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Original article

Ameliorating Effect of Pistachio Hydroalcoholic Extract on Cisplatin-Induced Nephrotoxicity in Mice

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Abstract

Background and objectives: Cisplatin-induced nephrotoxicity accompanies increased oxidative stress, leading eventually to kidney dysfunction. On the other hand, *Pistacia vera* nuts (pistachio) display multiple pharmacological effects such as antioxidant property. The present study investigated the effects of pistachio hydroalcoholic extract on nephrotoxicity induced by cisplatin in mice. **Methods:** Pistachios (100 g) were powdered and macerated in 1 L of ethanol (80%) for 72 h Then, dried with rotary evaporator apparatus**.** Forty male mice were divided into five groups: normal, cisplatin, cisplatin+DMSO, cisplatin+ pistachio hydroalcoholic extract 10, and cisplatin+ pistachio hydroalcoholic extract 100. Nephrotoxicity was induced by intraperitoneal injection of cisplatin (20 mg/kg/day) on the first day of the experiment. Pistachio hydroalcoholic extract (10 and 100 mg/kg/p.o) was administered for four consecutive days. The body weight and kidney function indices such as serum creatinine (Cr) and blood urine nitrogen (BUN) were measured. Also, the renal tissues were assessed for levels of malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx). **Results:** Cisplatin reduced animals' body weight. Also, cisplatin increased levels of Cr, BUN, and MDA, and decreased the activities of SOD, CAT, and GPx. Treatment with pistachio hydroalcoholic extract (100 mg/kg) reduced the levels of serum Cr, BUN, as well as renal MDA. Moreover, administration of 100 mg/kg pistachio hydroalcoholic extract to cisplatin-treated mice increased the body weight as well as CAT, GPx, and SOD activities. **Conclusion:** These results imply that pistachio hydroalcoholic extract treatment may diminish cisplatin-induced renal dysfunction through reduction of oxidative stress in the kidney tissue.

Keywords: cisplatin; mice; nephrotoxicity; oxidative stress; pistachio

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Introduction

Nephrotoxic drugs such as cisplatin are the main causes of acute renal failure cases in intensive care units [1]. Cisplatin is a platinum-based anticancer drug. It is extensively administered for different

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cancers such as head and neck, testicular, ovarian, and cervical carcinomas [2]. Nephrotoxicity is the main toxicity and dose-limiting side effect of this drug [3]. Cisplatin-induced nephrotoxicity causes renal dysfunction through overproduction of reactive oxygen species (ROS), DNA damage, and apoptosis [4]. On the other hand, it decreases the level and/or activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), and increases the level of malondialdehyde (MDA) in renal tissue [4]. These disturbances in oxidative status leads to renal dysfunction and elevates the blood urea nitrogen (BUN) and creatinine (Cr) [5]. Many studies reported that oxidative stress is the most important factor in renal injuries of cisplatin, and agents with antioxidant properties attenuates these lesions [6,7].

Pistachio nut (*Pistacia vera*, belonging to the Anacardiaceae family) has been known for its medicinal properties since ancient times [8]. Pistachios are unique sources of different compounds such as unsaturated fatty acids, β-carotene, α-tocopherol, flavonoids, and lutein with several effects [9]. Moreover, it has been demonstrated that pistachio has antioxidant and anti-inflammatory properties [10,11]. It has been proven that the pistachio diet significantly enhances oxidative status and decreases circulating inflammatory biomarkers [12].

As above-mentioned, inflammation and reactive oxygen species (ROS) play important roles in the pathophysiology of cisplatin-induced nephrotoxicity [13]; therefore, administration of natural compounds with antioxidant and anti-inflammatory properties may induce ameliorative effects. Based on the evidence above, we aimed to study the effects of hydroalcoholic extract of pistachios against cisplatin-induced nephrotoxicity in mice.

Materials and Methods

Ethical considerations

The Ethics Committee of Rafsanjan University of Medical Sciences approved this research (IR.RUMS.REC.1399.086). All experiments were performed in line with the guidelines set by the ethical committee of Rafsanjan University of Medical Sciences and the European Communities Council Directive 86/609/EEC of 24 November 1986.

Plant material

Dried pistachios long Akbari species (genetic code: M30), collected from Rafsanjan, Iran, were used in this study. In order to prepare the extract, the pistachios were powdered (100 g) and macerated in 1 L of ethanol (80%) for 72 h. Then, dried with rotary evaporate apparatus. The pistachio extract was stored at -20 °C [14]. For administration, the frozen extract was freshly dissolved in dimethyl sulfoxide 10% (DMSO, Sigma-Aldrich, Germany).

Animals

Forty male mice (30±2 g) were obtained from the animal house of Rafsanjan University of Medical Sciences. Animals were housed in polycarbonate cages (four per cage) at room temperature (21±1 °C) with a 12 h light/dark cycle and ad libitum access to food and water.

Experimental design

Animals were separated into five groups as follows (n=8): normal group: healthy animals without any treatment; cisplatin group: received cisplatin at the dose of 20 mg/kg on the first day of the experiment; cisplatin+DMSO group: received cisplatin on the first day of the experiment and DMSO 10% orally for four days; cisplatin+PE 10 group: received cisplatin on the first day of the experiment and pistachio hydroalcoholic extract orally at the dose of 10 mg/kg for four days [15]; Cisplatin+PE 100 group: received cisplatin on the first day of the experiment and pistachio hydroalcoholic extract orally at the dose of 100 mg/kg for four days. Pistachio hydroalcoholic extract and cisplatin dosages were selected from previous investigations [14,16].

Sample collection

Twenty-four h after the last administration of extract or vehicle, the animals were anesthetized with diethyl ether and the blood samples were collected from the orbital sinus. The blood samples were centrifuged at 3000 rpm for 15 min to separate the serum. The serum samples were kept at -20 °C for measurement of BUN and Cr. After anesthesia and blood sample collection, mice were killed by rapid decapitation and one kidney was immediately removed, then homogenized (1/10 w/v) in ice-cold Tris-HCl

buffer (100 mM, pH 7.4), centrifuged at 6000 rpm for 20 min, and the supernatant was collected and stored at -80 °C for estimating oxidative parameters.

Biochemical parameters

The serum levels of Cr and BUN were measured using a biochemical autoanalyzer (MINDRAY, Guangzhou, China) with respective commercial kits (ParsAzmoon Co., Tehran, Iran) [17].

Oxidative parameters

The renal activities of CAT, SOD, and GPx, as well as the MDA levels, were measured using commercially available kits (ZellBio, Germany) according to the manufactures' guidelines.

Statistical analysis

Statistical analysis was carried out via the GraphPad Prism program (ver. 6.01, GraphPad Software, USA). Results were expressed as mean ± SEM. The differences between the groups were tested using one-way ANOVA, followed by Tukey's post-hoc test. Statistical significance was defined as $p<0.05$.

Results and Discussion

As previously mentioned, pistachio extract has shown anti-inflammatory and antioxidant properties [18]. Hence, this study aimed to investigate the potential effects of hydroalcoholic extract of pistachios on cisplatin-induced nephrotoxicity.

Both the initial and final body weights of all animals were measured and presented in Table 1. The results of this study showed that a significant decrease in the final body weights was observed in animals treated with cisplatin and cisplatin+DMSO compared with the normal group ($p<0.001$). It seems that the reduction in body weight following cisplatin treatment may possibly be due to cytotoxic effects on the gastrointestinal tract [19]. Moreover, treatment with 10 and 100 mg/kg of pistachio hydroalcoholic extract resulted in a significant increase in the final body weight compared with the cisplatin group ($p<0.001$).

Our data demonstrated that in animals getting cisplatin alone and cisplatin+DMSO, BUN and Cr concentrations increased significantly as compared with the normal group ($p<0.001$ and $p<0.01$, respectively) (Figure 1). Treatment with 10 mg/kg of pistachio hydroalcoholic extract resulted in a significant decrease in BUN concentration compared with the cisplatin group ($p<0.05$). Furthermore, treatment with 100 mg/kg of pistachio hydroalcoholic extract significantly decreased the serum levels of BUN and Cr in comparison with the cisplatin group ($p<0.001$ and $p<0.01$, respectively). Cisplatin, as a potent antineoplastic agent, has some deleterious effects such as renal toxicity [20,21]. Cisplatin has induced renal toxicity via increasing lipid peroxidation, which leads to oxidative stress in renal tissue [22]. These phenomena induced histological lesions as well as functional disorders that are characterized by increased BUN and Cr levels [5,23]. On the other hand, it has been confirmed that herbal extracts or agents with antioxidant effects can protect kidneys from injuries of cisplatin. For example, ellagic acid and lycopene suppressed the stated increase in serum levels of cisplatin-treated animals [24]. Furthermore, the renoprotective effects of *Stachys pilifera* Benth hydroalcoholic extract and a well-known traditional Chinese herbal formula, Guizhi Fuling Wan, have been demonstrated in nephrotoxicity induced with cisplatin [25,26]. Previous reports have shown the potent antioxidant properties of pistachios [11,27,28].

Table 1. Effects of pistachio extract on initial and final body weights

Groups	Initial body weight	Final body weight
Normal	30.57±0.52	33.71±0.52
Cisplatin	30.20±0.48	21.80±0.58***
Cisplatin+DMSO	30.17±0.87	22.67±0.84***
Cisplatin+PE 10	30.00±1.08	29.75±1.31###
Cisplatin+PE 100	30.17±0.83	35.83±1.30###

Data were presented as the mean±SEM (n=8); * significant difference in comparison with the normal group (***p<0.001); # significant difference in comparison with the Cis group (###p<0.001); PE: pistachio hydroalcoholic extract.

Moreover, Ehsani et al. revealed that treatment with pistachio extract reduced serum levels of Cr and BUN in gentamicin-induced nephrotoxicity [14].As shown in Figure 2A, CAT activity in the kidney tissues of animals receiving cisplatin alone and cisplatin+DMSO group were significantly less than normal animals ($p<0.01$). Pistachio hydroalcoholic extract at the dose of 100 mg/kg significantly increased CAT activity compared with the cisplatin group ($p<0.05$). The activity of GPx was significantly decreased in the cisplatin group and cisplatin+DMSO group compared with the normal group ($p<0.001$) (Figure 2B).

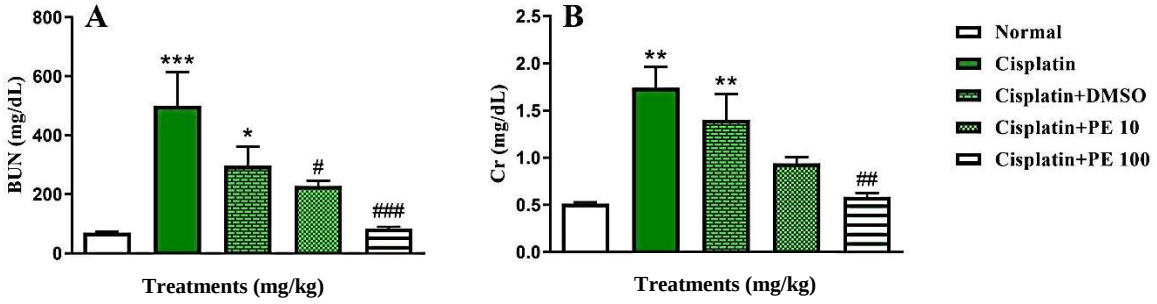

Figure 1. The effect of treatment with pistachio extract (PE) on BUN (A) and Cr (B) concentrations in cisplatin (Cis)-induced kidney toxicity. Data are expressed as mean ± SEM (n=8); ***** significant difference in comparison with the normal group (*p<0.05, **p<0.01, and ***p<0.001); # significant difference in comparison with the Cis group (#p<0.05, ##p<0.01, and ###p<0.001); PE: pistachio hydroalcoholic extract.

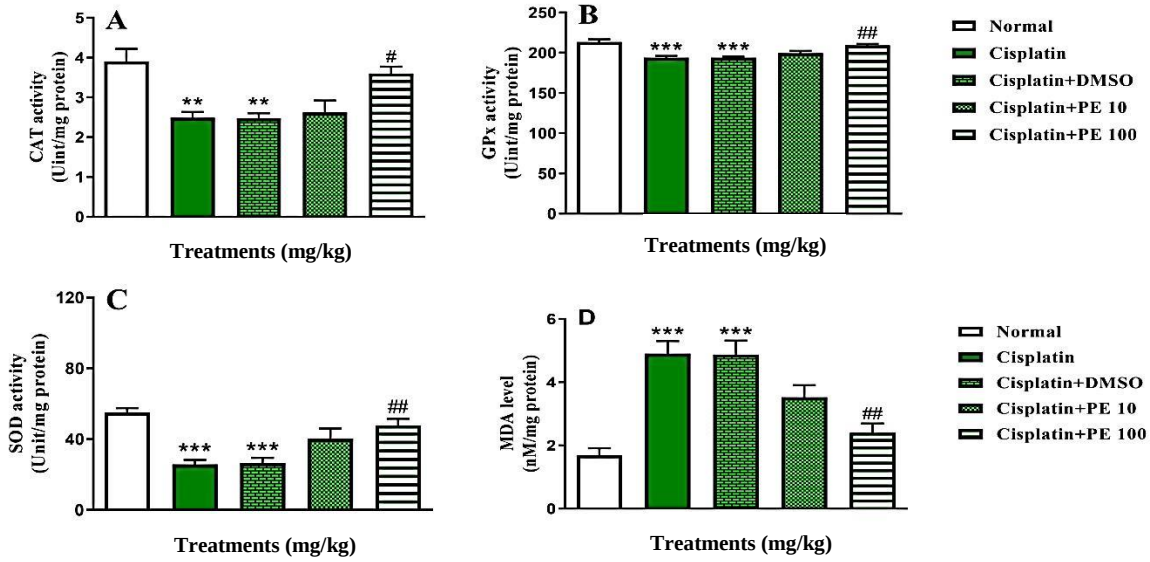

Figure 2. The effect of treatment with pistachio extract (PE) on CAT (A), GPx (B), and SOD (C) activities and MDA level (D) in cisplatin (Cis)-induced kidney toxicity. Data are expressed as mean ± SEM (n=8); * significant difference in comparison with the normal group (**p<0.01 and ***p<0.001); # significant difference in comparison with the Cis group (#p<0.05 and ##p<0.01); PE: pistachio hydroalcoholic extract.

Treatment with extract at the dose of 100 mg/kg significantly increased GPx activity compared with the cisplatin group (p<0.01). Moreover, SOD activity significantly decreased in the cisplatin group and cisplatin+DMSO group compared with the normal group (p< 0.001) (Figure 2C). Treatment with pistachio hydroalcoholic extract at the dose of 100 mg/kg significantly increased SOD activity compared with the cisplatin group (p<0.01). Furthermore, cisplatin significantly increased the MDA level in the kidney tissue compared with the normal group (p<0.001) (Figure 2D). Pistachio hydroalcoholic extract (100 mg/kg) resulted in a significant reduction in this variable (p<0.01).

Cisplatin is an alkylation agent, and despite potent therapeutic effects, has some deleterious effects such as nephrotoxicity [29]. Oxidative stress has critical role in the pathogenesis of kidney injury induced by cisplatin via reducing and/or inhibiting antioxidant enzymes as well as stimulating the generation of ROS [30]. On the other hand, pistachios are rich in campesterol, stigmasterol, and β-sitosterol, implying that pistachios have potent antioxidant effects [31]. It has been shown that in healthy volunteers, consumption of

pistachios reduces oxidative stress [32]. Moreover, it has been reported that methanolic extract of pistachios has hepatoprotective effects via scavenging of ROS as well as reducing lipid peroxidation [33]. These observations confirm that the protective effects of pistachio hydroalcoholic extract might be attributed to antioxidant activity and/or increasing the capacity of the antioxidant system.

Conclusion

We showed that treatment with pistachio hydroalcoholic extract could significantly reduce the cisplatin-induced renal damage in mice. Hence, these protective effects of pistachios, at least partially, may be related to their antioxidant property.

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Author contributions

Iman Fatemi and Ayat Kaeidi conceived the study idea; Elham Hakimizadeh, Jalal Hassanshahi and Mohammadreza Rahmani conducted the data collection; Mehrzad Mehrbani and Ayat Kaeidi analyzed the data; Elham Hakimizadeh and Iman Fatemi wrote the manuscript. All authors read, critically revised, and approved the final manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

Cr: creatinine; BUN: blood urine nitrogen; MDA: malondialdehyde; CAT: catalase; SOD: superoxide dismutase; GPx: glutathione peroxidase; ROS: reactive oxygen species; DMSO: dimethyl sulfoxide