

Renoprotective Effect of *Plantago Major* Against Nephrotoxicity and Oxidative Stress Induced by Cisplatin

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Introduction. The aim of this study was to investigate the possible renoprotective effect of *Plantago major* extract against cisplatin-induced nephrotoxicity in rats.

Materials and Methods. Rats were divided into 6 groups. The first group was the control, group 2 was treated with cisplatin (7 mg/kg, single dose), and groups 3 to 6 received cisplatin with vitamin E (100 mg/kg) and *Plantago major* extract at doses of 300 mg/kg, 600 mg/kg, and 1200 mg/kg, for 20 days.

Results. On day12, serum concentration of urea, creatinine, and potassium significantly increased and sodium concentration significantly decreased in the cisplatin group compared with the control rats. However, serum creatinine, urea, and potassium concentrations were significantly lower in all of the *Plantago major* groups compared to the cisplatin group. Also, there was a significant elevation in serum sodium concentration in the *Plantago major* 600 mg/kg group compared to the cisplatin group on day12. Injection of cisplatin caused a significant elevation in malondialdehyde concentration but a significant decrease in catalase activity and total thiol content compared to the control group. *Plantago major* extract at 1200 mg/kg significantly improved malondialdehyde concentration and total thiol content compared to the cisplatin group. Catalase activity with *Plantago major* significantly increased at all doses compared to the cisplatin group.

Conclusions. The current study suggests that *Plantago major* extract and vitamin E are able to improve kidney function as well as oxidative stress in cisplatin-induced renal toxicity in the rat.

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INTRODUCTION

Cisplatin is one of the most potent and important chemotherapy drugs that is now being used for the treatment of many types of tumors, including nonsmall cell lung carcinoma and head and neck, testicular, ovarian, and cervical malignancies.¹ Unlike the most antineoplastic agents, cisplatin is a simple inorganic molecule.² The mechanism of the cytotoxic activity of cisplatin is not fully

understood, but it is possibly due to inter- and intra-strand cross-links in DNA that result in inhibition of DNA synthesis and replication.³ Nevertheless, 2 main factors including severe side effects in normal tissues and acquired resistance to cisplatin have limited the use of this drug.²

Nephrotoxicity is one of the main side effects of cisplatin. Previous studies have shown that cisplatin enters renal epithelial cells via organic

cation transporter 2.^{3,4} Evidence indicates that the generation of reactive oxygen species (ROS), accumulation of lipid peroxidation products in epithelial tubular cells, and depletion of antioxidant systems are the main mechanisms associated with nephrotoxicity induced by cisplatin.⁵ Inside the cell, ROS may attack and injure multiple molecules including DNA, lipids, and proteins and also activate some important signaling pathways, which lead to necrosis and apoptosis.² Therefore, ROS may be considered as an early factor responsible for the activation of signaling pathways, which finally leads to cell death, kidney injury, and kidney failure in nephrotoxicity induced by cisplatin. Ramesh and Reeves have also shown that inflammation, mainly via tumor necrosis factor- α production, amplifies the injury and has an important role in pathogenesis of cisplatin-induced nephrotoxicity.⁶

Plantago major L (*P major*) is a perennial plant that belongs to the *Plantaginaceae* family. This plant is one of the most widely distributed medicinal herbs in all over the world.⁷ *P major* is presented to the Nordic countries parallel to the presentation of the first primitive cultivated fields in the stone age nearly 4000 years ago.⁸ Although, *P major* is regarded as a weed by many people, it is a traditional medicinal plant with many pharmacologic effects. This plant is known as *groblad* meaning “healing leaves” in Swedish and Norwegian and the Indians called it “white man’s footprint.”⁷ In traditional medicine, *P major* has been used as astringent, anesthetic, anti-inflammatory, antitumor, analgesic, antiviral, analeptic, and anti-ulcer.⁷ Caffeic acid derivatives, polysaccharides, lipids, iridoid glycosides, terpenoids, and flavonoids are some of the biologically active compounds of *P major*. A wide range of pharmacologic effects including immunomodulatory, anticancer, antimicrobial, antiulcer, analgesic, and antioxidant properties have been demonstrated from this plant.⁹⁻¹⁴ The aim of this study was to investigate the possible protective effects of *P major* on cisplatin-induced nephrotoxicity and oxidative stress in the rat.

MATERIALS AND METHODS

Chemicals

Cisplatin was purchased from the Mylan Company (Greece, Drugbank ID: DB00515). Vitamin E was purchased from the Osveh Company (Iran). 5,5'-dithiobis-2-nitrobenzoic acid (DTNB),

2-thiobarbituric acid, trichloroacetic acid, Tris, hydrogen chloride, and potassium chloride were obtained from the Merck Company (Germany). *P major* whole plant was obtained from Medicinal Plants Division of Imam Reza Pharmacy. All other reagents and solvents were of a high analytical grade.

Extract Preparation

For the preparation of the hydroalcoholic extract, *P major* whole plant was washed, dried, and powdered. Fifty grams of the powder was extracted in a Soxhlet extractor with ethanol (70% v/v). After the extraction, the solution was purified using a rotary vacuum evaporator and kept at 4°C until use.

Animals

A total of 60 adult male Albino Wistar rats weighing 250 g to 320 g were obtained from the Central Animal House of the School of Medicine, Mashhad University of Medical Sciences. The rats were housed at 22 ± 2°C and standard condition of 12-hour light-dark cycle with free access to food pellets and water. All experiments were performed under the authority of the Mashhad University of Medical Sciences and conformed the international animal ethics.

Experimental Protocol

The animals were randomly assigned into 6 groups of 10 each, to receive normal saline intraperitoneally on the 6th day of experiment (control group); cisplatin (7 mg/kg BW) intraperitoneally on the 6th day of experiment (cisplatin group); vitamin E (100 mg/kg BW) intraperitoneally for 20 consecutive days and cisplatin on the 6th day of experiment (vitamin E group); *P major* extract (300 mg/kg BW) in drinking water for 20 consecutive days and cisplatin on the 6th day of experiment (*P major* 300 group); and similarly, *P major* at doses of 600 mg/kg and 1200 mg/kg and cisplatin (*P major* 600 and *P major* 1200 groups).

Blood samples were collected in heparinized tubes from the orbital sinus under light ether anesthesia on days 0, 12, and 21. Twenty-four-hour urine samples from the rats were taken on days 0, 12, and 21 of the study, while each animal was separately housed in metabolic cage. Serum was separated by centrifugation at 4000 g for 10

minutes and was stored at -20°C until assayed. All animals were humanely killed 21 days after cisplatin injection and both kidneys were quickly removed. The left kidneys were stored at -70°C for subsequent measurement of total thiol content, malondialdehyde concentration, and catalase activity.

Serum Biochemical Parameters

Urea and creatinine concentrations were measured colorimetrically by using a photometer (Convergys 100 Biochemistry Analyser, Germany) and commercial diagnostic kits (Pars Azmoon, Tehran, Iran). Osmolarity was determined by a cryoscopic osmometer (Osmomat 030, Germany). Sodium and potassium concentrations were measured by using an electrolyte analyzer (AC 9800, China).

Estimation of Lipid Peroxidation

The kidney tissue malondialdehyde concentration was measured by the method by Uchiyama and Mihara. Briefly, the tissue samples were homogenized in cold potassium chloride solution (1.5%) to give a 10% homogenate and centrifuged at 1500 g for 10 minutes. The reaction mixture contained 15g of trichloroacetic acid, 0.375 g of 2-thiobarbituric acid, and 2 mL of hydrogen chloride. Two milliliter of this mixture was added to 1 mL of supernatant and the mixture was kept for 50 minutes in boiling water. The absorbance of colored layer was read at 535 nm. Malondialdehyde concentration was calculated from the following equation¹⁵:

Malondialdehyde concentration = absorbance / 1.56×105

Estimation of Total Thiol Content

Total thiol groups content of the renal tissue was estimated using the method explained by Sedlak and Lindsay. Briefly, the tissue samples were homogenized in cold potassium chloride solution (1.5%) to give a 10% homogenate and centrifuged at 1500 g for 10 minutes. Then, 50 µL of the supernatant was added to 1 mL of Tris-EDTA buffer (pH, 8.6), and absorbance was read at 412 nm (A1). After 10 minutes, 20 µL of DTNB reagent was added to the mixture and the absorbance was read again (A2). The absorbance of DTNB reagent was also read as a blank (B). Total thiol content

(mM) was calculated from the following equation¹⁶:

Total thiol content = $(A2 - A1 - B) \times 1.07/0.05 \times 13.6$

Estimation of Catalase Activity

The kidney tissue catalase activity was determined spectrophotometrically at 240 nm in tissue homogenates by the method of Aebi with hydrogen peroxide (30 mM) as the substrate.¹⁷

Statistical Analysis

All values are presented as mean ± standard error of mean. Homogeneity of variance was tested using the Levene test. The difference between means was analyzed by using the 1-way analysis of variance followed by the LSD test. A *P* value less than .05 was considered significant.

RESULTS

Serum Biochemical Parameters

Serum biochemical parameters were measured on the 1st, 12th, and 21st days of the experiment. There was no significant alteration in serum parameters between the cisplatin group and other treated animals on the 21st day (data are not shown). Serum urea and creatinine concentrations in the cisplatin group was significantly higher than in the control group on the 12th day (*P* < .001; Figures 1 and 2). However, both of these markers showed a significant decrease on the 12th day in the vitamin E (*P* < .001), as well as the *P major* 300, *P major* 600, and *P major* 1200 groups (*P* < .05, *P* < .001, and *P* < .001, respectively) as compared with the measurements

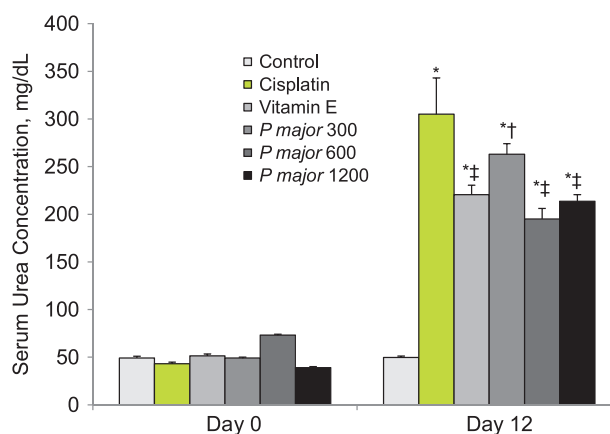


Figure 1. Serum urea concentration on days 0 and 12 in the rat experimental groups.

**P* < .001 compared to the control group on day12

†*P* < .05 compared to the cisplatin group on day12

‡*P* < .001 compared to the cisplatin group on day12

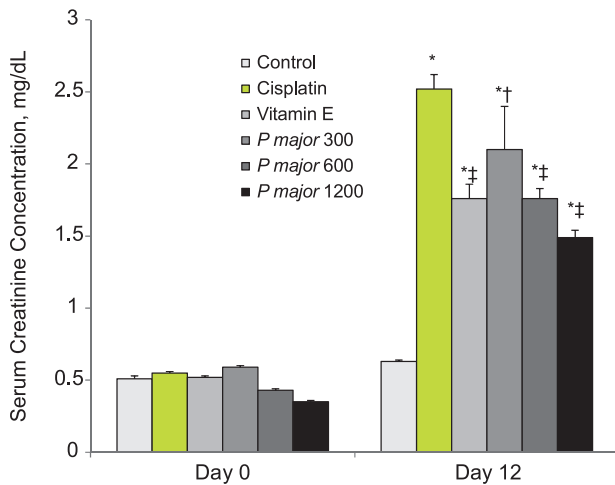


Figure 2. Serum creatinine concentration on days 0 and 12 in the rat experimental groups.

* $P < .001$ compared to the control group on day12
 † $P < .05$ compared to the cisplatin group on day12
 ‡ $P < .001$ compared to the cisplatin group on day12

of the same day in the cisplatin group (Figures 1 and 2). Serum potassium concentration showed a significant rise in the cisplatin group as compared with the control group ($P < .01$) on the 12th day of the experiment (Figure 3). Administration of vitamin E and *P major* extract at 300 mg/kg, 600 mg/kg, and 1200 mg/kg significantly reduced the serum potassium concentration on the 12th day as compared with the same day in the cisplatin group ($P < .001$; Figure 3). On day 12, serum sodium concentration was significantly lower in the cisplatin group than the control animals on the same day

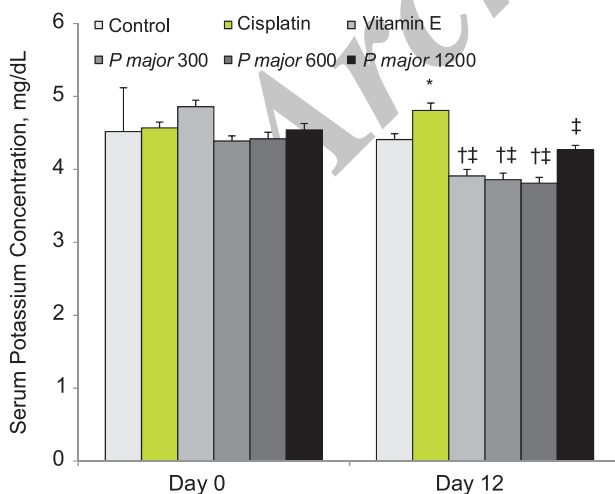


Figure 3. Serum potassium concentration on days 0 and 12 in the rat experimental groups.

* $P < .01$ compared to the control group on day12
 † $P < .001$ compared to the control group on day12
 ‡ $P < .001$ compared to the cisplatin group on day12

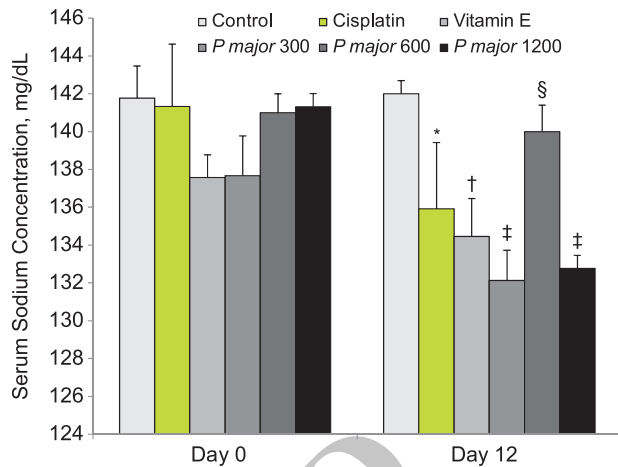


Figure 4. Serum sodium concentration on days 0 and 12 in the rat experimental groups.

* $P < .05$ compared to the control group on day12
 † $P < .01$ compared to the control group on day12
 ‡ $P < .001$ compared to the control group on day12
 § $P < .05$ compared to the cisplatin group on day12

($P < .05$; Figure 4). However, administration of *P major* extract at 600 mg/kg significantly increased this parameter on the 12th day as compared with the same day in the cisplatin group.

Biomarkers of Oxidative Stress

As seen in the Table, there was a significant increase in malondialdehyde concentration, but a significant reduction in total thiol content and catalase activity in the renal tissue of the cisplatin group as compared to the control animals ($P < .05$, $P < .01$, and $P < .01$, respectively). Administration of vitamin E and *P major* extract significantly reduced malondialdehyde concentration in the vitamin E and *P major* 1200 groups as compared with the cisplatin group ($P < .001$ and $P < .05$, respectively; Table). Furthermore, total thiol content in the *P major* 1200 group was significantly higher than in the cisplatin group ($P < .01$; Table). Additionally, catalase activity showed a significant increase in the vitamin E ($P < .001$), *P major* 300 ($P < .001$), *P major* 600 ($P < .01$), and *P major* 1200 ($P < .001$) groups as compared with the cisplatin-treated animals (Table).

DISCUSSION

Cisplatin, classified as an alkylating agent, is an effective anticancer drug.¹ The toxic effect of cisplatin, most notably nephrotoxicity, restricted its use.¹⁸ The exact mechanism of cisplatin-induced nephrotoxicity is not well known but several

Effect of *P Major* Extract on Malondialdehyde, Thiol, and Catalase Activity on the Kidney Tissue of Rat Experimental Groups

Parameter	Rat Groups					
	Control	Cisplatin	Vitamin E	<i>P major</i> 300	<i>P major</i> 600	<i>P major</i> 1200
Malondialdehyde, nmol/g tissue	19.2 ± 1.5	29.4 ± 4.3*	13.0 ± 1.3#	26.0 ± 5.4	22.0 ± 3.5	17.0 ± 1.5‡
Catalase activity, U/mL	18.5 ± 1.6	9.4 ± 1.2†	18.5 ± 0.7#	18.8 ± 1.7#	17.1 ± 1.6§	17.6 ± 2.1#
Thiol content, mM	0.58 ± 0.06	0.28 ± 0.01†	0.32 ± 0.12	0.28 ± 0.08	0.26 ± 0.06	0.73 ± 0.04§

**P* < .05 compared to the control group
 †*P* < .01 compared to the control group
 ‡*P* < .05 compared to the cisplatin group
 §*P* < .01 compared to the cisplatin group
 #*P* < .01 compared to the cisplatin group

mechanisms including DNA damage, inflammation, apoptosis, and lipid peroxidation have been implicated in pathogenesis of nephrotoxicity induced by cisplatin.³ Several investigations have reported that ROS are considered to be important mediators of nephrotoxicity associated with cisplatin.^{5,19} Although many studies focus on the mechanisms of kidney failure induced by cisplatin, strategies to protect patients from nephrotoxicity are limited. In recent years, the use of natural compounds as potential therapeutic agents for drug- or toxin-induced nephrotoxicity has been developed. *Plantago major* is a herb with a wide range of biological activities and various pharmacologic effects including wound healing activity, anti-inflammatory, analgesic, and antioxidant properties.⁷

In the current study, the possible protective effect of *P major* extract against cisplatin-induced nephrotoxicity and oxidative stress has been investigated. The present work demonstrated that cisplatin administration caused renal damage as was evident from an elevation in serum urea and creatinine concentration. This finding confirmed the accuracy of our experiment in induction of kidney dysfunction which is in agreement with investigations of Hosseinian and coworkers and Palipoch and coworkers.^{20,21} The present results also showed that administration of *P major* extract at 300 mg/kg, 600 mg/kg, and 1200 mg/kg significantly decreased serum urea and creatinine concentrations. There are points of evidence indicating that ROS increase the production of vasoconstrictor substances including isoprostane, endothelin, and thromboxane that result in glomerular vasoconstriction and glomerular filtration reduction. In addition, ROS decrease the GFR through the increase of resistance of afferent and efferent arterioles.^{3,22} In this regard,

the protective effect of *P major* extract in reduction of serum creatinine and urea concentrations may be associated with its antioxidant effect.¹⁴

Furthermore, the present results showed that beneficial effects of *P major* extract on serum urea and creatinine concentration were more evident at doses of 600 mg/kg and 1200 mg/kg compared to 300 mg/kg, which might be due to dose-dependency of *P major* extract against cisplatin-induced nephrotoxicity. Also, our results demonstrated that cisplatin caused significant alterations in serum sodium and potassium concentrations. The possible mechanism by which cisplatin exerts these toxic effects might be an interference in mitochondrial function and inhibition of adenosine triphosphatase production in the renal cells.³ Also, direct interaction of cisplatin with cytoplasmic part, C45 loop, of sodium-potassium adenosine triphosphatase pump in the renal tubular cells might be involved.^{23,24} Possibly, the antioxidant action of *P major* extract is responsible for the amendment of the alterations in serum sodium and potassium concentrations induced by cisplatin.¹⁴ There are numerous reports that indicate that cisplatin induces oxidative stress in renal tubular cells and many of cellular changes induced by cisplatin are a consequence of ROS production.²⁵⁻²⁷ It has also been shown that cisplatin inhibits the activity of antioxidant enzymes including catalase, glutathione peroxidase, and superoxide dismutase.²⁸ In our work, in agreement with the previous studies,²⁹⁻³¹ administration of cisplatin induced a significant increase in malondialdehyde concentration as a marker of lipid peroxidation, but a significant reduction in total thiol content and catalase activity of the renal tissue. However, administration of *P major* extract to the experimental groups was able to amend these alterations. In this regard, *P major* extract at 1200 mg/kg significantly decreased

malondialdehyde concentration but caused a significant elevation of total thiol content in the kidney tissue. In fact, *P major* extract at 300 mg/kg, 600 mg/kg, and 1200 mg/kg significantly reversed the reduction in catalase activity following the injection of cisplatin. Our findings also indicate that the beneficial actions of *P major* extract on cisplatin-induced nephrotoxicity are comparable with vitamin E. Thereby, protective effects of *P major* extract on cisplatin-induced renal injury, at least in part, can be due to antioxidant and free radical scavenging effects of this plant.¹⁴ However, more investigations are needed to elucidate the exact mechanisms of *P major* action on cisplatin-induced renal toxicity.

CONCLUSIONS

The current investigation demonstrates that *P major* extract and vitamin E are able to ameliorate the nephrotoxicity induced by cisplatin, possibly via their antioxidant properties. However, future studies are required to determine the exact mechanisms involved in *P major* effects on cisplatin-induced kidney toxicity.

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CONFLICT OF INTEREST

None declared.

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