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ORIGINAL ARTICLE

Ameliorating effect of troxerutin in unilateral ureteral obstruction induced renal oxidative stress, inflammation, and apoptosis in male rats

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Abstract

Unilateral ureteral obstruction (UUO) induces renal injury and troxerutin attenuates the inflammatory parameters and decreases oxidative stress. Accordingly, this study explored the renoprotective effect of troxerutin in UUO-induced renal oxidative stress, inflammation, and apoptosis in male Wistar rats. Animals were randomly separated into five groups ($n = 8$): control, UUO, and three UUO groups treated with troxerutin (1, 10, and 100 mg/kg). UUO-induced and vehicle/troxerutin administration was continued for 3 days. Then serum creatinine, mean arterial pressure (MAP), renal perfusion pressure (RPP), renal vascular resistance (RVR), and renal blood flow (RBF) were measured. Superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase activities, total antioxidant capacity (TAC), and malondialdehyde (MDA) levels as some oxidative stress parameters were measured in the left kidney. The immunoblotting method was applied to evaluate the cleaved caspase-3 Bax, Bcl-2, and TNF-α proteins level. The hematoxylin and eosin method was used to assess the kidney tissue damage score (KTDS). In 3 days, UUO significantly increased serum creatinine level, KTDS, RVR, MDA, Bax, cleaved caspase-3, and TNF-α protein levels ($p < 0.05$); and decreased RBF, TAC, SOD, catalase, GPx activity levels and Bcl-2 protein expression level in the left kidney ($p < 0.05$). Troxerutin (100 mg/kg) significantly attenuates the indicators alteration induced by UUO. Our findings represented that the renoprotective effect of troxerutin may be related to its anti-oxidative stress, anti-inflammation, anti-apoptosis, and RBF improver properties.

Keywords Unilateral ureteral obstruction · Troxerutin · Oxidative stress · Apoptosis · Inflammation · Rat

Introduction

Obstruction of the urinary tract is considered as a common clinical situation which can be conducive to kidney parenchymal injury (Vaughan et al. 2004). Unilateral ureteral obstruction (UUO) as an experimental model increases the renal vascular resistance (RVR) progressively (Chevalier 2006) and then decreases the renal blood flow (RBF), and glomerular filtration rate (GFR) via afferent and efferent arterioles constrict elevation (Hassanshahi et al. 2017). After UUO, various factors leading to vasoconstriction in the ipsilateral kidney (Chevalier 2006) predisposes the ipsilateral kidney to permanent hypoxia and tubulointerstitial fibrosis (Sun et al. 2012). Then renal fibrosis and renal microvasculature damage as two main factors induce kidney injury (Kim et al. 2006). After a short time, UUO increases histopathological changes in the ipsilateral kidney (Taal et al. 2011) and eventually induces

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obstructive nephropathy (Alpern and Hebert 2007). Moreover, UUO increases the macrophages infiltration and cytokine production (Thornhill et al. 2005), and upregulates the expression of tumor necrosis factor- , (TNF-) in the ipsilateral kidney (Mezzano et al. 2001; Rüster and Wolf 2006). These mediators can induce renal inflammation and fibrosis (Thornhill et al. 2005). In addition, UUO increases oxidative stress, via reactive oxygen species (ROS) formation (Felsen et al. 2003). Collectively, all these factors activate the apoptotic pathway via upregulation of caspase protein and downregulation of anti-apoptotic Bcl-2 protein then induce renal apoptosis (Zhang et al. 2001; Gulmi et al. 2002; Docherty et al. 2006). If UUO is not treated, it can eventually induce kidney injury (Hammad et al. 2014). For this reason, today, clinical urologists try to find a therapeutic strategy for treatment of UUO patients (Gulmi et al. 2002). In this regard, it has revealed that any mediator involved in obstructed kidney injury can be applied as one therapeutic target (Ucero et al. 2010). In addition, it has been shown that the natural compounds are useful in decreasing kidney injury because their pharmacological properties are acceptable and their side effects are very low (Naso et al. 2016); so, the natural compounds are more attractive against synthetic products for many researchers (El-Razek 2007; Naso et al. 2016). Troxerutin (vitamin P4) as a natural bioflavonoid compound exists in fruits, vegetables, and cereals (Yang et al. 2006). Troxerutin has a vasoprotective effect against hemorrhoidal disease and modifies the microcirculation (Sumboonnanonda and Lertsithichai 2004). Moreover, troxerutin has strong antioxidant properties (Sampath and Karundevi 2014), so that it significantly attenuates the kidney injury via radical scavenging mechanism in aged animal (Fan et al. 2009). In addition, it has been reported that troxerutin modulates the inflammatory parameters and inhibits the oxidative stress mediators in the animal brain (Lu et al. 2011; Lu et al. 2013). Also, troxerutin ameliorates the vascular abnormalities via inhibition of lipid peroxidation and oxidative stress parameters (Badalzadeh et al. 2017). Furthermore, it has been revealed that troxerutin can decrease heart injury through its anti-apoptotic effect in the heart ischemia-reperfusion model (Badalzadeh et al. 2017) and has a nephron-protective effect via its antioxidant and anti-inflammatory activities (Fan et al. 2009). Totally, troxerutin is a suitable agent for medical research due to its wide pharmacological effects (Yang et al. 2006), since the previous investigations have been revealed that continuing UUO decreases RBF and GFR and induces oxidative stress in the obstructed kidney (Quinlan et al. 2008). Moreover, oxidative stress directly by damage to pivotal structures of renal cells leads to apoptosis and cell death (Chevalier et al. 2010; Yeh et al. 2011). Also, ROS generation indirectly leads to rising in pro-inflammatory cytokines such as TNF-α and can induce apoptosis (Rüster and Wolf 2006; Chung et al. 2012); accordingly, this exploratory study was designed to evaluate the renoprotective effect of troxerutin following UUO in male rats with emphasis on renal hemodynamic, function, oxidative stress, inflammation, apoptosis, and histopathologic parameters.

Material and methods

Animals

In this experimental study, forty male Wistar rats (8 weeks old) weighing 213 ± 5 g were supplied by Rafsanjan University of Medical Sciences Animal House, Rafsanjan, Iran. The Wistar rats were housed in a temperature-controlled room (23 ± 1 °C) with 12 h light/dark cycle, and humidity 50%. Rats were fed by a standard rodent chow diet with free access to tap water. The Ethics Committee of Rafsanjan University of Medical Sciences approved the study (Ethics No. IR.RUMS.REC.1397.079). All experimental study was done in accordance with the guidelines for animal care and use of laboratory animals (National Institutes of Health Publication No. 85-23) revised in 2010.

Experimental groups and UUO model

After a week of adaptation to their environment, Wistar rats were weighed by a blinded person to the study and were randomly divided into 5 groups ($n = 8$ per group) including the following:

Group 1 (Sham + Saline): this group was considered as control (Liu et al. 2015). In this group, rats were subjected to sham-operated model and then received saline by intraperitoneal (i.p.) injection once-daily for 3 days.

Group 2 (UUO + Saline): UUO rats received troxerutin vehicle (saline, i.p.) 1 h after UUO for 3 days (once-daily).

Groups 3, 4, and 5 (UUO + TXR 1, 10, and 100): UUO groups received troxerutin (10 and 100 mg/kg, i.p.) 1 h after UUO for 3 days (once-daily).

Each rat was anesthetized with 450 mg/kg chloral hydrate (i.p.) (Sigma St. Louis, USA), and then under sterile condition, laparotomy was done on the left quadrant of the abdomen. The left ureter was exposed and ligated with a 4-0 silk suture (groups 2–5). After the formation of UUO or sham-operated models, rats were permitted to recuperate from the general anesthesia. A similar operation was done in the Sham + Saline group without UUO creation (group 1). The ligation or sham-operated patterns continued until 3 days after surgery (Hassanshahi et al. 2018). Synchronically, troxerutin (Sigma Aldrich, St. Louis, MO, USA) was dissolved in saline solution and then vehicle or troxerutin (1, 10, 100 mg/kg; i.p.) was administered to animals 1 h after UUO for 3 days (once-daily). All rats were administered with equivalent volumes of vehicle

or troxerutin. Then all animals were weighted and entered into the experimental protocol.

Experimental protocol

Hemodynamic parameters measurement

Urethane at a dose of 1.7 g/kg body weight (i.p.) (Sigma, St. Louis, USA) was applied to anesthetize each rat. Then the animal's body temperature was sustained at 37 °C helping a heated platform throughout the experimental protocol cycle. A polyethylene tube (Microtube Extrusions, Australia) was used for tracheal intubation to facilitate breathing (Hassanshahi and Nematbakhsh 2018). Also, the left carotid and femoral arteries were exposed and separated from surrounding tissues (Samimiat et al. 2018). Then these arteries were catheterized by a polyethylene tube and jointed to a bridge amplifier helping transducer cable, connected to power lab hardware (ADInstrument, Australia) and lab chart program 6 software for assessment of mean arterial pressure (MAP) and renal perfusion pressure (RPP) respectively. Moreover, the ipsilateral kidney was exposed and the left renal artery was isolated, then RBF was obtained as perfusion units (PU) helping needle-type laser Doppler probe (DP4s, Moor Instruments, UK) linked to a laser Doppler perfusion monitor instrument (DRT4, Moor Instruments, UK) (Hantz et al. 2001). The hemodynamic parameters recording was continued for 30–45 min to stabilize the parameters, then the last 5 min of equilibrium time was extracted for data analysis (Hassanshahi et al. 2018). RVR was obtained from the RPP to RBF ratio (mm Hg/perfusion units) (Hantz et al. 2001).

Serum creatinine measurement

At the end of the equilibrium period, the blood samples were collected from each rat via heart puncture then centrifuged at 6000*g* for 15 min. The serum creatinine (Cr) level was detected via quantitative diagnostic kits (Pars Azmoon, Iran).

Oxidative stress parameters measurement

Following the collection of the blood sample, animals were decapitated in deep anesthesia and the left kidney was harvested immediately and the dissected half of kidney tissue was frozen in liquid nitrogen and stored at − 80 °C until measurement procedures. To assess the superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) activities, total antioxidant capacity (TAC), and malondialdehyde (MDA) levels as some oxidative stress parameters were measured in the kidney tissue via commercial assay kits (ZellBio, Germany).

Immunoblotting analysis

The immunoblotting method was used to examine the protein levels involved in inflammation (TNF-α) and apoptosis (cleaved caspase-3, Bax, and Bcl-2) in the left kidney tissue. Briefly, the collected protein samples were separated by electrophoresis on 12.5% polyacrylamide gel and then transferred to nitrocellulose membrane. The membranes were incubated overnight at 4 °C in Tris-buffered saline as well as Tween 20 (TBST) (20 mM Tris–HCl, 0.1%Tween20, 150 mM NaCl, pH 7.4) with 5% nonfat milk. Afterward, the nitrocellulose membranes were incubated with monoclonal rabbit anti-TNF-α (Abcam, ab205587, USA, 1:1000), anti-caspase-3, (Cell signaling, 9664, USA, 1:1000), anti-Bax (Abcam, ab182733, USA, 1:1000), and anti-Bcl-2 (Cell signaling, 3498, USA, 1:1000) antibodies for 3 h at room temperature (RT). For dilution of all antibodies, the blocking buffer was used. Then the blots were washed three times with TBST and incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody (Abcam, ab205718, USA, 1:5000) for 1 h at RT. The enhanced chemiluminescence method was applied to the blots detection. The analyzing software (ImageJ) was used for band densitometry. Beta-actin (β-actin) immunoblotting (Abcam, ab115777, USA, 1:5000) was applied as a loading control. We presented the ratio of each protein band densitometry to its β-actin band in each sample for all experimental groups.

Histopathological assessments

The other half of ipsilateral kidney was fixed in buffered natural formalin solution (10%). Then all kidney tissues were cut into 6-μm sections. Subsequently, hematoxylin and eosin (H&E) method was applied for stained the kidney tissues samples. The kidney tissue damage score (KTDS) was investigated by a pathologist (who was blinded to the study) via H&E staining and evaluated semi-quantitatively, as described in previous studies (Wang et al. 2016; Sancak et al. 2017). Briefly, ten tubulointerstitial fields (magnification × 100) were randomly selected and the degree of tubulointerstitial injury was graded from 0 to 3 according to components including: tubular cell vacuolization, tubular dilatation and debris, tubular atrophy, hyaline cast, interstitial infiltration, interstitial edema, and fibrosis in the left kidney tissue. The kidney tissue sample was graded from 0 to 3 based on intensity of damage for each sample (0–0.5 = normal kidney without damage, 1 = minor damage, 2 = moderate damage, 3 = severe damage).

Statistical analysis

All animal data were statistically analyzed helping GraphPad Prism version 6.01 for Windows (GraphPad Software, USA) and are shown as mean ± SD (standard deviation). One-way

ANOVA followed by the post hoc Tukey test was applied for comparison between the groups in each quantitative data. However, the KTDS between the groups were compared by the Kruskal-Wallis test. Differences at the 95% confidence interval were considered statistically significant.

Results

The effect of troxerutin on body weight, hemodynamic parameters, and serum creatinine level

The result of this exploratory study showed that at the end of the study, the animal's body weight was not any significant change in all groups with each other (Table 1), and also, it has not changed significantly compared to the baseline (at the first of study) in all groups. Moreover, UUO for 3 days does not alter significantly the MAP and RPP indicators between the groups to each other (Table 1), while it significantly reduces the RBF (ANOVA, F (4, 15) = 10.62, $p < 0.001$; followed by Tukey's post hoc test, $p < 0.001$) and increases the RVR in UUO + Saline group versus Sham + Saline group (ANOVA, F (4, 15) = 6.38, $p < 0.01$; followed by Tukey's post hoc test, $p < 0.01$). However, in our study, troxerutin could increase the RBF (Tukey's post hoc test, $p < 0.05$) and decrease the RVR (Tukey's post hoc test, $p < 0.05$) in the UUO + 100 mg/kg TXR group versus UUO + Saline group. Also, 3 days UUO significantly increased the serum creatinine level as a main renal function parameter (ANOVA, F (4, 15) = 5.44, $p < 0.01$; followed by Tukey's post hoc test, $p < 0.01$). In addition, troxerutin could decrease the serum creatinine level in the UUO + 100 mg/kg TXR group compared with UUO + Saline group (Tukey's post hoc test, $p < 0.05$) (Table 1).

The effect of troxerutin on oxidative stress parameters (SOD, catalase, and GPx activities, total antioxidant capacity, and MDA level)

As illustrated in Fig. 1a, the oxidative stress status was evaluated in the ipsilateral kidney tissue 3 days after UUO. According to the results, the ANOVA analysis of MDA indicator showed a significant difference between the experimental groups (F (4, 35) = 6.51, $p < 0.001$). So that UUO increases the lipid peroxidation (increase ipsilateral kidney MDA level) significantly in UUO + Saline group versus Sham + Saline group (Tukey's post hoc test, $p < 0.01$). Also, troxerutin at the dose of 100 mg/kg could significantly attenuate the MDA level in the UUO + 100 mg/kg TXR group compared with UUO + Saline group (Tukey's post hoc test, $p < 0.05$), while lower doses of troxerutin did not have any significant effect.

Moreover, 3 days UUO significantly reduced the SOD (ANOVA, F (4, 35) = 5.45, $p < 0.01$; followed by Tukey's post hoc test, $p < 0.05$), GPx (ANOVA, F (4, 35) = 6.67, $p < 0.001$; followed by Tukey's post hoc test, $p < 0.01$), and catalase (ANOVA, F (4, 35) = 6.52, $p < 0.001$; followed by Tukey's post hoc test, $p < 0.01$) activities level as three main antioxidant enzymes in the left kidney tissue in UUO vehicle-treated group versus Sham + Saline rats. Also, troxerutin at dose of 100 mg/kg could increase significantly the SOD (Tukey's post hoc test, $p < 0.05$), GPx (Tukey's post hoc test, $p < 0.01$), and catalase (Tukey's post hoc test, $p < 0.05$) activity levels in the UUO + 100 mg/kg TXR group when compared with UUO + Saline group. Moreover, troxerutin also increased the GPx activity at the dose of 10 mg/kg (Tukey's post hoc test, $p < 0.05$) (Fig. 1b, c, d).

Also, the results of ANOVA analysis for TAC indicator showed a significant differences between the animal groups (ANOVA, F (4, 35) = 5.59, $p < 0.01$). So that UUO decreases the TAC indicator significantly in the ipsilateral kidney tissue after 3 days in UUO + Saline group

Table 1 The body weight, serum creatinine level, and hemodynamic parameters values at 3 days after vehicle/troxerutin administration in all experimental groups. Each data value is represented as mean ± SD. $n = 8$/group. Statistical evaluation was obtained by ANOVA followed by Tukey. $^{**}p < 0.001$ and $^{***}p < 0.001$ compared with Sham + Saline group. $^{\#}p < 0.05$ compared with UUO + Saline group. UUO, unilateral ureteral obstruction; TXR, troxerutin; MAP, mean arterial pressure; RBF, renal blood flow; RPP, renal perfusion pressure; RVR, renal vascular resistance; Cr, creatinine

Groups	Body weight (g)	Hemodynamic parameters				Functional parameter
		(mm Hg)	RPP (mm Hg)	PBF (PU)	RVR (mm Hg/PU)	Cr (mg/day)
Sham + Saline	212 ± 4.76	102 ± 6.51	89 ± 2.50	151 ± 11.18	0.59 ± 0.05	0.61 ± 0.04
UUO + Saline	211 ± 4.17	106 ± 4.58	95 ± 7.89	107 ± 7.14***	0.89 ± 0.14**	0.86 ± 0.09**
UUO + 1 mg/kg TXR	210 ± 3.34	103 ± 5.69	91 ± 5.98	113 ± 8.53	0.81 ± 0.03	0.72 ± 0.06
UUO + 10 mg/kg TXR	208 ± 5.85	104 ± 5.21	92 ± 6.11	121 ± 13.03	0.76 ± 0.11	0.75 ± 0.13
UUO + 100 mg/kg TXR	210 ± 5.23	102 ± 7.38	92 ± 4.17	135 ± 13.14#	0.68 ± 0.05#	0.62 ± 0.07#

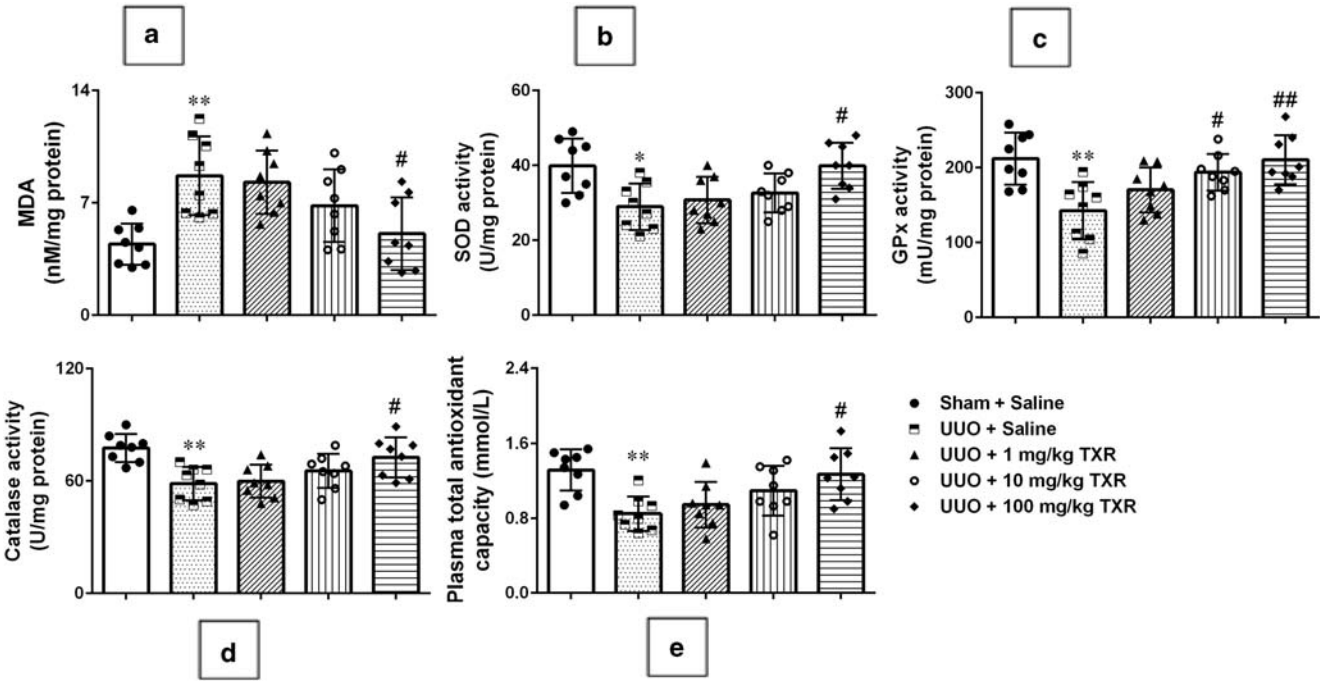

Fig. 1 The oxidative stress parameters values (SOD, catalase and GPx activities, total antioxidant capacity, and MDA level) 3 days after vehicle/troxerutin administration in all experimental groups. Each data value is represented as mean ± SD. $n = 8$/group. Statistical evaluation was obtained by ANOVA followed by Tukey. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with Sham + Saline group. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared with UUO + Saline group. UUO, unilateral ureteral obstruction; TXR, troxerutin; MDA, malondialdehyde; SOD, superoxide dismutase; GPx, glutathione peroxidase

compared with Sham + Saline group (Tukey's post hoc test, $p < 0.01$), while troxerutin administration could significantly increase the TAC level in the UUO + 100 mg/kg TXR group compared with UUO + Saline group (Tukey's post hoc test, $p < 0.05$) (Fig. 1e).

The effect of troxerutin on TNF-α protein expression level

As shown in Fig. 2, the immunoblotting analysis revealed that TNF-α protein expression level increases significantly in the

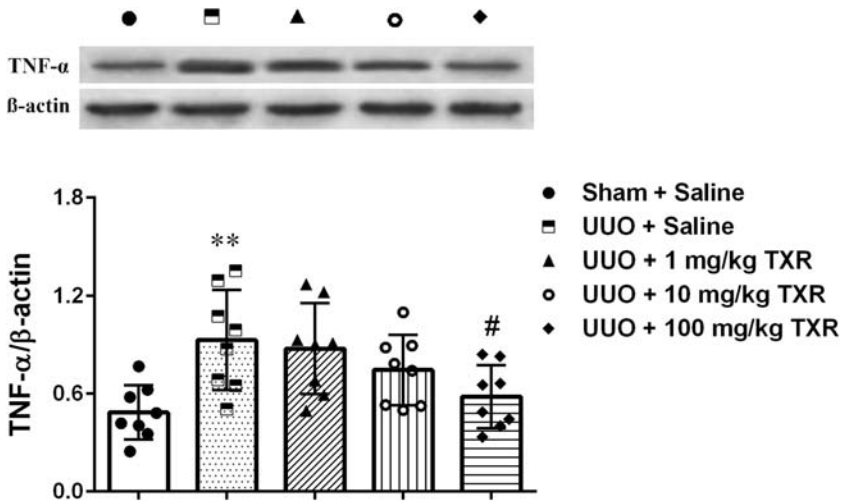

Fig. 2 Immunoblotting analysis of the TNF-α protein levels in obstructed kidney tissue at 3 days after vehicle/troxerutin administration in all experimental groups. Each data value is represented as mean ± SD. $n = 8$/group. Statistical evaluation was obtained using ANOVA followed by Tukey. $^{**}p < 0.01$ compared with Sham + Saline group. $^{\#}p < 0.05$ compared with UUO + Saline group. UUO, unilateral ureteral obstruction; TXR, troxerutin; TNF-α, tumor necrosis factor alpha

left kidney 3 days after obstruction in UUO + Saline group versus Sham + Saline group (ANOVA, F (4, 35) = 5.02, $p < 0.01$; followed by Tukey's post hoc test, $p < 0.01$). In addition, troxerutin at dose of 100 mg/kg could decrease significantly the TNF-α protein expression level as a main inflammatory indicator from 0.93 ± 0.31 value (TNF-α/β-actin) to 0.58 ± 0.19 in the UUO + 100 mg/kg TXR group compared with UUO + Saline group (Tukey's post hoc test, $p < 0.05$) (Fig. 2).

The effect of troxerutin on renal tissue apoptosis

The immunoblotting analysis also revealed that the 3 days UUO significantly increased the expression of the cleaved caspase-3 (ANOVA, F (4, 35) = 7.54, $p < 0.001$; followed by Tukey's post hoc test, $p < 0.01$) and Bax (ANOVA, F (4, 35) = 6.76, $p < 0.001$; followed by Tukey's post hoc test, $p < 0.01$) proteins and also decreased the expression of Bcl-2 protein (ANOVA, F (4, 35) = 3.86, $p < 0.01$; followed by Tukey's post hoc test, $p < 0.05$) in the ipsilateral kidney in UUO + Saline rats versus Sham + Saline group (Fig. 3). Furthermore, as shown in Fig. 3 a and b, troxerutin (100 mg/kg) significantly decreased the cleaved caspase-3 (Tukey's post hoc test, $p < 0.05$) and Bax (Tukey's post hoc test, $p < 0.05$) proteins expression level in the UUO + 100mg/kg TXR group when compared with UUO + Saline group. In addition, troxerutin has a statistically significant effect on the cleaved caspase-3 protein expression level at the dose of 10 mg/kg in the UUO + 10 mg/kg TXR group when compared with UUO + Saline group (Tukey's post hoc test, $p < 0.05$). However, troxerutin did not have any significant effect on Bcl-2 protein expression level in all UUO-treated groups compared with UUO + Saline group (Fig. 3c). As shown in Fig. 3d, troxerutin (100 mg/kg) could significantly decrease the Bax:Bcl-2 ratio in UUO + 100 mg/kg TXR group versus UUO + Saline group (ANOVA, F (4, 35) = 10.46, $p < 0.001$; followed by Tukey's post hoc test, $p < 0.01$).

The effect of troxerutin on renal tissue damage

According to the hematoxylin and eosin staining results, no pathologic finding was seen in Sham + Saline group (Fig. 4a, b). Also, it was found that the renal tissue damage increased 3 days after UUO, so that severe kidney damage was observed in UUO + Saline rats versus Sham + Saline group (ANOVA, F (4, 35) = 13.06, $p < 0.01$; followed by Tukey's post hoc test, $p < 0.001$) (Fig. 4a), and troxerutin (100 mg/kg) could decrease the damage induced by UUO (Fig. 4a, b). Moreover, KTDS decreased significantly in UUO + 100 mg/kg TXR group versus UUO + Saline group (Tukey's post hoc test, $p < 0.05$) (Fig. 4b).

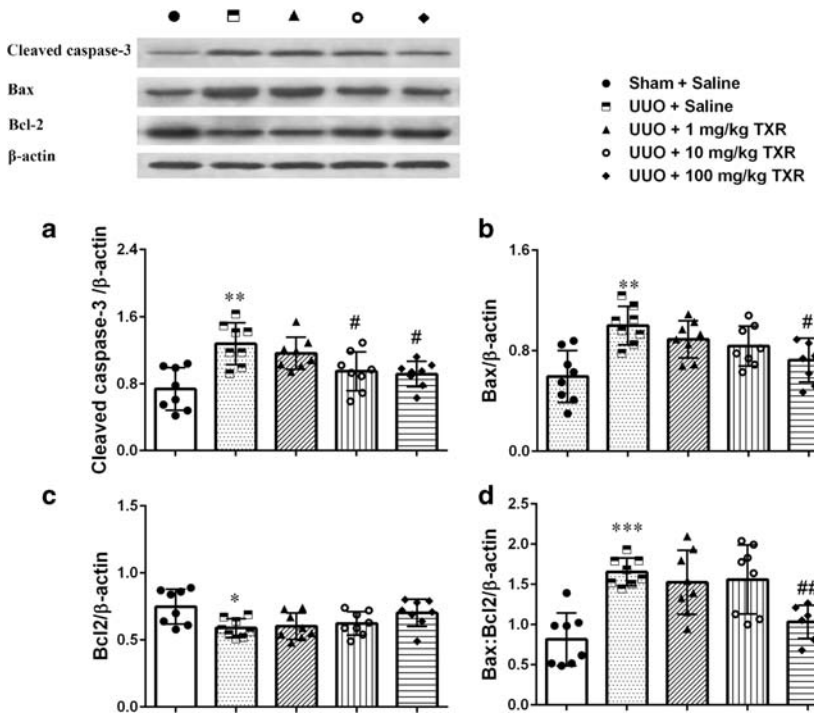

Fig. 3 Immunoblotting analysis of the cleaved caspase-3 (**a**), Bax (**b**), Bcl-2 (**c**), and Bax:Bcl-2 ratio (**d**) proteins in obstructed kidney tissue 3 days after vehicle/troxerutin administration in all experimental groups. Each data value is represented as mean ± SD. $n = 8$/group. Statistical evaluation was obtained by ANOVA followed by Tukey. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared with Sham + Saline group. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared with UUO + Saline group. UUO, unilateral ureteral obstruction; TXR, troxerutin

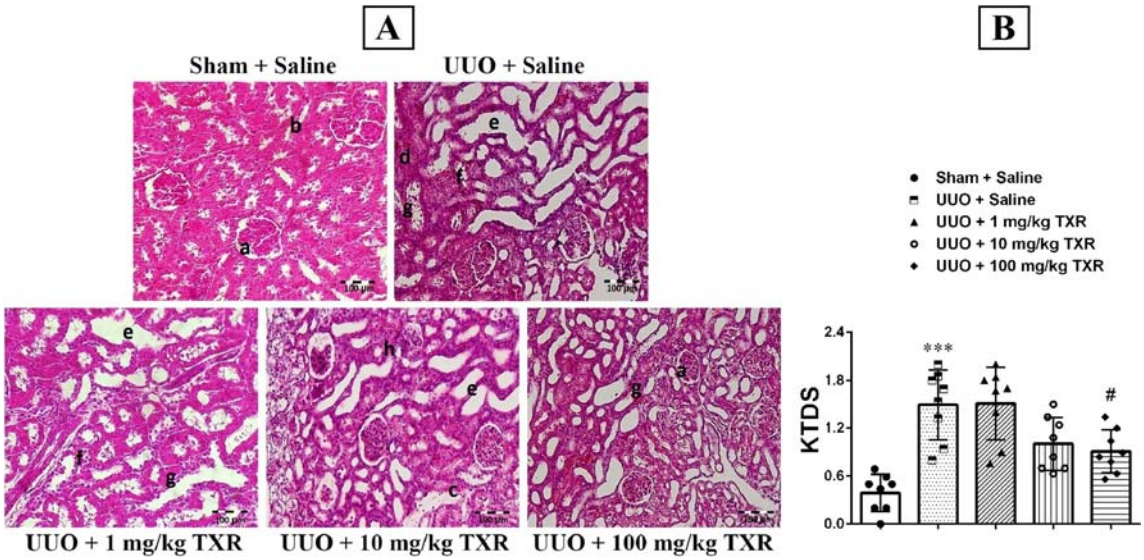

Fig. 4 The hematoxylin and eosin stained sections (magnification × 100) (**a**) and kidney tissue damage score (**b**) in the obstructed kidney 3 days after vehicle/troxerutin administration in all experimental groups. Scores 0 to 0.5 were considered as normal and scores 1 to 3 were considered as the presence of damage in kidney tissue. Each data value is represented as mean ± SD. $n = 8$/group. Statistical evaluation was obtained using Kruskal-Wallis test. $^{***}p < 0.001$ compared with Sham + Saline group. $^{\#}p < 0.05$ compared with UUO + Saline group. "a" indicates normal glomeruli, "b" indicates normal tubules, "c" indicates renal edema and fibrosis, "d" indicates interstitial infiltration of inflammatory cells, "e" indicates tubular dilatation and debris, "f" indicates tubular atrophy, "g" indicates cellular casts in some renal tubules, and "h" indicates renal tubular vacuolization in the left kidney tissue. TXR group. UUO, unilateral ureteral obstruction; KTDS, kidney tissue damage score; TXR, troxerutin

Discussion

The findings of the current study showed that 3 days UUO had no significant effect on the MAP and RPP parameters (Table 1). This finding is concordant with our previous studies which revealed that blood pressure does not increase significantly in a 3-day UUO model (Hassanshahi et al. 2018; Hassanshahi and Nematbakhsh 2018). Moreover, the current study represented that 3 days UUO significantly increases the RVR and reduces the RBF in the left kidney and subsequently increases the serum creatinine level (Table 1). In line with our study, it has been reported that the plasma creatinine value can increase in a 24-h UUO model (Li et al. 2003; Jin et al. 2017). Moreover, Martin Østergaard et al. (2013) showed that 3 days UUO increases the plasma creatinine concentration (Østergaard et al. 2013). On the other hand, contrary to our study, Watatani et al. (2014) reported that UUO for 3 days did not change the plasma creatinine level (Watatani et al. 2014). According to the results of the present study, troxerutin at the dose of 100 mg/kg could attenuate the pathologic alteration of the RBF and RVR indicators induced by the obstruction (Table 1). In addition, troxerutin (100 mg/kg) could reduce the serum creatinine level in UUO-treated group versus UUO + Saline group (Table 1). In this regard, it has been revealed that troxerutin improves microcirculation and increases the formation of collateral circulation (Panat et al. 2016). Also, troxerutin has a capillary protective effect (Gohel and Davies 2009). In addition to the troxerutin vascular protection effects, it seems that troxerutin can increase RBF by direct and indirect mechanisms affecting the vessels. Our results showed that UUO increases the MDA level as a lipid peroxidation indicator in the left kidney tissue and troxerutin (100 mg/kg) administration markedly could decrease this oxidative stress parameter (Fig. 1a). In agreement with our study, Adam et al. (2005) reported that troxerutin has a significant protective effect against MDA formation induced by coumarin in rat liver mitochondria (Adam et al. 2005). Furthermore, our study showed that UUO for 3 days induced oxidative stress via reducing the SOD, GPx, and catalase activities in the left kidney tissue (Fig. 1b, c, d), whereas troxerutin (100 mg/kg) could increase the activity levels of these three antioxidant enzymes in kidney tissue (Fig. 1b, c, d). In line with our study, Fan et al. (2009) showed that troxerutin protects the kidneys from injury caused by D-galactose via increasing the SOD, CAT, and GPx activities level and scavenging the free radical (Fan et al. 2009). Also, Shan et al. (2017) showed that troxerutin increases the antioxidative enzymes including SOD, catalase, and GPx and suppresses the oxidative stress of renal cells induced by BDE-47 in mice (Shan et al. 2017). This condition can inhibit the renal apoptosis pathway (Shan et al. 2017). Because the troxerutin compound can decrease kidney damage in the oxidative situation, it seems that troxerutin can be used in UUO situation. Also, our finding represented that the TAC is reduced in the kidney tissue suffering from UUO and administration of troxerutin (100 mg/kg) can modify this change (Fig.

1e). Generally, since the ROS have an inflammatory effect and contribute to tissue injury (Fan et al. 2009) and apoptotic cell death (Liu et al. 2010), antioxidant system reinforcement is necessary. So, troxerutin could nearly ameliorate the oxidative stress via renewing the reinforced the antioxidant enzymes in UUO-treated rat kidney. In addition, it is specified that oxidative stress activates the inflammatory parameters; then microvasculature permeability is increased and elements of blood leak into the interstitial spaces and inflammation is progressed (Guardia et al. 2001; Selloum et al. 2003). According to our immunoblotting results, 3 days UUO increases the level of TNF-α protein expression in the left kidney, and troxerutin (100 mg/kg) can attenuate this inflammatory marker in treated rats (Fig. 2). In this regard, Misseri et al. (2004) represented that UUO model increases the TNF-α mRNA expression level in kidney tissue (Misseri et al. 2004). It is also noted that troxerutin reduces markedly the TNF-α protein level as an important inflammatory indicator and exerts its own cardioprotective effects in myocardial cells in a heart ischemia-reperfusion model (Badalzadeh et al. 2017). It seems that troxerutin has renoprotective effect at least by inhibiting TNF-α protein expression level. Interestingly, in another part of the current study, we found that 3 days UUO induces renal apoptosis via increasing the Bax and cleaved caspase-3 and also decreasing the Bcl-2 proteins expression level in the left kidney tissue (Fig. 3). In addition, our result revealed that troxerutin can decrease the Bax and cleaved caspase-3 proteins expression level as two apoptosis indicators in the left kidney tissue of treated rats (Fig. 3a, b). Moreover, troxerutin (100 mg/kg) can decrease the Bax:Bcl-2 ratio in the left kidney tissue (Fig. 3d). In this regard, it has been reported that troxerutin has an anti-apoptotic effect via ameliorating oxidative stress (Liu et al. 2010). Also, Shu et al. (2017) revealed that troxerutin has a protective effect against ischemia-reperfusion-induced heart injury via modifying the apoptotic rate (Shu et al. 2017). These findings offered a renoprotective role for troxerutin against apoptosis induced by 3 days UUO. In the present study, H&E staining was used to evaluate kidney tissue damage. However, it is better to evaluate the fibrosis and interstitial infiltration by using specific staining, but besides histopathologic scoring parameters in H&E staining (such as tubular dilatation, debris, interstitial edema), the fibrosis and interstitial infiltration criteria can be used (Wang et al. 2016; Sancak et al. 2017; Zou et al. 2017). Moreover, previous studies showed that H&E staining can be used for showing the fibrosis and interstitial infiltration (Zou et al. 2017; Xia et al. 2018; Wang et al. 2019). The H&E staining of our study showed that the 3 days UUO induces renal tissue damage (Fig. 4a), and administration of troxerutin (100 mg/kg) can attenuate the kidney damage induced by UUO (Fig. 4b). In accordance with our study, Zhang et al. (2009) reported that troxerutin effectively inhibits the histopathologic changes especially leukocyte infiltration and parenchymal damage induced by D-galactose in mice liver (Zhang et al. 2009). This finding suggests that troxerutin may possibly protect the ipsilateral kidney against 3 days of UUO-induced renal parenchymal injury and dysfunction. The present study did not evaluate the probable troxerutin toxicity effects on animal organs. However, previous studies have shown that troxerutin has no notable side effects on experimental animals' body tissues such as the liver and kidney tissue in the same or higher doses used in our study (Zhang et al. 2009, Liu et al. 2010, Elangovan and Pari 2013, Shan et al. 2017).

Conclusion

The findings of the current study showed that troxerutin (100 mg/kg) can decrease the level of pro-apoptotic indicators expression in the ipsilateral kidney in a 3-day UUO model. Moreover, the renal hemodynamic parameters status (RBF and RVR) is better than the untreated-UUO situation. Moreover, troxerutin could decrease lipid peroxidation level and decreases the TNF-α expression level and also scavenges free radicals in obstructed kidney tissue in a 3-day UUO model. In addition, troxerutin could modify the KTDS in the ipsilateral kidney. In fact, our study shows that troxerutin decreases kidney injury and improves renal dysfunction caused by 3 days UUO. Collectively, it can be concluded that troxerutin may be considered in the future as an ameliorating substance in kidney injury induced by UUO. However, further in vivo and in vitro studies are needed to evaluate the other mechanisms of troxerutin effects in UUO model.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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