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Research Article

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Effects of *Pistacia atlantica* Subsp. *Kurdica* on Cadmium-Induced Oxidative Damage in Rat Kidney

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Abstract

Background: *Pistacia atlantica kurdica* has recently been shown to possess free radical scavenging ability. The current study aims to investigate the protective effect of this plant against cadmium-induced nephrotoxicity.

Methods: Thirty-six rats were divided into 6 groups (6 in each), and treated as follows: group 1 received normal saline (control group), group 2 (positive control) received cadmium by drinking water (100 mg/L/d), group 3 received 200 mg/kg of *P. atlantica* extract, and groups 4-6 received cadmium as well as 50, 100 and 200 mg/kg/d of *P. atlantica* extract (orally), respectively. After 2 weeks, oxidative damage and renal function markers were assayed by standard methods.

Results: In cadmium group, a significant increase was observed in serum blood urea nitrogen (BUN) ($P<0.01$) and lipid peroxidation (LPO) level of renal tissue ($P<0.001$) and a remarkable decrease was found in total thiol molecules (TTM) of the kidney ($P<0.001$). Despite the decreased renal antioxidant capacity, these changes were not significant. *P. atlantica* extract improved the LPO, TTM, and histopathological changes in renal tissue.

Conclusion: In this study, although the *P. atlantica* extract did not have a significant effect on cadmium-induced renal dysfunction, it did improve the oxidative/antioxidant balance in renal tissue.

Keywords: *Pistacia atlantica*, Cadmium, Nephrotoxicity, Oxidative stress

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Introduction

Cadmium is one of the most important environmental and food contaminants that threaten animal and human health. The primary sources of exposure to this metal are contaminated drinking water, tobacco, and industrial activities (1). One of the harmful features of this metal is that its biological half-life is very long (10-30 years) (2). Additionally, the renal tissue is one of the critical targets for cadmium (3). Following chronic exposure to cadmium, the glomerular filtration rate (GFR) decreases significantly, which eventually leads to renal failure (4). Several studies indicated that oxidative stress and increased generation of inflammatory cytokines are effective in the pathogenesis of cadmium nephrotoxicity (5,6). In contrast, anti-inflammatory and antioxidants agents have protective effects on renal failure induced by cadmium (7, 8). *P. atlantica* subsp. *kurdica* belongs to the Anacardiaceae family, which is frequently grown in different parts of Iran. Our previous study showed the presence of active compounds in this plant such as α-pinene, bornyl acetate, camphene, myrcene, and various phenolic components (9). In folk medicine, *P. atlantica* gum extract is used to treat neurological disorders, digestive problems, hepatic diseases, and respiratory disorders (10).

Previous studies revealed that *P. atlantica* possess anti-inflammatory and antioxidant properties (11-13). Therefore, the current study was conducted to evaluate the protective effect of *P. atlantica* against renal dysfunction in rats exposed to cadmium toxicity.

Materials and Methods

Chemicals

Cadmium chloride monohydrate ($CdCl_2. H_2O$) was obtained from Merck Company (Darmstadt, Germany). 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB), N-(1-naphthyl) ethylenediamine dihydrochloride (NED), bovine serum albumin (BSA), 2-thiobarbituric acid (TBA), and 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ) were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA).

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Plant Materials: Collection and Extraction Procedure

Pistacia atlantica was gathered from the Hamadan province, Iran. This plant was identified by Herbarium Unit of the School of Pharmacy, Hamadan University of Medical Sciences (HUMS), with herbarium number 403. The collected samples were dried and powdered. The extraction process was done by methanol, which lasted for 48 hours. After filtration, the resulting extract was condensed with a rotary evaporator and maintained at 4°C.

Animals

Male Wistar rats (250 ± 20 g) were obtained from the animal house of HUMS, Hamadan, Iran, and quarantined for seven days before use. The animals were kept in a suitable laboratory condition (12 h light/dark at 22–25°C) and fed with the standard diet and water *ad libitum*. It should be noted that the research protocol was approved by HUMS (IR.UMSHA.REC.1394.585).

Animal Treatment

A total of 36 rats were divided into 6 groups and treated as follows: group 1 received normal saline, group 2 received cadmium by drinking water (100 mg/L/d), group 3 received 200 mg/kg of *P. atlantica* extract, and groups 4-6 received cadmium (100 mg/L/d by drinking water) as well as 50, 100 and 200 mg/kg/d of *P. atlantica* extract (orally), respectively. After 2 weeks, the animals were anaesthetized by ketamine/xylazine and blood specimens were obtained from their heart. Then, the blood sample was centrifuged and its serum was kept at -20°C for biochemical analyses. Besides, the left kidney was isolated from rats and homogenized in phosphate buffer (pH = 7, 100 mM). After centrifugation of homogenized tissue at 5000 rpm for 15 minutes at 4°C, its supernatant was removed for biochemical examinations.

Biochemical Analysis

Serum renal markers, including blood urea nitrogen (BUN) and creatinine levels, were measured using colorimetric assay kits based on the manufacturer's recommendation (Pars Azmon, Iran). Thiobarbituric acid-reactive substances method was used for the determination of lipid peroxidation (LPO), as described earlier with some modifications (14). Briefly, homogenized renal tissue was mixed with TBA (0.2%) in H_2SO_4 (0.05 M) and heated for 45 minutes in a boiling water bath. Byproducts of LPO were extracted by n-butanol and its absorbance was assayed at 532 nm. Total antioxidant capacity (TAC) of renal tissue was measured by determining their ability to reduce Fe^{3+} to Fe^{2+}. The reaction between TPTZ reagent and Fe^{2+} gives a blue color with an optimum absorbance at 593 nm (8). Total thiol molecules (TTM) were determined spectrophotometrically, as described by Navaei-Nigjeh et al. Briefly, the DTNB reagent reacts with the thiol groups to yield a yellow colored complex, which has the maximum absorbance at 412 nm (15). At the end of each experiment, protein content was assayed in the crude homogenate of renal tissue using the Bradford technique (16).

Histopathologic Examination

The right kidney of rats was fixed in formalin 10% solution. After preparing paraffin-embedded block via automatic tissue processor, the renal tissue was cut into 4-6 µm thick sections by a rotary microtome. Finally, the tissue samples were dyed by hematoxylin and eosin and pictured using a microscope camera for histopathological analysis.

Statistical Analysis

The data were expressed as the mean ± standard error of the mean (SEM) and analyzed by Graph Pad Prism software version 6.0. The statistical difference of the values was determined by one-way analysis of variance (ANOVA) with Tukey test for multiple comparisons. A *P*-value of less than 0.05 was considered statistically significant.

Results

Effect of Pistacia atlantica Extract on Body and Kidney Weight

In this study, there were no remarkable differences between control and treatment groups in gaining weight. The kidney weight in different groups has no significant alteration as compared with control (Table 1).

The Effects of Pistacia atlantica Extract on Renal Function

Although cadmium could significantly increase serum BUN level ($P < 0.01$), no significant difference was observed in serum creatinine level. Moreover, no significant difference was observed in serum renal markers following the administration of different doses of *P. atlantica* extract to animals treated with cadmium (Table 2).

Table 1. Body and Kidney Weight Changes in Studied Groups

Groups	D_1W (g)	$D_{14}W$ (g)	KW (g)
Control	231.8±12.1	259.7±8.9	1.10±0.08
Cadmium	206.4±14.5	290.1±11.7	1.10±0.04
P. atlantica extract (200 mg/kg)	241.5±14.8	312.3±7.3	1.15±0.08
Cadmium + *P. atlantica* extract (50 mg/kg)	224.8±15.4	302.1±21.6	1.15±0.07
Cadmium + *P. atlantica* extract (100 mg/kg)	236.8±14.6	290.8±16.5	1.06±0.09
Cadmium + *P. atlantica* extract (200 mg/kg)	237.3±12.7	292.1±23.1	1.14±0.12

Data were analyzed using one-way ANOVA with Turkey test. The values were expressed as mean ± SEM, n=6 for each group. Cadmium was administered at a dose of 100 mg/L/d.
D_1W: day 1 weight; $D_{14}W$: day 14 weight; KW: kidney weight.

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Table 2. Effect of *P. atlantica* Extract on Renal Function Markers of Cadmium-Exposed Rats

Groups	BUN (mg/dL)	Creatinine (mg/dL)
Control	2.63±0.19	0.39±0.03
Cadmium	3.51±0.11**	0.45±0.05
P. atlantica extract (200 mg/kg)	2.89±0.11	0.46±0.03
Cadmium + *P. atlantica* extract (50 mg/kg)	2.94±0.20	0.41±0.04
Cadmium + *P. atlantica* extract (100 mg/kg)	2.93±0.08	0.43±0.02
Cadmium + *P. atlantica* extract (200 mg/kg)	3.07±0.17	0.41±0.03

Values were expressed as mean ± SEM, n = 6 for each treatment group. Data were analyzed using one-way ANOVA with Tukey post hoc test. Cadmium was administered at a dose of 100 mg/L/d.
** $P < 0.01$ vs control group.

The Effects of Pistacia atlantica Extract on Oxidative Stress Markers

Following cadmium administration, a significant increase was observed in LPO level of renal tissue ($P<0.001$). *P. atlantica* extract was able to decrease the level of LPO at the dose of 200 mg/kg in comparison with cadmium group ($P<0.05$). Cadmium was able to significantly reduce TTM ($P<0.001$). In addition, despite the decrease in renal TAC of cadmium-treated animals, these changes were not significant compared to the control group. *P. atlantica* extract improved renal TTM at doses of 50, 100, and 200 mg/kg in cadmium-treated rats ($P<0.05$, $P<0.01$, and $P<0.05$, respectively) (Figure 1).

The Effects of Pistacia atlantica Extract on Histopathological Changes

The findings of the microscopic examination indicated the structure of kidney cortex in control and *P. atlantica* extract groups with normal renal corpuscle, distal convoluted tubules, and proximal convoluted tubules. In the cadmium-treated rats, the hydropic swelling and hypertrophy of proximal tubular cells, severe vascular congestion, and obvious accumulations of inflammatory cells were observed when compared to the control group. *P. atlantica* (at doses of 100 and 200 mg/kg) prevented some of the histopathological changes of cadmium such as vascular congestion and accumulations of inflammatory cells (Figure 2).

Discussion

The present study provided more evidence to confirm the influence of oxidative stress in the pathogenesis of cadmium-renal failure. Moreover, our data revealed the relationship between antioxidant properties of *P. atlantica* extract and its therapeutic potential against renal oxidative injuries caused by the cadmium.

It has been supposed that elevated levels of the BUN and creatinine are related to renal failure (8). Urea is the main nitrogenous end product of protein degradation and BUN is an indirect and rough estimation of renal function that measures the level of urea nitrogen in blood and is directly associated with the excretory function of the kidney (8,17). Creatinine is a product formed in muscle by creatine metabolism (18). In the current study, cadmium leads to a notable increase in serum BUN level that might indicate the inability of the kidney to excrete this waste product. However, due to minor changes in serum creatinine level in cadmium-treated animals, it can be concluded that this metal is able to cause moderate to mild renal failure. In line with our findings, the destructive effects of cadmium on renal tissue have been reported in several studies (19,20). This evidence might be attributed to the alterations in the GFR, the threshold of tubular re-absorption, and renal blood flow. The administration of *P. atlantica* extract did not cause significant changes in renal indices, which may be related to the short duration of treatment and the slow recovery of renal function.

Oxidative stress is the outcome of an imbalance between antioxidant system and free radicals created in cellular

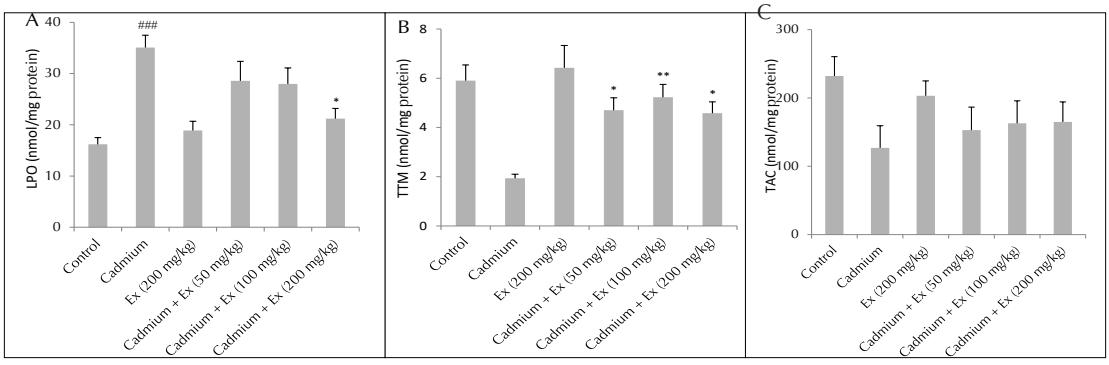

Figure 1. Effect of *P. atlantica* Extract on Renal Oxidative Stress Markers in Cadmium-exposed Rats.
The values were expressed as mean ± SEM, n = 6 for each treatment group. Data were analyzed using one-way ANOVA with Tukey post hoc test. *** $P<0.01$ vs control group; * $P<0.05$ and ** $P<0.01$ vs cadmium group. Cadmium was administered at a dose of 100 mg/L/day for two weeks. LPO: lipid peroxidation (A), TTM: total thiol molecules (B), TAC: total antioxidant capacity (C), Ex: *P. atlantica* extract.

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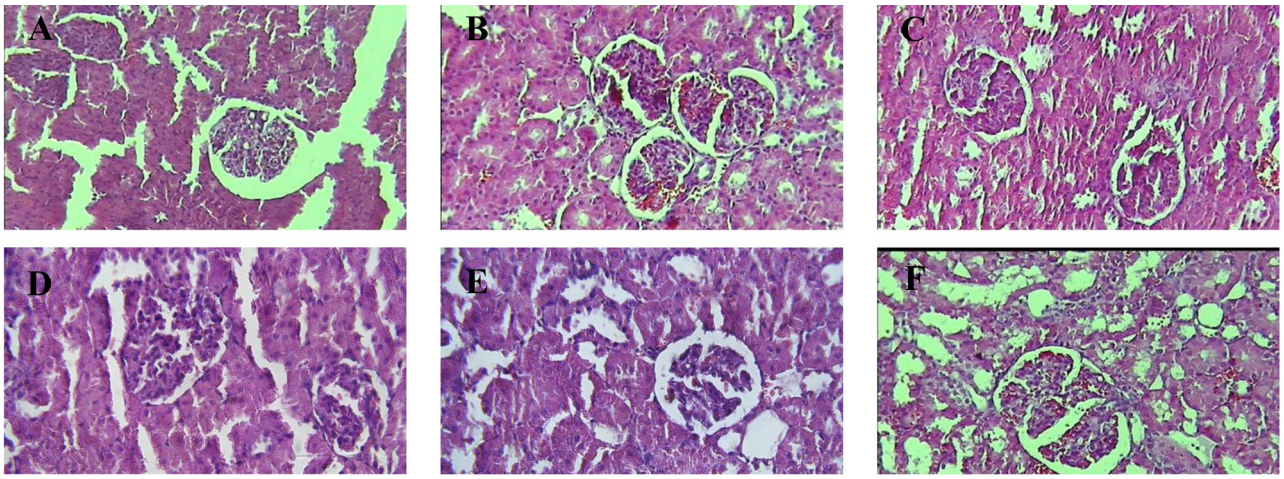

Figure 2. Effect of *P. atlantica* Extract on Renal Tissue of Cadmium-exposed Rats.
The histopathological changes (vascular congestion/accumulations of inflammatory cells) were evaluated in different groups; +Moderate, ++Severe, +++ Very severe. **A**: control group (-/-), **B**: cadmium group (+++/+++), **C**: *P. atlantica* extract (200 mg/kg) group (-/-), **D**: cadmium + *P. atlantica* extract (50 mg/kg) group (+++/+++), **E**: cadmium + *P. atlantica* extract (100 mg/kg) group (++/++), **F**: cadmium + *P. atlantica* extract (200 mg/kg) group (++/+). Original magnification of all images is ×40.

processes (21). Since this process exerts an important role in cadmium-induced nephrotoxicity, the oxidant/antioxidant markers such as LPO, TAC and TTM were assayed. In this study, a notable increase in LPO and a remarkable decrease in TTM were found in the cadmium group, indicating the induction of oxidative stress in renal tissue. In this regard, renal oxidative injury induced by cadmium has been reported in several studies (8,22,23).
LPO is a radical reaction that leads to the destruction of polyunsaturated fatty acids in lipid membranes and cell death (24). Following the administration of *P. atlantica* extract, renal LPO levels decreased, which could be attributed to the improvement of the thiol molecules in this tissue. Consistent with our findings, Heidarian et al assessed the effects of *P. atlantica* extract on gentamicin-induced renal failure in rats. Their findings showed that *P. atlantica* extract might reduce the level of oxidative and inflammatory markers by increasing the activity of antioxidant enzymes, such as catalase and superoxide dismutase, in gentamicin-treated rats (13). Besides, our previous study showed that the essential oil and hydro-alcoholic extract of *P. atlantica* hulls is a suitable source for phenolic and flavonoid compounds (9). The presence of these compounds may be related to the therapeutic and antioxidant properties of this plant.

Conclusion

Pistacia atlantica extract might improve the oxidative/antioxidant balance in kidneys by decreasing the LPO and restoring the antioxidant thiol molecules. However, further studies on the nutritional and therapeutic value of this plant should be conducted in the future.

Conflict of Interests

No conflict of interests is declared by the authors.

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