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# The beneficial effects of almond (*Prunus amygdalus* Batsch) hull on serum lipid profile and antioxidant capacity in male rats

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**Background/aim:** Almond hull is produced in high amounts and mostly used as livestock feed. This study was designed to examine the impact of almond hull powder (AHP) administration on lipid profile and antioxidant activities in plasma and liver in hyperlipidemic rats.

**Materials and methods:** As the first step, the antioxidant capacity and radical scavenging activity of AHP were determined using calorimetric methods. Then 36 adult male Wistar rats were randomly divided into 6 groups: group 1 with oral administration of 10% AHP, group 2 with oral administration of 20% AHP, group 3 with oral administration of 30% AHP, group 4 as a positive control with a high cholesterol diet, group 5 as a negative control with a normal diet, and group 6 as a sham group with a normal diet and 20% AHP. The rats were fed a hypercholesterolemic diet to create a hyperlipidemia rat model and then they received AHP for 30 days.

**Results:** Antioxidant and radical scavenging activity of the AHP extract showed a high content of antioxidants that exert potent radical scavenging activity. According to the results obtained, upon the administration of AHP the levels of cholesterol and triglycerides significantly decreased, while the antioxidant capacity of plasma increased.

**Conclusion:** AHP with bioactive compounds and fiber can reduce total cholesterol and triglycerides and improve serum antioxidant capacity.

**Key words:** Almond (*Prunus amygdalus* Batsch.), antioxidant capacity, cholesterol, hyperlipidemia, *Rosaceae*

## 1. Introduction

Today hyperlipidemia and atherosclerosis are the main causes of heart disease and death in the world (1,2). Different factors such as dietary fat, sedentary lifestyle, smoking, obesity, insulin resistance, and diabetes are known as the main risk factors (3). It has been well documented that there is a direct link between the risk of coronary heart disease (CHD) and the levels of cholesterol in the serum (4). Cholesterol plays an important role in the normal physiology of the human body (5). It is a basic compound for biological membranes, membrane fluidity regulation, synthesis of bile acids, and steroids (6). On the other hand, hypercholesterolemia causes the formation of plaque in the artery wall and subsequently creates atheromatous fibrous plaques (7). Many studies suggest that hypercholesterolemia on endothelial-dependent relaxation can cause damage to rabbit and human endothelia (8). Some of these investigations suggest that hyperlipidemia leads to atherosclerosis, which happens prior to cardiovascular dysfunction (2,9). For example, in a study, hypercholesterolemia caused the formation of sclerotic lesions and reduced endothelial-dependent relaxation in rabbits (10). Altogether, it seems that in these studies the effects of hyperlipidemia and atherosclerosis on vascular function or endothelial dysfunction have been studied more. Hyperlipidemia indirectly stimulates the production of oxygen-free radicals and consequently the formation and progression of atherosclerotic plaques in CHD (10–13). Some epidemiological studies have shown that the consumption of foods rich in natural antioxidants increases plasma antioxidant capacity and reduces the risk of heart disease and cancer (14–17). Several studies have shown that antioxidant activity may be ascribed more to compounds such as flavonoids, isoflavones, flavones,

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anthocyanins, catechins, and other phenols (14,16–21). Free radical scavenging and metal chelating are two main mechanisms involved in this process (13,22).

Almond (*Prunus amygdalus* Batsch.) belongs to the family *Rosaceae*, which also includes apples, pears, peaches, and raspberries (23). It is one of the most popular tree nuts in the world and is ranked number one among the products of tree nuts (24). It is typically used as ingredients and snack food (25). The fruit of the almond includes four distinct parts: the seed or edible meat, the inner brown skin, the middle shell, and the outer green layer as hull (23). At the end of the almond fruit maturation process, the hull splits open. After complete drying of the hull, it is easily separated from the inner shell (25). The commercial importance of almond is related to its edible seed (23). According to recent investigations, almond seed with green hull, the middle shell, and the brown skin shows a high capacity in scavenging of free radicals and this radical scavenging activity could be related to the presence of various antioxidant compounds such as terpenoids, flavonoids, and other phenolic acids, which are mainly concentrated in the agricultural byproducts of almond fruit (10,18,23,24,26–38). Many studies have shown that the hull of almonds is a rich source of phenolic compounds (31,39). Catechin, protocatechuic acid, benzoic acid, and 4-hydroxy benzoic acid have been found in almond hull (23,26,34–37). It is estimated that more than 6 million tons of almond hull per year are produced and used as livestock feed. Thus, it can be used as a potentially good source of natural antioxidants or other beneficial compounds such as fiber (32). To the best of our knowledge, the hypolipidemic effects of almond fruit byproducts have not been investigated comprehensively. This prompted us to study the possible nutritional effects of almond hull on the level of several blood biochemical factors in hyperlipidemic rats.

## 2. Materials and methods

### 2.1. Plant material

Almond (*Amygdalus communis* L.) fruits were collected from Esfahlan, located in East Azarbaijan Province of Iran, in September 2013. Green hull was obtained by separating it from the nut. After air drying, it was reduced to fine powder. This powder was stored at room temperature until use.

### 2.2. Chemicals and reagents

Folin–Ciocalteu reagent, cholesterol, and colic acid were obtained from Merck (Darmstadt, Germany). Quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), and gallic acid were purchased from Sigma–Aldrich (St. Louis, MO, USA).

### 2.3. Animals

Male Wistar rats (210–250 g) were purchased from the Pasture Institute of Iran, Tehran, Iran. Animals received standard pellet diet and water was available ad libitum. They were housed in the Animal House of Tabriz University of Medical Sciences at a controlled ambient temperature of 25 ± 2 °C with 50 ± 10% relative humidity and with a 12 h light/12 h dark cycle. This study was performed in accordance with the Guide for the Care and Use of Laboratory Animals of Tabriz University of Medical Sciences, Tabriz, Iran.

### 2.4. High-fat diet

A high-fat diet was used with some modifications (40). The high-fat pellet diet contained standard rodent chow powder (62.75%), cholic acid (0.25%), cholesterol (2%), lard oil (15%), wheat flour (10%), and sucrose (10%).

### 2.5. Extraction procedure

To prepare almond hull powder (AHP) extract, 1 g of AHP was mixed with 20 mL of pure methanol and the resulting mixture was stirred for 30 min using a magnetic stirrer (41). The yielded extracts were filtered through filter paper and stored at 4 °C until use.

### 2.6. Total phenolic content (TPC)

The TPC of the AHP extract was determined with Folin–Ciocalteu reagent according to the method described by Singleton and Rossi (42) with some modifications. Briefly, 0.5 mL of AHP extract was mixed with 2 mL of 7.5% sodium carbonate, and then the mixture was allowed to stand at room temperature for 2 min. After the addition of 2.5 mL of tenfold Folin–Ciocalteu reagent, the mixture was incubated in the dark for 30 min. The absorbance was measured at 720 nm using a spectrophotometer (T60, PG Instruments Ltd., UK). The concentration of phenolic compounds in the AHP extract was expressed as milligrams of gallic acid per gram of extract.

### 2.7. Total flavonoid content (TFC)

The TFC of the AHP extract was assayed by the colorimetric method described by Zhishen et al. (43) and Jahanban Esfahlan and Jamei (29) with minor modifications. AHP extract (250 µL) was mixed with 1.25 mL of distilled water and 75 µL of a 5% $NaNO_2$ solution. After 5 min, 150 µL of a 10% $AlCl_3.H_2O$ solution, 500 µL of 1 M NaOH, and 275 µL of distilled water were added to the mixture. The absorbance of the mixture was measured at 507 nm. The content of flavonoids was expressed as milligrams of quercetin per gram of extract.

### 2.8. Reducing power

The reducing power of the AHP extract was determined according to the method described by Oyaizu (44). Methanolic extract of AHP (1 mL), phosphate buffer (1 mL, 0.2 M, pH 6.6) and potassium ferricyanide (1.0 mL, 10 mg/mL) were mixed together and incubated at 50 °C for 20 min. Trichloroacetic acid (1.0 mL, 100 mg/mL) was added to the mixture and it was centrifuged at 13,400 × *g* for 5 min. The supernatant (1.0 mL) was mixed with distilled

water (1.0 mL) and ferric chloride (0.1 mL, 1.0 mg/mL). The absorbance of the samples was measured at 700 nm.

**2.9. FRAP assay**

The ferric reducing antioxidant power (FRAP) assay was performed according to the method described by Benzie and Strain (45) and Sadat Moosavi Dolatabadi et al. (46), with some modifications. The stock solutions were 300 mM acetate buffer (3.1 g of $C_2H_3NaO_2.3H_2O$ and 16 mL of $C_2H_4O_2$), pH 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ solution. The fresh working solution was prepared by mixing 25 mL of acetate buffer, 2.5 mL of TPTZ solution, and 2.5 mL of $FeCl_3.6H_2O$ solution. The AHP extract (50 µL) was allowed to react with 950 µL of the FRAP solution for 20 min in the dark. The absorbance of colored product (ferrous tripyridyltriazine complex) was recorded at 593 nm. A standard calibration curve was obtained using different concentrations of $FeSO_4.7H_2O$. The results were expressed as mg $FeSO_4$/g extract.

**2.10. DPPH radical scavenging activity**

DPPH radical scavenging activity was determined according to the method described by Brand-Williams et al. (47) and Sadat Moosavi Dolatabadi et al. (48) with some modifications. First 100 µL of AHP extract was added to 1 mL of DPPH solution (0.1 mM in methanol) and then the reaction mixture was shaken vigorously. After the incubation of that solution at room temperature for 10 min, its absorbance was recorded at 517 nm. Radical scavenging activity was calculated as the percentage of DPPH radical scavenging from the following equation:

$$RSA\% = (A_{blank} - A_{sample}) / A_{blank} \times 100$$

**2.11. Experimental protocol**

Thirty-six adult male Wistar rats were randomly divided into six groups (with six animals in each group): group 1 with oral administration of 10% AHP, group 2 with oral administration of 20% AHP, group 3 with oral administration of 30% AHP, group 4 as a positive control with a high cholesterol diet, group 5 as a negative control with a normal diet, and group 6 as a sham group with a normal diet and 20% AHP. After 2 weeks, the rats received a high cholesterol diet for 40 days in order to create a hyperlipidemic rat model; they were then treated with AHP for 30 days.

**2.12. Preparation of blood samples**

To prepare the blood samples, the rats did not receive any food for 12 h. Blood samples (2 mL) were collected from the tail vein at the end of the hyperlipidemia creation period and after treatment with AHP. In this investigation ether was used for mild anesthesia.

**2.13. Biochemical tests**

The levels of total cholesterol, triglycerides, and HDL in the serum were determined by spectrophotometry (Abbott model Alcyon 300, USA) using Pars Company kits (Iran). The levels of total antioxidant (TAO), glutathione peroxidase (GPX), and superoxide dismutase (SOD) enzymes were also measured using Pars Company kits.

**2.14. Determination of lipid peroxidation**

To measure the malondialdehyde (MDA) level as a marker of oxidative stress in the serum, the method described by Kei (49) was used. In this method, the MDA level was determined using thiobarbituric acid by recording the absorbance of samples with T70 UV/VIS spectrophotometer (PG Instruments Ltd, Lutterworth, UK). Lipid peroxidation was expressed as nanomole MDA production per milliliter of serum.

**2.15. Histological studies**

For histological study, biopsies of liver tissues (n = 3) of all groups were obtained and fixed in 10% neutral-buffered formaldehyde for 48 h, embedded in paraffin, and sectioned at 5 µm. The sections were stained with hematoxylin and eosin and then examined under light microscopy (400×).

**2.16. Statistical analysis**

All the assays were performed in triplicate. The data are presented as means ± SEM. Comparisons between groups were conducted by one-way ANOVA. If ANOVA analysis indicated significant differences, a Student–Newman–Keuls posttest was performed to compare mean values between the treatment and control groups.

## 3. Results

The Table shows the TPC, TFC, reducing power, FRAP assay of the AHP extract, and its DPPH radical scavenging activity. Phenolic compounds form a blue complex with

**Table.** Antioxidant content and radical scavenging activity of almond hull extract.

| Total phenolic content (TPC) | Total flavonoid content (TFC) | Reducing power | FRAP | DPPH |
|---|---|---|---|---|
| 398 ± 2.45[a] | 73.9 ± 3.25[b] | 0.651 ± 0.05 | 16.3 ± 0.25[c] | 74.8[d] |

[a] mg Gallic acid equivalents/g extract.
[b] mg Quercetin equivalents/g extract.
[c] mg $FeSO_4$.
[d] DPPH scavenging percentage.

phosphomolybdic acid and sodium tungsten phosphate. Hence, in the present study the TPC of the AHP extract was determined spectrophotometrically using Folin–Ciocalteu reagent. The TPC of the AHP extract was 398 ± 2.45 mg of gallic acid per gram of extract.

In order to determine the TFC of the AHP extract, another colorimetric method was employed using $AlCl_3$. In this method, the keto and hydroxyl groups in the rings A or C of flavonoids interact with $AlCl_3$ and generate stable complexes. In addition, the interaction of orthodihydroxyl groups in the rings A or B of flavonoids with $AlCl_3$ creates unstable acidic complexes. In the presence of flavonoid compounds, $AlCl_3$ shows high absorbance at 507 nm. Flavonoids with more functional groups have strong absorbance at this wavelength. The TFC of the AHP extract was 73.9 ± 3.25 mg of quercetin per gram of extract (Table).

The reducing power of the AHP extract was determined according to the method described by Oyaizu (44). The electron donating ability of the phenolic compound is an important mechanism of antioxidant activity. The oxidation and reduction of iron is an indicator in this method. The reducing power absorbance for almond hull extract was obtained as 0.651 ± 0.05 (Table).

In the FRAP method the antioxidant capacity of a sample can be evaluated by the ability of the extract to reduce tripyridyltriazine ferric ($Fe^{3+}$-TPTZ) to ferrous ($Fe^{2+}$) form at acidic pH. The absorbance of $Fe^{2+}$-TPTZ complex with the blue color can be recorded at 593 nm. According to the FRAP method, in our study the antioxidant activity of the AHP extract was obtained as 16.3 ± 0.25 mg of $FeSO_4$ per gram of extract (Table).

The antioxidant activity of the AHP extract was also measured in terms of its radical scavenging activity using DPPH free radicals. The methanolic extract of AHP showed good activity in DPPH radical scavenging (74.8%) (Table).

In comparison with the normal diet, the administration of a high-fat diet for 40 days significantly increased the serum level of total cholesterol and triglycerides ($P < 0.001$) (Figure 1). The administration of AHP in the test groups for 30 days, compared with the hypercholesterolemic rats, significantly decreased the serum level of total cholesterol and triglycerides ($P < 0.05$, $P < 0.01$, and $P < 0.001$). Additionally, AHP administration significantly increased the level of HDL in the serum of the test groups ($P < 0.05$, $P < 0.01$, and $P < 0.001$) (Figure 2).

The effects of AHP administration on the serum level of MDA are shown in Figure 3. After treatment with AHP, the serum level of MDA in all test groups significantly decreased ($P < 0.05$). Moreover, the administration of AHP reduced the serum level of MDA in the sham group ($P < 0.01$).

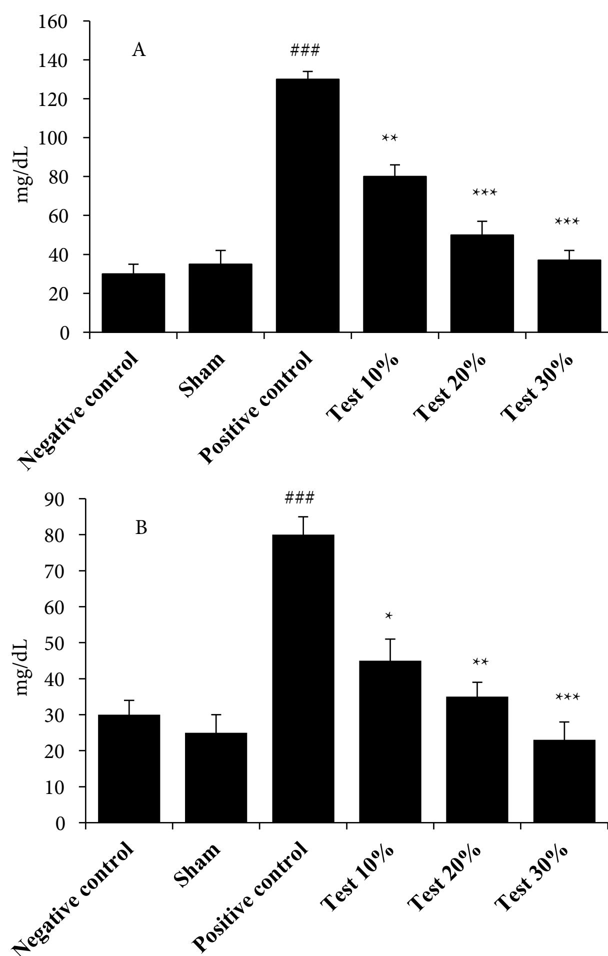


**Figure 1.** Effects of AHP administration for 30 days on the levels of total cholesterol (A) and triglycerides (B) in the serum of hypercholesterolemic and normal rats. Data are expressed as means ± SEM. n (number of rats per group) = 6. ###$P < 0.001$ compared with negative control group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with the positive hypercholesterolemic group using ANOVA.

Figures 4–6 show the effects of AHP administration on the levels of GPX and SOD (as two main antioxidant enzymes) and TAO. A significant increase was observed ($P < 0.05$ and $P < 0.01$) in the levels of GPX, SOD, and TAO in the test groups in comparison with the control group. However, a significant difference was not seen between the hypercholesterolemia and the control groups.

Figure 7 shows the liver tissue sections of the normal, hyperlipidemic, and test groups. In the histopathological studies, the liver tissue of the control group was normal (Figure 7A). While the hyperlipidemic group received only fat diet for 5 weeks, severe liver damage microvesicularly and macrovesicularly was observed (Figure 7B). Finally, in the test groups AHP administration significantly

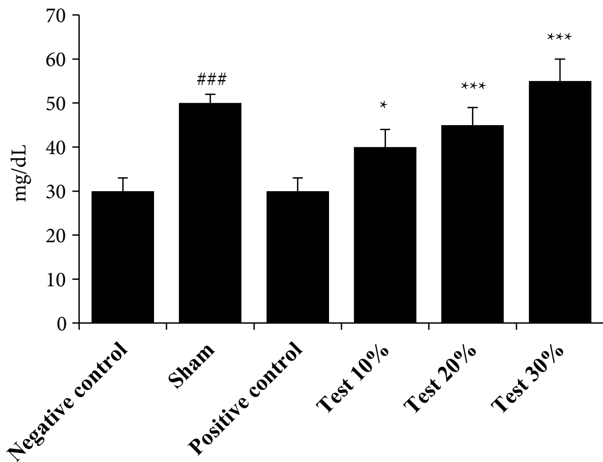


**Figure 2.** Effects of AHP administration for 30 days on the levels of HDL in hypercholesterolemic and normal rats. Data are expressed as means ± SEM. n (number of rats per group) = 6. ### $P < 0.001$ compared with the negative control group. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ compared with the positive hypercholesterolemic group using ANOVA.

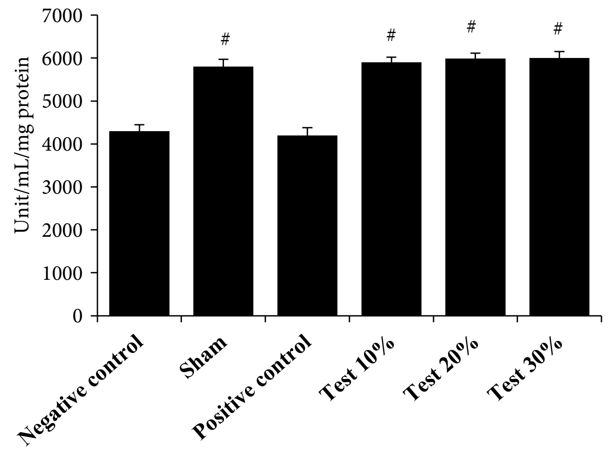


**Figure 4.** Effects of AHP administration for 30 days on the levels of GPX in hypercholesterolemic and normal rats. Data are expressed as means ± SEM. n (number of rats per group) = 6. #$P < 0.05$ compared with the negative control group.

decreased the lipid accumulation and abnormality in the liver tissue (Figure 7C).

## 4. Discussion

It is well known that free radicals cause oxidative damage to lipids, proteins, and nucleic acids (14). Although antioxidant enzymes such as SOD, catalase, and GPX in the body play an important role in scavenging oxidants and preventing damage to the cells, this defense mechanism is not sufficient. Therefore, cellular macromolecules are easily exposed to damage (50). Some epidemiological studies have shown that foods rich in natural antioxidants increase plasma antioxidant capacity and reduce the risk of heart disease and cancer (14). In addition to α-tocopherol, ascorbic acid, and carotenoids, various phenolic compounds are synthesized in plant tissues (51). Several studies have shown that the major part of the antioxidant activity may be related to the presence of compounds such as flavonoids, isoflavones, flavones, anthocyanins, catechins, and other phenols collecting free radicals and chelating metals as main mechanisms involved in this

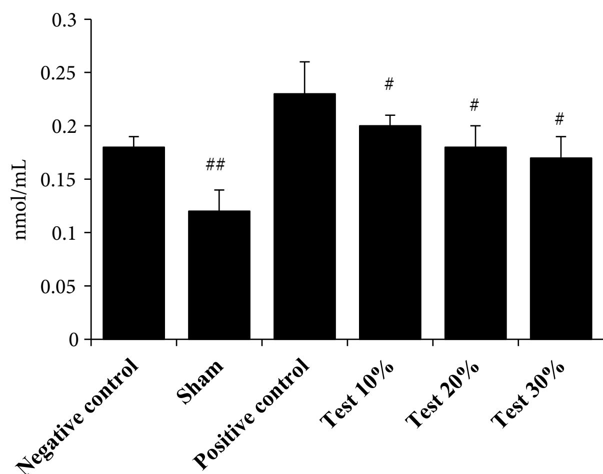


**Figure 3.** Effects of AHP administration for 30 days on the levels of MDA in hypercholesterolemic and normal rats. Data are expressed as means ± SEM. n (number of rats per group) = 6. #$P < 0.05$ and ##$P < 0.01$ compared with the negative control group.

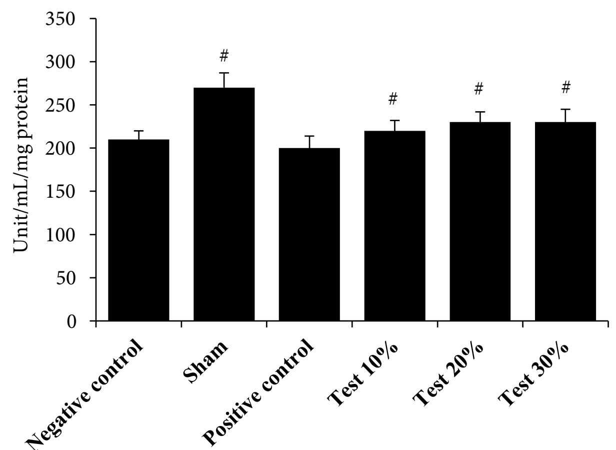


**Figure 5.** Effects of AHP administration for 30 days on the levels of SOD in hypercholesterolemic and normal rats. Data are expressed as means ± SEM. n (number of rats per group) = 6. #$P < 0.05$ compared with the negative control group.

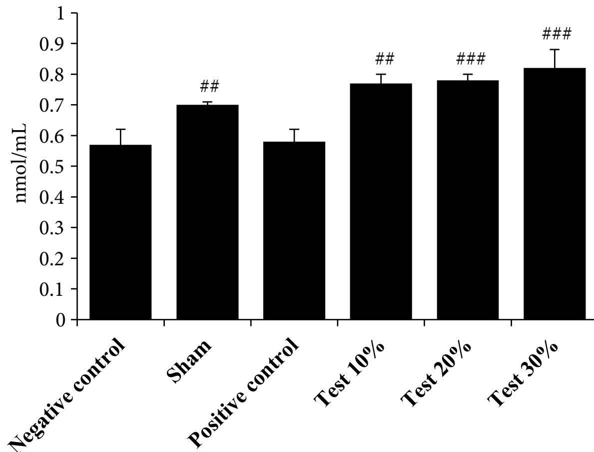


**Figure 6.** Effects of AHP administration for 30 days on the levels of TAO in hypercholesterolemic and normal rats. Data are expressed as means ± SEM. n (number of rats per group) = 6. ##P < 0.01 and ###P < 0.05 compared with the negative control group.

process (24). Many aromatic herbs, spices, and medicinal and other plants, such as nuts and beans, show antioxidant properties with protective effects (52). Nuts have been associated with the Mediterranean diet and their regular consumption in appropriate doses can reduce the risk of heart disease (37). Moreover, an anticancer activity of nuts has been demonstrated in animals. These beneficial effects are mainly related to lipid, arginine, fiber, vitamin E, and other antioxidant compounds such as polyphenols (23,53).

In the present study, the antioxidant content and radical scavenging activity of the AHP extract were determined as the content of phenols and flavonoids, and consequently radical scavenging activities. Our results indicated a high antioxidant content and powerful radical scavenging activity of the AHP extract. In another study by Jahanban Sfahlan et al. (24), the average of TPC for the AHP extract was 78.2 ± 3.41 mg of gallic acid per gram of extract. It can be seen that the measured TPC for the AHP extract in this study is higher than that of the above value. The average value for the reducing power of the AHP extract in the study by Jahanban Sfahlan et al. (24) was reported as 0.519, which is consistent with our results. The evaluation of the radical scavenging activity of the AHP extract by FRAP and DPPH assays indicated the high content of antioxidants in the extract of AHP.

Hyperlipidemia is one of the major risk factors for atherosclerosis and endothelial dysfunction (2,54). Decreasing the level of serum lipid plays an important role in reducing the risk of cardiovascular disease and increasing longevity (13,55). For example, it has been reported that a 10% reduction in the total cholesterol of serum may reduce the incidence of CHD by about 30% (55). In the present study, cholesterol (2%) and lard oil (15%) were added to the diet of rats for 40 days and significant increases in the serum levels of total cholesterol and triglycerides were obtained. These results, with some differences, are in agreement with the results of other studies in which an increase in rat dietary cholesterol intake resulted in plasma or serum cholesterol elevation (40). In addition to current drug therapies, the role of nutrition in controlling hyperlipidemia has been established (56). In this regard, plants are used as food to treat cardiovascular diseases (16). Recent investigations have shown that polysaccharides, flavonoids, polypeptides, steroids, alkaloids, and pectin in plants can reduce the risk factors of cardiovascular disease (57,58). It has been revealed that the consumption of almond kernels with or without brown skin is effective in reducing the level of total cholesterol and LDL (59). Almonds with skin, hull, and shell show a powerful capacity in collecting free radicals and this activity can be related to the presence of unsaturated fats, terpenoids, flavonoids, phenolic acids, and other beneficial compounds including fiber (18,23,24,26–30,32–38,60–63). Several studies have shown that the almond hull is a rich source of phenolic compounds and fiber (31,39,60,62). Thus, the considerable decrease in lipid parameters in the test groups could be due to the fiber content of AHP. Fibers can decrease plasma LDL levels by inhibiting the cholesterol and bile acid absorption and increasing LDL receptor activity. The decline in cholesterol level in the test groups showed the possible impact of AHP fiber. Moreover, dietary fibers affect the cholesterol absorption and enterohepatic bile circulation, and consequently reduce hepatic cholesterol. Additionally, regimes rich in fibers reduce TG levels by inhibiting hepatic lipogenesis. The results of the present study showed a significant reduction in the levels of total cholesterol and TG after treatment with AHP, as well as a considerable increase in HDL levels. Moreover, AHP administration improved the antioxidant capacity of plasma by enhancing the level of TAO and the content of antioxidant enzymes GPX and SOD. CAT, SOD, and GPx are known as the main enzymes present in the plasma and liver contributing in the antioxidant defense mechanism. In the present study, a reduction in the levels of the antioxidant enzymes SOD and GPx in hypercholesterolemic rats was observed, compared with the negative control group. Such decreases may be related to the generation of α- and b-unsaturated aldehydes during lipid peroxidation. These compounds enhance oxidative stress by promoting the cellular consumption of glutathione and inactivating selenium-dependent GPX. Considering the endogenous stress-related markers (SOD and GPx), our results indicate that AHP could improve the efficiency of the superoxide radical's conversion to

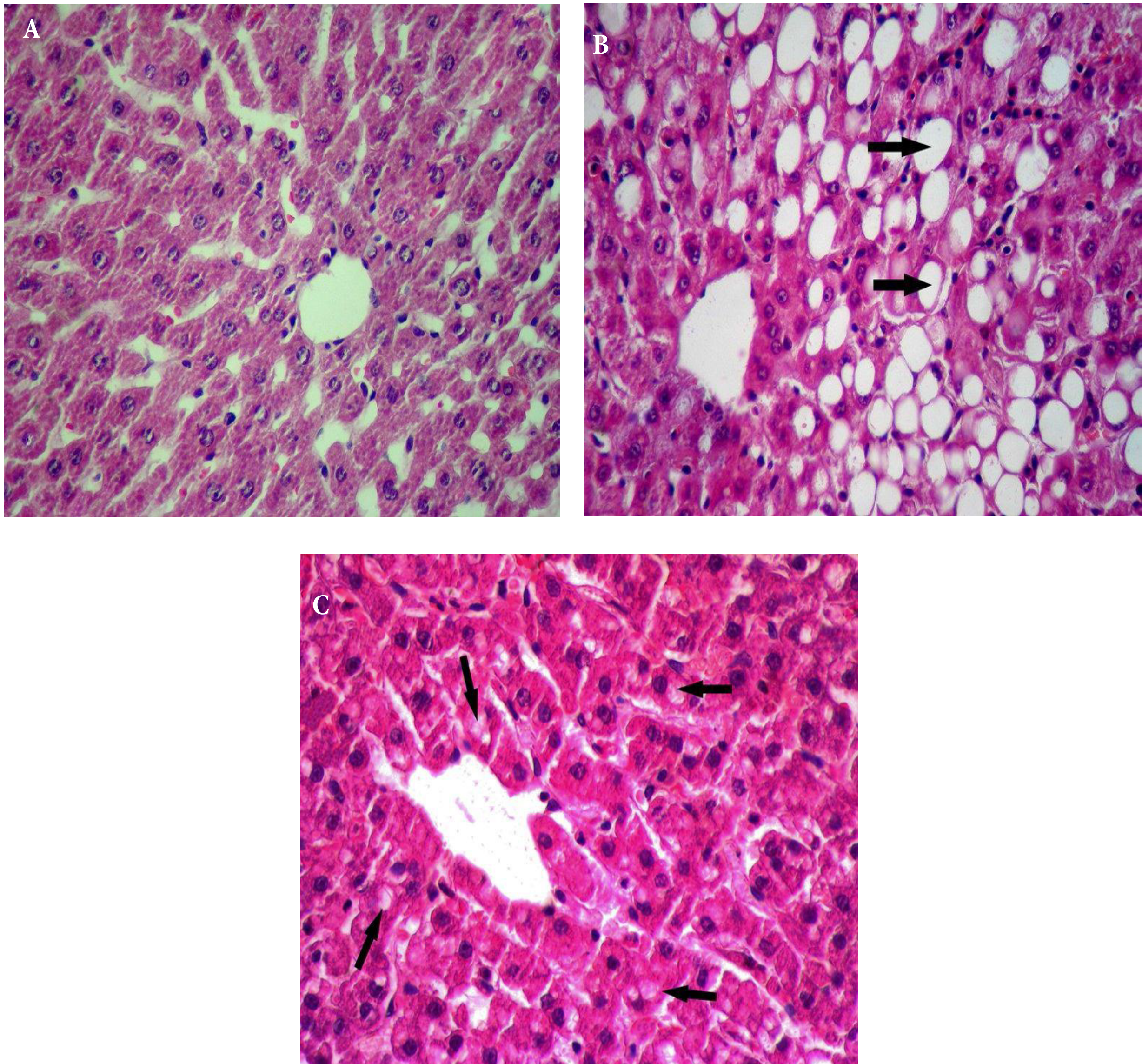


**Figure 7.** Liver tissue sections: (A) The liver tissue section from the negative control group shows the normal tissue structure. (B) The liver tissue section from the positive hypercholesterolemic group shows abnormal accumulation of lipids as steatosis within the cells. Lipids were accumulated in the cytoplasm of the cells and the nuclei were distorted. (C) The liver tissue section from the hypercholesterolemic group that received AHP shows reduction of lipid accumulation in the cytoplasm of the liver cells. Original magnification was 400× and H&E staining was used.

hydrogen peroxide and SOD activity in the test groups following deactivation of hydrogen peroxide by GPX. The increase in SOD activity might constitute protection against superoxide anion elevation. Because SOD catalyzes the decomposition of superoxide anions to hydrogen peroxide ($H_2O_2$), this enzyme prevents further production of free radicals. Superoxide radicals are converted by SOD to $H_2O_2$, which is broken down by catalase and GPx. GSH serves as a substrate for the enzyme GPx, and it has been proposed that, through its activity, GSH protects plasma against oxidative damage.

In a hypercholesterolemic regime, liver as the primary organ metabolizes the excess cholesterol, and thus is damaged by oxidative stress. Oxidative stress comes from an imbalance between the generation of free radicals and the effectiveness of the antioxidant defense system. In the liver cells, endogenous prooxidant conditions promote the development of atherosclerosis. Increased oxidant stress plays an important role in the chronic inflammatory responses to atherosclerosis and hypercholesterolemia. Thus, free radicals and lipid peroxidation in oxidative stress conditions lead to cell damage, including destruction of the cell membrane structure.

AHP showed powerful free radical scavenging capacity and this activity could be due to the presence of terpenoids, flavonoids, and other phenolic compounds. Almond hull, as an important agricultural byproduct of a fruit with valuable bioactive compounds and fiber, could reduce total cholesterol and triglycerides, and increase the plasma antioxidant capacity of hyperlipidemia rats. The effects of this agricultural byproduct of almond fruit on hyperlipidemic rats indicated that AHP with low economic value can be used for the treatment of hyperlipidemia. It is hoped that the results of the present study will open new horizons in the application of almond hull as an agricultural waste product in pharmaceutical and human nutrition.

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