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Original article

Protective Effect of Aqueous and Ethanolic Extracts of *Portulaca Oleracea* Against Cisplatin Induced Nephrotoxicity

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Abstract

Objective(s)
Portulaca oleracea L. is a herbaceous weed from portulacaceae family. It can be found in many parts of the world. Modern pharmacological studies have demonstrated that *P. oleracea* have antioxidant effects. The protective effect of aqueous and ethanolic extract of *P. oleracea* against cisplatin-induced renal toxicity was studied in rats.

Materials and Methods
Single intraperitoneal injection of 4 mg/kg cisplatin was administrated to rats. After 5 days, blood urea nitrogen (BUN) and serum creatinine (Scr) concentration were determined. Effect of aqueous and ethanolic extracts, before and after cisplatin injection on BUN and Scr, as well as morphological renal damage, was evaluated.

Results
It was indicated that treatment with aqueous and ethanolic extracts of *P. oleracea* in the highest dose (0.8 and 2 g/ kg), 6 and 12 hr before cisplatin injection reduced BUN and Scr. Tubular necrotic damage was not observed either.

Conclusion
Results suggest that *P. oleracea* extract may protect against cisplatin-induced renal toxicity and might serve as a novel combination agent with cisplan to limit renal injury.

Keywords: Antioxidants, Cisplatin, Kidney, Toxicity, *Portulaca oleracea*

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Introduction

Portulaca oleracea L. (Portulacaceae) is listed in the World Health Organization as one of the most used medicinal plants and it has been given the term 'Global Panacea' (1). *P. oleracea* is a summer annual which is grown as a vegetable in many parts of the world. This half-hardy low growing plant has slightly succulent leaves and stems that are used raw or cooked. There are green and yellow leaved forms; the green type has thinner leaves, is more vigorous and possibly better flavoured (2). It is used as a potherb in the Mediterranean, Central European and Asian countries. It is also referred to as the common Purslane (1).

In folk medicine, it is utilized as an antipyretic, anti-scorbutic, antiseptic, antispasmodic, diuretic, antihelmetic and for treatment of urinary disorders (3). The aerial parts of the plant are used medicinally for reducing pain and swelling (3). Recent pharmacological studies have shown muscle relaxant activity (4), reduction in locomotor activity, increased in the onset time of pentylenetetrazole-induced convulsion (5), analgesic, anti-inflammatory effects and antioxidant properties (3). It is reported that extracts of *P. oleracea* has inhibitory effect on lipopolysaccharide (LPS) and interferon-γ (IFN- γ) induced NO production (6).

It was shown that *P. oleracea* is a rich source of omega-3 fatty acids, gallotannins, kaempferol, quercetin, apigenin and glutathione (5, 7, 8). Aqueous extract of *P. oleracea* does not have any cytotoxicity or genotoxicity effect and it is safe for daily use (9).

Cisplatin is one of the most effective anticancer agents and it is used to treat solid tumors such as testicular and bladder, ovarian, breast and lung cancers (10). Nephrotoxicity is a main clinical problem and occurs in 25-35% of patients receiving a single dose of it (11). Several mechanisms have been considered for cisplatin nephrotoxicity including hypoxia, generation of free radicals, inflammation, lipid peroxidation and apoptosis (12). Also, cisplatin reduces activity of antioxidant enzymes and induced depletion of glutathion (GSH) (13). Due to the reported use of this plant in folk medicine for treatment of urinary disease and because of its antioxidant constituents and the lack of any report on its protective effect in nephrotoxicity in modern researches, this study was initiated.

Materials and Methods

Animals

The study was performed on male wistar rats, 250–300 g that were bred and kept at the animal center of School of Pharmacy, Mashhad. Animals were housed in a ventilated room under a 12/12 hr light/dark cycle at 24±2 °C and had free access to water and food.

Plant

Aerial parts of *P. oleracea* were collected from campus of Ferdowsi University of Mashhad and were identified by Ferdowsi University and voucher samples were preserved for reference in the herbarium of the Department of Pharmacognosy, School of Pharmacy, Mashhad, Iran (Voucher no. 2240-1615-12).

Preparation of Extract

The aerial parts of *P. oleracea* were cleaned, dried in shadow and powdered by mechanical grinder. Then the aerial parts powder (100 g) was defatted with petroleum ether (40-60 °C) using the soxhlet apparatus. For the ethanolic extract, powder was subsequently macerated in 800 ml ethanol (80%, v/v) for 3 days and the mixture was subsequently filtered and concentrated in vacuo at 40 °C. The residue was suspended in normal saline with tween 80 (1%). For the aqueous extract, 800 ml distilled water was added to 100 g aerial parts powder and filtered through cloth. The extract was then concentrated in vacuo to the desired volume.

Experimental Procedure

First, maximum tolerated dose (MTD) of extracts was determined. The experimental method is based on the modification of Baliga *et al* (14). One group (n= 5) received cisplatin (4 mg/kg) intraperitoneally and other groups of rats (n= 5) were used to study the effect of aqueous and ethanolic extracts of *P. oleracea* on cisplatin-induced renal toxicity and changes in renal function. Doses (0.2, 0.4, 0.8 g/ kg, i.p.) of aqueous and doses (0.5, 1, 2 g/ kg, i.p.) of ethanolic extracts, were injected 6 hr or 12 hr before cisplatin and the mentioned doses of aqueous and ethanolic extracts, were

injected 6 hr or 12 hr after cisplatin. Other groups received the same doses of aqueous and ethanolic extracts, 6 hr and 12 hr before cisplatin injection, and the same doses of aqueous and ethanolic extracts, 6 hr and 12 hr after cisplatin injection were administrated to next groups. One group acted as control (normal saline). Rats were sacrificed 5 days after cisplatin administration. The used protocols conformed to guidelines of the conduct of animal experiments issued by School of Pharmacy and were approved by the committee on the ethics of animal experiments in Mashhad University. Blood samples were collected and analyzed for blood urea nitrogen (BUN) and serum creatinine (Scr) by using the commercial kits (15). After bleeding, the kidneys were removed and fixed in 10% neutral buffered formalin for at least 24 hr. Tissues were processed for microscopical examination using a standard protocol and paraffin sections were stained with hematoxylin and eosin (16). The observed necrotic changes were limited to the tubulointerstitial areas and graded as mild, moderate and severe (17) as follows: Mild: areas of tubular epithelial cell swelling, necrosis and desquamation involving <50% of cortical tubules; Moderate: similar changes involving >50% but <75% of cortical tubules and Severe: similar changes involving >75% of cortical tubules.

Statistical analysis

The results are expressed as mean±SD. Data were analyzed by one-way analysis of variance. Sequential differences among means were calculated at the level of $P< 0.05$, using Tukey contrast analysis as needed.

Results

Cisplatin induced nephrotoxicity such as proximal and distal tubular necrosis, mainly in the paracortical region (corticomedullary) and intratubular casts in the outer stripe of the outer medulla (Figure 1B). Functional nephrotoxicity indicators such as BUN and Scr were elevated in cisplatin-treated rats compared with control. Treatment with aqueous and ethanolic extracts in the highest dose (0.8 and 2 g/ kg), 6 and 12 hr before cisplatin injection reduced BUN and Scr. Tubular necrotic damage was not observed either (Figure 1C). The protective effect of aqueous and ethanolic extracts before cisplatin injection were relatively similar and these effects were dose dependent (Table1).

Rats treated with aqueous and ethanolic extract, 6 and 12 hr after cisplatin injection had BUN and Scr levels significantly lower than those receiving cisplatin alone but mild to moderate cell injury was observed (Table 2, Figure 1D).

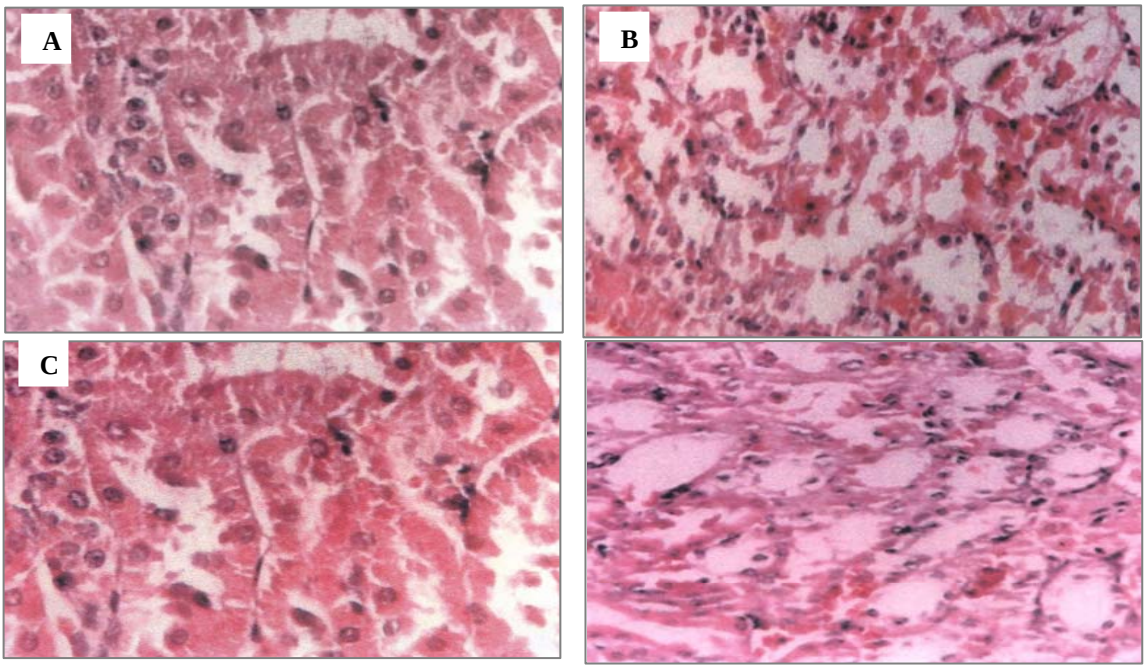

Figure 1. Histological examination. (A) Normal tubules. (B) Areas of severe necrosis of proximal tubules, 5 days after injection of 4 mg/ kg cisplatin. (C) No tubular necrosis after treatment with aqueous and ethanolic extracts (0.8 and 2 g/kg) 6 and 12 hr before cisplatin. (D) Mild to moderate tubular necrosis after treatment with aqueous and ethanolic extract (0.8 and 2 g/kg) 6 and 12 hr after cisplatin.

Table1. Effect of aqueous and ethanolic extract of *Portulaca oleracea* (0.8 and 2 g/Kg, i.p.) before injection of cisplatin (4 mg/ kg) on BUN and Scr concentration.

	BUN (mg/dl)	Scr (mg/dl)
Control	17.83±2*	0.58±0.3*
Cisplatin (Cis) 4 mg/Kg	70±8.8	1.7±0.23
Aqueous extract 0.8 g/Kg, 6 hr before Cis	27.2±6.97*	0.82±0.2*
Aqueous extract 0.8 g/Kg, 12 hr before Cis	32.2±9.2*	0.84±0.3*
Aqueous extract 0.8 g/Kg, 6 and 12 hr before Cis	21±6.78*	0.78±0.17*
Ethanolic extract 2 g/Kg, 6 hr before Cis	31.6±10.1*	0.94±0.2*
Ethanolic extract 2 g/Kg, 12 hr before Cis	35±10.83*	0.92±0.4*
Ethanolic extract 2 g/Kg, 6 and 12 hr before Cis	25±6.74*	0.82±0.1*

n= 5, mean±SD; *P< 0.05 significantly different compared with cisplatin-treated group.

Table 2. Effect of aqueous and ethanolic extract of *Portulaca oleracea* (0.8 and 2 g/Kg, i.p.) after injection of cisplatin (4 mg/ kg) on BUN and Scr concentration .

	BUN (mg /dl)	Scr (mg/ dl)
Control	17.83±2*	0.58±0.3*
Cisplatin (Cis) 4mg/Kg	70±8.8	1.7±0.23
Aqueous extract 0.8 g/Kg, 6 hr after Cis	39.2±7.72*	1±0.24*
Aqueous extract 0.8 g/Kg, 12 hr after Cis	42±7.77*	1.06±0.1*
Aqueous extract 0.8 g/Kg, 6 and 12 hr after Cis	32.2±11.73*	0.9±0.36*
Ethanolic extract 2 g/Kg, 6 hr after Cis	42±13.39*	1.02±0.3*
Ethanolic extract 2 g/Kg, 12 hr after Cis	46±9.38*	1.1±0.4*
Ethanolic extract 2 g/Kg, 6 and 12 hr after Cis	38±12.1*	1±0.5*

n= 5, mean±SD; *P< 0.05 significantly different compared with cisplatin-treated group.

Discussion

Cisplatin induced nephrotoxicity is considered to be a rapid process involving reaction with proteins in the renal tubules (18, 19) and is characterized by morphological destruction of intracellular organelles, GSH depletion, cellular necrosis and lipid peroxidation (13). This renal damage occurs within 1 hr after administration (20). It is important that the protective agent is present in renal tissue before damage occurs. This might explain why complete protection could not be observed when both extracts were given after injection of cisplatin and our results confirmed this notion. The acute renal failure indicated by increased Scr and BUN occurred before the development of tubular necrosis. These parameters are markers of glomerular filtration rate. Our results showed that extracts of *P. oleracea* reduced the rise of BUN and Scr induced by cisplatin as well as renal damage.

Free radicals formation is one of the mechanisms of nephrotoxicity induced by cisplatin and antioxidants have protective effect against renal toxicity induced by this drug (21). Constituents of *P. oleracea* such as flavonoids (quercetin), omega-3, ascorbic acid, β- carotene and glutathione have antioxidant activitiy (5, 7, 8), so this plant may inhibit lipid peroxidation by scavenging free radicals and increasing intracellular concentration of glutathione.

Our results are in agreement with previous studies that indicated silibinin (200 mg/kg, i.v.), one of the component of silymarin, which have antioxidant activity, 1 hr before the cisplatin (5 mg/kg, i.p.) prevented the effects of cisplatin on creatinine clearance and BUN and diminished morphological alteration in proximal tubules (22). Also milk thistle extract (0.6 g/kg, i.p.) 2 hr before cisplatin injection prevented the tubular damage and had protective effect against cisplatin-induced renal toxicity because of inhibitory effect on lipid peroxidation (17).

In addition, activation of pro-inflammation factors is another mechanism of nephrotoxicity induced by cisplatin and it was shown that *P. oleracea* has anti-infammatoty effect, so this plant may reduce renal damage (23-25).

Conclusion

The present finding suggests that *P. oleracea* protects against acute cisplatin nephrotoxicity and may be considered as a potentially useful

candidate in the combination chemotherapy with cisplatin. Protective effect of both extracts of this plant was more effective in 6 and 12 hr before cisplatin injection.

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