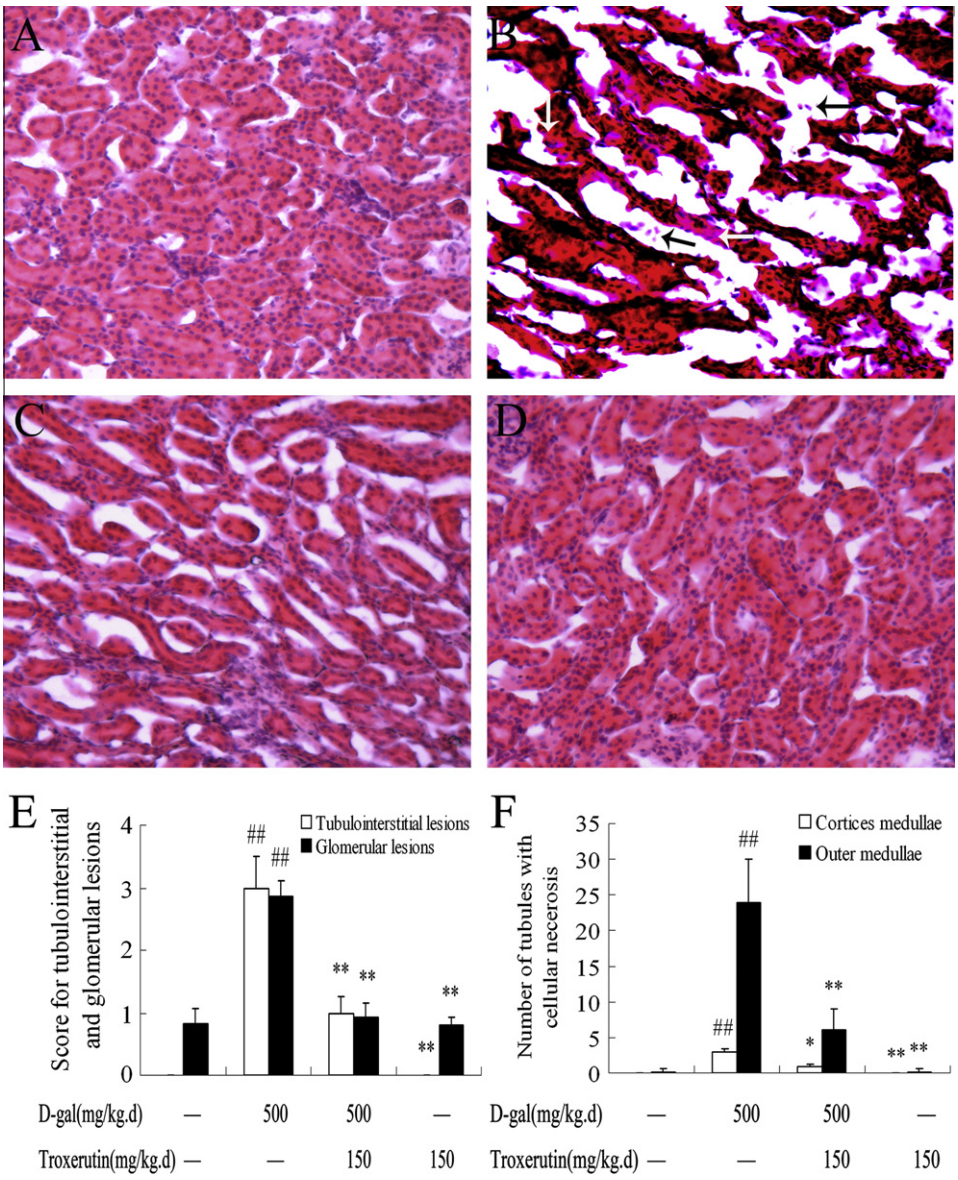

Fig. 2. Morphological and histological evaluation of kidney in mice. (A) Renal outer medulla of the vehicle control mouse; (B) renal outer medulla of D-gal-treated mouse; (C) renal outer medulla of D-gal-treated mouse fed with troxerutin; (D) renal outer medulla of mouse fed with troxerutin; (E) the score for tubulointerstitial and glomerular lesions; (F) the number of tubules with cellular necrosis from the renal cortices and outer medulla. The black arrow indicates necrosis or exfoliation of tubular cells. The white arrow indicates pyknosis or karyolysis of tubular epithelial nuclei. $^{\#\#}p < 0.01$, compared with the control group; $^{*}p < 0.05$ and $^{**}p < 0.01$, vs. D-gal-treated group. Original magnification, 10 × 10.

kidney of D-gal-treated mice. Interestingly, troxerutin could decrease ROS in the kidney by 30% [$F(3,8) = 39.086$, $p < 0.01$] as compared to the D-gal-treated mice (Fig. 3A). There was no significant difference in level of ROS among the control group, the troxerutin group and the troxerutin + D-gal group.

3.4. Effects of troxerutin on activity of NADPH oxidase in D-gal-treated mice kidney

NADPH oxidase is an important enzymatic source for the generation of reactive oxygen species (ROS) that damage cells in a variety of pathologic conditions (Wang et al. 2007). To determine whether troxerutin would confer protection against D-gal-induced kidney injury and whether ROS derived from NADPH oxidase played a role as an initiator of these putative protective effects, we examined the changes in the activity of NADPH oxidase. As shown in Fig. 3B, the activity of NADPH oxidase in D-gal-treated mice was significantly increased by 43% as compared with vehicle controls [$F(3,8) = 28.972$, $p < 0.01$]. However, the activity of NADPH oxidase in the mice cotreated with D-gal and troxerutin decreased by 23% as compared with the D-gal-treated mice [$F(3,8) = 28.972$, $p < 0.01$ vs. D-gal group]. No appearance difference could be observed in activities of kidney NADPH oxidase among the control group, the troxerutin group and the troxerutin + D-gal group (Fig. 3B).

3.5. Effects of troxerutin on activities of the antioxidant enzyme D-gal-treated mice kidney

In order to determine whether troxerutin can attenuate the oxidative damage in the kidney of D-gal-treated mice, we measured the activities of major antioxidant enzymes, including

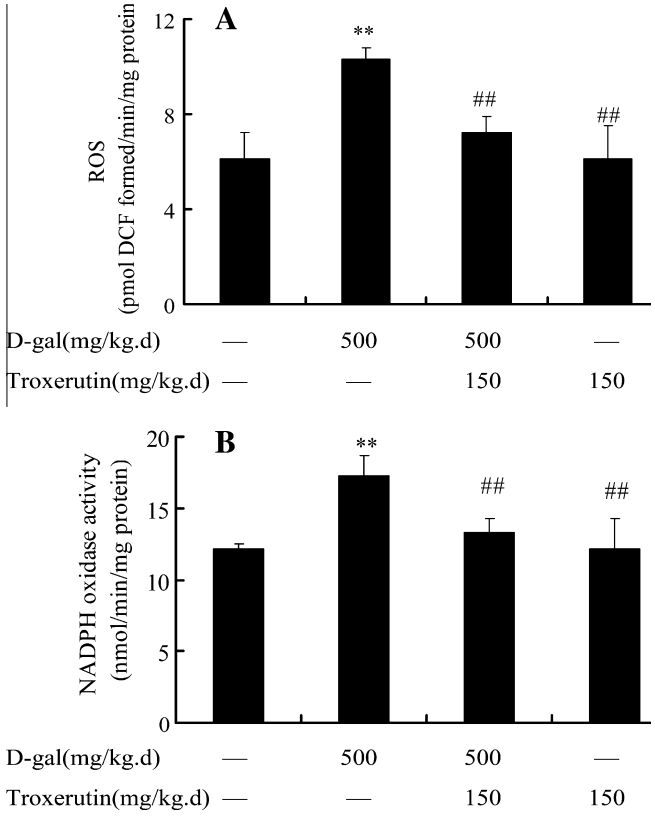

Fig. 3. Effect of troxerutin on the level of ROS and activity of NADPH oxidase in D-gal-treated mouse kidney. (A) Level of ROS; (B) activity of NADPH oxidase. Each value is expressed as mean ± S.E.M. ($n = 3$). $^{**}p < 0.01$, compared with the control group; $^{\#\#}p < 0.01$, vs. D-gal-treated group.

Cu/Zn-SOD, CAT and GPx in mouse kidney. The results showed that troxerutin could renew the activities of these antioxidant enzymes in the kidney of D-gal-treated mice (Fig. 4).

In D-gal-treated mouse, renal Cu/Zn-SOD activity was significantly decreased by 57% as compared with vehicle controls [$F(3,8) = 29.325$, $p < 0.01$]. Interestingly, the renal Cu/Zn-SOD activity of the troxerutin + D-gal-treated mice was markedly increased by 110% as compared with D-gal-treated mice [$F(3,8) = 29.325$, $p < 0.01$] (Fig. 4A). Renal CAT activity was markedly decreased by 32% in D-gal-treated mice as compared with that in the vehicle controls [$F(3,8) = 26.736$, $p < 0.01$]. In contrast, the treatment of troxerutin caused a dramatic increase by 44% in renal CAT activities of D-gal-treated mice [$F(3,8) = 26.736$, $p < 0.01$] (Fig. 4B). Renal GPx activities were significantly decreased (78%) in the D-gal-treated mice as compared with vehicle controls [$F(3,8) = 38.704$, $p < 0.01$]. However, troxerutin markedly increased the activities of GPx (320%) in D-gal-treated mice [$F(3,8) = 38.704$, $p < 0.01$] (Fig. 4C).

Interestingly, there were no significant difference in renal activities of Cu/Zn-SOD, CAT and GPx among the control group, the troxerutin group and the troxerutin + D-gal group.

3.6. Effects of troxerutin on level of 8-OHdG in the kidney of D-gal-treated mice

8-OHdG is one of such important oxidative DNA lesions formed by the oxidation of the C-8 position of 2′-deoxyguanosine, which has commonly been used as a biomarker of oxidative DNA damage (Toyokuni et al., 1995; Singh et al., 2007; Pilger and Rudiger, 2006; Chen et al., 2010). In order to determine whether troxerutin can

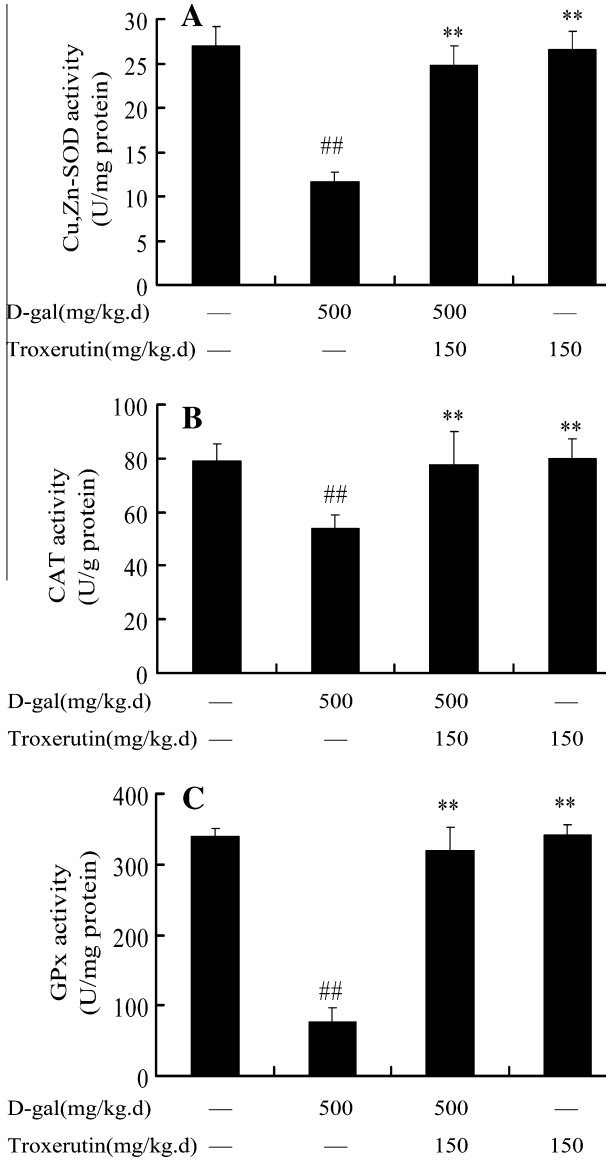

Fig. 4. Effect of troxerutin on the activity of antioxidant enzymes in D-gal-treated mouse kidney. (A) Cu/Zn-SOD activity; (B) CAT activity; (C) GPx activity. All values are expressed as mean ± S.E.M. $^{\#\#}p < 0.01$, compared with the control group; $^{**}p < 0.01$, vs. D-gal-treated group.

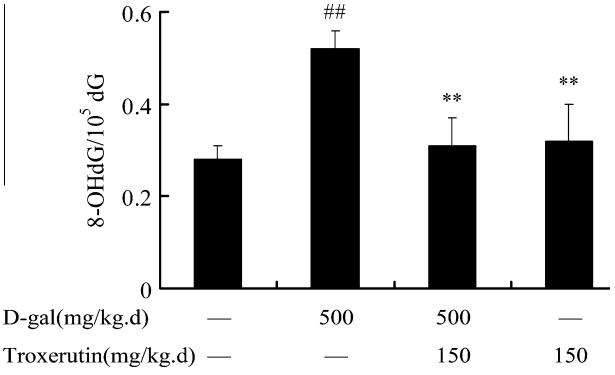

Fig. 5. Effect of troxerutin on the level 8-OHdG in D-gal-treated mouse kidney. Each value is expressed as mean ± S.E.M. ($n = 3$). $^{\#\#}p < 0.01$, compared with the control group; $^{**}p < 0.01$, vs. D-gal-treated group.

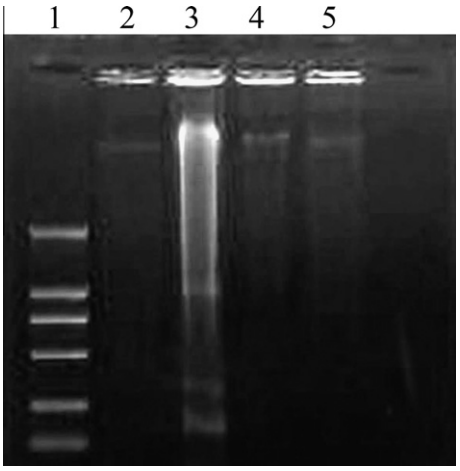

Fig. 6. Agarose gel electrophoresis of DNA fragments in mouse kidney. Lane 1: DNA marker; lane 2: The vehicle control mouse; lane 3: D-gal-treated mouse; lane 4: D-gal-treated mouse fed with troxerutin; lane 5: Mouse fed with troxerutin.

attenuate the kidney DNA damage in the D-gal-treated mouse, we measured the level of 8-OHdG. As shown in Fig. 5, the level of 8-OHdG in D-gal-treated mice was significantly increased by 86% as compared with vehicle controls [$F(3,8) = 19.987$, $p < 0.01$]. Interestingly, troxerutin could decrease the level of 8-OHdG in the kidney by 40% [$F(3,8) = 19.987$, $p < 0.01$] as compared to the D-gal-treated mice. There was no significant difference in level of 8-OHdG among the control group, the troxerutin group and the troxerutin + D-gal group (Fig. 5).

3.7. Effect of troxerutin on the DNA ladder fragmentation in the kidney of D-gal-treated mice

Orderly fragmentation of DNA in the form of a ladder due to endonucleolytic attack is reportedly considered as an apoptosis and DNA damage related event. DNA fragmentation is considered as one of the later steps in apoptotic program (Hickey et al., 2001). As shown in Fig. 6, clear DNA ladder formation was observed in kidney tissue of the D-gal-treated mice. Administration of troxerutin exerted a significant protective effect against D-gal-induced DNA damage as evidenced by markedly weaker DNA ladder formation (Fig. 6). No appearance difference could be observed in DNA ladder formation among the control group, the troxerutin group and the troxerutin + D-gal group.

3.8. Effect of troxerutin on the D-gal-induced apoptosis in mouse kidney

We used the TUNEL assay to investigate the effect of troxerutin on the D-gal-induced apoptosis (Fig. 7). The number of TUNEL-positive cells in the kidney of D-gal-treated mice was significantly increased [$F(3,8) = 45.626$, $p < 0.01$]. There were significantly fewer TUNEL-positive cells in the kidney of mice cotreated with D-gal and troxerutin as compared with the mice treated with D-gal [$F(3,8) = 45.626$, $p < 0.01$]. However, there was no significant difference in the number of TUNEL-positive cells in the kidney among the control group, the troxerutin group and the troxerutin + D-gal group (Fig. 7).

4. Discussion

The kidney is a highly specialized organ that maintains the internal environment of the body by selectively excreting or retaining various substances according to specific body needs. Alcohol, methamphetamine, glycerol, lipopolysaccharide, and many other drugs can induce renal injury (Rodrigo et al., 2002; Tokunaga et al., 2006; de Jesus Soares et al., 2007; Ueki et al., 2007; Fan et al., 2009). D-Gal is a reducing sugar and can be metabolized at normal concentration. Whereas, at high levels, D-gal can be converted into aldose and hydroperoxide under the catalysis of galactose oxidase, leading to the formation of a superoxide anion and oxygen-derived free radicals (Wu et al., 2008). D-Gal also reacted readily with the free amines of amino acids in proteins and peptides in vivo to form advanced glycation endproducts (AGEs). Evidence showed that AGEs could remarkably cause the accumulation of ROS (Zhang et al., 2005; Lu et al., 2007; Tian et al., 2005; Wu et al., 2008; Lu et al., 2010a,b; Fan et al., 2009). ROS are associated with the inflammatory response and frequently they contribute to the tissue damaging effects of inflammatory reactions (Cuzzocrea et al., 2000; Fan et al., 2009; Lu et al., 2010b). Our previously studies have demonstrated that D-gal can induce renal injury, including oxidative stress and inflammation in mice, which can lead to renal dysfunction (Fan et al., 2009). In the present study, we demonstrated long-term injection of D-gal in mouse kidney induces over-production of ROS and leads to renal oxidative damage. Whereas, troxerutin by oral gavage administration markedly decreased the level of ROS in D-gal-treated mice, which might be ascribed to its ability to scavenge and prevent free radical generation (Lu et al., 2007, 2010a,b; Fan et al., 2009). ROS could also cause the damages of DNA, proteins and lipids within cells, which led to tissue injury (Zhang et al., 2009; Fan et al., 2009; Liu et al., 2010). In nephrotoxic compounds-treated animal, the histological changes of kidney, such as structure damage, renal tubular epithelial cell necrosis, leukocyte infiltration, tubular epithelial cell pyknosis and glomerular lesions were observed (Fan et al., 2009; de Jesus Soares et al., 2007; Ueki et al., 2007). In this study, the results showed that troxerutin markedly decreased the levels of serum urea, uric acid and creatinine in D-gal-treated mice (Fig. 1) and improved the D-gal-induced histopathologic changes in mouse kidney (Fig. 2). These results suggest that troxerutin could protect mouse kidney against D-gal-induced renal dysfunction and histopathologic damage.

Recent studies confirmed an important role for NADPH oxidases as major sources of ROS involved in renal dysfunction. In particular, NADPH oxidases are specifically activated by many of the stimuli that are known to cause renal endothelial dysfunction (Etoh et al. 2003; Tian et al., 2008; Dworakowski et al., 2008; An et al., 2009; Xie et al., 2009; Kim et al., 2009; Jiang, 2009). In this study, we confirmed that D-gal administration significantly increased activity of NADPH oxidases in the kidney, which contributed to the increase of ROS. However, troxerutin could markedly inhibit the activities of NADPH oxidases in the kidney of D-gal-treated mice, which could be associated with decreased ROS levels in the kidney of D-gal-treated mice (Fig. 3). Therefore, potential mechanisms underlying the nephroprotective effect of troxerutin on the D-gal-treated mice might be ascribed at least in part to its ability to inhibit activity of troxerutin and prevent free radical generation.

Antioxidant enzymes can protect cellular compounds against damage induced by free radicals. Superoxide dismutases (SOD), catalase (CAT) and glutathione peroxidases (GPx) are important antioxidant enzymes (Boots et al., 2008). The SOD decomposes superoxide radicals (O_2^-) and produce H_2O_2. H_2O_2 is subsequently removed to water by CAT in the peroxisomes, or by GPx oxidizing GSH in the cytosol (Dröge, 2002; Lee and Choi, 2003). So the activities of these enzymes have been used to assess oxidative stress in cells (Fen et al., 2008). Many studies have shown that the excessive ROS induced by D-gal would break the balance between ROS production and antioxidant defenses. The antioxidant enzymes may be exhausted and its concentration depletions (Fan et al., 2009; Lu et al., 2007, 2010a,b; Wu et al., 2008; Shan et al., 2009). In the

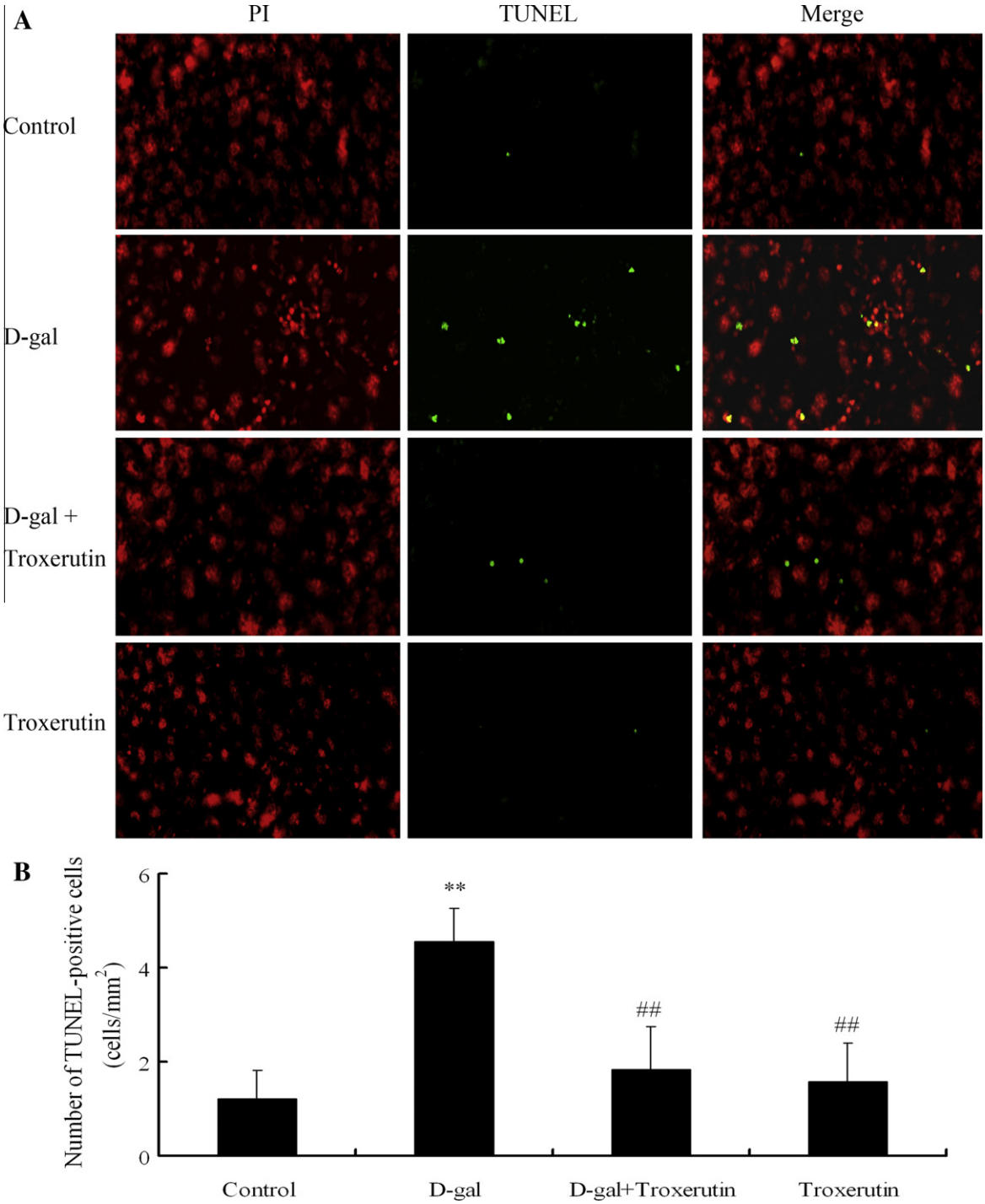

Fig. 7. A. In situ detection of fragmented DNA [deoxyribonucleotidyl transferase-mediated dUTP-FITC nick-end-labeling (TUNEL) assay] in the kidney of mice. The kidney tissues were processed for TUNEL and photographed using a fluorescence microscope with either propidium iodide (PI) filter alone (left) or an FITC filter alone (middle). The merged images show that apoptotic cells appear yellow and non-apoptotic cells appear red (right). Scale bars = 100 mm. B. The histogram showed the relative proportion of TUNEL-positive cells in the kidney of mice. All values are expressed as mean ± S.E.M. $^{\#\#}p < 0.01$, compared with the control group; $^{**}p < 0.01$, vs. D-gal-treated group. (For interpretation of the references in colour in this figure legend, the reader is referred to the web version of this article.)

present study, the activities of antioxidant enzymes in mouse kidney, including SOD, CAT and GPx, were dramatically decreased by the treatment of D-gal. It indicated that D-gal induced the oxidative stress by the inhibiting the activities of antioxidant enzymes (Fig. 4). Interestingly, troxerutin could markedly increase the activities of those antioxidant enzymes in the kidney of D-gal-treated mice (Fig. 4). It suggested that troxerutin could at least partly attenuate oxidative stress by renewing the activities of those antioxidant enzymes in D-gal-treated mouse kidney.

ROS has also been reported to injure DNA via causing single-strand breaks, DNA–protein cross-links and modification of base residues such as the introduction of a hydroxyl group (–OH) into the C-8 position of guanosine and guanine residues forming 8-OHdG and 8-hydroxyguanine being the widely used as a sensitive

biomarker of DNA oxidation (Valko et al., 2006; Cooke et al., 2006; Yoshioka et al., 2008; Chen et al., 2007; Gałaźyn-Sidorczuk et al., 2009). It was reported cadmium, potassium bromate ethanol, cigarette smoke, ferric nitrilotriacetate, arsenic trioxide, methylmercury, streptozotocin, dimethylarsinic acid, cyclosporine, carbon tetrachloride and other nephrotoxic compounds can induce increase of 8-OHdG levels in the kidney (Gałaźyn-Sidorczuk et al., 2009; Umemura et al., 2009; Chen et al., 2010; Iqbal et al., 2009; Li et al., 2010; Jin et al., 2008; Imaeda et al., 2002; Etoh et al., 2003; Shi et al., 2006; Vijayaraghavan et al., 2001; Ghee et al., 2008; Jeong et al., 2009). In this study, 8-OHdG level in the kidney was determined (Fig. 5). The level of 8-OHdG was increased markedly in the kidney of D-gal-treated mice, which correlated with the level of ROS, suggesting that DNA is a common target of ROS induced by D-gal in kidney. Whereas, treatment with troxerutin produced a significant decrease in the level of kidney 8-OHdG as compared with D-gal-treated mice. Our findings indicate that troxerutin may play an effective role in protecting kidney function from D-gal-induced oxidative DNA damage by decreasing the 8-OHdG level in kidney of D-gal-treated mice.

Apoptosis is the process of programmed cell death that may occur in multicellular organisms. Cell apoptosis or necrosis can inevitably affect glomerular filtration rate and endothelial function, resulting in renal failure (Bonegio and Lieberthal, 2002; de Vries et al., 2003; Favreau et al., 2010). Studies with D-gal-lesioned mice and D-gal-lesioned rats have shown that D-gal-induced oxidative stress is a potent inducer of apoptosis. Recent reports from our laboratory and others have also demonstrated that continuous subcutaneous injection of D-gal in mice induced an increase in cell karyopyknosis, apoptosis and levels of cleaved caspase-3 protein in hippocampal neurons (Cui et al., 2006; Lu et al., 2007, 2010c). In our present study, the renal apoptosis level was significantly higher in D-gal-treated group than in vehicle controls, which may lead to impaired renal function (Fig. 7). Several reports suggest that DNA becomes an easy target for both apoptosis and necrosis, and the stability of the cellular genomic apparatus is constantly challenged by a wide-spectrum of exogenous (high energy radiations, environmental toxicants) and endogenous (oxidative damage, factor imbalance) threats, which generate DNA lesions (Hickey et al., 2001; Maurya et al., 2005, 2004a,b). Internucleosomal DNA fragmentation is a significant feature of DNA damage and apoptotic cell death. As shown in Fig. 6, there was elevated DNA fragmentation in the kidney of D-gal-treated mice, associated with increased ROS. Whereas, administration of troxerutin effectively inhibited DNA ladder formation in the kidney of gal-treated mice (Fig. 6). Moreover, high ROS concentrations contribute to the apoptotic cell death whenever they are generated in the context of the apoptotic process (Slater et al., 1995; Lu et al., 2010a). Based on labeling of DNA strand breaks in the kidney of mice, the TUNEL method was used to analyze apoptotic cells in situ (Fig. 7). Our previous reports showed that D-gal administration can induce apoptosis of brain tissue (Lu et al. 2010b). The present study showed that D-gal increased the number of TUNEL-positive cells in the kidney of mice (Fig. 7). However, we found that troxerutin markedly decreased the number of TUNEL-positive cells in mice treated with D-gal. Thus, it suggests that troxerutin has a potential nephroprotective effect against D-gal-induced DNA damage and apoptosis by its antioxidant activity.

Until now, this is the first time where the nephroprotective effects of troxerutin on D-gal-induced oxidative DNA damage have been examined. Here we demonstrated that troxerutin administration attenuated D-gal-induced renal dysfunction and histopathologic changes. Troxerutin decreased the activity of NADPH oxidases, renewed the activities of the antioxidant enzymes, down-regulated the levels of ROS and 8-OHdG, and reduced the number TUNEL-positive cells and DNA ladder formation. Troxerutin consequently improved D-gal-induced oxidative DNA damage in the kidney of mice.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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References

Aebi, H., 1984. Catalase in vitro. Meth. Enzymol. 105, 121–126.
An, W.S., Kim, H.J., Cho, K.H., Vaziri, N.D., 2009. Omega-3 fatty acid supplementation attenuates oxidative stress, inflammation, and tubulointerstitial fibrosis in the remnant kidney. Am. J. Physiol. Renal Physiol. 297, 895–903.
Blasig, I., Lowe, H., Ebert, B., 1987. Radical trapping and lipid peroxidation during myocardial reperfusion injury-Radical scavenging by troxerutin in comparison to mannitol. Biomedica Biochimica Acta 46, S539–S544.
Blasig, I., Loewe, H., Ebert, B., 1988. Effect of troxerutin and methionine on spin trapping of free oxy-radicals. Biomedica Biochimica Acta 47, S252–S255.
Boisseau, M., Taccoen, A., Garreau, C., Vergnes, C., Roudaut, M., Garreau-Gomez, B., 1995. Fibrinolysis and hemorheology in chronic venous insufficiency: a double-blind study of troxerutin efficiency. J. Cardiovas. Surg. (Torino) 36, 369–374.
Bonegio, R., Lieberthal, W., 2002. Role of apoptosis in the pathogenesis of acute renal failure. Curr. Opin. Nephrol. Hypertens. 11, 301–308.
Boots, A.W., Haenen, G.R., Bast, A., 2008. Health effects of quercetin: from antioxidant to nutraceutical. Eur. J. Pharmacol. 585, 325–337.
Cai, W., He, J., Zhu, L., Lu, C., Vlassara, H., 2006. Advanced glycation end product (AGE) receptor1 suppresses cell oxidant stress and activation signaling via EGF receptor. Proc. Nat. Acad. Sci. 103, 13801–13806.
Chen, H.I., Liou, S.H., Ho, S.F., Wu, K.Y., Sun, C.W., Chen, M.F., Cheng, L.C., Shih, T.S., Lou, C.H., 2007. Oxidative DNA damage estimated by plasma 8-hydroxydeoxyguanosine (8-OHdG): INFLUENCE of 4,4'-methylenebis (2-chloroaniline) exposure and smoking. J. occup. Health 49, 389–398.
Chen, H., Chu, G., Cao, Z., Liang, J., Lu, J., Zhou, G., 2010. Magnetic bead-based approach to monitoring of cigarette smoke-induced DNA oxidation damage and screening of natural protective compounds. Talanta 80, 1216–1221.
Cooke, M.S., Olinski, R., Evans, M.D., 2006. Does measurement of oxidative damage to DNA have clinical significance? Clin. Chim. Acta 365, 30–49.
Cui, X., Zuo, P., Zhang, Q., Li, X., Hu, Y., Long, J., Packer, L., Liu, J., 2006. Chronic systemic D-galactose exposure induces memory loss, neurodegeneration, and oxidative damage in mice. protective effects of R-alpha-lipoic acid. J. Neurosci. Res. 83, 1584–1590.
Cuzzocrea, S., McDonald, M.C., Filipe, H.M., Costantino, G., Mazzon, E., Santagati, S., Caputi, A.P., Thiemermann, C., 2000. Effects of tempol, a membrane-permeable radical scavenger, in a rodent model of carrageenan-induced pleurisy. Eur. J. Pharmacol. 390, 209–222.
de Jesus Soares, T., Volpini, R.A., Francescato, H.D., Costa, R.S., da Silva, C.G., Coimbra, T.M., 2007. Effects of resveratrol on glycerol-induced renal injury. Life Sci. 81, 647–656.
de Vries, B., Matthijsen, R.A., van Bijnen, A.A., Wolfs, T.G., Buurman, W.A., 2003. Lysophosphatidic acid prevents renal ischemia-reperfusion injury by inhibition of apoptosis and complement activation. Am. J. Pathol. 163, 47–56.
Dröge, W., 2002. Free radicals in the physiological control of cell function. Physiol. Rev. 82, 47–95.
Dworakowski, R., Alom-Ruiz, S.P., Shah, A.M., 2008. NADPH oxidase-derived reactive oxygen species in the regulation of endothelial phenotype. Pharmacol. Rep. 60, 21–28.
Etoh, T., Inoguchi, T., Kakimoto, M., Sonoda, N., Kobayashi, K., Kuroda, J., Sumimoto, H., Nawata, H., 2003. Increased expression of NAD(P)H oxidase subunits, NOX4 and p22phox, in the kidney of streptozotocin-induced diabetic rats and its reversibity by interventive insulin treatment. Diabetologia 46, 1428–1437.
Fan, S.H., Zhang, Z.F., Zheng, Y.L., Lu, J., Wu, D.M., Shan, Q., Hu, B., Wang, Y.Y., 2009. Troxerutin protects the mouse kidney from D-galactose-caused injury through anti-inflammation and anti-oxidation. Int. Immunopharmacol. 9, 91–96.
Favreau, F., Zhu, X.Y., Krier, J.D., Lin, J., Warner, L., Textor, S.C., Lerman, L.O., 2010. Revascularization of swine renal artery stenosis improves renal function but not the changes in vascular structure. Kidney Int. (in press).
Francescato, H.D., Costa, R.S., Scavone, C., Coimbra, T.M., 2007. Parthenolide reduces cisplatin-induced renal damage. Toxicology 230, 64–75.
Gałaźyn-Sidorczuk, M., Brzóska, M.M., Jurczuk, M., Moniuszko-Jakoniuk, J., 2009. Oxidative damage to proteins and DNA in rats exposed to cadmium and/or ethanol. Chem. Biol. Interact. 180, 31–38.

Gandhi, N., Maurya, D., Salvi, V., Kapoor, S., Mukherjee, T., Nair, C., 2004. Radioprotection of DNA by glycyrrhizic acid through scavenging free radicals. J. Radiat. Res. (Tokyo) 45, 461–468.

Ghee, J.Y., Han, D.H., Song, H.K., Kim, W.Y., Kim, S.H., Yoon, H.E., Choi, B.S., Kim, Y.S., Kim, J., Yang, C.W., 2008. The role of macrophage in the pathogenesis of chronic cyclosporine-induced nephropathy. Nephrol. Dial. Transplant. 23, 4061–4069.

Gohel, M.S., Davies, A.H., 2009. Pharmacological agents in the treatment of venous disease: an update of the available evidence. Curr. Vasc. Pharmacol. 7, 303–308.

Helbock, H.J., Beckman, K.B., Shigenaga, M.K., Walter, P.B., Woodall, A.A., Yeo, H.C., Ames, B.N., 1998. DNA oxidation matters: the HPLC-electrochemical detection assay of 8-oxo-deoxyguanosine and 8-oxo-deoxyguanine. Proc. Nat. Acad. Sci USA 95, 288–293.

Hickey, E.J., Raje, R.R., Reid, V.E., Gross, S.M., Ray, S.D., 2001. Diclofenac induced in vivo nephrotoxicity may involve oxidative stress-mediated massive genomic DNA fragmentation and apoptotic cell death. Free Radic. Biol.Med. 31, 139–152.

Imaeda, A., Kaneko, T., Aoki, T., Kondo, Y., Nagase, H., 2002. DNA damage and the effect of antioxidants in streptozotocin-treated mice. Food Chem. Toxicol. 40, 979–987.

Iqbal, M., Okazaki, Y., Okada, S., 2009. Curcumin attenuates oxidative damage in animals treated with a renal carcinogen, ferric nitrilotriacetate (Fe-NTA): implications for cancer prevention. Mol Cell Biochem. 324, 157–164.

Jeong, K.H., Lee, T.W., Ihm, C.G., Lee, S.H., Moon, J.Y., Lim, S.J., 2009. Effects of sildenafil on oxidative and inflammatory injuries of the kidney in streptozotocin-induced diabetic rats. Am. J. Nephrol. 29, 274–282.

Jiang, F., 2009. NADPH oxidase in the kidney: a Janus in determining cell fate. Kidney Int. 75, 135–137.

Jin, X.L., Chan, H.M., Lok, E., Kapal, K., Taylor, M., Kubow, S., Mehta, R., 2008. Dietary fats modulate methylmercury-mediated systemic oxidative stressand oxidative DNA damagein rats. Food Chem. Toxicol. 46, 1706–1720.

Kessler, M., Ubeaud, G., Walter, T., Sturm, F., Jung, L., 2002. Free radic scavenging and skin penetration of troxerutin and vitamin derivatives. J. Dermatolog. Treat. 13, 133–141.

Kim, J., Park, J.W., Park, K.M., 2009. Increased superoxide formation induced by irradiation preconditioning triggers kidney resistance to ischemia-reperfusion injury in mice. Am. J. Physiol. Renal Physiol. 296, 1202–1211.

Lee, P.J., Choi, A.M.K., 2003. Pathways of cell signaling in hyperoxia. Free Radic. Biol. Med. 35, 341–350.

Li, Z.Y., Piao, F.Y., Liu, S., Wang, Y., Qu, S.X., 2010. Subchronic exposure to arsenic trioxide-induced oxidative DNA damage kidney tissue of mice. Exp.Toxicol. Pathol. (in press).

Liu, C.M., Zheng, Y.L., Lu, J., Zhang, Z.F., Fan, S.H., Wu, D.M., Ma, J.Q., 2010. Quercetin protects rat liver against lead-induced oxidative stress and apoptosis. Environ. Toxicol. Pharmacol. 29, 158–166.

Lu, J., Zheng, Y.L., Wu, D.M., Luo, L., Sun, D.X., Shan, Q., 2007. Ursolic acid ameliorates cognition deficits and attenuates oxidative damage in the brain of senescent mice induced by D-galactose. Biochem. Pharmacol. 74, 1078–1090.

Lu, J., Wu, D.M., Hu, B., Cheng, W., Zheng, Y.L., Zhang, Z.F., Ye, Q., Fan, S.H., Shan, Q., Wang, Y.J., 2010a. Chronic administration of troxerutin protects mouse brain against D-galactose-induced impairment of cholinergic system. Neurobiol. Learn. Mem. 93, 157–164.

Lu, J., Wu, D.M., Zheng, Y.L., Hu, B., Zhang, Z.F., Ye, Q., Liu, C.M., Shan, Q., Wang, Y.J., 2010 (b). Ursolic Acid Attenuates D-Galactose-induced inflammatory responsein mouse prefrontal cortex through inhibiting AGEs/RAGE/NF-kB pathway activation. Cerebral Cortex. (in press).

Lu, J., Wu, D.M., Zheng, Y.L., Hu, B., Zhang, Z.F., 2010c. Purple sweet potato color alleviates D-galactose-induced brain aging in old mice by promoting survival of neurons via PI3K pathway and inhibiting cytochrome C-mediated apoptosis. Brain Pathol. 20, 598–612.

Maurya, D.K., Salvi, V.P., Nair, C.K.K., 2004a. Radioprotection of normal tissues in tumour-bearing mice by troxerutin. J. Radiat. Res. 45, 221–228.

Maurya, D.K., Salvi, V.P., Nair, C.K.K., 2004b. Troxerutin as a radioprotector. Ind. J. Radiat. Res. 1, 28–38.

Maurya, D.K., Balakrishnan, S., Salvi, V.P., Nair, C.K.K., 2005. Protection of cellular DNA from gamma-radiation induced damages and enhancement in DNA repair by troxerutin. Mol. Cell. Biochem. 280, 57–68.

McCord, J.M., Fridovich, I., 1969. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J. Biol. Chem. 244, 6049–6055.

Paglia, D.E., Valentine, W.N., 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 70, 158–169.

Pilger, A., Rudiger, H.W., 2006. 8-Hydroxydeoxyguanosine as a marker of oxidative damage related to occupational and environmental exposure. Int. Arch Occup. Environ. Health 8, 1–15.

Ramesh, T., Begum, V.H., 2008. Protective effect of sesbania grandiflora against cigarette smoke-induced oxidative damage in rats. J. Med. Food 11, 369–375.

Rodrigo, R., Trujillo, S., Bosco, C., Orellana, M., Thielemann, L., Araya, J., 2002. Changes in (Na+K)-adenosine triphosphatase activity and ultrastructure of lung and kidney associated with oxidative stress induced by acute ethanol intoxication. Chest 121, 589–596.

Sarkar, A., Biswas, N., Kapoor, S., Mahal, H.S., Nair, C.K.K., Mukherjee, T., 2005. One-electron redox reactions of troxerutin in aqueous solutions. Res. Chem. Intermed. 31, 857–866.

Shan, Q., Lu, J., Zheng, Y., Li, J., Zhou, Z., Hu, B., Zhang, Z., Fan, S., Mao, Z., Wang, Y.J., Ma, D., 2009. Purple sweet potato color ameliorates cognition deficits and attenuates oxidative damage and inflammation in aging mouse brain induced by D-galactose. J. Biomed. Biotechnol. 2009, 1–9.

Shi, Z.M., Feng, P., Jiang, D.Q., Wang, X.J., 2006. Mistletoe alkali inhibits peroxidation in rat liver and kidney. World J. Gastroenterol. 12, 4052–4055.

Shih, W., Hines, W.H., Neilson, E.G., 1988. Effects of cyclosporine A on the development of immune-mediated interstitial nephritis. Kidney Int. 33, 1113–1118.

Shinomol, G.K., Muralidhara, 2007. Differential induction of oxidative impairments in brain regions of male mice following subchronic consumption of Khesari dhal (*Lathyrus sativus*) and detoxified Khesari dhal. Neurotoxicology 28, 798–806.

Singh, R., Sram, R.J., Binkova, B., Kalina, I., Popov, T.A., Georgieva, T., Garte, S., Taioli, E., Farmer, P.B., 2007. The relationship between biomarkers of oxidative DNA damage, polycyclic aromatic hydrocarbon DNA adducts, antioxidant status and genetic susceptibility following exposure to environmental air pollution in humans. Mutat Res. 620, 83–92.

Slater, A.F.G., Stefan, C., Nobel, I., Van Den Dobbelsteen, D.J., Orrenius, S., 1995. Signalling mechanisms and oxidative stress in apoptosis. Toxicol. Lett. 82, 149–153.

Tian, J., Ishibashi, K., Reiser, K., Grebe, R., Biswal, S., Gehlbach, P., Handa, J.T., 2005. Advanced glycation endproduct-induced aging of the retinal pigment epithelium and choroid: a comprehensive transcriptional response. Proc. Nat. Acad. Sci. 102, 11846–11851.

Tian, N., Moore, R.S., Phillips, W.E., Lin, L., Braddy, S., Pryor, J.S., Stockstill, R.L., Hughson, M.D., Manning Jr., R.D., 2008. NADPH oxidase contributes to renal damage and dysfunction in Dahl salt-sensitive hypertension. Am. J. Physiol. Regul. Integr. Comp. Physiol. 295, 1858–1865.

Tokunaga, I., Kubo, S., Ishigami, A., Gotohda, T., Kitamura, O., 2006. Changes in renal function and oxidative damage in methamphetamine-treated rat. Leg. Med. 8, 16–21.

Toyokuni, S., Okamoto, K., Yodoi, J., Hiai, H., 1995. Persistent oxidative stress in cancer. FEBS Lett. 358, 1–3.

Ueki, M., Taie, S., Chujo, K., Asaga, T., Iwanaga, Y., Ono, J., Maekawa, N., 2007. Urinary trypsin inhibitor reduces inflammatory response in kidney induced by lipopolysaccharide. J. Biosci. Bioeng. 104, 315–320.

Umemura, T., Tasaki, M., Kijima, A., Okamura, T., Inoue, T., Ishii, Y., Suzuki, Y., Masui, N., Nohmi, T., Nishikawa, A., 2009. Possible participation of oxidative stress in causation of cell proliferation and in vivo mutagenicity in kidneys of gpt delta rats treated with potassium bromate. Toxicology 257, 46–52.

Valko, M., Rhodes, C.J., Moncol, J., Izakovic, M., Mazur, M., 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem. Biol. Interact. 160, 1–40.

Vijayaraghavan, M., Wanibuchi, H., Karim, R., Yamamoto, S., Masuda, C., Nakae, D., Konishi, Y., Fukushima, S., 2001. Dimethylarsinic acid induces 8-hydroxy-2′-deoxyguanosine formation in the kidney of NCI-Black-Reiterrats. Cancer Lett. 165, 11–17.

Wang, Q., Sun, A.Y., Simonyi, A., Kalogeris, T.J., Miller, D.K., Sun, G.Y., Korthuis, R.J., 2007. Ethanol preconditioning protects against ischemia/reperfusion-induced brain damage: role of NADPH oxidase-derived ROS. Free Radic. Biol. Med. 43, 1048–1060.

Wenisch, C., Biffignandi, P., 2001. Effect of bioflavonoids (trihydroxyethylrutin and disodium flavodate) in vitro on neutrophilreactive oxygen production and phagocytic ability assessed by flow cytometry. Curr. Med. Res. Opin. 17, 123–127.

Wu, J., Chen, X., Xie, Y., Yamanaka, N., Shi, S., Wu, D., Liu, S., Cai, G., 2005. Characteristics and risk factors of intrarenal arterial lesions in patients with IgA nephropathy. Nephrol. Dial. Transplant. 20, 719–727.

Wu, D.M., Lu, J., Zheng, Y.L., Zhou, Z., Shan, Q., Ma, D.F., 2008. Purple sweet potato color repairs D-galactose-induced spatial learning and memory impairment by regulating the expression of synaptic proteins. Neurobiol. Learn. Mem. 90, 19–27.

Xie, X.S., Liu, H.C., Yang, M., Zuo, C., Deng, Y., Fan, J.M., 2009. Ginsenoside Rb1, a panoxadiol saponin against oxidative damage and renal interstitial fibrosis in rats with unilateral ureteral obstruction. Chin. J. Integr. Med. 15, 133–140.

Yoshioka, N., Nakashima, H., Hosoda, K., Eitaki, Y., Shimada, N., Omae, K., 2008. Urinary excretion of an oxidative stress marker, 8-hydroxyguanine (8-OH-Gua), among nickel-cadmium battery workers. J. Occup. Health 50, 229–235.

Zhang, Q., Li, X., Cui, X., Zuo, P., 2005. D-Galactose injured neurogenesis in the hippocampus of adult mice. Neurol. Res. 27, 552–556.

Zhang, Z.F., Fan, S.H., Zheng, Y.L., Lu, J., Wu, D.M., Shan, Q., Hu, B., 2009. Troxerutin protects the mouse liver against oxidative tress-mediated injury induced by D-galactose. J. Agric. Food Chem. 57, 7731–7736.