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# *Tribulus terrestris* Hydroalcoholic Extract Effect on Cisplatin-Induced Apoptosis in Mice Kidney

## Efecto del Extracto Hidroalcohólico de *Tribulus terrestris* sobre la Apoptosis Inducida por Cisplatino en el Riñón de Ratones

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**GHANBARI, A.; ZARE, F.; KHAZAEI, M.; MORADI, M. & RAOOFI, A.** *Tribulus terrestris* hydroalcoholic extract effect on cisplatin-induced apoptosis in mice kidney. *Int. J. Morphol., 34(2)*:713-718, 2016.

**SUMMARY:** Cisplatin (EBEWE Pharma, Unterach, Austria) is an anti-cancer drug used in chemotherapy. One of the limiting major side effects of cisplatin is nephrotoxicity. *Tribulus terrestris* (TT) has been used as an synthetic or herbal protective agents for kidney disorders. The present study amid to investigate the *Tribulus terrestris* Hydroalcoholic extract effect on cisplatin-induced apoptosis in mice kidney. Male adult mice (n= 30) were divided into control group and 4 experimental groups (n= 6). Control group received saline, the first experimental group received cisplatin (5.5 mg/kg) and other three experimental groups received cisplatin (5.5 mg/kg) and different doses of hydroalcoholic extact of TT (100, 300 and 500 mg/kg i.p) respectively. The kidneys were removed after 4 days of injections, and TUNEL assay on mice's kidneys were performed. Weights of body and kidneys and apoptotic index were assessed. Data analysis was performed using one-way ANOVA followed by Tukey's post hoc test. The results showed that cisplatin lead to a reduction in the weight of body and kidney ($P < 0.01$), and increased apoptotic index significantly compared to the control group ($P < 0.001$), while in treated groups with TT, the weights of body and kidney were significantly higher compared with cisplatin group, but apoptotic index did not show significant differences. These parameters reached normal range after administration of fruit extracts of TT for 4 days. The study demonstrates that extract of TT could have protective effect on cisplatin- induced apoptosis of kidney. This may be related to the presence of antioxidant components acting via a multitude of central and peripheral mechanisms.

**KEY WORDS: Cisplatin; Nephrotoxicity; *Tribulus terrestris*; Kidneys; Protective effect.**

## INTRODUCTION

Cisplatin (cis-diamminedichloroplatinum II) (CP), a most effective chemotherapeutic agent, has been successfully used in treatment of a variety of solid tumors, including ovary, testis, bladder, head and neck, lung, cervix, and endometrium (Baek *et al*., 2003; Hadjzadeh *et al*., 2012). It is an efficient platinum-derived alkylating agent that acts in unspecific phases of the cellular cycle against proliferating and resting cells (Lirdi *et al*., 2008) However, the use of dose intensification CP is limited by major side effects such as nephrotoxicity, peripheral neuropathy, ototoxicity, azoospermia, sperm morphology and motility alterations in normal tissues (Lirdi *et al*.; Pabla & Dong, 2008). CP nephrotoxicity pathological feature, the major adverse effect of CP in approximately one third of patients during chemotherapy in clinic, it has been the cause of renal cell damage, cell death and the loss of renal function or acute renal failure, especially in renal tubules (Jiang *et al*., 2009; Ramesh & Reeves, 2002; Sheikh-Hamad *et al*., 2004; Arjumand *et al*., 2011; Kang *et al*., 2011; Arany & Safirstein, 2003; Pabla & Dong). Oxidative agents, principally involving reactive oxygen species (ROS) in renal tubular cells, are considered as the main mechanisms of nephrotoxicity of CP. The interaction of ROS with cellular components may lead to injury DNA, proteins and lipids (An *et al*., 2001; Matsushima *et al*., 1998). Depending on CP administration dose and cellular status, tubular cell death has been known in the forms of both apoptosis and necrosis, albeit the mechanism basic cisplatin-induced tubular cell death is not thoroughly discerned (Pabla & Dong). To avoid this side effect,

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prescription of complementary synthetic or herbal protective agents has been investigated against CP-induced nephrotoxicity (Saleh *et al*., 2009; Naqshbandi *et al*., 2012; Ashrafi *et al*., 2012; Pérez-Rojas *et al*., 2011). *Tribulus terrestris* L (popularly known as puncture vine) is an annual herb of the Zygophyllacea family about 30–70 cm high and has pinnate leaves (of unequal length), yellow flowers and characteristic stellate shaped carpel fruit (Rajendar *et al*., 2011; Singh, *et al*., 2012; Sharma *et al*., 2013). *Tribulus terrestris* L grows with a wide distribution in tropical and moderate areas, including the US and Mexico, the Mediterranean region, and throughout Asia (Phillips *et al*., 2006; Chu *et al*., 2003). A fruit of traditional Chinese medicine (Sugunavarman *et al*., 2010), the plant extract is mainly used for kidney disorders. Different parts of *Tribulus terrestris* L have been used traditionally in treating a variety of diseases including hypertension and coronary artery diseases, diabetes and hyperlipidemia, as well as fungal diseases (Sharma *et al*.; Phillips *et al*.; Chu *et al*.; Sugunavarman *et al*.). In addition it has been shown that *Tribulus terrestris* L extract has antioxidative, apoptosis inhibitory, and vasodilator properties (Shalaby & Hammouda, 2014; Kavitha *et al*., 2011). The fruit removes gravel from the urine and stone in the bladder (Kavitha & Jagadeesan, 2006). *Tribulus terrestris* L is also reported to have cooling, diuretic, tonic and aphrodisiac effects (Sugunavarman *et al*.). Accordingly, it is of particular interest to discover the effects of *Tribulus terrestris* L on cisplatin-induced apoptosis in mice kidney.

## MATERIAL AND METHOD

**Collection and preparation of aqueous extract**. Whole plants were purchased from a traditional medicine center in June 2013 and identified and authenticated by a botanist. Then, fresh fruits of *T. terrestris* L were dried in shade at room temperature (25±2 ºC) and its extract was obtained through the percolati on method. Extracting method was described previously (Keshtmand *et al*., 2015). With this method, approximately 200 g of the dry plant specimen was ground and added to 400 mL of 70 % ethanol and were left to macerate at room temperature for 4 h. Then, the tinctured grains were extracted by percolation method and extract concentrated in a vacuum and dried in the flat surface. The obtained extract weight, 6.5 g was expressed as mg/kg of body weight for 4 days followed by CIS injection. After dissolving the extract in distilled water, extract was immediately administered interaperitoneally to mice.

**Drugs**. Cis-diammineplatinum (II) dichloride (cisplatin) from Ebeve Pharma (Austria) was purchased. Ten to fifteen minutes before use, CP was dissolved in saline in darkness. At one day of the experiment an intraperitoneal injection (5.5 mg/kg) was given (Raoofi *et al*., 2015).

**Animal experiment and Groups**. Thirty male Balb/c mice (weight, 25 g to 30 g) were procured from animal house of Kermanshah University of Medical Sciences. The mice were maintained in a 12-h light/dark cycle in a temperature and humidity controlled facility without any stressful stimuli. The animals were provided with standard diet pellets and water ad libitum. Experiments were started after one week of adaptation. Research protocols were approved by the local ethics committee. Mice were distributed at random into five groups (each of six mice) and individually put in metabolic cages and treated as follows: (i) control group (C) received normal saline (0.9 % NaCl); (ii) Experimental groups Consist of cisplatin-treated group (E1): one injection of cisplatin (5 mg/kg body weight, i.p), *T. terrestris* -cisplatin-treated groups (TT + CIS): CIS + 100 mg/kg extract of TT (E2), CIS + 300 mg/kg extract of TT (E3), CIS + 500 mg/kg extract of TT (E4). Body weights of the mice were recorded initially and at the end of the experimental procedure (day 5). Weights of the kidneys were also noted.

**TUNEL Method**. The DNA 30-end labeling of apoptotic cells was detected by the terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick end-labeling (TUNEL) assay by using In situ Cell Death Detection Kit, AP (Roche Diagnostics Deutschland GmbH, Germany; 17848651). Sections were dewaxed with xylene and rehydrated by standard methods and washed in deionized water. Nuclei in the tissue sections were excluded from protein by incubating with 50 µL of proteinase K (10 mg/mL) for 20 min at 37 ˚C. After washing with phosphate buffered saline PBS (10 min, twice), the slides were incubated with TUNEL reaction mixture in a humidified chamber at 37 °C for 60 min, followed by rinsing with PBS for (10 min, three times). The sections were counterstained with propidium iodide solution diluted to 1 µg/mL in PBS (15 min), and then washed in deionized water (5 min). Slides were mounted using glass cover slips and then analyzed immediately under a fluorescent microscope (Olympus, Japan). Apoptotic index (AI) was calculated by dividing the number of TUNEL positive cells to total number of the cells in randomly selected fields, and the result was multiplied by 100 (Lirdi *et al*.).

**Data analysis**. In this experimental study all Data were recorded as Mean±SE. the one way analysis of variance with Tukey's post hoc test was used to determine differences between control and experimental groups. $P < 0.05$ was regarded as statistically significant.

## RESULTS

After 4 days of injections CIS in a dose of 5.5 mg/kg (E1) (P= 0.004) and CIS + 100 mg/kg extract of TT (E2) (P= 0.011) to male mice of the experiment induced significant decreases in weight of the total body when compared to the normal control group (Fig. 1). The weight of the kidneys in comparison with control group were significantly decreased in E1 (P= 0.002) group but there was not changed in other ones (P >0.05) (Fig. 2).

Renal tubular apoptosis has been suggested as a mechanism of cisplatin-induced acute kidney injury. The TUNEL method detects DNA fragmentation associated with late apoptosis. The TUNEL staining sections of the kidneys revealed that apoptosis occurred in both cortex and medulla of CP involved groups. Calculated apoptotic index based on these sections showed this occurrence significantly increased in E1group (P <0.001) and CIS + 100 mg/kg extract of TT groups (P <0.01) (Figs. 3 and 4). As shown in Figure 3, in the CIS + 300 TT and CIS + 500 TT treatment groups there were no significant changes in renal tubular apoptosis (Figs. 3 and 4).

## DISCUSSION

The present study demonstrated *T. terrestris* hydroalcoholic extract effect on cisplatin-induced apoptosis in mice kidney. Treatment of mice with CP resulted in renal damage, showing a significant decline in the weight of animals treated with CP. These data supported other studies that indicated reductions in body weight could be attributable to toxic side effect of chemotherapeutic drugs and it suggests that *T. terrestris* relieves the adverse effects of CP (Raoofi *et al.*). In the present study, we determined DNA fragmentation in kidney using the TUNEL technique. A single dose of CP caused apoptosis in kidney. It seems that inflammatory responses with regard to the weight of animals are acute consequence of CIS administration that occurs during 3–4 days after exposure (Ueki *et al.*, 2013). In vivo administration of nephrotoxic doses of CP produces a large increase in apoptosis in the kidney (Ramesh & Reeves, 2004). Previous studies showed that CP upregulates the expression of TNF-α in mouse kidney and TNF-α stimulates an inflammatory response *in vivo* which exacerbates cisplatin nephrotoxicity (Ramesh & Reeves, 2003). Treatment of cisplatin-treated mice with *T. terrestris* significantly improved renal dysfunction, reducing tubular cell damage, oxidative stress and apoptosis. Thus, together with other herbal extracts, *T. terrestris* reduces apoptosis

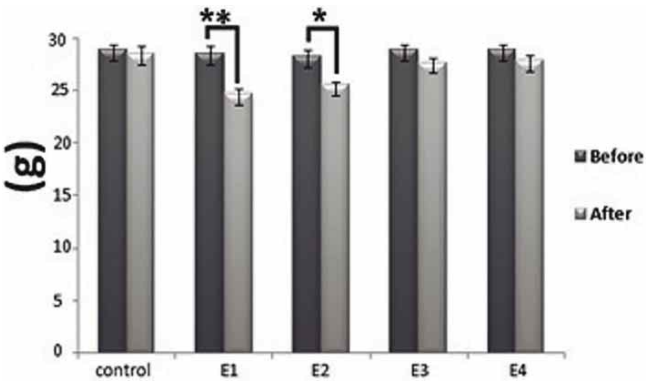



Fig. 1. The effect of toxic dose of cisplatin (CIS) (5.5 mg/kg) and different doses of *Tribulus Terrestris* (TT) on total body weights in the mice (left bar of the pairs is initial weights and the right bar is end weights). The groups (X axis) are control: not treated, E1: CIS, E2: CIS + TT (100 mg/ kg), E3: CIS + TT (300 mg/kg), E4: CIS + TT (500 mg/kg). *= P <0.05, **= P < 0.01.

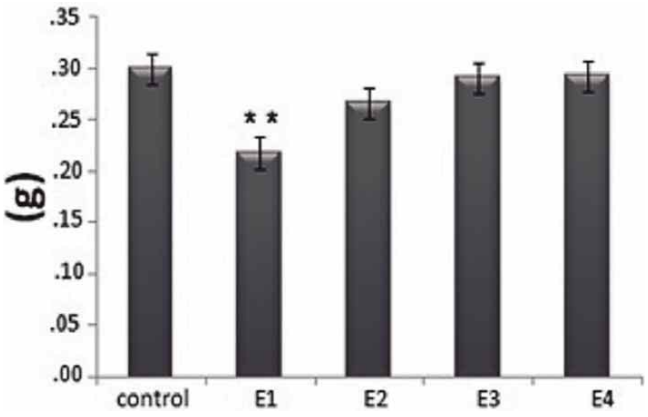



Fig. 2. The effect of toxic dose of cisplatin (CIS) (5.5 mg/kg) and different doses of *Tribulus terrestris* (TT) on the kidney weights. The groups (X axis) are Control: not treated, E1: CIS, E2: CIS + TT (100 mg/kg), E3: CIS + TT (300 mg/kg), E4: CIS + TT (500 mg/kg). **= P <0.01 in comparison with the control group.

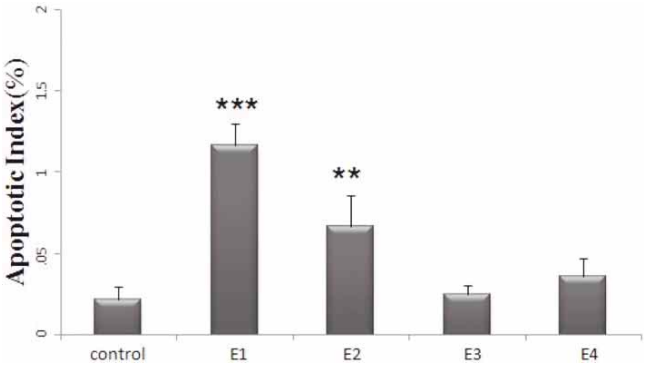
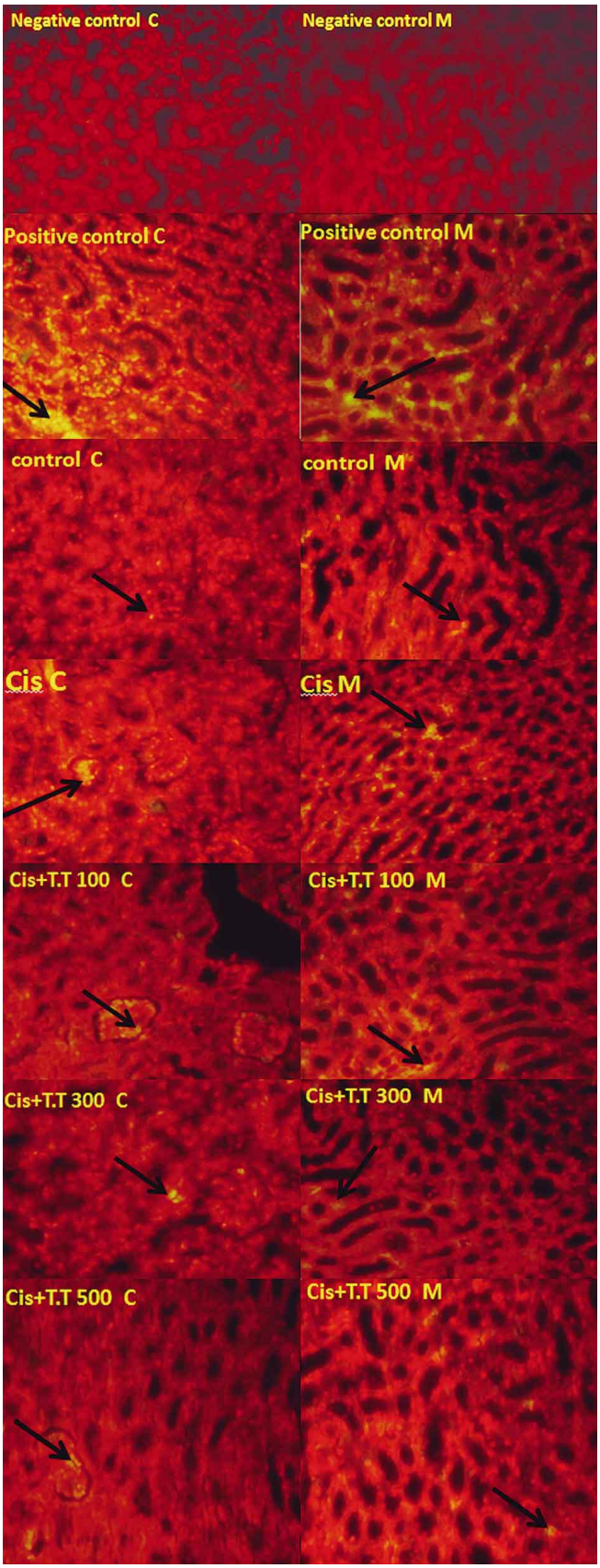



Fig. 3. The effect of toxic dose of cisplatin (5.5 mg/kg) and different doses of *Tribulus terrestris* (TT) on apoptotic index of kidneys. The groups (X axis) are Control: not treated, E1: CIS, E2: CIS + TT (100 mg/kg), E3: CIS + TT (300 mg/kg), E4: CIS + TT (500 mg/kg). **= P <0.01, ***= P <0.001 in comparison with the control group.




of CP by decreasing the number of apoptotic cells that can be considered as the antioxidant activity or diuretic effect of *T. terrestris*. The antioxidant activity of *T. terrestris* could be attributed to its flavonoid content (Harborne & Williams, 2000). A number of studies have shown that flavonoids have the ability to regulate a variety of enzyme systems involved in cell division, proliferation, detoxification, inflammation and immune response (Choi *et al*., 2004; Di Carlo *et al*., 1999; Hollman & Katan, 1999). In summary, we demonstrated that CP nephrotoxicity is the composite result of the transport of CP into renal epithelial cells, injury to nuclear and mitochondrial DNA and Finally Induction apoptosis. In conclusion, our previous and present studies indicate that the antioxidant action of *T. terrestris* prevents of renal cell death by apoptosis and necrosis. Treatment of male mice with extract of *T. terrestris* showed obvious effects on the kidney studied.

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**GHANBARI, A.; ZARE, F.; KHAZAEI, M.; MORADI, M. & RAOOFI, A.** Efecto del extracto hidroalcohólico de *Tribulus terrestris* sobre la apoptosis inducida por cisplatino en el riñón de ratones. *Int. J. Morphol., 34(2)*:713-718, 2016.

**RESUMEN:** El cisplatino (EBEWE Pharma, Unterach, Austria) es un medicamento contra el cáncer utilizado en quimioterapia. Uno de los principales efectos secundarios limitantes del cisplatino es la nefrotoxicidad. *Tribulus terrestris* (TT) ha sido utilizado como agente protector sintético o herbal para los trastornos renales. El objetivo fue investigar el efecto del extracto hidroalcohólico de TT sobre la apoptosis inducida por cisplatino en el riñón de ratones. Se utilizaron ratones adultos machos (n= 30), que fueron divididos en 4 grupos, un control y tres grupos experimentales (n= 6). El grupo control recibió solución salina; el primer grupo experimental recibió cisplatino (5,5 mg/kg) y los otros tres grupos experimentales recibieron

Fig. 4. The effect of toxic dose of cisplatin (5.5 mg/kg) and different doses of *Tribulus terrestris* on cortex and medulla of the kidneys of male balb/c mice (TUNEL staining, Arrows show TUNEL positive cells, Photos represent X200 magnification).

cisplatino (5,5 mg/kg) con diferentes dosis de extracto hidroalcohólico de TT (100, 300 y 500 mg/kg vía ip) respectivamente. Los riñones fueron retirados después de 4 días de aplicadas las inyecciones, y se realizó el ensayo TUNEL en los riñones. Se evaluó el peso corporal de los ratones, el peso de los riñones y el índice de apoptosis. El análisis de datos se realizó mediante ANOVA de un factor seguido por la prueba post hoc de Tukey. Los resultados mostraron que el cisplatino con plomo provocó una reducción en el peso corporal y el riñón (P <0,01) y un aumento significativo del índice de apoptosis en comparación con el grupo control (P <0,001), mientras que en los grupos tratados con TT, los pesos corporales y de los riñones fueron significativamente mayores en comparación con el grupo de cisplatino, pero el índice de apoptosis no mostró diferencias significativas. Estos parámetros alcanzaron niveles normales después de la administración de extracto de TT durante 4 días. El estudio demuestra que el extracto de TT podría tener un efecto protector sobre la apoptosis inducida por cisplatino en el riñón, que podría estar relacionado con la presencia de componentes antioxidantes que actúan a través de múltiples mecanismos centrales y periféricos.

**PALABRAS CLAVE: Cisplatino; Nefrotoxicidad; *Tribulus terrestris*; Riñones; Efecto protector.**

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