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Protective effect of hydro alcoholic extract of *Pistacia atlantica* against acetaminophen-induced hepatotoxicity and oxidative stress in male rats

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Pistacia atlantica (p.atlantica) **has antioxidant, anti-inflammatory and anti-viral properties. This study was undertaken to evaluate the protective effect of *P.atlantica* against acetaminophen-induced hepatotoxicity in male rats. Forty eight male wistar rats were used in this study. The rats were randomly divided into six groups. Group 1, the control group; group 2, the rats that received acetaminophen only; group3, the rats that received acetaminophen plus silymarin; and groups 4, 5 and 6 were treated with acetaminophen plus *p.atlantica* extract (200, 400, 800 mg/kg, respectively). At the end of study, the blood specimens and liver samples were examined. In the acetaminophen-only treated group, the levels of serum HDL and liver CAT, SOD and vitamin C significantly decreased compared to control animals. Also, in this group, total cholesterol, TG, LDL, VLDL, protein carbonyl (PC), MDA, serum TNF-α and TNF-α gene expression significantly increased compared to the control group. Treatment with *Pistacia atlantica* resulted in a significant increase in CAT, SOD, vitamin C , HDL and a significant decrease in the level of MDA, PC, TG, LDL, VLDL, total cholesterol, serum TNF-α, and gene expression of TNF-α compared to the acetaminophen-only treated group. The present study suggests that p.atlantica leaves has protective effects against Acetaminophen-induced hepatotoxicity and oxidative stress.**

INTRODUCTION

Acetaminophen (paracetamol) is one of the most common drugs used as an anti-fever and analgesics that is completely safe in its therapeutic dose but it is responsible for liver toxicity in high dose (1). Toxicity with acetaminophen causes damage to the kidney and liver cell membrane, and studies have shown that kidney injury caused by acetaminophen, is significantly related to oxidative stress (1, 2). Acetaminophen overdose causes excessive production of its metabolite (N-acetyl-p-benzoquinone imine), which inhibits the mitochondrial oxidative phosphorylation, which results in inhibition the synthesis of ATP and reducing the membrane potential and inducing oxidative stress (1). Previous studies have shown that overdose with acetaminophen results in the release of antioxidants and increased free radicals and cell death (3). Medicinal plants are known to be natural antioxidants worldwide, which have beneficial effects on various diseases, such as cancer and liver diseases (4). Medicinal plants today are inexpensive and valuable resources that are widely used to progress in the treatment of various liver diseases (5, 6). *Pistacia atlantica* (*p.atlantica*) is one of the traditional herbs in the treatment of liver, kidney and digestive diseases in Iran, Algeria, Turkey, Jordan, and Greece (7). *P.atlantica* has antioxidant, antiviral and anti-inflammatory properties (8, 9). The aim of this study was evaluate the effects of the hydro alcoholic extract on hepatotoxicity induced by acetaminophen in male rats.

MATERIALS AND METHODS

Chemicals

Acetaminophen was purchased from the company Sigma. The kits for evaluation Plasma triglyceride (TG), total cholesterol (TC), glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), urea and creatinine were purchased from the Pars Azmoon Company. Nitro blue tetrazolium, 2,4,6-Tripyridyl-s-triazine (TPTZ), and 2,4-dinitrophenylhydrazine (2,4-DNPH) prepared from Sigma company. hydrogen peroxide, 2-Thiobarbituric acid, sodium acetate, Coomassie brilliant blue G250 and ferric chloride (Fecl3,6H2O) was purchased from Merck Company. SYBR® Green PCR Master Mix was provided by Qiagen Co. All other chemicals were analytical grade.

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Extraction method

P.atlantica **(**herbarium number:93) was prepared from the Zagros mountains in southwest of Iran. The plant was dried at room temperature in the vicinity of the air, then crushed and was extracted with ethanol: water (70:30). Finally, the extracts were stored at 5°C.

Experimental animals

48 male Wistar rats (200 ± 20 gr) were used in this study. All rats were preserved in standard conditions (humidity 55 ± 5 and 12 hours in darkness - brightness and 23 ± 2 ° C, with enough water, and sufficient food). The rats were randomly divided into six groups as following; group 1, the control group, received intraperitoneally serum physiology, group 2 received intraperitoneally acetaminophen at dose of 835 mg/kg (10), group 3 (positive control) received intraperitoneally acetaminophen at dose of 835 mg/kg (10) and one hour later silymarin at dose of 50 mg/kg was given orally (11), The groups 4, 5 and 6 were injected intraperitoneally with 835 mg/kg of acetaminophen (10) and after an hour received p.atlantica (200, 400 and 800 mg/kg respectively) orally (12). After 7 days, the rats were anesthetized fasting and blood specimens were collected by cardiac puncture and centrifuged at 3000 rpm for 15 min. Plasma and serum were prepared from the blood samples for different biochemical analyses. Each liver sample was divided to determine liver catalase (CAT), superoxide dismutase (SOD), tumor necrosis factor-α (*TNF-α*) gene expression, and to conduct histopathological examinations.

Biochemical analysis

Serum levels of GOT, GPT, TG, TC, LDL-C, HDL-C were measured by an enzymatic method using an auto analyzer (BT 3000, France). Serum TNF-α levels were measured by ELISA kit.

Measurement of tissue and serum malondialdehyde

The tissue and serum MDA levels were measured according to the Agarwal protocol by high-performance liquid chromatography (HPLC) (13). According to this method 1.1, 3.3 tetraethyl propane was used for MDA standards and the results were expressed as micro molar (µM).

Measurement of plasma antioxidant capacity

Plasma antioxidant capacity was measured with FRAP (Ferric Reducing Ability of Plasma) assay using TPTZ and according to previous methods (14).

Measurement of liver catalase and SOD activities

Tissue SOD activity was measured based on inhibion of NBT recovery at 560nm using Beauchamp and Fridivich method (15). Liver catalase activity was also measured according to previous methods (16) The total protein was calculated according to the Bradford method (17).

Determination of vitamin C level in the liver

Vitamin C was measured by Stanley and Omaye method using 2,4 dinitrophenylhydrazine (18). In this method 100 mg of liver tissue was homogenized in 900 µl of trichloroacetic acid 5% and was centrifuged at 3500g for 20 minutes. Then, 500µl supernatant was mixed with 100µl 2,4 dinitrophenylhydrazine /thiourea/copper (DTC) and incubated at 37 ° C for 3 hours. Then, 750 µl of sulfuric acid 65% was mixed to the reaction and incubated at 25 Centigrade degree for 30 minutes. The absorbance was measured at 520 nm and the standard curve was plotted at 0-20µ g/µl for vitamin C.

Serum Protein carbonyl Measurement

Serum protein carbonyl was measured according to the Reznick and Packer method was used for measurement ofr Serum protein carbonyl content (19), using Guanidinium 6M. The content of protein carbonyl was shown as nmol/mg and total protein was measured by Bradford method (17).

Real-time quantitative PCR (RT-qPCR)

The liver mRNA was isolated using the Thermo Scientific kit according to the manufacturer's protocol. Quantity and quality of total RNA were done by absorbance of 260/280 nm using a spectrophotometer by the Nanodrop2000 (Thermo USA) (20). cDNA measurement was done by the PrimeScript^{TM} reagent Kit (Takara Bio Inc. Japan) and in accordance with the instructions. Then cDNA amplified according to RT-qPCR and using SYBR® Green PCR Master Mix in the presence of specific primers for *TNF-α* (forward: 5´-CTGGCGTGTTCATCCGTTC-3´, reverse: 5´-GGCTCTGAGGAGTAGACGATAA-3´) and *18s* (forward: 5´CGCAAATTACCCACTCCCGAC-3´, reverse: 5´GCGTTTAATGGGTGAGGGCTG-3´) genes. Primers after design with Oligo 7.0 software was approved using Blast-Nucleotide (NCBI). Primers were synthesized from the pioneer company. PCR carried out in primary denaturation at 95 ° C for 10 minutes. RT-qPCR was performed in 40 cycles (including secondary denaturation at 15 seconds at 95 °C, 20 seconds at 60 °C for annealing, 25 seconds for 72 °C for extension). *18s* gene was used as an internal control gene to control the expression of mRNA.

Histopathological studies

After scarifying the rats, the rat liver was fixed in 10% formaldehyde solution and then by microtome was selected in 5µm pieces and stained with hematoxylin and eosin (21). Tissue changes were observed with the optical microscope.

Ethical principles

This article has been adapted from a research project approved by the Ethical committee approval code: IR.SKUMS.REC.1394.300. This research was financially supported by Shahrekord University of Medical Sciences, Shahrekord, Iran (grant no. 1587).

Statistical analysis

The results for each group analyzed and compared with different groups by the SPSS software version 20. One-way ANOVA was used to compare the mean of the groups and Tukey test was used to compare the groups. In our research, $P < 0.05$ was statistically considered significant.

RESULTS

Effect of hydro alcoholic extract of *P.atlantica* leaves on serum parameters

Consumption of Acetaminophen for 7 days led to a noticeable elevation (P<0.05) in serum total cholesterol, triglyceride, LDL-C, and VLDL-C compared to the control group (Table 1). Treatment with hydro alcoholic extracts of *P.atlantica* leaves at doses of 400 and 800 mg/kg of body weight reduced the lipid profile in dose dependent manner compared with the group receiving acetaminophen alone. The decrease of lipid profiles in the receiving group of 800 mg/kg of the hydro alcoholic extract of *P.atlantica* leaves was significant (P<0.05) compare to the acetaminophen-only treated group. However, treatment with this extract at dose of 200 mg/kg did not significantly change in lipid profiles. Receiving of silymarin in the third group resulted in a significant

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Table 1 Effects of *Pistacia atlantica* on serum lipid profile in different groups of rats

Parameters (mg/dl)	Group1	Group2	Group3	Group4	Group5	Group6
TG	113.87±3.52	140.25±5.52[a]	113.87±3.75[b]	133.75±3.61[ac]	128.87±2.47[abc]	110.75±2.05[bde]
TC	45.62±3.42	57.87±2.41[a]	51.37±1.84[ab]	56.50±2.90[ac]	52.75±1.83[abd]	50.25±1.66[bd]
HDL-C	34.52±2.39	28.80±1.60[a]	34.35±2.24[b]	28.67±2.26[ac]	31.24±1.86	33.06±1.32[bd]
LDL-C	9.78±0.809	15.52±0.98[a]	11.6±0.74[ab]	13.6±1.03[ac]	13.14±0.86[ab]	10.82±0.48[bde]
VLDL	22.77±0.704	28.05±1.104[a]	22.77±0.751[b]	26.95±0.85[ac]	25.73±0.582[abc]	22.15±0.410[bde]

Triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), very low density lipoprotein cholesterol (VDL-C)

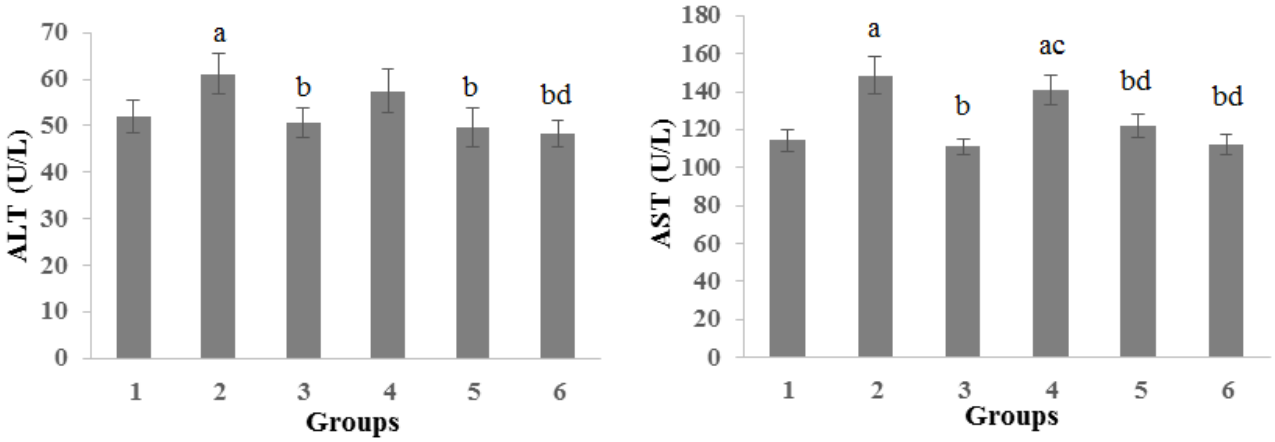

Figure 1 Effect of *P.atlantica* extracts on ALT and AST activities. Group 1 was the control; group 2 was acetaminophen-only treated; group 3 was treated with acetaminophen plus silymarin; groups 4, 5 and 6 were treated with acetaminophen plus *Pistacia atlantica* hydro alcoholic leaves extract at the doses of 200, 400, and 800 mg/kg, respectively. [a]p<0.05 compared to the control group (group 1). [b]p<0.05 compared to the acetaminophen-only treated group (group 2). [c] p<0.05 compared to the group treated with acetaminophen plus silymarin (group 3). [d]p<0.05 compared to the group treated with acetaminophen plus *Pistacia atlantica* hydro alcoholic leaves extract at doses of 200mg/kg (group 4). [e]p<0.05 compared to the group treated with acetaminophen plus *Pistacia atlantica* hydro alcoholic leaves extract at doses of 400mg/kg (group 5).

decrease (P <0.05) in the lipid profile compared to the acetaminophen-only treated group.

Effects of hydro alcoholic extract of P.atlantica leaves on serum ALT and AST

The results indicated that administration of acetaminophen for 7 days resulted in a significant increase (P<0.05) in AST and ALT in rats receiving acetaminophen compared to the control group (Figure 1). Treatment with hydro alcoholic extracts of *P.atlantica* leaves in doses of 200, 400 and 800 mg/kg reduced the serum levels of AST and ALT in a dose-dependent manner compared to the animals receiving acetaminophen alone. AST and ALT levels decreased significantly (P <0.05) in the doses of 400 and 800 mg/kg, compared with those receiving acetaminophen alone. Administration of silymarin in group 3 resulted in a significant decrease (P <0.05) in serum AST and ALT levels compared to the acetaminophen-only treated group.

The effects of hydro alcoholic extract of P.Atlantica leaf on the CAT activity in liver tissue

Liver toxicity induced by acetaminophen resulted in a significant decrease (p <0.05) in catalase activity in the acetaminophen-only treated group compared to the control group (Table 2). However, treatment with the hydro alcoholic extract of P.atlantica leaf at doses of 400 and 800 mg/kg significantly increased catalase activity compared to the acetaminophen-only treated group. The catalase tissue levels in the silymarin treated group increased significantly compared to the acetaminophen-only treated group. Treatment with dose of 800 mg/kg restored the catalase tissue level at the control group level.

The effects of Hydro alcoholic Extract of P.atlantica leaves on SOD Activity in liver Tissue

In table 2 by comparing the level of SOD activity in different groups, it was found that the activity of CAT in the acetaminophen-only treated group decreased significantly (P<0.05) compared to the control group. Treatment with the hydro alcoholic extract of *Pistacia atlantica* leaves at doses of 400 and 800 mg/kg significantly increased (P <0.05) the activity of SOD compared to the group receiving acetaminophen alone. However, in the group receiving a dose of 800 mg/kg the level of SOD was higher (P <0.05) than the group receiving a dose of 400 mg/kg. Administration of silymarin caused a significant increase in SOD level compared to the acetaminophen-only treated group.

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Table 2 Effect *Pistacia atlantica* extract on catalase (CAT) and superoxide dismutase (SOD) activities liver Vittamine C and protein carbonyl (PC)

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
CAT (U/mg protein)	50.85±1.92	32.16±1.94[a]	49.56±1.84[b]	30.27±1.40[ac]	43.96±1.29[abcd]	50.71±2.44[bde]
SOD (U/mg protein)	17.84±2.15	6.90±1.48[a]	17.06±2.79[b]	7.19±1.39[ac]	11.69±2.01[abcd]	16.06±1.15[bde]
Vitamin C (mg/g liver)	11.05±0.42	8.21±0.49[a]	10.71±0.32[b]	9.29±0.49[abc]	10.52±0.31[bd]	11.16±0.26[bde]
PC (nmol DNPH/mg protein)	5.85±1.06	11.86±1.81[a]	4.52±1.58[b]	9.98±1.53[ac]	7.36±1.33[b]	3.89±1.20[bde]

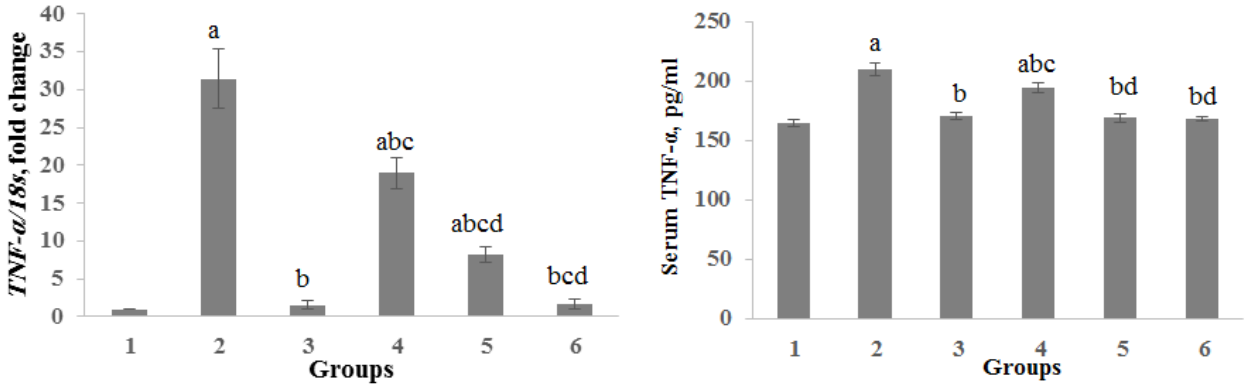

Figure 2 The effects of Hydro alcoholic Extract of *Pistacia atlantica* leaves on on serum TNF-α and expression of TNF-α. Group 1 was the control; group 2 was acetaminophen-only treated; group 3 was treated with acetaminophen plus silymarin; groups 4, 5 and 6 were treated with acetaminophen plus *Pistacia atlantica* hydro alcoholic leaves extract at the doses of 200, 400, and 800 mg/kg, respectively. [a]p<0.05 compared to the control group (group 1). [b]p<0.05 compared to the acetaminophen-only treated group (group 2). [c] p<0.05 compared to the group treated with acetaminophen plus silymarin (group 3). [d]p<0.05 compared to the group treated with acetaminophen plus *Pistacia atlantica* hydro alcoholic leaves extract at doses of 200mg/kg (group 4). [e]p<0.05 compared to the group treated with acetaminophen plus *Pistacia atlantica* hydro alcoholic leaves extract at doses of 400mg/kg (group 5).

The Effects of Hydro alcoholic Extract of P.atlantica leaves on Vitamin C Level in liver Tissue

The level of vitamin C in the acetaminophen-only treated group decreased significantly (p<0.05) compared to the control group (Table2). Treatment with doses of 200, 400 and 800 mg/kg hydro alcoholic extract of *P.atlantica* leaves showed a significant increase (p<0.05) in the vitamin C level in dose dependent manner compared to the acetaminophen-only treated group.

The effect of hydro alcoholic extract of P.atlantica leaves on the serum protein carbonyl

The serum protein carbonyl level in the acetaminophen-only treated group indicated a significant increase (p<0.05) compared to the control group (Table 2). The results demonstrated that administration of hydro alcoholic extract of *P.atlantica* leaves at doses of 200, 400 and 800 mg/kg reduced the level of protein carbonyl in comparison with the acetaminophen-only treated group. This decrease in the levels of PC in groups receiving extract of *P.atlantica* at doses of 400 and 800 mg/kg was significant (p <0.05) compared to the acetaminophen-only treated group. The serum level of PC in the silimarin treated group decreased significantly (P <0.05) in comparision with the acetaminophen-only treated group.

The effects of hydro alcoholic extract of P.atlantica leaves on serum and tissue MDA levels

Hepatotoxicity induced by acetaminophen in the acetaminophen-only treated group resulted in a significant increase in serum and tissue malondialdehyde (p<0.05) compared to the control group (Table 3). In groups treated with hydro alcoholic extract of *P.atlantica* leaves at doses of 200, 400 and 800 mg/kg the MDA levels in serum and liver significantly decreased (P<0.05) compared to the acetaminophen-only treated group. Also, treatment with alcoholic extract of *P.atlantica* at dose of 800 mg/kg could result in a further reduction in the MDA levels of serum and liver compared to the groups receiving hydro alcoholic extract of *P.atlantica* at doses of 200 and 400 mg/kg. The MDA levels of serum and liver in the silymarin treated group decreased significantly (P<0.05) compared to the acetaminophen-only treated group.

The effect of hydro alcoholic extract of P.atlantica leaves on plasma antioxidant capacity

Table 3 shown that administration of acetaminophen in the acetaminophen-only treated group caused a significant reduction (p<0.05) in serum antioxidant capacity compared to the control group. Treatment with hydro alcoholic extract of *P.atlantica* leaves with doses of 400 and 800 mg/kg significantly increased (P<0.05) plasma

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Table 3 Serum and tissue liver malondialdehyde (MDA) and plasma FRAP levels

(Parameters μM)	Group1	Group2	Group3	Group4	Group5	Group6
Serum MDA	26.03±1.98	59.08±2.02^{a}	26.22±3.43^{b}	38.98±0.57^{abc}	32.54±1.08^{abcd}	27.44±2.19^{bde}
Liver MDA	25.3±2.27	51.90±7.61^{a}	26.98±2.63^{b}	35.79±3.23^{abc}	32.20±0.88^{abc}	26.48±1.28^{bde}
FRAP	635.67±61.3	440.45±23.17^{a}	611.20±89.21^{b}	447.95±39.26^{ac}	540.7±35.64^{bd}	870.69±68.98^{abcde}

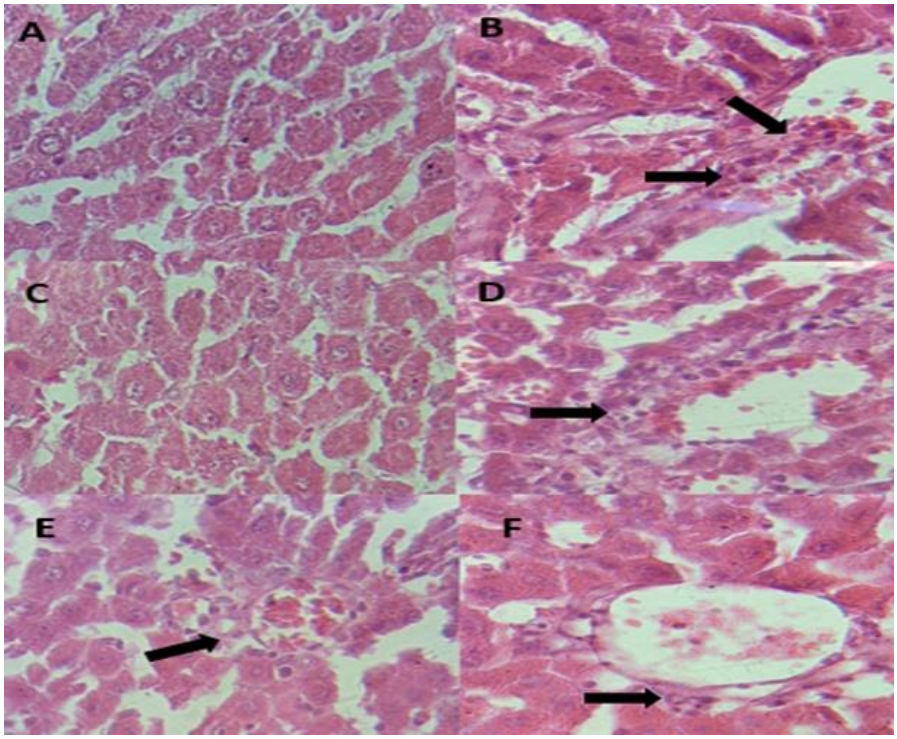

Figure 3 Effects of hydro alcoholic extract of *Pistacia atlantica* leaves on the liver histology of experimental groups. (A) Control group (group 1); (B) Acetaminophen-only treated rats (group 2); (C) Acetaminophen-administered rats supplemented with silymarin (group 3); (D), (E) and (F), Acetaminophen-treated rats supplemented with 200, 400, and 800 mg/kg of hydro alcoholic extract of *Pistacia atlantica* leaves, respectively (groups 4, 5 and 6).

antioxidant capacity compared to the acetaminophen-only treated group. Significant difference (P <0.05) in serum antioxidant capacity between rats receiving hydro alcoholic extract of *P.atlantica* leaves in two doses of 400 and 800 mg/kg was found. Plasma antioxidant capacity in the silymarin treated group increased significantly (P <0.05) compared to the acetaminophen-only treated group. Treatment with a dose of 800 mg/kg caused a significant increase (P <0.05) at the level of antioxidant capacity of plasma compared to the silymarin treated group.

The effect of hydro alcoholic extract of P.atlantica leaves on serum TNF-α level

Acetaminophen in the acetaminophen-only treated group caused significant increase (P<0.05) in serum TNF-α levels in comparison with the control group (Figure 2). However, in groups treated with the hydro alcoholic extract of *P.atlantica* leaves at doses of 400 and 800 mg/kg the serum level of this protein significantly (P <0.05) decrease compared to the acetaminophen-only treated group. Serum levels of TNF-α in the silymarin treated group indicated a significant decrease compared to the acetaminophen-only treated group. There was no significant difference between the dose of 400 and 800 mg/kg body weight of the hydro alcoholic extract of *P.atlantica* leaves in comparison with the control group.

The effect of hydro alcoholic extract of P.atlantica leaves on TNF-α gene expression

Figure 2 indicate the *TNF-α* gene expression in the studied groups. The results of this study demonstrated that gene expression of TNF-α in the acetaminophen-only treated group increased in comparison with the control group and this increase was significant (p<0.05). On the other hand, in the groups treated with hydro alcoholic extract of *P atlantica* leaves in doses 200, 400 and 800 mg/kg the expression of this gene decreased significantly (P<0.05) compared to the acetaminophen-only treated group. The expression of TNF-α in the silymarin treated group indicated a significant decrease (P<0.05) compared to the acetaminophen-only treated group.

Histopathological study

Figure 3 indicated the histopathological studies in all experimental groups. Examination with the optical microscope showed normal hepatocytes in the control group (Figure 3A). The administration of

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acetaminophen in acetaminophen-only treated group led to infiltration of lymphocyte cells in comparison with the control group (Figure 3B). Treatment with hydro alcoholic extracts of *P.atlantica* leaves at dose of 200 mg/kg decreased cell infiltration compared to the acetaminophen-only treated group (Figure 3D). Liver degeneration and lymphocytic cell infiltration were significantly reduced in groups treated with extracts of *P.atlantica* leaves at doses of 400 and 800 mg/kg compared to the extracts of *P.atlantica* leaves group (Figure 3(E&F)). Also, in the silymarin treated group normal hepatocytes were observed (Figure 3C).

The data are expressed as mean ± SD and n=8 in each group. Group 1 was the control; group 2 was acetaminophen-only treated; group 3 was treated with acetaminophen plus silymarin; groups 4, 5 and 6 were treated with acetaminophen plus *Pistacia atlantica* hydro alcoholic leaves extract at the doses of 200, 400, and 800 mg/kg, respectively. $^{a}p<0.05$ compared to the control group (group 1). $^{b}p<0.05$ compared to the acetaminophen-only treated group (group 2). $^{c}p<0.05$ compared to the group treated with acetaminophen plus silymarin (group 3). $^{d}p<0.05$ compared to the group treated with acetaminophen plus *Pistacia atlantica* hydro alcoholic leaves extract at doses of 200mg/kg (group 4). $^{e}p<0.05$ compared to the group treated with acetaminophen plus *Pistacia atlantica* hydro alcoholic leaves extract at doses of 400mg/kg (group 5).

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DISCUSSION

In the present study, acetaminophen caused hyperlipidemia, especially TC, TG, LDL-C, and VLDL-C which is agreement with previous report (22). It is reported that hyperlipidemia is due to effect of drugs on acetyl-CoA carboxylase 1 (lipogenic isoform in liver and adipose tissues) and fatty acid synthesis gene expression that are related to lipogenesis (23). On the other hand, treatment with *P.atlantica* (800, 400 mg/kg) or silymarin result in the decrease of hyperlipidemia intensity which is in line with other studies (6, 24, 25). Several reports have demonstrated that natural compounds can reduce hyperlipidemia (24, 25), which is in agreement with the results of this study. Therefore, based on the results of this study, the reduction of the serum lipid levels is resulted from the antioxidant properties of *P.atlantica*.

Acetaminophen-induced liver toxicity reported in humans and animals (26). In this study, acetaminophen induced an increase in ALT and AST compared to the control group, which is in according to the previous studies (27). However, *P.atlantica* extract, due to its antioxidant properties, could markedly decrease ALT and AST. Previous studies has demonstrated that *P.atlantica* has antioxidant and anti-inflammatory properties and antioxidants can protect cell membranes (8, 24, 25). *P.atlantica* contains phenolic compounds, which protect against oxidative stress (28). Therefore, probably the presence of phenolic compounds in *P.atlantica* is responsible for the protective properties against oxidative stress induced by acetaminophen in this study.

MDA level and protein carbonyl can be markers of oxidative stress (29). In the present study, the levels of MDA and serum protein carbonyl were significantly increased in acetaminophen-only treated rats compared to the control group which is in according to previous studies (30). In our study, *P.atlantica* was able to reduce the levels of MDA in serum and liver tissue at doses of 200, 400 and 800 mg/kg and decrease levels of carbonyl protein at doses of 400 and 800 mg/kg compared to the second group. In previous study, protective effect of moringa peregrina leaves was indicated to reduce the level of MDA in hepatotoxicity induced by acetaminophen (31). Previous research has also shown that *P. atlantica* has antioxidant properties and has been used in traditional medicine (32, 41). In this study, *P. atlantica* not only decreased serum and tissue MDA but also increased plasma FRAP concentration. Therefore, the increase in FRAP is due to the antioxidant properties of this extract.

The studies have demonstrated that oxidative stress plays an important role in the production of free radicals and hepatotoxicity induced by acetaminophen (33). In this study, acetaminophen reduced the enzymatic antioxidants (CAT, SOD), and non-enzymatic antioxidants (vitamin C), which is in according to previous studies (34, 35). However, *P.atlantica* extract resulted in elevated levels of vitamin C, SOD, and CAT in *P.atlantica* extract treated groups compared to the acetaminophen-only treated rats. In addition, previous studies have demonstrated that acetaminophen can cause histopathological changes in the liver tissue (36). Therefore, in this study, the increase in vitamin C, catalase and SOD was considered as a reason for reducing ROS, reducing serum and liver MDA, protein carbonyl and damaging the liver structure in the groups treated with *P. atlantica*, which led to improvment histopathological alteration of the liver.

TNF-α is an inflammatory cytokine and it is one of the most important signs of oxidative stress-induced inflammation that can cause apoptosis (37, 38). Several studies have demonstrated that acetaminophen-induced hepatotoxicity is associated with inflammation that increases TNF-α (39). The current study indicated that expression of liver TNF-α and serum TNF-α level significantly increased in acetaminophen-only treated rats compared to the control group. In this study, treatment with the extract of *P.atlantica* or silymarin significantly reduced serum TNF-α and the expression of the genes in the liver tissue compared to the acetaminophen-only treated rats. Therefore, the reduction of serum TNF-α and expression of its gene in liver tissue is other evidence for the protective effects of *P. atlantica* due to its anti-inflammatory and antioxidant properties.

Acetaminophen may cause apoptosis and autophagy by increasing levels of caspase-3 (40). In this study, the effects of *P. atlantica* extract on apoptosis and apoptosis factors such as NF-KB and P53 have not been studied. These factors can increase cell apoptosis. Therefore, future studies can be based on the anti-apoptotic effects of *P.atlantics* extract.

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CONCLUSION

The results of this study showed that *P.atlantica* extract protects the liver against the toxicity of acetaminophen in the rat. The protective effects of *P. atlantica* extract can be related to its antioxidant and anti-inflammatory properties. Therefore, *P.atlantica* extract can be considered as a protective agent against acetaminophen-induced oxidative stress and liver toxicity.

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Conflict of interest
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