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Protective Effects of *Tribulus Terrestris* L Extract Against Acute Kidney Injury Induced By Reperfusion Injury in Rats

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Original Paper

Introduction. This study aimed to investigate the protective effect of aerial parts of the *Tribulus terrestris* L extract on acute kidney injury (AKI) induced by ischemia for 30 minutes and reperfusion for 24 hours in rats.

Materials and Methods. Ten male Sprague-Dawley rats in the AKI and 10 in the *Tribulus terrestris* groups received the extract solvent and extract of the plant (11 mg/kg), respectively, for 13 days (oral administration). On day 14, ischemia for 30 minutes and reperfusion for 24 hours were induced on the rats. In the last 6 hours of the reperfusion period (24 hours), urine samples were collected in metabolic cages. At the end of this period, blood samples were also taken to determine plasma urea nitrogen, creatinine, and electrolyte concentrations. The kidney tissues were collected for measuring the level of oxidative stress and histological studies. They were compared with the sham operation group and a control group with normal diet and no operation.

Results. In the *Tribulus terrestris* group, the increase in plasma creatinine and urea nitrogen concentrations was significantly less following reperfusion, and their values reached the same level as that in the sham group. Creatinine clearance and urine osmolarity in the *Tribulus terrestris* group was higher in comparison with the AKI group, whereas sodium absolute excretion, fractional excretion of potassium, oxidative stress, and cellular damages were less.

Conclusions. Oral administration of *Tribulus terrestris* extract for 2 weeks can decrease kidney functional disturbance, oxidative stress, and cellular damages following reperfusion injury in rats.

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INTRODUCTION

Tribulus terrestris L is a herb (*Zygophyllaceae* family) with a wide distribution in the south of Europe, south of Asia, Africa, and north of Australia. In traditional medicine, different parts of *Tribulus terrestris* are applied to the treatment of hypertension and coronary artery diseases, diabetes and hyperlipidemia, and fungal diseases.[1-5] In addition, it has been shown that *Tribulus terrestris* possesses diuretic, aphrodisiac, lithontriptic, and antibacterial effects.[6-8] Recent studies have also indicated that *Tribulus terrestris* extract has antioxidative, apoptosis inhibitory, and vasodilator properties,[9-14] as well as protecting renal epithelial cells against damages due to oxalates and ethylene glycol consumption.[15,16]

Various conditions may result in acute kidney injury (AKI), the most common of which is reperfusion injury. Pathophysiological damages due to reperfusion injury include vascular endothelium,

tubular epithelium, and inflammation.[17-19] These damages through induction of oxidative stress, necrosis, and cellular apoptosis, as well as sustained reduction of renal blood flow (RBF), lead to further damage to kidney function.[17] It has already been proven that *Tribulus terrestris* extract possesses antioxidant and vasodilator properties, and that it can protect renal epithelial cells. The aim of this study was to investigate the protective effects of *Tribulus terrestris* extracts against AKI induced by ischemia-reperfusion.

MATERIALS AND METHODS

Rats

In this experimental study, 40 male Sprague-Dawley rats weighing 280 g to 350 g were supplied and divided into 4 groups of 10 each: control (no operation and no injection), sham (sham operation), AKI, and AKI receiving *Tribulus terrestris* extract (TT). These animals were placed in controlled conditions of temperature (23 ± 2°C) and 12-hour light-dark cycles. They were provided with standard rat food and water ad libitum.

Induction of Ischemia-Reperfusion

Ischemia-reperfusion was used as the model of inducing AKI. In the AKI and TT groups, the rats were first anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg to 60 mg/kg) and a longitudinal incision was made in the linea alba. Then, the arteries and veins of both kidneys were simultaneously clamped for 30 minutes. After the 30-minute period, obstruction was removed and the surgical area was sutured with 3-zero silk. The rats were returned to the cages to spend the 24-hour reperfusion period. During this period, they had free access to water and food. The same protocol was followed for the sham group; however, no occlusion was done. The control group did not go through anesthesia or any operation.

Extraction Method

Tribulus terrestris was obtained from pharmaceutical plants farm of Arak University. Then, aerial parts of the plant were dried in shade and its extract was obtained through the percolation method. The solvent used for extracting was 70% ethanol. After filtering, the resulting extract was concentrated through rotavapor and dried in freeze drier.[20]

Study Protocol

The rats in the AKI and TT groups received the extract solvent and *Tribulus terrestris extract* at 11 mg/kg dose of body weight, respectively (a total volume of 5 mL through gavage), for 13 days.[21] At the beginning of day 14, they underwent surgery and ischemia (30 minutes) as well as reperfusion for 24 hours. Then at the beginning of the last 6 hours of the reperfusion period, the rats were transferred to a metabolic cage and their urine samples were collected. At the end of this period, the rats were anesthetized again and blood samples were obtained from their descending aorta. Next, the amount of oxidative stress in the right kidney was measured and the left kidney was extracted for histological studies. The rats in the sham group received the solvent of *Tribulus terrestris* extract and underwent the same procedure. The control group, however, received a normal diet without operation and underwent measurements.

Measurements

Plasma creatinine and urea nitrogen concentrations were measured by an RA-1000 autoanalyzer (Technicon, Tarry Town, USA). An EasyLyte (Medica, Bedford, USA) was used for measurement of sodium and potassium concentrations in urine and plasma samples, while urine and plasma osmolarity were measured using an osmometer (Osmomat 010, Gonotec, Germany).

For evaluation of the status of oxidative stress, malondialdehyde and ferric reducing/antioxidant power (FRAP) values in kidney tissue samples were measured. These parameters were measured as explained in our previous studies,[22-24] through Ohkawa and Benzie methods, respectively.[25,26] The removed segments of the kidneys were fixed in 10% formalin and placed in paraffin to be studied histologically. After providing slides stained with hematoxylin-eosin, the following were measured: the degree of renal histopathological damages in terms of increase in Bowman space, decrease in the number of erythrocytes in glomerular capillaries, tubular cells necrosis and their exfoliation into tubular lumens, vacuole formation within the cells, vascular congestion, and intratubular proteinaceous casts.[22-24]

Statistical Analysis

All data were presented as mean ± standard error of mean. For analyzing data on kidney

function parameters and oxidative stress values, the 1-way analysis of variance and the Duncan post hoc test were used. The comparison of total histopathological scores between the groups was made using the nonparametric the Kruskal-Wallis and the Mann-Whitney tests. A *P* value less than .05 was considered as the level of significance.

RESULTS

For evaluating the induction of reperfusion injury, plasma creatinine and urea nitrogen concentrations were determined, which did not differ between the sham and control groups (Table 1). Ischemia for 30 minutes and reperfusion for 24 hours resulted in increases in plasma creatinine (2.9 times) and urea nitrogen (3.3 times) concentrations in the AKI group compared with the sham group ($P < .001$). However, plasma creatinine and urea nitrogen concentrations in the TT group significantly decreased in comparison with the AKI group ($P < .001$), and creatinine concentration value reached the same level as that in the sham group.

Reperfusion in the AKI group increased the absolute excretion of sodium and fractional excretion of potassium by 2.6 and 4.9 times, respectively, in comparison with the sham group ($P < .001$). Administration of *Tribulus terrestris* extract in the TT group caused significant decreases in the absolute excretion of sodium and fractional excretion of potassium levels compared with those in the AKI group, in a way that they were not significantly different from their corresponding values in the sham group (Table 1).

Figure 1 shows that renal reperfusion injury decreased creatinine clearance in the AKI group by 65% in comparison with that in the sham group ($P < .01$), whereas administration of *Tribulus terrestris* extract in the TT group increased it by 1.9 times as compared with the AKI group ($P < .05$).

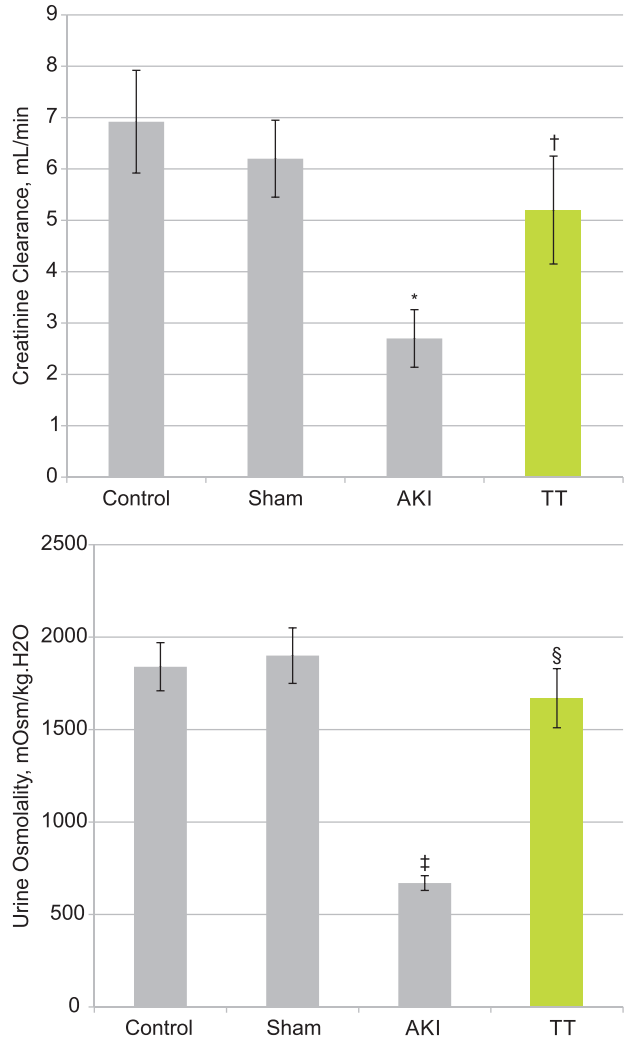

Figure 1. Mean levels of creatinine clearance and urine osmolality after the reperfusion period in rats with induced acute kidney injury (AKI) with and without pretreatment with saline (AKI) or Tribulus terrestris (TT), compared with rats in the sham operation and control groups.
*$P < .01$ in comparison with the sham group
†$P < .05$ in comparison with the AKI group
‡$P < .001$ in comparison with the sham group
§$P < .001$ in comparison with the AKI group

Table 1. Effects of Oral Administration of *Tribulus Terresteris* (TT) Extract on Kidney Function Parameters After Acute Kidney Injury (AKI) Induced by Ischemia-Reperfusion

Parameters	Animal Groups			
	Control	Sham	AKI	TT
Plasma creatinine, mg/dL	0.86 ± 0.01	0.94 ± 0.05	2.74 ± 0.14*	1.00 ± 0.06†
Plasma urea nitrogen, mg/dL	18.90 ± 1.00	18.80 ± 1.18	62.50 ± 1.90*	32.00 ± 2.60†‡
Absolute excretion of sodium, mmol/min.kg body weight	4.20 ± 0.84	3.92 ± 0.43	10.20 ± 1.60*	3.10 ± 0.24§
Fractional excretion of potassium, %	15.60 ± 2.00	14.20 ± 1.60	69.60 ± 9.03*	21.00 ± 3.60†

*$P < .001$ in comparison with the sham group
†$P < .001$ in comparison with the AKI group
‡$P < .01$ in comparison with the sham group
§$P < .01$ in comparison with the AKI group

In addition, urine osmolarity in the AKI group decreased to less than its corresponding value in the sham group ($P < .001$). Administration of *Tribulus terrestris* extract in the TT group significantly increased urine osmolarity compared with the AKI group ($P < .001$), as such it reached the same level as the sham group (Figure 1).

Tissue malondialdehyde in the sham group was 8.5 ± 1.1 nmol/g kidney weight (Figure 2), which increased to 34.3 ± 2.3 nmol/g in the AKI group ($P < .001$). Although treatment with *Tribulus terrestris* extract could decrease malondialdehyde value to 20.1 ± 2.9 nmol/g ($P < .01$), it was still 2.3 times more than that in the sham group ($P < .01$). Moreover, tissue FRAP in the sham group was 12.5 ± 1.6 µmol/g kidney weight that decreased to 6.4 ± 0.8 µmol/g following reperfusion ($P < .01$). In the TT group, FRAP level was 11.8 ± 1.5 µmol/g that was significantly higher than its corresponding value in the AKI group (Figure 2).

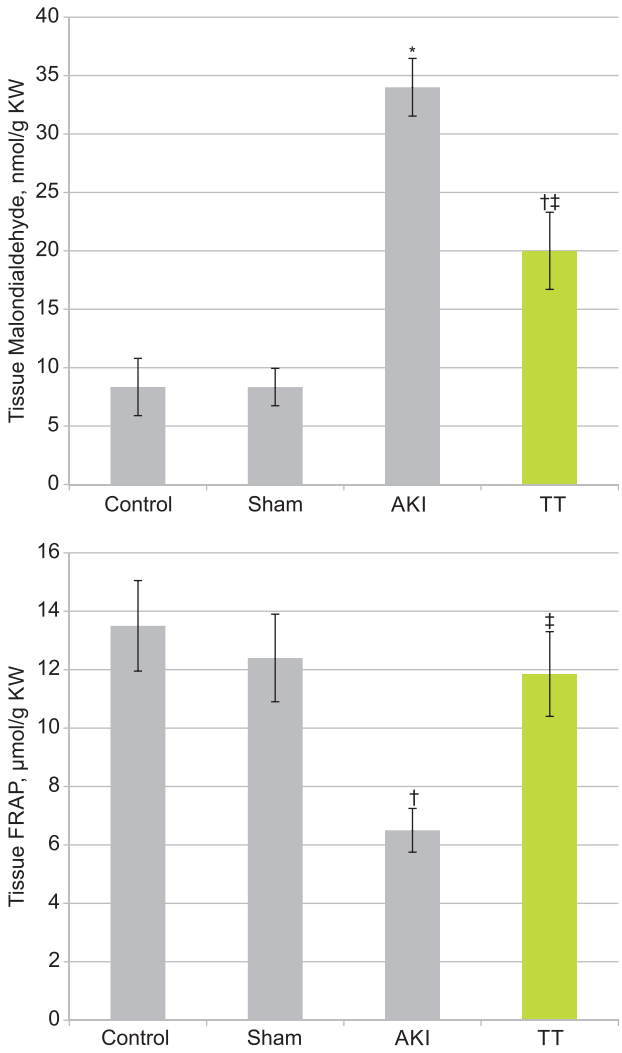

Figure 2. Mean levels of renal tissue malondialdehyde and ferric reducing/antioxidant power (FRAP) after the reperfusion period in rats with induced acute kidney injury (AKI) with and without pretreatment with saline (AKI) or Tribulus terrestris (TT), compared with rats in the sham operation and control groups.
*$P < .001$ in comparison with the sham group
†$P < .01$ in comparison with the sham group
‡$P < .01$ in comparison with the AKI group

As Table 2 shows, in the cortical region of the kidneys, the Bowman space increased (grade 5) and the number of erythrocytes decreased in the glomerular capillaries (grades 5) and the cells were vacuolated (grade 4) in the AKI group. In addition, cells in the proximal tubule walls and the thick ascending limb of loop of Henle were damaged (grades 3 and 2, respectively). All these damages were partially improved with treatment with *Tribulus terrestris* in the TT group (Figure 3). In the external medulla, reperfusion induced damage in the tubular segments of Pars Recta (S3) and the thick ascending limb of loop of Henle (grade 3), vascular congestion (grade 5), and intratubular proteinaceous casts (grade 4). All these damages decreased in the TT group as compared with the AKI group (Figure 4). In the internal medulla, the values for vascular congestion and intratubular proteinaceous casts in the TT group relatively decreased as compared with the AKI group (Table 2). The total histopathologic score in the AKI

Table 2. Histopathologic Scores in Rats With Oral Administration of Tribulus Terresteris (TT) Extract After Acute Kidney Injury (AKI) Induced by Ischemia-Reperfusion

Histopathology	Animal Groups		
	Sham	AKI	TT
Cortex			
Bowman space enlargement	0	V	I
Proximal tubule injury	0	III	I
Thick ascending limb injury	0	II	I
Reduced number of erythrocytes in glomerular capillaries	0	V	II
Intracellular vacuolization	0	IV	I
Outer medulla			
Pars recta (S3) injury	0	III	I
Thick ascending limb injury	0	III	I
Vascular congestion	0	V	II
Intratubular proteinaceous casts	0	IV	II
Inner medulla			
Vascular congestion	0	III	II
Intratubular proteinaceous casts	0	IV	I
Total histopathologic score	0	41*	15*†

*$P < .001$ in comparison with the sham group
†$P < .01$ in comparison with the AKI group

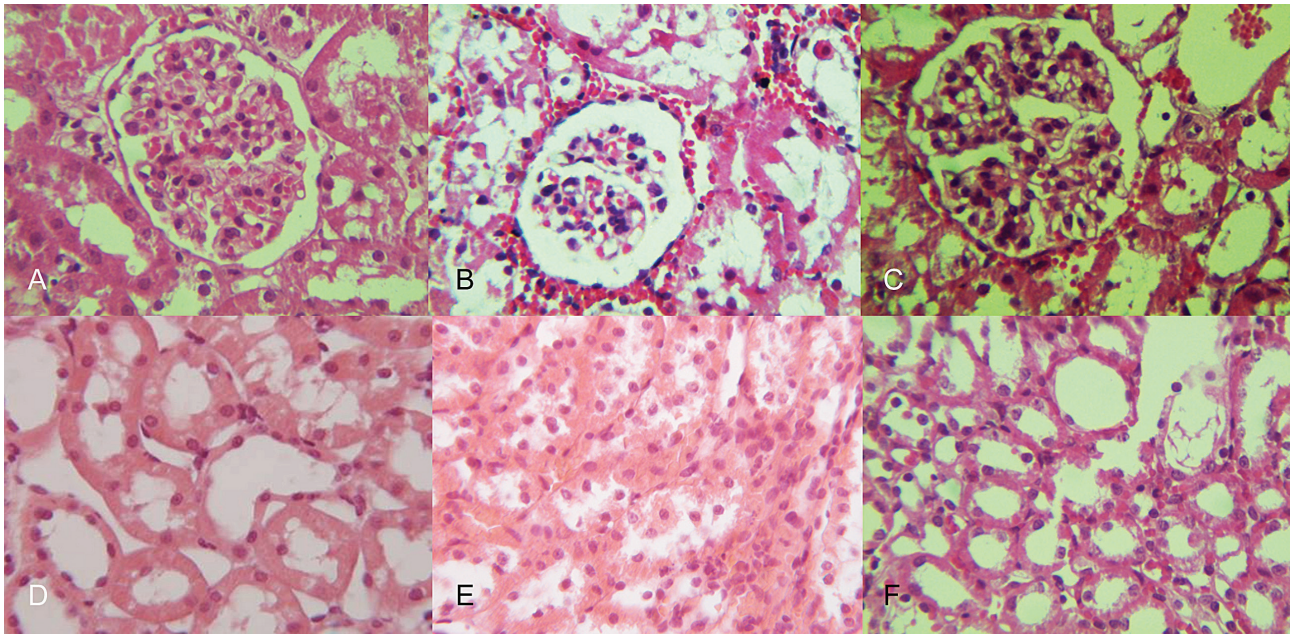

Figure 3. A to C, Bowman space widening in the sham, acute kidney injury, and *Tribulus terrestris* groups, respectively. **D to F**, Outer medulla for tubular cells necrosis in the sham, acute kidney injury, and *Tribulus terrestris* groups, respectively (hematoxylin-eosin, × 400).

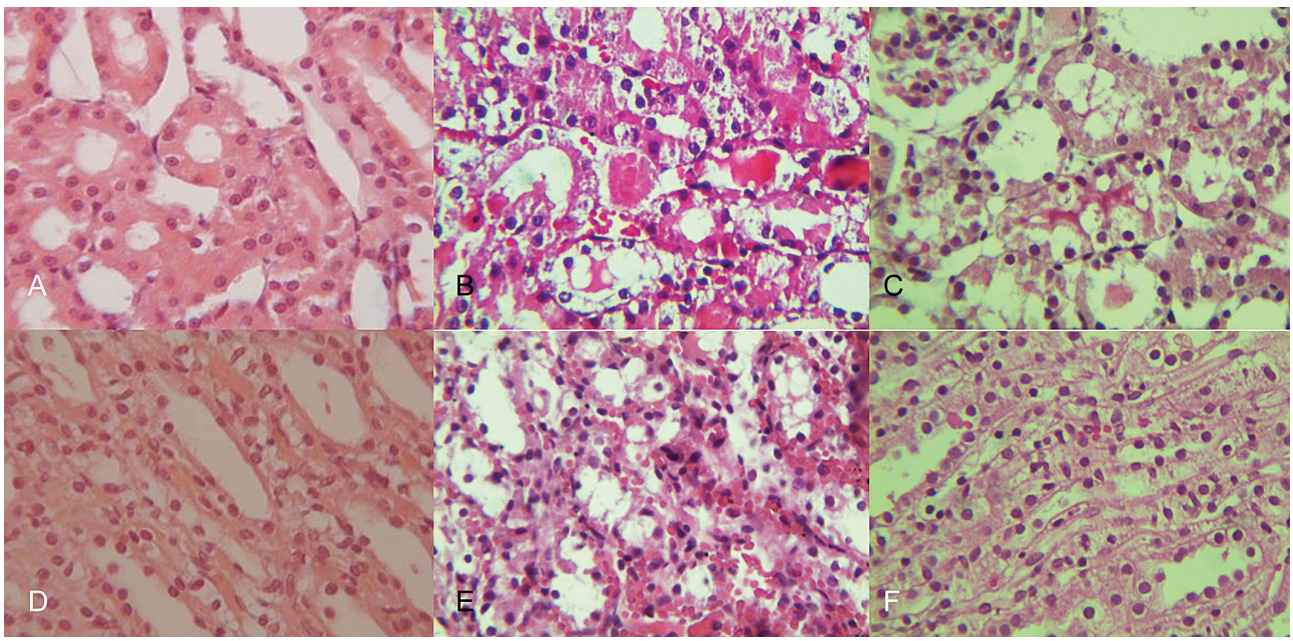

Figure 4. A to C, Outer medulla showing intratubular casts in the sham, acute kidney injury, and *Tribulus terrestris* groups, respectively. **D to F**, Outer medulla showing vascular congestion in the sham, acute kidney injury, and *Tribulus terrestris* groups, respectively (hematoxylin-eosin, × 400).

group was 41, while with treatment with *Tribulus terrestris* extract it was significantly lower in the TT group ($P < .01$).

DISCUSSION

This study showed that the oral administration of *Tribulus terrestris* extract decreases histological damages, oxidative stress, and as a result, improves kidney function following bilateral renal ischemia for 30 minutes and reperfusion for 24 hours. In the AKI group, creatinine clearance as the index of glomerular filtration rate significantly decreased,

which caused significant increase in plasma creatinine and urea nitrogen concentrations. Several studies have shown that renal reperfusion injury by intrarenal vasoconstriction and increasing the bonding molecules leads to linking leukocytes, platelets, and erythrocytes to each other and permanent reduction in RBF during the reperfusion period.[17,27] In the present study, the decreased number of erythrocytes in glomerular capillaries and vascular congestion in medullae verified that.

Reduction in RBF together with increased pressure in the Bowman space and increased back leak through the damaged epithelial layer of tubules result in significant decreases of glomerular filtration rate in AKI.[17] Various studies have shown that *Tribulus terrestris* fruit extract has vasodilator properties.[1,14] In their study on rats, Sharifi and coworkers reported that consumption of *Tribulus terrestris* fruit extract led to vasodilatations through reducing the activity of angiotensin-converting enzyme.[14] Other studies have shown that *Tribulus terrestris* extract through increasing nitric oxide release from vascular endothelium and its hyperpolarization as well as its direct effect leads to vasodilatation.[1,28,29] Thus, it seems that *Tribulus terrestris* extract can resolve the vasoconstriction due to renal reperfusion injury, due to its inhibitory effect on the activity of angiotensin-converting enzyme, stimulation of nitric oxide release from vascular endothelium, hyperpolarization of the vascular smooth muscles, and its direct effect, and as a result, improve plasma parameters, such as creatinine and urea nitrogen.

Histological study showed that following reperfusion injury, the degree of damage to cells in the tubules walls, and their exfoliation into the lumens along the proximal tubules and the thick ascending limb significantly increased. During ischemia, severe reduction of adenosine triphosphate results in the complete loss of cell metabolic activity. This leads to the development of mitochondrial damage, increased production of reactive oxygen species, and increased concentration of intracellular calcium, which result in disruption of proteins, DNA, and cell membrane, through activating phospholipases, proteases, endonucleases, and lipid and protein peroxidation, that induce and develop damage to cells and stimulate inflammation.[17,30,31] Zhang and colleagues indicated that *Tribulus terrestris* extract reduced oxidative stress and cell apoptosis in heart muscles of rats following reperfusion injury.[10] They showed that *Tribulus terrestris* extract resulted in the reduction of proapoptotic proteins such as Bax and caspase-3 through activating protein kinase C and increased the level of Bcl-2 anti-apoptotic protein. They also stated that *Tribulus terrestris* extract reduced oxidative stress in heart muscles. Hence, *Tribulus terrestris* extract maybe exerts sedative effects on cellular damages and oxidative stress in kidney tissues (as in heart muscles) through a similar mechanism or other mechanisms that need to be investigated in future studies.

CONCLUSIONS

Overall, it can be concluded that *Tribulus terrestris* extract increases RBF during the reperfusion phase through its vasodilatory effects, which in turn, returns glomerular filtration rate to its normal level. As a result, it leads to relative improvement of the plasma parameters indicative of kidney function. In addition, through reduction of cell destructive damages and oxidative stress, *Tribulus terrestris* can prevent the development and progression of cellular damages. Clearly, determining the exact mechanism of each of these effects in kidney requires further investigation.

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CONFLICT OF INTEREST

None declared.

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