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Nephroprotective and hepatoprotective effects of *Tribulus terrestris* L. growing in Saudi Arabia

[Efectos nefroprotector y hepatoprotector de *Tribulus terrestris* L. que crece en Arabia Saudita]

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Abstract

Context: *Tribulus terrestris* (Zygophyllaceae) is a popular leafy prostrate branching herb used in folk medicine as a diuretic and urinary antiseptic.

Aims: To evaluate the hepatoprotective and nephroprotective activities of the ethanolic plant extract and petroleum ether, dichloromethane and aqueous methanol fractions against CCl₄ induced toxicity in adult Wistar rats.

Methods: The total 95% ethanol extract at 200 and 400 mg/kg and petroleum ether, dichloromethane and aqueous methanol at 200 mg/kg was administered p.o. for seven days followed by one dose of CCl₄ (1.25 mL/kg, p.o.) at day six. Serum and tissue parameters for both liver and kidney functions were measured. Histopathological study of both tissues was conducted. Results were compared with normal rats, negative controls receiving only CCl₄ and positive controls treated with silymarin (10 mg/kg, p.o.).

Results: Effect of the total 95% ethanol extract at 400 mg/kg on serum and tissue liver parameters were weak. However, protective effect on kidney was promising. The best effect was observed on the urea and creatinine levels. Both malondialdehyde and non-protein sulfhydryl groups in kidney tissues were improved to levels comparable with those obtained by silymarin.

Conclusions: The current study confirmed the positive effect of the plant on the kidney tissues and function. The activity was trapped to the dichloromethane fraction that could provide pure active compounds.

Keywords: Biochemical parameters; creatinine; ethanol extract; rats; urea.

Resumen

Contexto: *Tribulus terrestris* (Zygophyllaceae) es una hierba rastrera frondosa ramificada que se usa en la etnomedicina como diurético y antiséptico urinario.

Objetivos: Evaluar las actividades hepatoprotectora y nefroprotectora del extracto etanólico de la planta y las fracciones de éter de petróleo, diclorometano y metanol acuoso contra la toxicidad inducida por CCl₄ en ratas Wistar adultas.

Métodos: El extracto total en etanol 95% a 200 y 400 mg/kg y las fracciones de éter de petróleo, diclorometano y metanol acuoso a 200 mg/kg fueron administradas p.o. por siete días seguidas por una dosis de CCl₄ (1.25 mL/kg, p.o.) en el día seis. Se midieron los parámetros séricos y tisulares para las funciones hepáticas y renales. Se realizó el estudio histopatológico de ambos tejidos. Los resultados se compararon con ratas normales, los controles negativos recibieron sólo CCl₄ y los controles positivos se trataron con silimarina (10 mg/kg, p.o.).

Resultados: El efecto del extracto total de etanol 95% (400 mg/kg) sobre los parámetros séricos y tisulares del hígado fue débil. Sin embargo, el efecto protector renal fue superior. El mejor efecto se observó en las concentraciones de urea y creatinina. Tanto los grupos sulfhidrilo no proteicos como malondialdehído en los tejidos renales mejoraron a niveles comparables con los obtenidos por la silimarina.

Conclusiones: El presente estudio confirma el efecto positivo de la planta en el tejido y la función renal. La actividad se atribuye a la fracción de diclorometano que podría proporcionar compuestos activos puros.

Palabras Clave: Creatinina; extracto etanólico; parámetros bioquímicos; ratas; urea.

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INTRODUCTION

Genus *Tribulus* family Zygophyllaceae is presented in Saudi Arabia by about eight species (Collenette, 1999). *Tribulus terrestris* is native to warm temperate and tropical regions of the old world in southern Europe, southern Asia, throughout Africa, and in northern Australia. It can thrive even in desert climates and poor soil (Parker, 1972; GRIN, 2000).

Tribulus terrestris is a popular leafy prostrate branching herb with deep yellow flowers and stems up to 30 cm. Fruits are hairy with small narrow spines (Collenette, 1999). The plant known as “Qutiba” or “Darisa” is used in folk medicine for various purposes. It has long been used as a tonic, aphrodisiac, astringent, analgesic, stomachic, antihypertensive, diuretic and urinary anti-septic (Ody, 2000). Local Bedouin in Saudi Arabia use the plant to treat the urinary disorder (Al-Asmari et al., 2014). Hot or cold-water extract prepared from the plant is used as a beverage in Iraq (Al-Ali et al., 2003). In Turkish folk medicine, the plant is used as diuretic in the management of colic, hypertension and hypercholesterolemia (Arcasoy et al., 1998). The wide range of use in different countries as well as the LD₅₀ of 19.8 g/kg (Al-Ali et al., 2003) indicated the high safety of the plant and did not support the single case report for intoxication with a high dose of the plant water extract (Talasaz et al., 2010). This intoxication may result from the overdose or due to other contamination since the used extract was not subjected to any analyses.

Several pharmacological actions have been reports for the plant extracts and constituents. The dry fruits extract of *T. terrestris* exhibited free radical scavenging activity (Vangalapati, 2014). The methanol extract of the fruits provided protection against the mercuric chloride induced nephrotoxicity in the mice (Kavitha and Jagadeesan, 2006). The plant extract also has diuretic, aphrodisiac, antiurolithic, immunomodulatory, antidiabetic, absorption enhancing, hypolipidemic, cardiotonic, hepatoprotective, anti-inflammatory, analgesic, antispasmodic, antibacterial, anthelmintic, larvicidal and anticariogenic activities (Chhatre et al., 2014, El-Shaibany et al., 2015). The plant saponins were effective in improving myocardial energy metabolism and lactic acid metabolism in hyperlipidemic rats with acute

myocardial infarction (AMI) (Jiang et al., 2006). Saponins from the plant also increase hepatic lipase (HL) and lipoprotein lipase (LPL) activity in liver tissue and decrease the ratio of LPL activities between the adipose tissue and the muscular tissue in lipid-metabolic disordered mice, which correlated with the reduction of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels in plasma and anti-atherosclerosis (Li et al., 2007). They can protect myocardial injury caused by hypoxia-reoxygenation. Morphological change shows that saponins have cytoprotective bioactivity (Zhang et al., 2010). The spirostanol-based steroidal saponins from *T. terrestris* exhibited remarkable activity against fungal organisms (*Candida albicans* and *Cryptococcus neoformans*) and cancer cell lines such as human malignant melanoma, human oral epidermoid carcinoma, human breast carcinoma, and human ovary carcinoma (Bedir et al., 2002).

The current study was conducted to evaluate the hepatoprotective and nephroprotective of the total ethanol extract and fraction of liquid-liquid partition against CCl₄ induced toxicity in rats.

MATERIAL AND METHODS

Chemicals

Silymarin, ethylenediamine tetraacetic acid (EDTA), Folin reagent, Mayer hematoxylin, Eosin-phloxine, trichloroacetic acid, 5, 5'-dithio-bis-(2-nitrobenzoic acid), 2-thiobarbituric acid and paraform (Sigma-Aldrich, St. Louis, MO, USA). All solvents used were of analytical grade.

Plant material

Plants of *Tribulus terrestris* L. (Zygophyllaceae) were collected from Almajmah, Saudi Arabia, in February 2015. The plants were identified by Dr. Mohammad Atiqur Rahman, taxonomist of the Medicinal, Aromatic and Poisonous Plants Research Center (MAPPRC), College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. A voucher specimen (No. 16151) was deposited at the herbarium of this center.

Extraction and fractionation

The dried ground whole plants (1000 g) were extracted to exhaustion by percolation at room temperature with 95% ethanol (12 L), and the extract was evaporated *in vacuo* to leave a dark green residue. A portion of this residue (73 g) was dissolved in 1200 mL of 20% aqueous methanol and defatted with petroleum ether (500 mL \times 3) to yield 21.2 g petroleum ether soluble fraction. The aqueous methanol fraction was diluted with water until a 40% aqueous methanol mixture was produced and this was partitioned with dichloromethane (500 mL \times 3) to yield 1.8 g of dichloromethane soluble fraction and 50 g of the aqueous methanol soluble fraction.

Animals

Male Wistar albino rats (150-200 g) of approximately the same age (8-10 weeks), obtained from the Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh were used. The animals were housed under constant temperature ($22 \pm 2^\circ\text{C}$), humidity (55%) and light/dark conditions (12/12 h). They were provided with Purina chow (LabDiet, St. Louis, USA) and free access to drinking water *ad libitum* (Abdel-Kader et al., 2010). The experiments and procedures used in this study were approved by the Ethical Committee of the College of Pharmacy, Prince Sattam Bin Abdulaziz University, according to existing international regulations for the handling and care of laboratory animals.

Hepatoprotective and nephroprotective activity

Male Wistar rats were divided into eight groups of five animals each. Group I received (1 mL, p.o.) normal saline solution and was kept as a control group. Groups II - VIII received a single dose p.o. of CCl_4 (1.25 mL/kg body weight). Group II received only CCl_4 treatment. Group III was administered silymarin at a dose of 10 mg/kg p.o. (20.7 $\mu\text{mol/kg}$, p.o.). Groups IV - V were treated p.o. with 200 and 400 mg/kg of the total ethanolic extract. Groups VI - VIII were treated p.o. with 200 mg/kg of aqueous methanol soluble fraction, dichloromethane soluble fraction and petroleum ether soluble fraction, re-

spectively. Treatment started five days before CCl_4 administration and continued until day six. After 24 h, following CCl_4 administration in day seven the animals were sacrificed using ether anesthesia. Blood samples were collected by heart puncture, and the serum was separated for evaluating the biochemical parameters.

Determination of biochemical enzymatic parameters

The biochemical parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltranspeptidase (GGT), alkaline phosphatase (ALP) and total bilirubin were estimated by reported methods (Edwards and Bouchier, 1991). The enzyme activities were measured using diagnostic strips (Reflotron[®], Roche, Basel, Switzerland) and were read on a Reflotron[®] Plus instrument (Roche, Basel, Switzerland). Serum creatinine and blood urea were assayed using Randox Diagnostic kits (Randox Laboratories Ltd., Crumlin, U.K.) by the reported method (Varley and Alan, 1984), creatinine kinase and uric acid were determined using Reflotron, Roche kit, and lactate dehydrogenase (LDH) was estimated using Human diagnostic kit with UV-VIS Spectrophotometer (Shimadzu, Japan) (Merdes et al., 1985; Braun et al., 1987). Potassium level was measured using diagnostic strips (Reflotron[®], Roche) while the photometric determination of sodium level was done using Mg-uranyl acetate method (Henry et al., 1974) and calcium was determined by colorimetry (Moorehead & Biggs, 1974).

Determination of non-protein sulfhydryl groups, malondialdehyde, and total protein

The livers and kidneys samples were separately cooled in a beaker immersed in an ice bath. The tissues were homogenized in 0.02 M EDTA in a Potter-Elvehjem type C homogenizer (Sigma-Aldrich, St. Louis, MO, USA). Homogenate equivalent to 100 mg tissues was used for the measurements. Non-protein sulfhydryl groups (NP-SH) were quantified by mixing homogenate with 4 mL of distilled water and 1 mL of 50% trichloroacetic acid (TCA) in 15 mL test tubes. The tubes were shaken intermittently for 10-15 min and centrifuged for 15 min at approximately 3000 rpm to precipitate the protein. Two mL of the supernatant were mixed with 4 mL of 0.4 M

of Tris buffer, pH 8.9 and 0.1 mL of 0.01 M of DTNB [5,5'-dithio-bis-(2-nitrobenzoic acid)] and the samples were shaken. The absorbance was measured spectrophotometrically within 5 min of addition of DTNB at 412 nm against a reagent blank with no homogenate (Sedlak and Lindsay, 1968).

Concentrations of malondialdehyde (MDA) were determined by incubation of the homogenate at 37°C for 3 h in a metabolic shaker. Then 1 mL of 10% aqueous TCA was added and mixed. The mixture was then centrifuged at 800 rpm for 10 min. One millilitre of the supernatant was removed and mixed with 1 mL of 0.67% 2-thiobarbituric acid in water and placed in a boiling water bath for 10 min. The mixture was cooled and diluted with 1 mL distilled water. The absorbance of the solution was then measured at 535 nm. The content of MDA (nmol/g wet tissue), as an index of the magnitude of lipid peroxidation, was then calculated, by reference to a standard curve of MDA solution (Utley et al., 1967).

For total protein (TP) determination, parts of the homogenate were treated with 0.7 mL of Lowry's solution, mixed and incubated for 20 min in dark at room temperature. Diluted Folin's reagent (0.1 mL) was then added, and samples were incubated at room temperature in the dark for 30 min. The absorbance of the resulted solutions was then measured at 750 nm (Lowry et al., 1951).

Histopathology

The fixed liver and kidney samples were placed in cassettes and loaded into automated vacuum tissue processor (ASP 300 S, Leica Biosystems, Nussloch, Germany). The samples were embedded and blocked in paraffin wax, and thin sections (3 µm) were made using microtome (TBS SHUR/cut 4500, Triangle Biomedical Sciences, Durham, North Carolina, USA). Sections were stained with Mayer's hematoxylin solution and counterstained in eosin-phloxine solution (Prophet et al., 1994). Slides were examined using Slide Scanner (SCN 400 F Leica Microsystems, Wetzlar, Germany) for slide observation and imaging on the magnification of 10x and 40x objectives

Statistical analysis

Results were expressed as mean \pm standard error of mean (SEM). Statistical analysis was performed, using one-way analysis of variance (ANOVA). When the F-value was found statistically significant ($p < 0.05$), further comparisons among groups were made using Dunnett's multiple comparisons test. All statistical analyses were performed using SPSS software 17.0 (Released Aug. 23, 2008), Chicago, USA.

RESULTS AND DISCUSSION

Total extract of *T. terrestris* was subjected to biological testing for evaluation of the hepatoprotective and nephroprotective activities using silymarin as standard. The use of silymarin, at a dose of 10 mg/kg before the administration of CCl₄ resulted in a significant decrease in the elevated AST, ALT, GGT, ALP, and bilirubin level in rats. The total extract of *T. terrestris* showed a weak protective effect at the two used doses (200 and 400 mg/kg) (Table 1). Effect on the levels of MDA, NP-SH, and total protein even with the higher dose was also weak and not highly significant (Table 2). Consequently, further investigation for liver protection was discontinued.

The kidney regulates plasma ionic composition including sodium, potassium, calcium, magnesium, chloride. It is also concerned with the removal of nitrogenous metabolic waste products such as urea, creatinine and uric acid (Pocock and Richards, 2006). Elevations of serum electrolytes, urea and creatinine are considered reliable parameters for investigating drug-induced nephrotoxicity in animals and man (Adelman et al., 1981).

The toxicity of CCl₄ resulted in a significant impairment of kidney functions. CCl₄ is metabolized by cytochrome P-450, and this process generates a highly reactive free radical, initiates lipid peroxidation of the cell membrane of the endoplasmic reticulum and causes a chain reaction. These reactive oxygen species can cause oxidative damage to DNA, proteins and lipids (Melin et al., 2000). This toxicity is reflected as the elevation of the serum levels of LDH, creatinine-kinase, urea, uric acid, creatinine, sodium, potassium and calcium (Tables 3, 4). The level of MDA in kidney tissues was elevated while

NP-SH and total protein levels were decreased (Table 5). The protective effect of silymarin reversed these effects with different degrees. The extract of *T. terrestris* showed a dose-dependent effect and highly significant reduction in the urea and creatinine levels (Table 3). Improvement in the levels of sodium, potassium and calcium was also observed (Table 4). The level of MDA was reduced to 3.59 ± 0.17 and 2.59 ± 0.15 nmol/g with the use of 200 and 400 mg/kg extract, respectively. At the higher dose, the level of NP-SH was restored to 5.72 ± 0.71 nmol/g close to the control value 6.46 ± 0.25 nmol/g. Effect on total protein was not statistically significant (Table 5).

Histopathological study revealed the normal renal architecture in animals in the control group (Fig. 1-A). Kidney cells of rats treated with CCl₄ showed dramatic histopathological changes (Fig. 1-B). Kidney cells of rats treated with CCl₄ and *T. terrestris* showed few signs of toxicity. Most of the cells were protected and appeared as normal while the damaged cells were few (Fig. 1-D). The results support the ability of the plant extract to protect against CCl₄ induced toxicity. Previous studies have also demonstrated the safety of this plant. None of acute, subacute or chronic toxicological studies

have indicated any toxicity (Zarkova, 1983; Gendzhev, 1985; Vir and Sandeep, 2015).

The petroleum ether, dichloromethane, and aqueous methanol fractions were tested for their nephroprotective activity at 200 mg/kg. Three parameters: serum creatinine, urea, and uric acid were measured (Table 6). The dichloromethane fraction was the most active where significant improvements were observed in the used parameters (55, 27, 51% reduction in the levels of serum creatinine, urea, and uric acid, respectively) compared to silymarin (60, 45, 55% reduction). The aqueous methanol fraction was less active while the petroleum ether fraction showed very weak effect. The results pointed out to some active compounds present in the dichloromethane and aqueous methanol fractions. Some studies have reported the identification of tribulusamides A and B, tigogenin, neotigogenin, terrestrosid F, and gitonin in *Tribulus terrestris* extracts (Kostova and Dinchev, 2005) to which could be attributed part of the effects found in this study (Li et al., 2007) but the isolation and identification of these or other compounds, in the present extract or fractions, is one of our future research plans.

Table 1. Effect of *T. terrestris* total ethanol extract (ETT) on serum marker enzymes of control and experimental rats.

Treatment	Dose (mg/kg)	AST		ALT		GGT		ALP		Bilirubin	
		(IU/L)	%	(IU/L)	%	(IU/L)	%	(IU/L)	%	(mg/dL)	%
Control	1 mL	100.20 ± 6.89	-	30.07 ± 3.82	-	3.57 ± 0.26	-	363.75 ± 13.44	-	0.54 ± 0.0	-
CCl ₄	1.25 mL/kg	314.75 ± 13.30 ^{***a}	-	277.75 ± 8.91 ^{***a}	-	10.72 ± 0.37 ^{***a}	-	657.00 ± 21.06 ^{***a}	-	2.57 ± 0.17 ^{***a}	-
Sil + CCl ₄	10	136.00 ± 10.00 ^{***b}	57	94.27 ± 6.10 ^{***b}	66	5.82 ± 0.33 ^{***b}	46	416.00 ± 8.72 ^{***b}	37	0.89 ± 0.08 ^{***b}	65
ETT + CCl ₄	200	285.00 ± 8.44 ^b	9	275.25 ± 11.30 ^b	-	9.62 ± 0.42 ^b	10	668.85 ± 15.28 ^b	-	2.22 ± 0.12 ^b	14
ETT + CCl ₄	400	225.75 ± 12.97 ^b	28	242.25 ± 7.33 ^{*b}	13	8.75 ± 0.47 ^{*b}	18	566.00 ± 17.93 ^{*b}	14	1.83 ± 0.08 ^{*b}	29

All values represent mean ± SEM of n = 5. *p<0.05; **p<0.01; ***p<0.001; ANOVA, followed by Dunnett's multiple comparison test.

^a As compared with Control group (administered with a solution of 0.9% sodium chloride). ^b As compared with CCl₄ only group.

Sil: Silymarin; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyltranspeptidase; ALP: Alkaline phosphatase.

% Represents % of change respect to CCl₄ group.

Table 2. Effect of *T. terrestris* total ethanol extract (ETT) on MDA, NP-SH and total protein in liver tissue of control and experimental rats.

Treatment	Dose (mg/kg)	MDA (nmol/g)	NP-SH (nmol/g)	Total protein (g/L)
Control	1 mL	0.40 ± 0.03	7.78 ± 0.66	112.57 ± 4.68
CCl ₄	1.25 mL/kg	4.26 ± 0.58 ^{***a}	3.90 ± 0.39 ^{***a}	60.47 ± 4.62 ^{***a}
Sil + CCl ₄	10	1.09 ± 0.07 ^{***b}	6.71 ± 0.67 ^{***b}	88.62 ± 4.68 ^{***b}
ETT + CCl ₄	200	3.51 ± 0.19 ^b	4.85 ± 0.36 ^b	61.67 ± 2.99 ^b
ETT + CCl ₄	400	2.50 ± 0.06 ^{***b}	5.51 ± 0.31 ^{***b}	68.86 ± 2.04 ^b

All values represent mean ± SEM of n = 5. *p<0.05; **p<0.01; ***p<0.001; ANOVA, followed by Dunnett's multiple comparison test.

^a As compared with Control group (administered with a solution of 0.9% sodium chloride). ^b As compared with CCl₄ only group.

NP-SH: Non-protein sulfhydryl groups; MDA: Malondialdehyde; Sil: Silymarin.

Table 3. Effect of *T. terrestris* total ethanol extract (ETT) on LDH, creatinine-kinase, urea, uric acid and creatinine of control and experimental rats.

Treatment	Dose (mg/kg)	LDH		Creatinine-kinase		Urea		Uric acid		Creatinine	
		IU/L	%	IU/L	%	(nmol/L)	%	(mg/dL)	%	(mg/dL)	%
Control	1 mL	121.25 ± 9.69	-	164.00 ± 7.22	-	37.60 ± 1.77	-	1.95 ± 0.14	-	0.98 ± 0.06	-
CCl ₄	1.25 mL/kg	207.25 ± 9.15 ^{***a}	-	275.00 ± 11.94 ^{***a}	-	189.50 ± 7.68 ^{***a}	-	6.32 ± 0.27 ^{***a}	-	3.82 ± 0.18 ^{***a}	-
Sil + CCl ₄	10	135.00 ± 13.54 ^{***b}	35	211.25 ± 6.30 ^{***b}	23	119.65 ± 9.48 ^{***b}	37	2.42 ± 0.26 ^{***b}	62	1.79 ± 0.13 ^{***b}	53
ETT + CCl ₄	200	199.25 ± 5.45 ^b	4	286.75 ± 6.90 ^b	4	159.50 ± 6.02 ^{***b}	16	5.53 ± 0.3 ^b	12	2.48 ± 0.23 ^{***b}	35
ETT + CCl ₄	400	170.25 ± 4.64 ^{***b}	19	243.50 ± 5.95 ^{***b}	11	148.50 ± 5.97 ^{***b}	26	4.85 ± 0.12 ^{***b}	23	2.15 ± 0.15 ^{***b}	44

All values represent mean ± SEM of n = 5. *p<0.05; **p<0.01; ***p<0.001; ANOVA, followed by Dunnett's multiple comparison test.

^a As compared with Control group (administered with a solution of 0.9% sodium chloride). ^b As compared with CCl₄ only group

% Represents % of change respect to CCl₄ group.

Sil: Silymarin; LDH: Lactate dehydrogenase.

Table 4. Effect of *T. terrestris* total ethanol extract (ETT) on sodium, potassium and calcium of control and experimental rats.

Treatment	Dose (mg/kg)	Sodium		Potassium		Calcium	
		(mEq/L)	%	(mEq/L)	%	(mg/dL)	%
Control	1 mL	98.15 ± 3.53	-	4.40 ± 0.21	-	4.53 ± 0.34	-
CCl ₄	1.25 mL/kg	158.39 ± 1.75 ^{***a}	-	13.45 ± 1.09 ^{***a}	-	23.61 ± 0.21 ^{***a}	-
Sil + CCl ₄	10	129.43 ± 1.73 ^{***b}	18	6.45 ± 0.25 ^{***b}	52	13.69 ± 1.55 ^{***b}	42
ETT + CCl ₄	200	152.06 ± 0.91 ^{***b}	4	10.05 ± 0.56 ^{***b}	25	21.07 ± 2.18 ^b	11
ETT + CCl ₄	400	143.19 ± 2.84 ^{***b}	10	9.22 ± 0.37 ^{***b}	31	18.69 ± 1.05 ^{***b}	21

All values represent mean ± SEM of n = 5. *p<0.05; **p<0.01; ***p<0.001; ANOVA, followed by Dunnett's multiple comparison test.

^a As compared with Control group (administered with a solution of 0.9% sodium chloride). ^b As compared with CCl₄ only group.

% Represents % of change respect to CCl₄ group.

Sil: Silymarin.

Table 5. Effect of *T. terrestris* total ethanol extract (ETT) on MDA, NP-SH and total protein in kidney tissue of control and experimental rats.

Treatment	Dose (mg/kg)	MDA (nmol/g)	NP-SH (nmol/g)	Total protein (g/L)
Control	1 mL	0.29 ± 0.01	6.46 ± 0.25	141.91 ± 4.15
CCl ₄	1.25 mL/kg	4.42 ± 0.28 ^{***a}	3.91 ± 0.19 ^{***a}	64.07 ± 4.82 ^{***a}
Sil + CCl ₄	10	1.24 ± 0.02 ^{***b}	6.41 ± 0.44 ^{***b}	104.19 ± 4.42 ^{***b}
ETT + CCl ₄	200	3.59 ± 0.17 ^{*b}	4.72 ± 0.33 ^b	66.46 ± 3.70 ^b
ETT + CCl ₄	400	2.59 ± 0.15 ^{***b}	5.72 ± 0.71 ^{*b}	74.85 ± 5.56 ^b

All values represent mean ± SEM of n = 5. *p<0.05; **p<0.01; ***p<0.001; ANOVA, followed by Dunnett's multiple comparison test.

^aAs compared with Control group (administered with a solution of 0.9% sodium chloride). ^bAs compared with CCl₄ only group.

NP-SH: Non-protein sulfhydryl groups; MDA: Malondialdehyde; Sil: Silymarin.

Table 6. Effect of *T. terrestris* fractions on serum creatinine, urea, and uric acid in control and experimental rats.

Treatment	Dose (mg/kg)	Creatinine		Urea		Uric acid	
		(mg/dL)	%	(nmol/L)	%	(mg/dL)	%
Control	1 mL	0.82 ± 0.04	-	40.80 ± 2.82	-	1.91 ± 0.09	-
CCl ₄	1.25 mL/kg	3.37 ± 0.30 ^{***a}	-	148 ± 5.90 ^{***a}	-	6.12 ± 0.17 ^{***a}	-
Sil + CCl ₄	10	1.34 ± 0.13 ^{***b}	60	82.07 ± 7.12 ^{***b}	45	2.73 ± 0.17 ^{***b}	55
AM + CCl ₄	200	1.92 ± 0.18 ^{*b}	43	128.50 ± 6.30 ^{*b}	13	3.67 ± 0.15 ^{***b}	40
DCM + CCl ₄	200	1.52 ± 0.06 ^{**b}	55	108.00 ± 1.58 ^{***b}	27	3.02 ± 0.16 ^{***b}	51
PE + CCl ₄	200	2.54 ± 0.27 ^b	25	142.00 ± 5.32 ^b	4	4.73 ± 0.21 ^{**b}	23

All values represent mean ± SEM of n = 5. *p<0.05; **p<0.01; ***p<0.001; ANOVA, followed by Dunnett's multiple comparison test.

^aAs compared with Control group (administered with a solution of 0.9% sodium chloride). ^bAs compared with CCl₄ only group.

% Represents % of change respect to CCl₄ group.

Sil: Silymarin; AM: Aqueous methanol fraction; DCM: Dichloromethane fraction; PE: Petroleum ether fraction.

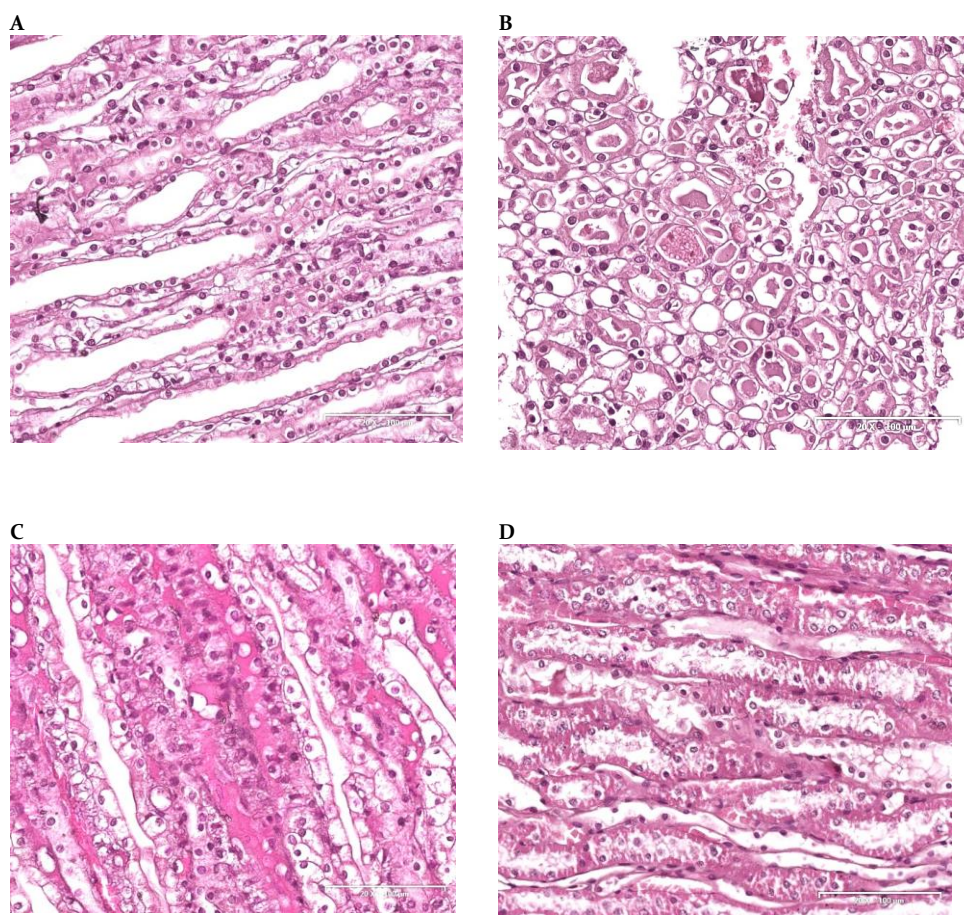


Figure 1. Histopathological appearance of kidney cells; (A) normal cells; (B) kidney cells of rats treated with CCl_4 showed Abnormal cellular structures with degenerated corpuscle. Abnormal glomeruli, reduced in size, the almost absence of Bowman space, degenerated and convoluted necrotic tubules. Inflammatory infiltrate, abnormal calyx obstructed by hyaline and granulated materials. (C) Kidney cells of rats treated with CCl_4 and silymarin (10 mg/kg) showed some abnormal cortex, corpuscle, convoluted tubules with glomerulus architecture disruption, reduced in size, reduced Bowman space, degeneration, and necrosis in both glomerulus and tubules. (D) Kidney cells of rats treated with CCl_4 and *T. terrestris* total ethanol extract (400 mg/kg) showed corpuscle contain normal and few abnormal glomeruli. Almost normal glomerulus, slight degeneration in Bowman capsule and tubules, necrosis in few tubules. Medulla shows mildly degenerated tubules obstructed with hyaline and cellular materials. Calyx with the abnormal presence of the hyaline material. Magnification 20x, 100 μm .

CONCLUSIONS

The obtained results indicated the extract of *Tribulus terrestris* has significant nephroprotective activity as claimed in folk medicine. After liquid-liquid fractionation, the activity was trapped to the dichloromethane fraction followed by the polar aqueous methanol fraction. Further chromatographic investigation directed by biological evaluation are needed to identify the active principle of this plant.

<http://jppres.com/jppres>

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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