Amelioration of Cisplatin-Induced Liver Toxicity Through Antioxidative Properties of Hydroalcoholic Extract of Pistachio Nuts in Mice

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Abstract

Introduction: Pistachio are known for their medicinal properties and have many pharmacological effects such as antioxidant activity. This study aimed to investigate the effects of hydroalcoholic pistachio extract (PE) in the pathogenesis of cisplatin (Cis)-induced liver toxicity.

Materials and Methods: In this experimental study, 35 male mice were assigned randomly into four groups. Liver toxicity was induced in mice by intraperitoneal injection of Cis (20 mg/kg/day on the first day of the experiment). PE (10 and 100 mg/kg; p.o) was administered on day 1 (1 h before cisplatin injection) and continued for three consecutive days. At the end of the experiment, blood samples were obtained to measure serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). Moreover, the activities of catalase (CAT) and glutathione peroxidase (GPx) in the liver tissue were evaluated

Results: Cis increased the serum levels of AST, ALT, and ALP as well as decreasing the activities of CAT and GPx. Administration of PE at the dose of 10 mg/kg for four days showed a significant decline in the serum levels of AST and ALT. Treatment of Cis-treated mice with 100 mg/kg PE decreased the serum levels of AST, ALP, and ALT as well as increasing CAT and GPx activities in the hepatic tissue.

Conclusion: These findings demonstrate that PE could be an effective remedy for the prevention of cisplatin-induced liver dysfunction and oxidative damage in mice.

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1. Introduction

Cisplatin (Cis) is a chemotherapeutic drug that is used to treat solid organ cancers such as ovarian and testicular [1, 2]. The liver is one of the organs where Cis accumulates and eventually leads to liver toxicity [3, 4]. It has also been shown that Cis can directly induce damage to the liver through different mechanisms especially oxidative stresses [5]. Several previous reports have indicated that overproduction of free radicals and reactive oxygen species (ROS) in cisplatin-induced hepatotoxicity is associated with increased lipid peroxidation besides decreased levels of protein-bound sulfhydryl groups and glutathione [6]. On the other hand, Cis induces the depletion of hepatic antioxidant enzymes like glutathione peroxidase (GPx) and catalase (CAT) [7]. The rate of interest is expanding in the use of herbal plants for decreasing the toxicity of chemical drugs and increasing the clinical usages [8]. Pistacia vera (*P. vera*), belonging to the family *Anacardiaceae*, has been known for its medicinal properties since ancient times [9]. Its nut (pistachio) is a unique source of various compounds such as unsaturated fatty acids, β-carotene, α-tocopherol, flavonoids, and lutein [10]. In addition, it has been shown that pistachio have many pharmacological effects such as antioxidant [11], antimicrobial [12], anti-hyperlipidemia [13], anti-nociceptive, hepatoprotective effect [14], and anti-inflammatory [15]. The radical scavenging activity of the pistachio extract have been shown previously by using the stable 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) [16]. A recent human study, found that a diet of pistachio nuts significantly enhanced oxidative status and attenuated circulating inflammatory biomarkers [17]. As above-mentioned, ROS play an important role in the pathophysiology of Cis-induced liver injury [18]. Therefore, administration of natural compounds displaying antioxidant properties could have ameliorative effects. As a result, this study was undertaken to investigate the effects of hydroalcoholic pistachio extract (PE) on the side effects of cisplatin in mouse liver.

2. Materials and Methods

2.1. Animals

The study was performed on 35 male mice (20-25 g) that were obtained from the animal house of Rafsanjan University of Medical Sciences, Rafsanjan, Iran. Animals were kept in plastic cages under controlled conditions: temperature of 20–23 °C with a 12 h light/dark cycle. Mice were provided with a standard commercial mouse diet and distilled water. All experiments were carried out in line with the guidelines established by the ethical committee of Rafsanjan University of Medical Sciences (Ethics number: IR.RUMS.REC.1399.052) and the European Communities Council Directive 86/609/EEC of 24 November 1986.

2.2. Pistachio extraction

In order to prepare PE, we used dried Akbari pistachio (long species, genetic code: M30), collected from Rafsanjan region, Iran. 100 g of the pistachio were powdered and macerated in 1 L of ethanol (80%) for 72 h. We used rotary evaporation to remove the vehicle; then, kept the extract at -20°C [19].

For oral administration, the frozen extract was freshly dissolved in dimethyl sulfoxide 10% (DMSO, Sigma-Aldrich, Germany).

2.3. Experiment design

Animals were randomly divided into five groups as follows (n=7): (1) Normal: healthy group without any treatment; (2) Cis: received Cis (20 mg/kg/i.p.) on the first day of the experiment; (3) Cis+PE 10: received Cis on the first day of the experiment and PE orally at the dose of 10 mg/kg for four days; (4) Cis+PE 100: received Cis on the first day of the experiment and PE orally at the dose of 100 mg/kg for four days; and (5) Cis+DMSO: received Cis on the first day of the experiment and DMSO 10% orally for four days. Cis (CISPLATIN MYLAN®) was obtained from MYLAN Pharmaceuticals (France). Cis and PE dosages were selected from previous investigations [19, 20].

2.4. Sample collection

24 h after the last administration of PE, the animals were anesthetized with diethyl ether, and the blood samples were collected from the orbital sinus. Then, the blood samples were centrifuged at 3000 rpm for 15 min. The separated sera were stored at -20°C for measurement of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). After that, the mice were killed. The livers were removed quickly and resuspended at 50 mg/mL in phosphate-buffered saline containing 1X butylated hydroxytoluene. Homogenization and centrifugation at 6000 rpm for 20 min followed to collect the supernatant for determination of CAT and GPx activities.

2.5. Biochemical analysis

2.5.1. Liver function

The serum levels of ALT, AST, and ALP were evaluated by using a biochemical autoanalyzer (MINDRAY, Guangzhou, China) with respective commercial kits (ParsAzmoon Co., Tehran, Iran).

2.5.2. CAT and GPx assays

The liver CAT and GPx activities were evaluated by using commercially available kits (ZellBio, Germany) according to the manufacturer's guidelines.

2.6. Statistical analysis

Statistical analysis was performed using GraphPad Prism 6.01 (GraphPad Software, USA). Results are expressed as mean± SEM. The normal distribution of the results was checked by the Kolmogorov-Smirnov test. For parametric results we used one-way ANOVA followed by the Tukey post-hoc test, and for nonparametric results, Kruskal-Wallis followed by Dunn's post-hoc test were used. Statistical significance was defined as $P<0.05$.

3. Results

3.1. Effect of PE on liver function

As shown in Figure 1, levels of AST, ALT, and ALP were significantly ($P<0.01$, $P<0.01$, and $P<0.05$, respectively) increased in the Cis group compared with the normal group. Administration of PE at the dose of 10 mg/kg significantly decreased the levels of AST and ALT significantly compared with the Cis group ($P<0.001$ and $P<0.05$, respectively). Furthermore, in animals treated with 100 mg/kg PE, the levels of AST, ALT, and ALP were significantly decreased compared with the Cis group ($P<0.001$, $P<0.01$, and $P<0.001$, respectively).

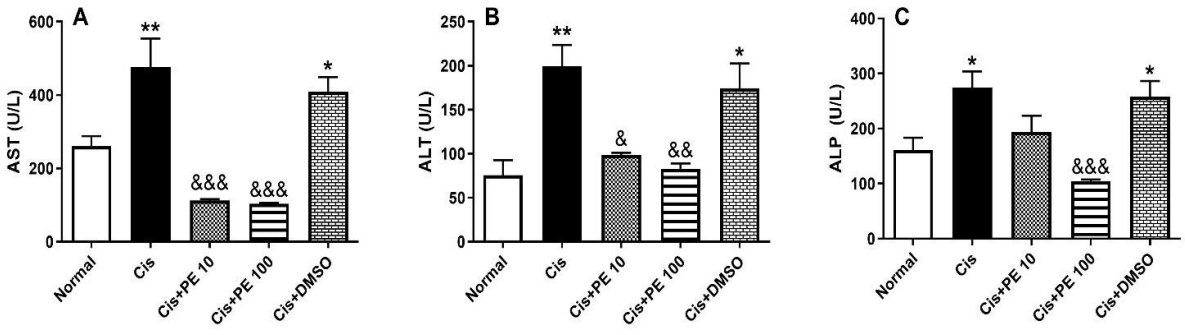

Figure 1. The effect of treatment with pistachio extract (PE) on AST (A), ALT (B), and ALP (C) levels in cisplatin (Cis)-induced liver toxicity. Data are expressed as mean ± SEM (n=7). * Significant difference in comparison with the normal group (*P<0.05 and **P<0.01); & Significant difference compared with the Cis group (&P<0.05, &&P<0.01 and &&&P<0.001).

3.2. Effect of extract of pistachio on antioxidant activity

The effects of Cis on the activity of antioxidant enzymes CAT and GPx are illustrated in Figure 2. The activities of CAT and GPx were significantly decreased in the Cis group compared with the normal group (all P<0.01). Treatment with 100 mg/kg PE significantly increased CAT and GPx activity compared with the Cis group (all P<0.05).

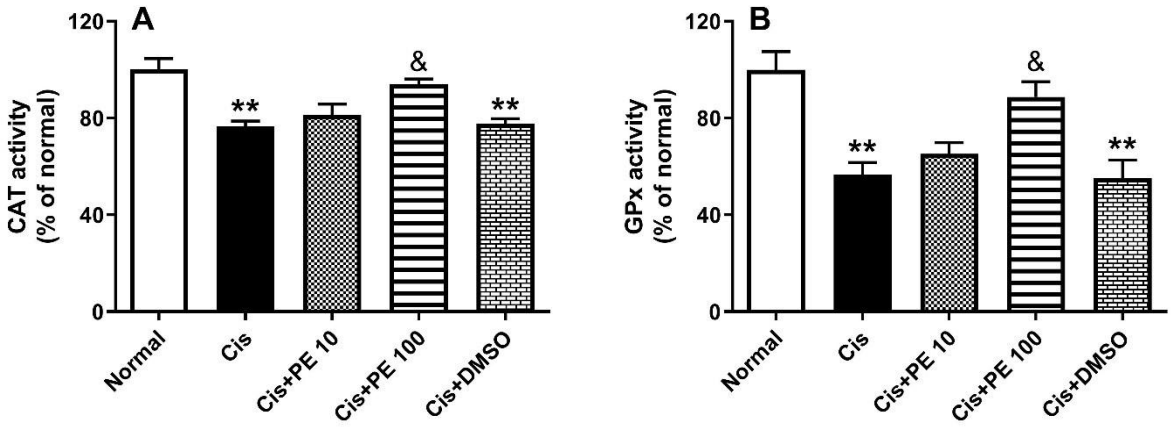

Figure 2. The effect of treatment with pistachio extract (PE) on CAT (A) and GPx (B) activities in cisplatin (Cis)-induced liver toxicity. Data are expressed as mean± SEM (n=7). *Significant difference in comparison with the normal group (**P<0.01); & Significant difference compared with the Cis group (&P<0.05).

4. Discussion

In this study, the effect of PE on Cis-induced liver toxicity in mice was examined. Our results indicated that intraperitoneal administration of Cis significantly increased serum levels of ALT, AST, and ALP, as well as decreasing the hepatic activities of CAT and GPx, which was consistent with previous reports [21]. We also found that administration of PE (more potentially at the dose of 100 mg/kg) produced a significant decrease in the serum levels of ALT, AST, and ALP as well as increasing the activities of CAT and GPx in the liver tissue compared with the normal group.

In line with our finding, it is well established that Cis-induced liver injuries elevated the liver function enzymes. Kim et al. showed that a single dose of Cis in mice could cause liver function impairment, which is characterized by elevation of AST and ALT activities [22]. These liver injuries can be directly due to the effects of Cis on the liver cells [5], or indirectly, as a consequence of kidney damages [23, 24]. On the other hand, our results indicated that treatment with PE decreased the serum levels of ALT, AST, and ALP. The present study is the first to indicate that its outcomes show that oral administration of PE had a significant and, in some measure, dose-dependent protective effects on Cis-induced liver toxicity in mice.Cis-induced liver injury has been verified by several studies [21, 22, 25]. Martins et al. stated that Cis induced lipid peroxidation and oxidative damage in the liver [5]. In another study, Liao et al., showed that Cis modified liver function of mice via a mechanism of oxidative stress caused by raised lipid peroxidation and reduced antioxidant capacity [25]. On the other hand, the antioxidant system plays an important role in protecting cells against the harmful effects of reactive oxygen species. In accordance with earlier findings, our results also indicated that administration of Cis produced a significant decrease in the activities of CAT and GPx in the liver tissue compared with the normal group [26]. Pistachio nut has been rated among the top 50 antioxidant-rich foods [27]. Pistachio contain several components with high antioxidant activity such as polyphenols, tocopherols, lutein, phytosterols, vitamin B6, gallic acid, and carotenoids [11].

Kocyigit et al. have shown that consumption of pistachio significantly decreases oxidative stress and enhances plasma lipid profile in healthy volunteers [28]. Moreover, in a new study in humans, it was established that the pistachio nut significantly improves the oxidative status and reduces the circulating inflammatory biomarkers in inflammatory bowel diseases [17]. Furthermore, we indicated that PE increased activities of CAT and GPx in the liver tissue. On the other hand, it is well established that administration of several herbal extracts is useful for the prevention or amelioration of drug-induced hepatotoxicity [29-31].

The hepatoprotective actions of these plants have been attributed to their antioxidant properties. These observations endorse the hypothesis that the protective effects PE might be attributed to reinforcement of the antioxidant system.

5. Conclusions

This study is the first to find that treatment with extract of pistachio reduced levels of AST, ALT, and ALP and intensified activities of antioxidant enzymes such as CAT and GPx. The present results suggest that PE can be an effective agent against the toxic effects of Cis in the liver tissue.

Conflict of interest

The authors declare no conflicts of interest.

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