ISSN: 0976-3104
SUPPLEMENT ISSUE: Biological Science

ARTICLE

EXTRACT OF *PISTACIA ATLANTICA* L. ON HYPERLIPIDEMIA AND BIOMARKERS OF OXIDATIVE STRESS IN RATS FED A HIGH-FAT DIET AND HYPOGLYCEMIC EFFECT IN DIABETIC RATS INDUCED WITH ALLOXAN

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ABSTRACT

Cardiovascular disorders are considered one of the greatest widespread diseases of human life. herbal cures were used traditionally to treat or prevent such of chronic disease and many of them have been indicated precise effect on hyperlipidemia. This study was performed to determined Pistacia atlantica leaf extract on lipid profile. 80 rates divided in 8 groups of 10 randomly, which feeds by normal diet, high fat diet (3 groups), high fat diet plus 200mg/kg and 400mg/kg of hydroalcoholic extract of pistachia atlantica, high fat diet plus 10 mg/kg atorvastatin respectively in prevention phase. After 90 days, low-density lipoproteins (LDL), High-density lipoproteins (HDL), Triglyceride, Fast blood sugar, C - Reactive Protein, malondialdehyde (MDA), Acyl-Coenzyme A, acetyltransferase (ACAT), Glutathione S-Transferase(GST), Catalase, superoxide dismutase (SOD), Total glutathione (GSH) and triglyceride and histopathologic test of liver and aortic arch were assessed. Then 3 groups of hyperlipidemic rats remains for 30 days feeding normal diet, normal diet plus 400mg/kg pistachia atlantica extract and normal diet plus 10mg/kg atorvastatin in treatment phase. All the tests were repeated in 120th day of experiment. After prevention phase, glucose, cholesterol, TG, LDL and HLDL in groups fed by herbal extract were decreased, also HDL increased significantly specialy in 400mg/kg dose (p=0.000). in addition, plant diet reducing MDA, OxLDL, plasma carbonyl, NO and increased catalase, SOD, thiol groups of plasma. Moreover, pathologic studies determined atherosclerotic plaques formed by 90 days high fat diet that prevented in groups fed by both doses of pistachia atlantica. In treatment phase, 400mg/kg of pistachia atlantica caused absolute decrease in GL, TC, LDL, VLDL, ALT, MDA and liver weight in hyperlipidemic group. Consequently, Pistachia atlantica leaf significantly decreased lipid profile and atherosclerotic biomarkers in 90-day prevention and 30-day treatment phases.

KEY WORDS
leaf extract, Pistacia atlantica L, hyperlipidemia, alloxan

Received: 6 March 2017
Accepted: 4 April 2017
Published: 16 April 2017

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INTRODUCTION

Nowadays, cardiovascular disease is the most prevalent cause of mortality which is closely associated with formation of atherosclerotic plaques, which in turn is derived from certain stressors such as hyperlipidemia, hypertension, diabetes, and even fatty liver [1, 2]. Among these factors, hyperlipidemia, particularly high blood LDL level, contributes more fundamentally and markedly to pathogenesis of the disease [3, 4]. Severity and type of dyslipidemia alongside risk factors associated with bad habits such as over-eating, obesity, physical inactivity, and adverse high-fat diets are considered to be predisposing factors for formation of atheroma plaques in the vessel wall, particularly coronary arteries [5]. Many drugs are used to treat dyslipidemia. Despite high efficacy of these drugs, lack of appropriate overlapping effects on both cholesterol and triglyceride, relatively prevalent complications (increasing liver enzymes, rhabdomyolysis, allergic complications,etc.) as well as high interaction with other drugs and contraindications in many physiological states and diseases have encouraged the researchers to seek out better drugs [6,7].

Medicinal plants have long been investigated for treatment of dyslipidemias, and over 200 plants have been demonstrated to be effective in decreasing lipidemia (8). However, there are certain plants that are considered to be effective in decreasing lipidemia according to popular belief, are used as additives in foods and are recommended to study. Pistacia atlantica leaf is an Iranian traditional plant which has long been used a sweetener and antibacterial agent. P. atlantica tree can reach a height of 9 m and its tree is mainly used. Another product of this tree is gum which is green when it exudes from the trunk and is considered an oleoresin in terms of chemical compounds and physical characteristics. Unfortunately, most studies have been conducted on P. atlantica fruit and no study has yet investigated on P. atlantica leaf [9, 10]. The aim of this study was to investigate the effects of hydroalcoholic P. atlantica leaf extract on lipid profile, glycemia, and atherosclerosis-inducing biomarkers.

MATERIALS AND METHODS

Preparation, extraction, and selection of appropriate solvent

P. atlantica was identified and then collected from Shirmard village in Iran. After the pharmacognosy expert of the Isfahan University of Medical Sciences approved the primary identification and the code 461 of the Herbarium was specified to P. atlantica sample, it was shadow-dried. Then, botanolic, hexane, methanolic, and methanolic/aqueous extracts were obtained from the plant and the hydroalcoholic extract

(80:20) selected according to the assays of phenolic, flavonoid, and flavnolic groups and prepared by percolation. Finally, the extract was evaporated in an evaporator and dried by an oven at 37 °C [11].

Selection of dose

Using the method of acute toxicity study and Foreman (OECD guideline) and the Software AOT 425 (determining UP and DOWN method), and no mortality in 5000m/kg, LD50 of this plant, was considered to be higher than 5000 mg/kg. Then, the doses 25, 50, 100, 200, 400, and 800 mg/kg of the plant were examined for chronic toxicity within 28 days. Regarding the conducted experiments, we found no chronic toxicity. According to two doses of 200mg/kg and 400mg/kg, it was considered effective and safe dose [12].

Formulation of atherogenic diet

In the light of hyperlipidemic food ingredients for human, we attempted to prepare a diet with greatest similarity. In this formulation, a combination of cow fat, impure saturated fat palm oil, egg cholesterol, and sugar (20%) were used. An emulsion (1 cc) was prepared to gavage according to the suitable dose of cholesterol 25mg/kg [13].

Study design

In this study, the effects of P. atlantica were investigated in prevention and treatment phrases.

Prevention phase

Eighty male Wistar rats weighing approximately 200-250 G and aged approximately eight weeks were divided into eight groups of 10 each as follows:

Group 1: Fed with normal food;
Group 2: Fed with fat only;
Group 3: Fed with fat and 200mg/kg of P. atlantica;
Group 4: Fed with fat and 400mg/kg of P. atlantica;
Group 5: Fed with fat and atorvastatin (10mg/kg);
Group 6: Fed with fat alone;
Group 7: Fed with fat alone; and
Group 8: Fed with fat alone.

On day 90, the rats in the groups 1-5, fed with water only for 12 h, were anesthetized, their blood samples taken, and then their liver, kidney, and aortic arch isolated and kept in formalin 9%.

Biochemical tests were conducted on lipid profile, hepatic profile, renal profile, and inflammatory biomarkers. Furthermore, the weights of the rats were measured and recorded on days 1, 10, 20, 30, 40, 50, 60, 70, 80, and 90.

Treatment phase

In the remaining three groups (6-8) that were fed with fat alone until the day 90, after the blood samples were taken from the eyes, a veterinary participated in the second phase of the study as follows: (The duration of the study in the second phase was 30 days and biochemical tests were repeated).

Group 6: Previously hyperlipidemicized rats with normal diet;
Group 7: Previously hyperlipidemicized rats with normal diet and atorvastatin 10mg/kg; and
Group 8: Previously hyperlipidemicized rats with normal diet and 400mg/kg of P. atlantica (because this dose had better effect on lipid profile in phase 1).

On the day 120, the rats were fed with water only for 12 h and the whole procedure in the prevention phase was duplicated. The weights of the rats were recorded on the days 100, 110, and 120.

Study of hypoglycemic effects of P. atlantica

Regarding that P. atlantica was found to have reducing effects on fasting glycemia in the prevention and treatment phases, the groups below were studied to establish this effect using subcutaneous administration of alloxan120mg/kg:

10 control rats fed with normal diet;
10 control rats fed with normal diet and 1 cc normal saline subcutaneously administered;
10 rats diabetized with alloxan120mg/kg
10 rats diabetized with alloxan and 200mg/kg hydroalcoholic P. atlantica extract.
10 rats diabetized with alloxan and 400mg/kg hydroalcoholic P. atlantica extract.
10 rats diabetized with alloxan120mg/kg and metformin (100 mg/kg).

On the day 30, the rats were anesthetized after 12-h fasting, blood samples were taken, and the relevant tests conducted.

Measurement of antioxidant activity and total phenolic and flavonoid contents of the plant

To measure antioxidant activity, Khalighi et al's method was used [14]. According to the inhibition rate of free radical DPPH and the solution absorption, antioxidant activity was measured by UV spectrophotometer at 517-nm wavelength after 30-min presence in a dark environment, and RSA measured.

To measure total phenolic and flavonoid contents, Kim and Khalighi method was used [15]. Total flavonoid content was measured according to rutin equivalent amount (1 mg) and P. atlantica extract and dried powder.

Measurement of total plasma antioxidant capacity

To measure total plasma antioxidant capacity, FRAP (the ratio of changes to the measurement and inside measurement weights, 3.3% and 1.18% respectively) offered by Benzik et al was used [16].

Measurement of NO

To measure NO amount, Barkel et al's method according to Griess reaction was conducted using spectrophotometry.

Briefly, from 0.1 M sodium nitrite, 100 micromole of solution was prepared and serial triple concentrations (as standard concentrations to plot standard curve) were prepared from this concentration.

1000 microL of the serum sample was introduced, as paired, into 96-well plates. One hundred microL of sulfanilamide solution (1 g sulfanilamide in 100 cc of phosphoric acid 5%) was introduced into all wells containing the sample and standards. The plate was incubated at room temperature in the dark for 5-10 min. One hundred microL of N-(1-Naphthyl) ethylenediamine dihydrochloride (NED) was introduced and the plate was incubated at room temperature in the dark for 5-10 min again. Half an hour later, maximom optical absorbance was read using spectrophotometer at 530-nm wavelength and the amount of nitrite in the samples determined with reference to the standard curve [17].

Plasma carbonyl

Levin et al's method was used to measure plasma carbonyl groups. Reagent 2,4 dinitrophenyl hydrazine created Schiff base with carbonyl groups in the proteins and a yellow complex was formed with the color intensity measured spectrophotometrically at 380-nm wavelength [18].

Plasma thiol

Hu's method of colorimetry was used to measure plasma thiol [19]. The Ellman's reagent dTNB formed a yellow complex with thiol-containing groups with maximum absorbance at 412-nm wavelength.

Measurement of superoxide dismutase (SOD) enzymatic activity

SOD was measured according to Carrillo et al's method using xanthine oxidase as an oxygen delivery system for infants with nitro-blue tetrazolium (NBT) indicator. To identify other enzyme types, KCN was used [20].

Measurement of catalase enzymatic activity

Catalase was investigated according to Carrillo's method. Catalase enzymatic activity was measured at 37°C temperature and 240-nm wavelength and reported [21]

The data were analyzed by one-way ANOVA, paired t-test, and Tukey's post-hoc test in SPSS 21. $P < 0.05$ was considered to be the level of significance.

RESULTS

Mean weight of the animals in 10 time periods (first day, 10th day, 20th day, 30th day, 40th day, 50th day, 60th day, 70th day, 80th day and 90th day) of the studied groups were different in phase 1 of the study [Fig. 2].

Table 2-3: Comparison of variables in the studied group of phase I.

Groups	Normal	Hyper	Hyper+ atorvastatin	Hyper+ *P.a*.200mg/kg	Hyper+ *P.a* 400mg/kg	P-value
GL	103.79±11.53	142.08±25.44	9.27 109.43±	59.44±6.67	56.27±3.79	0.000
TC	59.89±12.27	134.30±26.48	80.00±7.11	82.03±4.93	75.83±7.16	0.000
TG	38.33±4.87	109.40±45.47	61.75±12.70	39.05±4.22	29.13±8.14	0.000
LDLC	18.78±5.19	48.92±5.75	27.51±1.66	39.36±1.63	33.13±11.75	0.000
VLDL	7.67±0.97	21.88±9.09	12.32±2.51	7.82±4.13	5.82±2.00	0.000
HDL	23.86±6.15	22.29±4.93	13.23±2.51	31.20±4.26	32.29±7.19	0.000
OXLDL	3806.89±526.79	4592.30±241.24	4103.12±229.60	3837.00±379.98	3714.83±321.81	0.000
ALT	369.11±51.00	451.80±129.63	315.42±80.27	334.26±13.81	307.20±20.12	0.000
UA	2.76±0.23	4.87±2.88	2.24±0.38	1.26±0.23	1.80±0.41	0.002
CR	0.77±0.09	0.97±0.13	0.69±0.08	0.63±0.09	0.60±0.05	0.000
GPT	49.44±5.98	51.00±13.47	41.25±11.97	46.29±2.12	43.20±4.28	0.000
UREA	47.33±11.80	45.10±18.65	35.62±8.25	13.20±3.17	11.02±8.43	0.000
CAT	8.42±0.31	5.02±0.71	5.74±0.40	6.35±0.47	6.71±0.77	0.000
SOD	42.36±5.57	35.61±3.29	36.34±4.34	41.20±5.17	42.10±2.70	0.000
THIOL	202.04±13.02	164.00±24.09	203.92±14.04	200.10±4.16	201.31±11.02	0.000

CARBO	0.77±0.09	0.87±0.04	0.62±0.07	0.51±0.07	0.49±0.08	0.000
MDA	2.33±0.48	6.31±0.76	3.03±0.43	2.71±0.37	239±0.60	0.000
DPPH	266.57±80.22	301.18±39.25	403.67±18.49	413.21±7.18	428.16±20.29	0.000
Liver Weight	4.40±0.34	7.21±0.90	4.95±0.18	4.94±0.31	4.49±0.30	0.000
NO	3.59±0.64	11.26±1.80	3.78±0.64	3.78±0.39	3.69±0.26	0.000

Table 5: Camparison of variables in 91th and 121th days of each group in phase II

variables	Days of sampling	Hyper+normal diet	Hyper+Atorvastatin	Hyper+P. a 400mg/kg
GL	91th day 121th day p- vaue	135.38±32.00 136.75±39.50 0.666	135.50±59.03 137.10±61.91 0.345	124.20±10.48 89.51±13.24 0.004
TC	91th day 121th day p- value	159.12±41.37 139.62±50.19 0.297	117.70±30.80 110.40±26.51 0.004	127.12±17.15 89.17±28.76 0.018
TG	91th day 121th day p- value	80.12±18.51 74.38±22.37 0.588	98.90±36.13 79.60±41.20 0.310	107.18±22.39 59.15±12.31 0.000
LDLC	91th day 121th day p- value	71.84±15.10 69.21±19.61 0.617	65.35±12.61 59.57±12.32 0.389	85.30±12.17 49.34±10.24 0.002
VLDL	91th day 121th day p- value	17.62±4.02 14.85±4.50 0.174	17.98±4.39 13.18±5.63 0.012	21.04±6.21 11.81±7.20 0.000
HDL	91th day 121th day p- value	17.06±7.70 18.90±7.50 0.116	11.69±3.75 13.28±3.54 0.328	11.98±5.17 10.30±2.84 0.853
OXLDL	91th day 121th day p- value	4090.62±872.60 3786.50±722.96 0.452	3811.00±451.62 3801.10±700.07 0.953	4051.73±156.80 3919.22±503 0.236
ALT	91th day 121th day p- value	386.88±44.61 384.12±33.85 0.746	453.90±136.71 528.60±228.93 0.252	583.31±200.38 529.13±101.69 0.795
UA	91th day 121th day p- value	2.30±0.59 2.18±0.50 0.083	4.15±0.29 4.22±0.37 0.566	4.20±0.52 4.13±0.45 0.767
CR	91th day 121th day p- value	0.74±0.12 0.65±0.05 0.087	0.91±0.20 0.90±0.16 0.910	1.10±0.12 0.90±0.18 0.168
GPT	91th day 121th day p- value	140.00±8.32 138.75±7.29 0.774	111.70±39.49 112.62±39.22 0.965	141.31±29.17 129.17±23.11 0.159
UREA	91th day 121th day p- value	33.50±3.63 33.62±2.77 0.946	58.60±8.98 58.10±6.56 0.875	64.10±13.42 57.10±4.75 0.164
GOT	91th day 121th day p- value	113.50±18.02 110.88±10.29 0.643	132.70±25.32 130.40±25.03 0.828	160.50±44.24 123.30±12.87 0.020
CAT	91th day 121th day p- value	4.57±0.95 4.82±0.74 0.351	4.73±0.89 5.08±0.59 0.424	4.40±0.21 5.12±0.01 0.004
SOD	91th day 121th day p- value	33.64±2.47 34.03±2.44 0.048	33.80±1.65 35.36±2.12 0.086	33.17±2.78 37.21±7.04 0.043
THIOL	91th day 121th day p- value	157.38±22.86 166.50±17.22 0.346	151.40±18.29 182.40±9.73 0.001	157.23±17.41 182.95±11.41 0.001
CAR	91th day 121th day p- value	0.88±0.05 0.82±0.08 0.045	0.91±0.06 0.76±0.09 0.002	0.98±0.04 0.73±0.06 0.000
MDA	91th day 121th day p- value	6.17±0.57 5.89±0.65 0.285	6.03±0.81 4.29±0.33 0.000	6.28±1.03 4.12±0.35 0.000
FRAP	91th day 121th day p- value	274.04±39.13 294.28±38.68 0.225	280.38±37.30 331.31±53.15 0.018	281.06±29.24 362.43±27.38 0.000
WLIV	91th day 121th day p- value	7.09±0.56 6.59±0.46 0.008	7.14±0.68 5.84±0.45 0.000	7.26±0.40 5.32±0.43 0.000

Table 6: Glycemic variations in the studied rats

Number	Day	Control	Treatment with *P.a*	Treatment with *P.a*	Diabetic	Diabetic and treated with metformin	Diabetic and treated with 200 mg/kg *P. M.*	Diabetic and treated with 400 mg/kg *P.M.*	One-way ANOVA
1	0	115.1±7/8	109.4±5.4	120.6±3.9	121.4±2.9	128.7±3.1	127/5±5/1	131/2±4/9	P<0.05
2	7	118/1±1/7	120/2±4/1	116.9±4.9	383/5±18.2	413.2±21.1	407/7±21/3	411/5±31/6	P<0.05
3	30	122.4±5.7	104.3±2.7	89.4±7.2	320.7±5.9	301±7.3	281.3±11.6	254.3±31.3	P<0.05

- The values were expressed as mean (±standard deviation) for each group.
 * Significant difference from healthy controls (P<0.05).

According to [Table 1], both the 200 mg/kg and 400 mg/kg of P. atlantica could remarkably reduce weights of rats feed by fat and plant diet. Meanwhile 400mg/kg indicated better effect in reducing weight than 200mg/kg (p< 0.05). In addition cross relation between treating group and weight in time was observed (F= 84.413, DF= 12.714, p=0.000).

Means of variants, glucose, cholesterol, TG, LDL and HLDL in groups fed fat and plant (both 200mg/kg and 400mg/kg) decreased in comparison with groups just getting fat diet, also HDL increased significantly. 400mg/kg dose of P. atlantica in reducing glucose, cholesterol and elevating HDL was more effective than 200mg/kg dose (p=0.000). Moreover, both doses of plant diet triggered reducing MDA, OxLDL, plasma carbonyl, NO and increased catalase, SOD, thiol groups of plasma; which significantly differed from control group.

Plasma antioxidant capacity was noticibly different in groups which received P. atlantica diet than groups feed fat diet; 400mg/kg dose showed better effects (p=0.000).
To be noticed the data of weight loss, lose abdomen fat, decreasing fat profile and increasing HDL, effect of 400 mg/kg dose on inflammation factors was determined in phase 2 of this study. Also pathologic studies indicated that high fat diet in phase 1 (90 day) caused formation of atherosclerotic plaques and 90 day treating by both 2 doses of plant inhibited formation of atheroma plaques.

Phase 2 (determination of therapeutic effects of plant in three studied group from 91th till 120th day)
In duration of 91th and 120th days, mean variation of gradual weight loss in hyperlipidemic rats with normal diet and 400mg/kg of plant comparing to hyperlipidemic rats with normal diet demonstrated significant difference (p=0.000). Also remarkable decreasing of GL, TC, LDL, VLDL, ALT, MDA and liver weight was observed in hyperlipidemic group feed with 400mg/kg of P. atlantica during phase 2 (91th till 121th day) and plasma antioxidant capacity, catalase, plasma thiol and SOD were increased (p=0.000). Furthermore decreased blood sugar was presented in Alloxan induced diabetic rats fed with plant diet while 400mg/kg dose of plant indicated more evident hypoglycemic effects (p<0.05).

DISCUSSION

This study demonstrated that P. atlantica leaf, in both 90-day prevention and 30-day treatment phases, could decrease lipid profile and exerted optimal effects on atherosclerotic biomarkers. Potent antioxidant compounds and properties of P. atlantica have caused decrease in oxidative stress. In previous studies, the cholesterol-lowering effect of P. atlantica essential oil was attributed to decreased PAP (22). The findings of this study indicated the presence of one or more active compounds in P. atlantica such that this plant caused increase in cholesterol in all lipoprotein fractions in the short term.

Besides that, this increasing effect was not observed on cholesterol in the long term. This finding can be due to the essential fatty acids abundantly found in P. atlantica, linoleic acid and linolenic acid that cause decrease in cholesterol in the long term. Because phosphatidate phsphohydrolase contributes importantly to development of fatty liver, and P. atlantica was found to cause decline in the activity and synthesis of this enzyme in the long term, researchers have recommended that P. atlantica be used to treat fatty liver.
In the present study, which was conducted on P. atlantica leaf, the cholesterol-lowering effects of this plant were obviously observed. Moreover, decreased ALT, AST, and liver weight as well as increased SOD, catalase, GSH, and GPX confirmed the protective effects of P. atlantica against fatty liver and increase in insulin sensitivity.

SOD is a metalloenzyme that helps regulate master eukoryotic free radicals. SOD causes free radicals to convert into OH. In addition, catalase decomposes hydrogen peroxide and therefore protects the tissues against highly active hydroxyl radicals. Catalase is an antioxidant enzyme which is extensively spread in animal tissues and is mostly active in liver and red blood cells. Glutathione is an important antioxidant which plays a fundamental role in removing the system of toxic peroxides and aldehydes, and indirectly causes increase in and survival of vitamins C (water-soluble antioxidant) and E (adipose-soluble

antioxidant). Conversion into water is the fundamental role of gluthatione and catalase. This conversion appears to occur in these steps [23].

NO levels are subjects with metabolic diseases and are negatively correlated with body mass index (BMI), blood pressure and triglyceridemia. Abnormal lipid metabolism, lipid peroxidation (oxidized low-density lipoprotein, ox-LDL) plays an important role in the formation and progression of arteriosclerosis. Its content responds to the speed and intensity of lipid peroxidation and indirectly responds to the damage severity of free radical. Malondialdehyde (MDA), the product of lipid peroxidation, has one of the greatest toxic actions [24, 25, 26].

In this study, decreased MDA and OxLDL, and modified NO caused prevention of lipid peroxidation and cell damage induced by the plant.
In addition, P. atlantica, in both study phases on lipid profile and study on rats with alloxan-induced diabetes, caused decrease in fasting blood sugar. In this study, this inconsistency in the findings was resolved.

In two phases, prevention and treatment, P. atlantica leaf was found to cause decrease in oxidative stress, and an independent study on rats with alloxan-induced diabetes confirmed the significantly reducing effects of this plant on fasting blood sugar ($P<0.05$).

Therefore, regarding to potent antioxidant activity of P. atlantica, its optimal effects on oxidative stress and in preventing atherosclerotic plaques as well as its low toxicity, clinical trials should be conducted on the effects of P. atlantica in decreasing cholesterolemia and blood sugar.

CONFLICT OF INTEREST
There is no conflict of interest.

ACKNOWLEDGEMENTS
This article was derived from the Ph.D. thesis of the first author and funded by the Research and Technology Deputy of the Shahrekord University of Medical Sciences, Iran.

FINANCIAL DISCLOSURE
None

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