

The Regulatory Effect of Hydrolyzed Protein from *Pistachio Vera* on Serum Lipid Levels

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Information	Abstract
<p>Article Type: Original Article</p>	<p>Background & Aims: Various studies have investigated the effects of pistachio fat on serum lipid profile. We aimed to study the effect of the hydrolyzed protein of <i>Pistacia vera</i> kernel on the lipid profile of Wistar rats.</p> <p>Methods: Forty eight male Wistar rats were divided into 2 main groups as control groups (STD: fed with standard diet, STD 2: fed with standard diet and gavaged with 500 mg/kg pistachio hydrolysate) and target groups (HFD: fed with high-fat diet, HFD+5: fed with high-fat diet and gavaged with 5 mg/kg/day pistachio hydrolysate, HFD+50: fed with high-fat diet and gavaged with 50 mg/kg/day pistachio hydrolysate, HFD+500: fed with high-fat diet and gavaged with 500 mg/kg/day pistachio hydrolysate. After 56-day treatment, the serum level of Triglycerides (TG), Total cholesterol (TC), and High-density lipoprotein (HDL) were measured.</p> <p>Results: The mean levels of serum TC, and TG in different groups were significantly higher in HFD group compared with STD group ($P<0.01$ and $P<0.05$ respectively). Mean TC and TG levels were significantly lower in HFD+500 group than in HFD group ($P<0.001$ and $P<0.01$ respectively). Mean HDL was significantly lower in HFD group compared with STD group ($P<0.05$), and significantly higher in HFD+500 group compared with HFD group ($P<0.01$).</p> <p>Conclusions: The results of this study showed favorable effects of the pistachio hydrolyzed protein on reducing TC and TG levels, and increasing HDL level in the rats consuming high-fat diet. On the other words, the pistachio hydrolysate has regulatory effects on the lipid profile of consumers of high-fat diet.</p>
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1. Introduction

Currently, more than three million adults in the US and Europe were diagnosed with hyperlipidemia, and this number is estimated to increase at a drastic pace [1]. The term "hyperlipidemia" refers to a group of inherited and acquired illnesses characterized by excessively elevated blood lipid levels [2, 3]. The lipids typically mean cholesterol, chylomicrons, Apo lipoproteins, and lipoproteins (very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL)) [4]. Based on an objective definition, hyperlipidemia occurs when the levels of LDL, total cholesterol (TC), and triglyceride (TG) are higher than the 90th percentile compared with the general population, or in other words, the level of HDL is less than the 10th percentile in comparison to the general population [2].

Numerous research investigations and clinical trials have shown a clear correlation between hyperlipidemia and the risk of ischemia/reperfusion damage. Additionally, hyperlipidemia may reduce the effectiveness of heart-protective therapies such as ischemic pre- and post-conditioning. Moreover, it was frequently reported that hyperlipidemia plays a well-known role in developing atherosclerosis in blood vessels [5].

Initial treatment of hyperlipidemia is based on the modification of diet and lifestyle, although lipid-lowering medications are used if needed. A low-carbohydrate and low-fat diet, as well as elevated physical activity, is usually recommended. Based on American Heart Association's guidelines, consumption of saturated fat should be limited to about 5% of the daily calories, and uptake of trans-saturated fat should be restricted as much as possible [6-8].

Research indicates that increasing the amount of protein consumed via food may be very advantageous for either weight reduction or maintenance, enhancing the lipid/lipoprotein profile, reducing blood pressure, and ultimately promoting cardiovascular well-being [9, 10]. Different studies reported that animal proteins, such as casein or beef increase cholesterol level and atherosclerosis [11, 12], while it showed that the replacement of animal protein with plant protein significantly reduced blood cholesterol levels [13-16].

Pistachio Vera L. (Pistachio) belongs to Anacardiaceae family. The kernel of this nut possesses a high nutritional value. Similar to other edible nuts, pistachio is a rich source of protein, lipids, and vitamins [17]. While edible nuts contain approximately 75- 220 g/ kg protein (if a nitrogen-to-protein conversion factor of 5.3 is used), pistachio has a relatively high protein concentration of approximately 210 g/kg [18].

Various studies investigated the effects of pistachio fat (which is mainly composed of unsaturated fatty acids) on the lipid profile. Nevertheless, our search turned up no research on the impact of pistachio protein on lipid profiles. Therefore, the purpose of this research was to create pistachio kernel protein hydrolysates and assess any potential effects they could have on the lipid profile of rats fed a high-fat diet.

2. Materials and Methods

Preparation of pistachio hydrolyzate

The protein contents of various pistachio cultivars planted in Rafsanjan city of Iran are not considerably different. On the other hand, Ohadi cultivar covers the highest level of pistachio cultivation in Iran [19, 20]. Therefore, the Ohadi pistachio cultivar was selected and provided

from Rafsanjan pistachio gardens. Trypsin, pepsin, and chymotrypsin enzymes, as well as streptozotocin were purchased from Sigma Aldrich (Steinheim, Germany). Serum TG, TC, and HDL levels were measured using an auto-analyzer (Biotechnica BT1500 Chemistry, Italy).

Ohadi cultivar of pistachio was supplied from Rafsanjan, Iran. The pistachio hydrolyzate was prepared using enzymatic hydrolysis protocol described by Li *et al.* [21] with minor modifications. Initially, 90 mL of distilled water was used to pulverize and distribute 10 g of the dried pistachio kernels. The combination was then subjected to a 4-hour treatment with the pepsin enzyme (5000 U/g pistachio powder) at 37°C while maintaining a pH of 1.8. To stop hydrolysis reaction, the mixture was kept in a boiling water bath for 10 min. Enzymatic hydrolysis with the same concentration of trypsin enzyme was performed at 37° C and pH 7.8 for 6 h. Again, the hydrolysis process was terminated using the boiling water bath. Chymotrypsin was used for the final enzymatic digestion, which lasted four hours at 37°C and pH 8. Centrifugation was carried out at 9000 ×g and 4° C for 25 minutes after the mixture was heated in boiling water for 10 minutes. Finally, supernatant containing pepsin-trypsin-chymotrypsin hydrolyzate was separated. To determine the peptide concentration in the obtained hydrolyzate, Bradford test was applied. Thus, SDS-PAGE electrophoresis was used to ensure the function of the enzymes.

Animals and experimental design

In this experimental study, we followed the guidelines for the care and using laboratory animals as approved by Rafsanjani University of Medical Sciences (RUMS) under the ethics code of IR.RUMS.REC.1396.171. Based on previous studies, healthy adult Wistar rats (60 rats) weighing in the range of 250-300 g were kept in

polypropylene cages under normal light/dark conditions at 22±2°C. Because the research is longitudinal, the sample size was determined to be 48 [22] using the method for calculating the sample size for repeated measures or three times, or by utilizing the data from Pengli *et al.* (2014).

$$n = \frac{2 \left(z_{\alpha/2} + z_{\beta} \right)^2 \sigma^2 \{ 1 + (m - 1)\rho \}}{md^2}$$

Then, 48 male Wistar rats were randomly divided into 6 groups (N=8). Rats were divided into 2 main groups: 2 control groups and 4 target groups.

STD group: The first control group included 8 rats fed with a standard diet and gavaged with normal saline every day for 56 days.

STD 2 group: The second control group included 8 rats fed with a standard diet and gavaged with 500 mg/kg pistachio hydrolysate every day for 56 days.

HFD: The first target group included 8 rats fed with a high-fat diet (HFD) and gavaged with normal saline every day for 56 days.

HFD+5: The second target group included 8 rats fed with high-fat diet and gavaged with 5 mg/kg pistachio hydrolysate every day for 56 days.

HFD+50: The third target group included 8 rats fed with high-fat diet and gavaged with 50 mg/kg pistachio hydrolysate every day for 56 days.

HFD+500: The fourth target group included 8 rats fed with high-fat diet and gavaged with 500 mg/kg pistachio hydrolysate every day for 56 days.

The high-fat diet was purchased from Royan Institute (Isfahan, Iran) with a vitality of 5.2 kcal/g, including 24% protein, 26% carbohydrate, and 35% fat for 4 weeks [23, 24].

After 56 days, the rats were anesthetized with ketamine, and blood samples were taken from

the corners of their eyes, and sent to the laboratory of Rafsanjan University of Medical Science to measure TC, TG, and HDL levels.

Statistical analysis

The statistical analysis was conducted utilizing the software applications Excel and SPSS. Each value is presented as the mean \pm SD. By applying ANOVA followed by the Tukey post-hoc test, differences between the groups were ascertained. P values smaller than 0.05 were considered statistically significant.

3. Results

Figure 1 depicts the mean level of serum TC in different groups of rats. As the results show the mean TC was significantly higher in the HFD group than in the STD group ($P < 0.01$), while there was no significant difference between the STD and STD2 groups. The mean TC was significantly lower in the HFD+ 500 group than in the HFD group ($P < 0.001$). The decrease in the HFD+5 and HFD+50 groups was not significant (Figure 1).

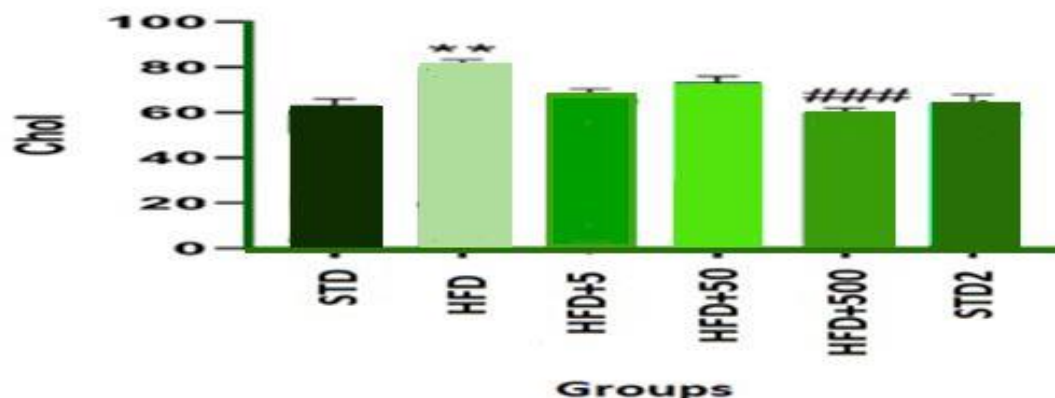


Figure 1. The mean level of serum TC in different groups of rats. Groups: standard diet (STD), standard diet and 500 mg/kg pistachio hydrolysate (STD 2), high-fat diet (HFD), HFD and 5 mg/kg pistachio (HFD+5), HFD and 50 mg/kg pistachio (HFD+50), HFD and 500 mg/kg pistachio (HFD+500). ** $P < 0.01$ Vs STD, ### $P < 0.001$ Vs HFD

Figure 2 presents the mean level of serum HDL in different groups of rats. The mean HDL was significantly lower in the HFD group than in the STD group ($P < 0.05$), while there was no significant difference between STD and STD2

groups. The mean HDL was significantly higher in the HFD+500 group than HFD group ($P < 0.01$). The level of HDL was higher in the HFD+5 and HFD+50 groups than in the HFD group, but it was not significant (Figure 2).

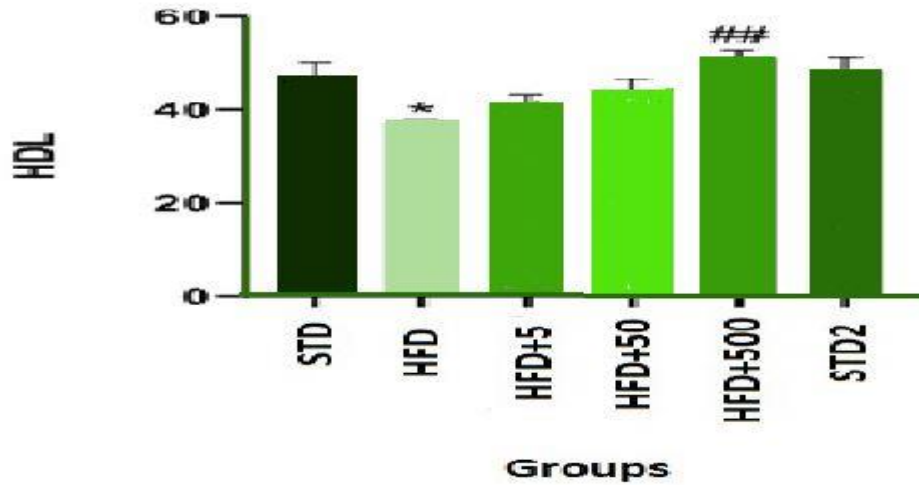


Figure 2. The mean level of serum HDL in different groups of rats. Groups: standard diet (STD), standard diet and 500 mg/kg pistachio hydrolysate (STD 2), high-fat diet (HFD), HFD, and 5 mg/kg pistachio (HFD+5), HFD and 50 mg/kg pistachio (HFD+50), HFD and 500 mg/kg pistachio (HFD+500). * $P < 0.05$ Vs STD, ## $P < 0.01$ Vs HFD

Figure 3 displays the mean level of serum TG in each of the rats' groups. There was no significant difference between the STD and STD2 groups, however the mean TG level was considerably greater in the HFD group compared to the STD group ($P < 0.05$). The mean

TG was significantly lower in the HFD+500 group than in the HFD group ($P < 0.01$). The level of TG was lower in HFD+5 and HFD+50 groups than HFD group but it was not significant (Figure 3).

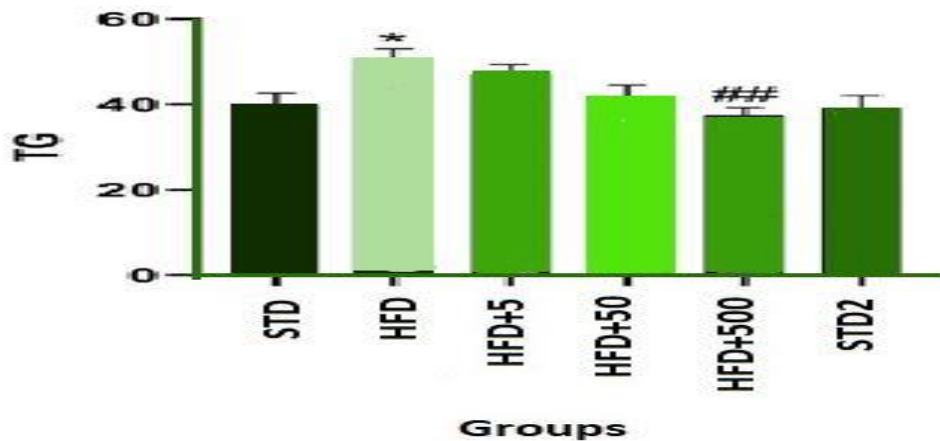


Figure 3. The mean level of serum TG in different groups of rats. Groups: standard diet (STD), standard diet and 500 mg/kg pistachio hydrolysate (STD 2), high-fat diet (HFD), HFD and 5 mg/kg pistachio (HFD+5), HFD and 50 mg/kg pistachio (HFD+50), HFD and 500 mg/kg pistachio (HFD+500). * $P < 0.05$ Vs STD, ## $P < 0.01$ Vs HFD.

4. Discussion

High dose of the pistachio hydrolysate showed significant effects on the lipid profile of the rats (TG, HDL, and TC). Pistachio has relatively little fat and among pistachio fatty acids, oleic and linoleic fatty acids, which are known to have protective properties against cardiovascular diseases, constitute more than 60% the total pistachio fat [18]. Bulló *et al.* (2015) reported that pistachios can improve health-related outcomes, and reduce the risk of cardiovascular diseases [18].

The maximum dose of pistachio hydrolysate (500 mg/kg/day) significantly decreased the cholesterol level of the rodents in the current study when compared to the control group. Consistent with the results reported in a separate investigation conducted by Nazifi *et al.* [25], this study also observed positive impacts of pistachio oil on the serum lipid profiles of rodents. Furthermore, a study on hypercholesterolemic rabbits showed the hypolipidemic effects of pistachio [26]. Thus, some other studies showed that pistachio consumption can decrease total cholesterol levels [27-30]. On the other hand, some other investigations have reported no significant effect of pistachio consumption in the daily diet (pistachio consumption as 15% of total caloric intake in the day) on total cholesterol in the hypercholesterolemia patients [31]. This inconsistency might be due to differences in study population (human vs animal).

Consumption of pistachio hydrolysate increased HDL levels, according to our findings. This result was in line with the results of additional research. An additional experimental investigation demonstrated a noteworthy impact of pistachios on HDL when incorporated into the diet [26, 32]. The results showed a significant

decrease in TG level. This finding was in line with other studies on rats [25], and rabbits [26]. On the other hand, another experimental investigation showed a significant increase in TG in rabbits which was not in line with our findings [32]. This difference in results can be due to different study methods and animals. Furthermore, another study by Kocyigit *et al.* found no significant effect of pistachio consumption on TG levels in the healthy participants [30]. Since the results of different studies were reported differently, it is recommended to conduct more studies in the future.

The use of *Pistachio Vera* hydrolyzed protein was the study's key strength. The absence of a serum LDL level evaluation was this study's most significant shortcoming.

5. Conclusion

The present study showed a favorable effect of the protein hydrolysates of pistachio kernels on the lipid profile of the HFD-fed rats. The protein hydrolysates can reduce TC and TG levels, and increase HDL levels in HFD-fed rats.

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conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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