

Survey on Effects of *Pistacia Atlantica* Gum Extract on Some Enzymes and Biochemical Factors Arising from "Cisplatin" in the Serum of Male Wistar Rats

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Information	Abstract
<p>Article Type: Original Article</p>	<p>Introduction: Cisplatin is an anticancer drug used for tumor treatment, causing free radicals and damaging the DNA. The higher doses of cisplatin induce toxicity in organs, such as the kidney and liver. Regarding antioxidant properties of <i>Pistacia Atlantica</i> gum containing flavonoid compounds, this study aims to investigate the protective effects of the gum on Wistar rat's liver and kidney cells against cisplatin toxicity.</p> <p>Materials and Methods: Emulsion was made from <i>Pistacia Atlantica</i> gum using the self-emulsification method to facilitate rats' gavage. Then, 24 male Wistar rats were randomly divided into 4 groups, including the control group (CG), extract group (EG), gum & cisplatin group (GCG), and cisplatin group (CiG). Subsequently, the concentration of malondialdehyde (MDA), total protein (TP), total antioxidant capacity (TAC), the activity of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX) were measured in rat's blood samples for 15 days. One-way ANOVA was used to demonstrate differences between groups by SPSS Ver.2018 software.</p> <p>Results: <i>Pistacia Atlantica</i> gum significantly halted the reduction effects of cisplatin on GPX, CAT, and SOD in group 3. It displayed a significant increase in TAC and a remarkable reduction in MDA.</p> <p>Conclusion: <i>Pistacia Atlantica</i> gum appeared to have protective and moderate effects against cisplatin-induced toxicity on the liver and kidney. Moreover, its antioxidant attributes caused a reduction in free radicals.</p>
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1. Introduction

Cisplatin is an anticancer drug used to treat solid tumors [1]. It negatively affects the DNA of tumor cells and reduces the rate of cell division [2]. The intracellular effects of cisplatin include impaired function of ATPase enzymes in interaction with mitochondria, as well as disruption of the cell division cycle and intracellular transport, ultimately leading to apoptosis or necrosis [3]. Cisplatin also results in free radicals that can activate the apoptosis signaling cascade, which is controlled by MAPK (mitogen-activated protein kinase) [4].

After cisplatin-induced necrosis, damaged cell membranes and intracellular liver enzymes, such as AST (aspartate aminotransferase), ALT (alanine aminotransferase), and LDH (lactate dehydrogenase), enter the bloodstream, increasing MDA level. Reduction in GPX, CAT, and SOD activity is also observed in cisplatin-exposed cells. Therefore, cisplatin, like other anticancer drugs, has some dangerous side effects, such as involving the SOX-2 and INOS genes, causing hepatotoxicity in long treatment periods [5]. In a similar study, injecting a single dose of cisplatin in rats has resulted in an increase in total bilirubin and a decrease in total protein [5, 6].

Free radicals are highly reactive, with a strong tendency to capture electrons. They break down other molecules that disrupt

oxidative reactions, which, in turn, damage DNA and intracellular proteins, leading to cardiovascular and hepatic hemorrhage [7]. *Pistacia Vera* and *Atlantica* are two pistachio genus species widely-grown in eastern Iran [8, 9].

Pistacia Vera gum is rich in flavonoids, saponins, and tannins [10]. Research shows flavonoids to have a protective effect on the liver [11]. Another study has shown that the *Pistacia Atlantica* methanolic extract (in vitro and in vivo in rats) has the highest amount of phytochemicals and antioxidant activity and leads to a significant reduction in MDA serum level and a significant increase in the CAT mean serum level [12]. Earlier research has shown the antioxidant activity of *Pistacia Lentiscus*, another species of *Pistacia*, to be equivalent to the synthetic antioxidant Torlex (vitamin E), which prevents fat peroxidation in rat liver [13]. Due to the antioxidant activity of natural flavonoids and their phenolic compounds, and since pistachio kernels are a great source of these natural substances, they can act as powerful scavengers of free radicals [14].

Anticancer chemotherapy drugs have devastating side effects, including weight loss, hair loss, weakened immune system, and reduced function of organs such as the liver and kidneys, during the treatment period depending on the doses used. Therefore, patients should always be under

special care and take supplements along with chemotherapy drugs. This study aims to investigate the effect of *Pistacia Atlantica* gum extract on the change of some biochemical factors in the serum of rats treated with cisplatin.

2. Materials and Methods

For this research, materials and methods were based on experimental studies in Rafsanjan Medical Science University and similar research [5, 15]. The study was performed under the ethical permits IR.RUMS.REC.1397.006. Span 80, tween 80, and solvent of acetone were prepared from Merck, Germany.

The preparation of gum emulsion:

Since the Pistachio gum is a honey shape liquid with high viscosity, it is rather challenging to dissolve it in water and gavage directly. In the present study, to tackle the problem mentioned above, the self-emulsification method was found to be most effective in preparing gum emulsion. This technique was performed in the following three stages:

A) Homogeneous organic solution composed of gum (40.3 g) and lipophilic surfactant (1.05 g Span 80) was prepared in a water-miscible solvent (33.7 ml acetone). The homogeneous aqueous phase was formed by water (140 ml) and hydrophilic surfactant (1.54 g tween 80).

B) Subsequently, the organic phase was added gently to the aqueous phase under magnetic stirring. The gum emulsion was

spontaneously formed by diffusion of organic solvent in the external aqueous phase, forming microdroplets. The magnetic stirring was performed for about half an hour to obtain equilibrium in the system.

C) The totality of water-miscible solvent was removed by evaporation for 40 min under reduced pressure. Microdroplets of gum were dispersed in an aqueous solution and hydrophilic surfactant. The milky and thinner liquid was obtained, which could be gavaged to rats easily.

Animals:

Twenty-four 4-5 week-old male Wistar rats with an average weight of 250 gr were procured from the animal reproduction center of the physiology department at Rafsanjan University of Medical Sciences. They were kept in special cages (three in each) at an average of 25 °C and 12:12 hours day-night light cycle for 15 days. All members of these groups fed the usual food and water during the period. They were divided into 4 equal groups randomly by the following arrangement:

Group 1: This was used as a control group and took nothing but food additives added to the gum of *Pistacia Atlantica*, which were 3.2 Span80 and 4.6 Tween80 (mg per kg weight of rat) for each rat; they were solved in enough amount of distilled water and gavaged to animals daily for 15 days.

Group 2: Each member of this group received 250 (mg per kg weight of rat) of *Pistacia Atlantica* gum by gavage daily for 15 days.

Group 3: Similar to group 2, members of this group received 250 (mg per kg weight of rat) of *Pistacia Atlantica* gum by gavage daily for 15 days, as well as 10 (mg per kg weight of rat) cisplatin by intraperitoneal injection in the 10th day.

Group 4: Group members only received 10 (mg per kg weight of rat) cisplatin (single dose) by intraperitoneal injection on the 10th day.

Sampling and analysis:

On the 16th day, the blood of all 24 male rats were taken under anesthesia, and samples were collected. Then, blood samples were centrifuged at 10000 rpm for 3 min; further, serums were divided and stored at -20°C until analyzing. For analysis, serums were temperate to 37 °C in a water bath; next, TAC, MDA, TP, SOD, TAC, and GPX were measured by assay kit from Navamdsalant Research Company. Data were then analyzed by SPSS 2018, being presented as mean±standard deviation.

The one-way ANOVA followed by Tukey post hoc test was used to compare serum levels across the study groups. A P-value < 0.05 was considered significant.

3. Results

Table 1 presents the mean GPX activity in the 4 groups. As seen, there is no significant difference between CG and EG; however, a significant difference is observed between CiG and the CG that reduces GPX activity (P< 0.001). Further, GCG, which received cisplatin and extract, has increased GPX activity compared to CiG.

According to Table 1, the mean MDA concentration in the 4 groups shows no significant difference between CG, EG, and GCG. Cisplatin can only cause a significant increase in the MDA concentration in group 4; however, the gum in GCG can reduce this concentration in the presence of cisplatin compared to CiG.

The TAC is significantly different between all groups. The most significant increase is observed in EG, whereas CiG has the lowest TAC (Table 1). Due to the presence of antioxidant compounds in the extract, group 3 could compensate for the decrease in cisplatin TAC.

Moreover, there is a significant difference among all groups with the highest amount of CAT in CG (71.25 nmol/min/ml) and the lowest in CiG (46.86 nmol/min/ml), indicating that the gum in combination with cisplatin is able to maintain the level of CAT in GCG. On the other hand, the lack of gum results in a considerable decrease in CAT for CiG.

There is a significant difference among all groups, with the highest amount of SOD in CG and GCG (20.12, 19.16 U/ml) and the lowest in CiG (13.92 U/ml), showing that the gum in combination with cisplatin can maintain the level of SOD in GCG. On the other hand, the lack of gum results in a considerable decrease in SOD for CiG (Table 1).

Further, according to the table, the mean TP concentration in the 4 groups shows no significant difference between CG, EG, and GCG. Cisplatin can only cause a significant decrease in the TP concentration in group 4; however, the gum in GCG can enhance this concentration in the presence of cisplatin compared to CiG.

Table 1: The comparison of the average of biochemical parameters in different groups of rats

Group	Mean ± Standard deviation					
	GPX (mu/ml)	MDA (nmol/ml)	TAC (nmol fe2+/L)	CAT (nmol/min/ml)	SOD (U/ml)	TP (mg)
Group1 (CG)	23.09 ^a	4.82 ^b	0.91 ^{bc}	71.25 ^a	20.12 ^a	7.81 ^a
Group 2 (EG)	22.3 ^{ab}	4.83 ^b	1.57 ^a	65.36 ^c	17.44 ^b	6.99 ^a
Group 3 (GCG)	21.74 ^b	5 ^b	0.992 ^b	69.33 ^b	19.16 ^a	7.02 ^a
Group 4 (CiG)	18.3 ^c	6.12 ^a	0.629 ^c	46.86 ^d	13.92 ^c	4.39 ^b

Data are analyzed using one-way ANOVA. In each factor, groups with different letters are statistically significantly different (P<0.05)

4. Discussion

The decrease in the activities of GPX, SOD, CAT, and TAC enzymes in CiG (group 4) is probably related to the side effects of the drug, disrupting the intracellular antioxidant system [16]. However, no significant change is observed in EG compared to CG, indicating that the gum consumption does not induce a disruption in GPX, SOD, CAT, and TAC enzymes. The similarity of GPX, SOD, CAT, and TAC enzyme

activity in GCG to that in the control group, besides its increase compared to group 4 (CiG), can be due to the existence of flavonoids abundantly in *Pistacia* gum with antioxidant properties [17]. MDA is measured to assess fatty liver disease as a biomarker of oxidative stress [18]. Decreased MDA levels in CiG (group 4) may indicate hepatotoxicity due to cisplatin toxicity. This toxicity is caused by a malfunction of the cellular antioxidant defense system. However, *Pistacia Atlantica* gum extract is able to

compensate for the oxidative effects of plant flavonoids and bring the level of MDA closer to CG than CiG. The decrease in total protein levels in CiG is due to cisplatin-induced nephropathy and kidney damage, proven in articles on similar topics earlier [19]. A disorder in the cell's antioxidant defense system leads to kidney damage in the renal tubular cells, gradually causing the protein to be excreted from the kidneys and the level of total serum protein to be decreased. This is the case in the EG (group 2), although not significant. Also, GCG (group 3) can reduce the excretion of total protein significantly. Its serum level does not decrease significantly compared to CG. This indicates less damage to the renal tubular cells, possibly due to the elimination of the antioxidant damage of flavonoids in *Pistacia Atlantica* gum [17]. In a similar study, Liu X-q et al. have examined the effects of cisplatin nephropathy in the presence of Rutaecarpine; they have linked its positive effects to the reduction of Rutaecarpine-induced oxidants in PDE4B, the major enzyme in the inflammatory pathway of renal cells [20]. In a study entitled "Protective effect of vitamin E and cod liver oil against acute cisplatin-induced kidney damage in rats," Azza MA Abo-Elmaaty et al. have reported vitamin E and cod liver oil to have beneficial effects in reducing cisplatin-side effects in rat's kidney cells. They examine the activity of

antioxidant defense enzymes, such as SOD and CAT, as well as the levels of MDA and GSH in the pathway of these enzymes [21].

5. Conclusions

According to the results, among six biochemical factors (GPX, CAT, SOD, MDA, TAC, and total protein), only cisplatin is significantly different in CiG from CG due to its dangerous side effects. However, *Pistacia Atlantica* gum in GCG can neutralize those side effects. The gum is not significantly different in EG compared to CG, showing that it has minor effects on normal cells. Therefore, the gum is a suitable alternative and supportive substance to reduce the side effects of anticancer chemotherapy drugs, such as cisplatin adjuvant ones. This research study has been performed in male rats; thus, further research in other cases is recommended to obtain more accurate conclusions.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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