Antioxidant effects of ethyl acetate extract of Akbari pistachio kernel on toxic effects arising from taking cisplatin (a chemotherapy medication) in rats

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Abstract

Background and purpose: Cisplatin is an important anticancer drug and despite its beneficial clinical effects in the treatment of cancer, it has toxic side effects owing to the production of free radicals. Pistachio is one of the plants having compounds with antioxidant properties and contains vitamins E and C, beta-carotene, various antioxidants, gamma tocopherol, quercetin, flavonoids, lutein, xanthine and minerals such as copper and selenium. Pistachio kernel extract contains multi-purpose phenolic compounds and can act as a free radical scavenger.

Method: This experimental study was conducted on 36 male Wistar rats for 15 days. The rats were randomly divided into six 6-rat groups. Group 1 was the control group including healthy rats receiving DMSO (Dimethyl sulfoxide). Group 2 was healthy rats receiving 200 mg/kg body weight of ethyl acetate extract of pistachio kernel for 15 days. Group 3 was the healthy rats receiving 400 mg/kg body weight of ethyl acetate extract of pistachio kernel for 15 days. Group 4 was rats receiving cisplatin at a dose of 10 mg/kg body weight by intraperitoneal injection on the tenth day of untreated experiment. Group 5 was the rats receiving cisplatin at a dose of 200 mg/kg body weight from ethyl acetate extract of pistachio kernel for 15 days. Finally, group 6 included the rats receiving cisplatin at a dose of 400 mg/kg body weight from ethyl acetate extract of pistachio kernel for 15 days. After 15 days, blood samples were taken from the corners of the rats' eyes and after isolating the serum was revived and concentration of Malondialdehyde (MDA), glutathione and the activity of enzymes including superoxide dismutase (SOD), catalase (Cat), glutathione peroxidase (Gpx) and total antioxidant capacity (TAC) was measured in the serum.

Results: In groups 2 and 3 receiving only the extract, a significant decrease in serum levels of SOD and Cat and a significant increase in TAC were observed compared to that of the control group ($P< 0.001$). Moreover, in group 4 receiving only cisplatin, a significant decrease was observed in serum levels of Gpx, Cat, SOD and TAC, and a significant decrease was also observed in MDA compared to that of the control group ($P< 0.001$). In addition, the high dose of the extract in group 3 led to a significant decrease in MDA compared to that of the control group, yet the low dose of extract in group 2 caused a significant decrease in Gpx enzyme compared to that of the control group ($P< 0.001$).

Discussion: The results of this study showed that the hydro-alcoholic extract of pistachio kernel has protective and modulating effects against cisplatin toxicity in rats and due to its antioxidant properties, it increased the activity of antioxidant enzymes.

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1. Introduction

Cancer is the spontaneous growth of a tissue [1]. Cancer is a chronic and non-communicable disease involving different parts of the body [2]. Cisplatin (cis diammine dichloroplatinum) is one of the most important anti-cancer drugs used in the treatment of many tumors such as the head and neck, ovaries, testes and lungs [3]. This drug reduces the rate of division of cancer cells by acting on their DNA [4]. The intracellular effects of cisplatin include decreased normal ATPase activity, mitochondrial damage, cell cycle arrest, and disruption of cellular transmission systems; all of these effects can lead to apoptosis or necrosis [5]. These effects, along with the involvement of pre-inflammatory genes such as SOX-2 and nitric oxide synthase (INOS), may play a major role in liver toxicity, and high doses of the drug or repeated use of low doses can cause liver toxicity [6]. Cisplatin disrupts the cell's antioxidant defense system and damages DNA. Moreover, it generates free radicals to activate cell death signaling cascades [7]. Cisplatin causes necrosis or damage to cell membranes and the release of the enzymes aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase into the bloodstream. It increases Malondialdehyde and decreases glutathione storage in liver tissue following cisplatin exposure. Peroxidation and reduction of regenerated glutathione also occur after treatment with cisplatin. Cisplatin can inhibit the activity of superoxide dismutase, catalase and glutathione peroxidase. Antioxidants prevent the leakage of enzymes into the cell by maintaining the integrity and stability of the membrane or regenerating damaged liver cells and causing the enzyme level to return to a normal level [8]. Free radicals are very strong reactors that strongly tend to capture electrons, thus damaging other molecules. Damage from oxidative reactions to DNA and proteins and other molecules can lead to the progression and exacerbation of cardiovascular, cancer, and liver diseases [9]. In general, antioxidant species are of three categories [10]:

1. Enzyme systems such as catalase, glutathione peroxidase, superoxide dismutase, Ceruloplasmin, etc.

2. Small molecules such as ascorbate, uric acid, vitamin E.

3. Proteins such as albumin, transferrin and metallothioneins

Pistachio is one of the plants having compounds with antioxidant properties. Pistachio kernels contain vitamins E and C, beta-carotene, various antioxidants, gamma tocopherol, quercetin, flavonoids, lutein, xanthine and minerals including copper, iron and selenium. Pistachio has liver protective properties and is rich in phenolic compounds that are powerful antioxidants. The antioxidants in pistachios can prevent the reduction of superoxide dismutase and catalase and glutathione peroxidase by eliminating free radicals; they ensure the survival of these enzymes [11]. Quercetin in pistachios reduces serum fat levels and liver fat accumulation, improves liver function, reduces liver transaminases and prevents the function of liver fibrosis agents. Quercetin has a significant growth inhibitory effect on cancer cells and tumors, and quercetin is used in chemotherapy with cisplatin [12]. Pistachio, as a rich source of antioxidants, can be a good option to counteract the toxic oxidative effects of cisplatin due to the reduction of side effects compared to chemical drugs [6]. Anti-cancer chemotherapy drugs have

devastating side effects depending on the dose used during treatment. Therefore, patients are always required to be under intensive care and take supplements in addition to chemotherapy drugs. This study investigates the antioxidant effects of ethyl acetate extract of Akbari pistachio kernel on the toxic effects of cisplatin (an anti-cancer drug).

2. Materials and methods

In this experimental study, which was conducted in Rafsanjan University of Medical Sciences in 2019, it was attempted to investigate the effect of ethyl acetate extract of pistachio kernel on 36 male Wistar rats. This study has been approved by the ethics committee of Rafsanjan University of Medical Sciences with the ethics code IR.RUMS.REC.1397.008.

Preparation of the extract: To prepare pistachio kernel extract, dried pistachio kernel powder was soaked in a ratio of 1 to 4 in 70% ethyl acetate solution at room temperature for 48 hours in the dark. The filtered mixture and the filtered solution were then frozen for 24 hours at a temperature of minus 20 °C. In order to remove the solvent, a Rotary evaporator-SB100 made by Eyela Company (Japan) was used, and it was then kept in the refrigerator until it was used. To use the extract, the dry powder of the extract was dissolved in 3% dimethyl sulfoxide (DMSO) [13].

The rats: As many as 36 male Wistar rats with a lifespan of 4-5 weeks and an average weight of 250 g were obtained from the Animal Reproduction Center, Department of Physiology, Rafsanjan University of Medical Sciences. They were kept in special three-in-three cages with an average temperature of 25 °C, 12:12 hours of circadian light cycle for 15 days. All members of these groups ate the usual food and water of that period.

These 36 male rats were randomly divided into 6 groups of 6 and then the treatment period for 15 days was as follows:

Group 1: Healthy rats received DMSO (Dimethyl sulfoxide) at a concentration of 3%, which was actually used as a kernel extract stabilizer, by oral gavage. This group was considered as the control group.

Group 2: Healthy rats receiving a dose of 200 mg/kg body weight of pistachio kernel extract containing 3% DMSO for 15 days as oral gavage.

Group 3: Healthy rats that received a dose of 400 mg/kg body weight for 15 days from pistachio kernel extract containing 3% DMSO as oral gavage.

Group 4: Rats receiving a single dose of cisplatin at a dose of 10 mg/kg body weight by intraperitoneal injection on the tenth day of the experiment.

Group 5: Rats receiving a single dose of cisplatin at a dose of 10 mg/kg body weight plus a dose of 200 mg/kg body weight of pistachio kernel extract containing 3% DMSO for 15 days by oral gavage.

Group 6: Rats receiving a single dose of cisplatin at a dose of 10 mg/kg body weight plus a dose of 400 mg/kg body weight of pistachio kernel extract containing 3% DMSO for 15 days by oral gavage.

The treated rats were anesthetized with ether on the 16th day and blood samples were then taken from the corners of their eyes and the serum was isolated by centrifuge model 5430

(Eppendorf company, Germany) at 3000 rpm for 15 minutes [14].

Serum levels of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX) and total antioxidant capacity (TAC) were evaluated for investigating the effects of cisplatin and the effect of pistachio kernel ethyl acetate extract on cisplatin. Moreover, the amount of Malondialdehyde (MDA) and reviving glutathione (GSH) was determined by calorimetric method (Quantitative) using the analysis kits of Navandsalamat Research Company.

The collected data were statistically analyzed by SPSS version 24. Results for quantitative data were reported as mean ± standard deviation. In order to compare the mean serum levels of the enzymes across the study groups, one-way analysis of variance (One-way ANOVA) followed by Tukey's multiple comparisons test was used. The normality of the frequency distribution of quantitative variables was assessed by the non-parametric Kolmogorov-Smirnov test and also by calculating the skewness and Kurtosis indices. Homogeneity of variances among groups was also assessed by Levene's test. Significance level in the tests was considered to be 0.05.

3. Results and analysis of results

According to the Kolmogorov-Smirnov test, the variables CAT, TP, SOD, GPX, TAC and MDA had normal distribution in each group (P >0.05). No casualties were observed during the treatment period. And data are allowed to be analyzed with "one-way ANOVA test" . According to the Levene's test, homogeneity of variances was confirmed among groups (GPX: P= 0.750, MDA: P= 0.236, TAC: P= 0.052, CAT: P= 0.153, SOD: P= 0.879, and TPro: P= 0.861). According to the one-way ANOVA, the means of variables were statistically significant across groups. Table 1 indicates the mean and standard deviation of GPX, CAT, SOD activity, MDA, TAC and total protein concentration in study groups.

Table 1. Comparison of mean variables across the study groups

Groups	Mean ± Standard deviation (SD)				
	GPX (Glutathione peroxidase) (U/ml)	MDA (Malondialdehyde) (nmol/ml)	TAC (Total antioxidant capacity) (nmol Fe^{2+}/L)	CAT (Catalase) (nmol/min/ml)	SOD (Superoxide dismutase
Group 1 (Control) (n=6)	23.20±0.62	4.60±0.24	0.92±0.03	71.60±1.22	20.00±0.96
Group 2 (Extract1) (n=6)	21.40±1.06	4.20±0.24	1.10±0.06	64.27±1.35	18.00±0.51
Group 3 (Extract 2) (n=6)	22.70±0.70	3.90±0.17	1.40±0.06	67.27±1.65	19.42±0.88
Group 4 (Cisplatin) (n=4)	18.30±0.48	6.12±0.38	0.63±0.03	46.86±0.97	13.92±0.52
Group 5 (Extract 1+Cis) (n=6)	20.30±1.12	5.73±0.22	0.97±0.03	69.40±2.03	19.23±0.97
Group 6 (Extract 2+Cis) (n=5)	21.90±0.57	5.20±0.24	1.00±0.03	70.10±2.54	20.10±0.51

For each variable, groups with different English letters are statistically different from one another ($p<0.05$).

4. Results analysis

GPX enzyme activity: In groups 2 and 3, which used pistachio kernel extract alone, only a dose of 200 mg/kg body weight of the extract significantly reduced the serum level of glutathione peroxidase compared to that of the control group (P< 0.001). In group 4, cisplatin significantly reduced serum levels of glutathione peroxidase compared to that of the control group (P< 0.001). In group 5, the animals received a dose of 200 mg/kg of extract in addition to cisplatin. Serum glutathione peroxidase level increased significantly compared to that of the cisplatin group and decreased significantly compared to that of the control group (P< 0.001).

CAT enzyme activity: The extract significantly reduced the serum level of catalase in groups 2 and 3 compared to that of the control group (P< 0.001). Moreover, in group 4, the serum level of catalase significantly reduced compared to that of the control group. In groups 5 and 6, there was no significant difference in serum level of catalase in the control group (P= 0.164), but there was a significant increase compared to the cisplatin group (P< 0.001).

SOD enzyme activity: In groups 2, 3, 4 and 5, serum levels of this enzyme were significantly reduced compared to that of the control group (P< 0.001), but in group 6 there was no significant difference compared to the control group (P= 0.082). In groups 5 and 6, a significant increase was observed compared to cisplatin group, and we also found a significant increase in serum levels of superoxide dismutase in group 6 compared to that of group 2 (P< 0.001).

TAC: In groups 2 and 3, the total antioxidant capacity significantly increased compared to that of the control group (P< 0.001), but in group 4, a significant decrease was observed compared to that of the control group. In groups 5 and 6, the TAC increased significantly compared to that of cisplatin group (P< 0.001).

MDA serum level: In group 3, the high dose of the extract caused a significant decrease in malondialdehyde compared to the control group (P< 0.001), but in group 4, cisplatin caused a significant increase in malondialdehyde level compared to that of the control group (P< 0.001). In groups 5 and 6, malondialdehyde levels significantly increased compared to that of the control group and significantly decreased compared to cisplatin group (P< 0.001).

5. Discussion

In this study, serum levels of superoxide dismutase, catalase, glutathione peroxidase and serum malondialdehyde levels and total antioxidant capacity were investigated and evaluated. The results of this study showed the toxic damage of cisplatin on the body of rats and the relative modulation of these destructive effects after consuming pistachio extract [15].

According to studies and available evidence, cisplatin increases the level of malondialdehyde and decreases glutathione peroxidase; this indicates that the oxidative stress caused by free radicals is one of the possible mechanisms in the pathophysiology of cisplatin toxicity. Research conducted by Yousef MI et al (2006) reported lipid peroxidation and degradation and resuscitation of glutathione and reduction of glutathione peroxidase after cisplatin treatment in rat liver tissue; this is in line with the results of the present study [16]. Decreased glutathione levels following a decrease in glutathione peroxidase can be a direct factor in cisplatin-induced fat peroxidation. Hepatotoxicity of cisplatin has been attributed to oxidative damage and production of reactive oxygen species.

Studies by Mansour et al (2006) indicated that cisplatin could inhibit glutathione peroxidase and catalase activity and increase malondialdehyde levels in rat serum. In this study, significant changes were observed in the antioxidant defense system and total antioxidant capacity and malondialdehyde content after cisplatin injection; this is in line with the results of other studies in this field [17]. The reason that the selected doses of the extract failed to significantly reduce the amount of malondialdehyde due to the use of cisplatin is the fact that the changes in malondialdehyde is likely to be dependent on the dose of pistachio extract.

In the present study, the results indicated that by increasing the dose of pistachio extract, the amount of malondialdehyde increased owing to the use of cisplatin decreased to a more acceptable level compared to that of the group receiving a low dose of pistachio extract; it is closer to the normal and acceptable level of the control group. This may indicate that if a sufficient amount of the extract is provided to the rat's body system, it can overcome the lipid peroxidation caused by cisplatin consumption. Such changes are similar to the case reported by Boroushak et al (2015) in rats treated with cisplatin and pomegranate seed oil; their results showed an increase in MDA concentration due to simultaneous intake of pomegranate seed oil and cisplatin [18].

Superoxide dismutase, catalase, glutathione peroxidase are antioxidant enzymes that form the defense system against reactive oxygen species [19]. Decreased serum superoxide

dismutase level is a sensitive indicator of liver cell damage. This enzyme is one of the most important factors in the enzymatic antioxidant defense system. Superoxide dismutase kills the superoxide anion by converting it to hydrogen peroxide, thus reducing its toxic effects [20]. Excessive formation of superoxide anions following cisplatin administration significantly reduces superoxide dismutase levels in cisplatin-receiving rats, leading to a significant reduction in the activity of hydrogen peroxide-inhibiting enzymes. This leads to a significant reduction in the activity of hydrogen peroxide inhibitory enzymes, that is catalase and glutathione peroxidase. In this study, the use of pistachio extract prevented the reduction of superoxide dismutase, catalase and glutathione peroxidase, and higher doses of the extract showed greater amounts of these conditions; this may be due to the removal of radicals by the extract, leading to preservation and survival of these enzymes. The difference in cisplatin-induced SOD enzyme activity is qualitatively similar to a similar study conducted on humans by Banci et al [21]. In another similar study conducted by Amleer et al, on the effect of cisplatin and olive leaf extract, olive leaf extract was found to have the ability to reduce oxidants in cisplatin-treated rat testicular tissue [22].

Catalase is an antioxidant enzyme that is widely distributed in animal tissues and is most active in the liver and red blood cells. Catalase breaks down hydrogen peroxide and protects tissues against hydroxyl free radicals [23]. Thus, decreased catalase activity may lead to the damaging effects of superoxide and hydrogen peroxide radicals.

Glutathione reductase is a hepatic cytosolic enzyme that is involved in the reduction of glutathione oxide as the end product of glutathione peroxidase activity on regenerative glutathione [24]. In this study, after cisplatin treatment, a significant reduction was observed in glutathione peroxidase; this is qualitatively similar to a similar study conducted by Basuony et al on cisplatin-treated humans and Panax ginseng root [25].

6. Conclusion

Given the design limitations and systemic differences between humans and rats, specific conclusions will be difficult, but in general, pistachio kernel extract along with cisplatin may increase glutathione peroxidase levels by increasing detoxification of destructive active metabolites. These results are in line with those of the other studies confirming the antioxidant properties of pistachios. The involvement of reactive oxygen species (ROS), nitrogen, and chlorine in the pathology and toxicity of cisplatin has been demonstrated in numerous similar studies conducted on rats or humans. By causing dysfunction of the antioxidant system, cisplatin leads to oxidative stress and a decrease in the total antioxidant capacity. In our study, with its antioxidant properties, pistachio kernel extract compensates for these reduced levels to an acceptable level. If further studies are conducted on other animal or human species, it can be introduced as a way to reduce the side effects of cisplatin.

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