International Journal of Phytomedicine 3 (2011) 285-293

http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Beneficial effects of artichoke on liver phosphatidate phosphohydrolase and plasma lipids in rats fed by lipogenic diet

Esfandiar Heidarian[1], Effat Jafari-Dehkordi[2], Ali Seidkhani-Nahal[3]

***Corresponding author:**

Dr. Esfandiar Heidarian
1 Clinical Biochemistry Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran.
Telephone +98 381 3346720
Fax +98 381 3346721
E-mail: heidarian_e@skums.ac.ir
Or
heidarian46@yahoo.com
2. Traditional medicine Ph.D. student, Tehran University of Medical Sciences, Tehran, Iran.
3. Master of Science in biochemistry, Ilam University of Medical Sciences, Ilam, Iran.

Abstract

Artichoke (Cynara scolymus L.) is full of natural antioxidants and has a lipid-lowering effect. The aim of this study was to investigate the effect of artichoke on the liver phosphatidate phosphohydrolase, plasma lipid levels, plasma malondialdehyde, and plasma antioxidant in rats fed by lipogenic diet. Male rats were fed by standard pellet diet (group I), standard diet supplemented with 10% artichoke (group II), lipogenic diet (containing sunflower oil, cholesterol and ethanol) plus 10% artichoke (group III) and only lipogenic diet (group IV). On day 60 of the experiment, liver phosphatidate phosphohydrolase activity, liver triglyceride, plasma lipids, plasma malondialdehyde, and plasma antioxidant levels were measured. Phosphatidate phosphohydrolase activity, liver triglyceride, the ratio of total cholesterol to high density lipoprotein cholesterol, plasma total cholesterol and triglyceride levels were significantly decreased due to artichoke treatment in groups II and III compared to groups I and IV, respectively. Significant reduction in plasma malondialdehyde and significant elevation in plasma antioxidant power observed in groups II and III compared to groups I and IV, respectively. The results clearly indicated that artichoke can be useful for the reduction of phosphatidate phosphohydrolase activity and liver triglyceride. Also, artichoke has beneficial effects in the controlling of hyperlipidemia, abnormalities in lipid profiles and oxidative stress in hyperlipidemic regimes.

Keywords: artichoke, hypercholesterolemia, liver triglyceride, plasma lipids, phosphatidate phosphohydrolase.

Introduction

Alterations in serum lipid and lipoprotein levels, especially hypercholesterolemia, result in a variety of chronic diseases such as coronary heart diseases and atherosclerosis [1-3]. Many studies have been conducted on plant flavonoids that might be beneficial in reducing the risk of obesity and its complications [4, 5]. In this respect, artichoke (Cynara scolymus L.) is introduced as new lipid-lowering therapeutic agent [6, 7]. Artichoke leaves were used in traditional medicine for a variety of diseases especially, hyperlipidemia. Hypolipidemic effects of artichoke have been documented in experimental and clinical studies [7, 8]. Also, artichoke is full of natural bioactive components, i.e., caffeic acid, chlorogenic acid, cynarin, and luteolin. These components reduce the production of reactive oxygen species (ROS), lipid peroxidation and the oxidation of low-density lipoproteins in vitro experiments [9-11]. Therefore, these properties of artichoke warrant its application in traditional medicine.

Phosphatidate phosphohydrolase (PAP, EC 3.1.3.4) catalyzes the dephosphorylation of phosphatidic acid to yield inorganic phosphate (Pi) and 1 ,2 diacylglycerol. This enzyme is a regulatory step in controlling the synthesis of glycerophospholipids and triacylglycerols [12]. The produced diacylglycerol serves as a precursor for the biosynthesis of major glycerolipids in animal cells [12, 13]. In addition, triglyceride (TG) serves as an important storage molecule that allows organism to survive periods of food deprivation. In human diseases, the regulation of TG storage is very important because both excessive and inadequate fat storage are accompanied with dyslipidemia, insulin resistance, and diabetes [14, 15]. In rat liver, two distinct forms of PAP have been reported based on N-ethylmaleimide (NEM) sensitivity [12, 16]. The NEM-sensitive form (PAP_1), located in cytosol and microsomal fraction, requires Mg^{2+} for its activity and is a regulatory enzyme in TG and phospholipids biosynthesis [12]. The second form is PAP_2. It presents in plasma membrane and does not require Mg^{2+} for its activity. This form is primarily involved in lipid signaling pathways by modulating the second messengers of diacylglycerol and phosphatidic acid [17, 18].

Most of the previous studies on artichoke have shown that artichoke has cholesterol and triglyceride-lowering effects [7, 8]. A study has shown the inhibition effect of artichoke on HMG-CoA reductase in the cholesterol biosynthesis pathway [19]. Nevertheless, most of the previous studies on artichoke focus less on enzyme involving in triglyceride metabolism, especially PAP enzyme, in details. To the best of our knowledge, there is no study investigating the effect of artichoke on PAP in hypercholesterolemic animals or humans. Therefore, the aim of this study was to determine the effects of dietary supplementation with artichoke on the liver PAP, plasma lipids, liver triglyceride content, plasma antioxidant, and malondialdehyde (MDA) levels in rats fed by lipogenic diet.

Materials and methods

Chemicals

Phosphatidic acid (sodium salt), dithiothreitol (DTT), 2,4,6-tripyridyl-s-triazine (TPTZ), and phenylmethylsulfunyl fluoride (PMSF) were purchased from Sigma (Sigma Chemical Co., USA). Sodium tetraborate, bovine serum albumin, Tris–HCl, ethylenediaminetetra acetic acid (EDTA), ethyleneglycol-bis (beta-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), sucrose, 2-thiobarbituric acid (TBA), and ferric chloride ($FeCl_3.6H_2O$) were provided from Merck (Germany). All other chemicals used were of analytical grade.

Preparation of artichoke

The artichoke used in our study was obtained from Isfahan Agricultural Research Center (Iran). Then, the 10% artichoke pellets were made by mixing 10 g of dried and crushed artichoke with 90 g of powdered standard rat pellet diet.

Animals and experimental design

Male Wistar albino rats (150-200 g) were maintained at approximately 22 °C with a 12 h light/12 h darkness cycle, and had free access to food and tap water. They were randomly divided into 4 diet groups (n = 6/ group) as below:

Group I, normal control rats which received standard pellet chow.

Group II, animal rats fed with a standard pellet chow supplemented with 10% artichoke.

Groups III and IV, the rats fed with a lipogenic diet containing standard pellet chow supplemented with 0.5% cholic acid, 20% sunflower oil, and 2% cholesterol for two wk to produce hyperlipidemia. Additionally, group III and group IV drank water containing 3% ethanol [20]. In group III, after 2 weeks, 10% artichoke was added into lipogenic regime for 45 days, whereas the rats in group IV were maintained on lipogenic diet (hyperlipidemic control group). On d 60 of the experiment, fasted animals anesthetized with chloroform and their blood samples were collected in test tubes containing EDTA through cardiac puncture. All plasma

specimens were separated by low speed centrifugation (2000g) for 10 min and were stored at –80 ºC until they were analyzed. All animal procedures were performed with regard to Iranian animal ethics society and local university rules.

Analytical procedures:

Total cholesterol (TC), plasma TG and high density lipoprotein cholesterol (HDL-C) levels were determined by enzymatic method (Pars Azmun kit, Iran) with JENWAY spectrophotometer (model 6105, England). Low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) were calculated with Fridewald formula [21]. Liver triglyceride was extracted from liver tissue by Folch-altered method which invented by Norman [22].

Preparation of rat liver homogenate:

The liver of each rat was perfused through the inferior vena cava with ice-cold saline (0.9%) to remove blood and inorganic phosphate from it to assess the liver PAP activity and the liver content triglyceride. A portion of perfused liver was homogenized in 4 volumes of ice-cold buffer (pH 7.4) containing 50 mM Tris–HCl, 0.25 M sucrose, 1 mM PMSF and 0.1 mM EDTA by homogenizer (Heidolph, Silentcrusher M model, Germany) at 8000 rpm at 4 ºC for 5 min [23]. The homogenate was centrifuged at 4500 rpm at 4 ºC for 10 min and then, the supernatant kept for the enzyme assay.

Determination of PAP activity:

PAP activity was measured in the assay buffer (250 µl) containing 50 mM Tris–HCl (pH 7.4), 1 mM EGTA, 1 mM DTT, 1 mM EDTA, 2 mM Mg Cl_2, 0.35 mM phosphatidate, and appropriate amount of the enzyme solution. After 10 min incubation at 37 °C, the reaction was stopped by addition of 0.5 ml trichloroacetic acid (10%). Hence, the released Pi was measured [23]. All PAP activity assays were linear in relation to the protein concentrations and the incubation time used in them. The release of 1 µmole of Pi per min was defined as one unit (U) of PAP activity. Specific activity was considered as units per mg protein. Protein concentration was determined by method of Bradford [24].

Measurement of malondialdehyde:

The plasma MDA level was determined using thiobarbituric acid according to the method of Ohkawa [25]. The plasma samples were incubated for 1 hour at 95°C with thiobarbituric acid, after the reaction of MDA with thiobarbituric acid, the reaction product was followed spectrophotometrically at 532 nm. The measurements were done in duplicates and the results were expressed in µM. MDA standards were prepared from 1,1,3,3-tetraethoxypropane (TEP).

Ferric reducing/antioxidant power (FRAP) assay:

The antioxidant capacity of each sample was measured according to the procedure described by Benzie and Strain [26]. In this method, the complex between Fe^{2+} and TPTZ gives a blue color with absorbance at 593 nm. $FeSO_4.7H_2O$ was used as a standard of FRAP assay at a concentration range between 100 to 1000 µM.

Table 1. The specific activity of PAP and liver triglyceride in experimental groups.

Groups	PAP activity (nmolPi/min/mg protein)	Liver triglyceride (mg/g tissue)
Group I (control)	9.41 ± 0.39	3.80 ± 0.39
Group II	8.52 ± 0.90*#	2.64 ± 0.35*
Group III	6.46 ± 0.61*	5.16 ± 0.10*#
Group IV	6.73 ± 0.27*	7.38 ± 0.52*

The data were expressed as Mean ± S.D.; n = 6 in each group. Normal control (I); control supplemented with10% artichoke (II); hyperlipidemic rats treated with 10% artichoke (III); hyperlipidemic rats without treatment (IV) groups.

*** P < 0.05 compared with the corresponding value for group I (normal control animals).**

 # P < 0.001 compared with the corresponding value for group IV (hyperlipidemic animals).

Table 2. Effect of artichoke on TC, TG, LDL-C, HDL-C, VLDL-C levels and atherogenic index in hyperlipidemic rats.

Groups	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDLC (mg/dl)	VLDL-(mg/dl)	Atherogenic index (units)	
						TC/HDL-C	LDL/HDL-C
I	87.71 ± 13.61	56.98±6.22	51.67±5.39	23.52±2.07	10.50±2.43	1.70±0.16	0.47±0.13
II	53.36 ± 7.34*	46.68±4.62	41.32±2.16*	4.94±1.82*	9.45±1.10	1.29±0.10*	0.11±0.05*
III	79.20 ± 6.75**	49.01±6.10**	52.13±2.87	17.43±2.51**	9.80±2.34**	1.52±0.03**	0.33±0.05**
IV	146.25 ± 29.93#	75.56±4.07#	58.41±8.72	70.67±10.81#	15.11±0.95#	2.42±0.26*	1.21±0.23*

The data are expressed as mean ± S.D.; n = 6 in each group. Normal control (I); control supplemented with 10% artichoke (II); hyperlipidemic rats treated with 10% artichoke (III); hyperlipidemic rats without treatment (IV) groups.

*** P < 0.001 compared with the corresponding value for group I (normal control animals).**
****P < 0.001 compared with the corresponding value for group IV (lipogenic regime).**
 # P < 0.001 compared with the corresponding value for groups I and II.

Statistical analysis

All data were expressed as mean ± S.D. The data were analyzed by SPSS software (version 11.5). Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey post hoc test for multiple comparison. Differences were considered significant at $P < 0.05$ level.

Results

Table 1 summarizes the effect of artichoke on the liver triglyceride and PAP activity in experimental groups. Group II showed a significant reduction ($P < 0.05$) in the liver PAP activity compared to group I. No significant change ($P > 0.05$) was observed in liver PAP activity of group IV compared to group III. Also, there was a noticeable reduction ($P < 0.05$) in PAP activity between groups III and IV compared to group I. Group IV showed a significant increase ($P < 0.001$) in the liver triglyceride in comparison with groups I and III. The liver triglyceride declined ($P < 0.05$) in groups II and III compared with groups I and IV, respectively.

Effect of artichoke on plasma lipid levels:

Table 2 shows the mean plasma levels of TG, TC, HDL-C, LDL-C, VLDL-C, and atherogenic index in experimental groups. The levels of plasma TG, TC, VLDL-C, and LDL-C in group IV (consuming lipogenic diet) were significantly increased ($P < 0.05$) compared to other groups. Additionally, the plasma HDL-C in group III had no significant change ($P > 0.05$) compared to group IV. In groups II and III, the plasma level of cholesterol significantly decreased ($P < 0.001$) in comparison with groups I and IV, respectively. The plasma level of TG in the rats which consumed standard diet supplemented with artichoke (group II) was lower than group I (control group) but it was not significant ($P > 0.05$). On the other hand, the plasma level of TG in group III (consuming oil and cholesterol diet supplemented with10% artichoke) significantly decreased ($P < 0.001$) compared to group IV. In group II the plasma levels of HDL-C and LDL-C significantly decreased ($P < 0.001$) compared to group I. VLDL-C was declined in group II in contrast with group I (control group) but it was not significant ($P > 0.05$). VLDL-C in group III showed an important reduction ($P < 0.001$) compared with group IV. There was a significant ($P < 0.001$) elevation in atherogenic index (TC/HDL-C and LDL/ HDL-C) of group IV with respect to group I while, a significant reduction ($P < 0.001$) was observed in groups II and III compared with groups I and IV respectively.

Effect of artichoke on the plasma level of MAD:

Figure 1 shows that plasma MAD was significantly increased ($P < 0.05$) in group IV after the consumption of lipogenic diet when compared with other groups. On the other hand, in group II the consumption of artichoke led to a significant ($P < 0.001$) reduction of plasma MAD in comparison with group I (control). Also, in group III a significant ($P < 0.001$) reduction of

plasma MAD was seen as compared with group IV.

Effect of artichoke on the plasma level of antioxidant power:

Figure 2 shows the plasma antioxidant values in each experimental group. At the end of the work, a significant increase ($P < 0.05$) was found in the FRAP values of group II compared to the groups I and IV. No significant change was observed in plasma level antioxidant power between groups II and III ($P > 0.05$). Also, there was a noticeable difference ($P < 0.01$) between groups III and IV.

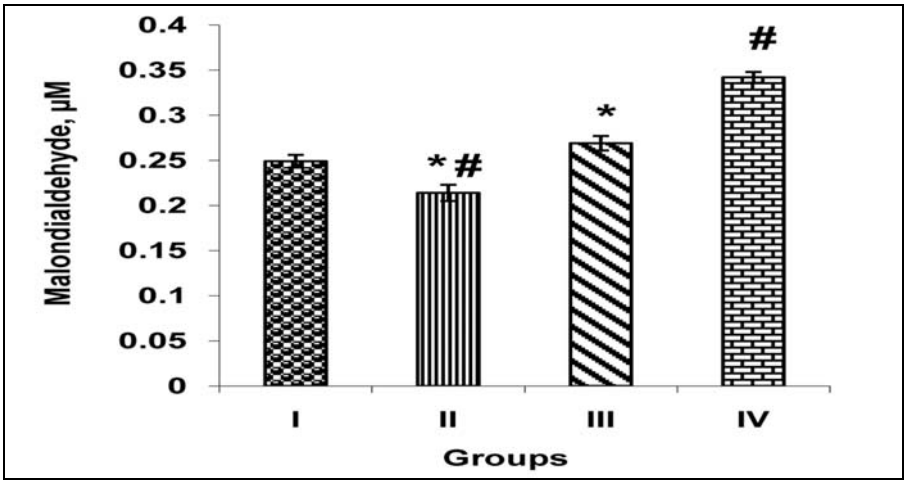

Figure 1. Plasma malondialdehyde level in normal diet (I); normal diet supplemented with 10% artichoke (II); hyperlipidemic rats treated with 10% artichoke (III); hyperlipidemic rats without treatment (IV) groups. The data are expressed as mean ± S.D, n = 6 in each group.

 # P < 0.001 compared with the corresponding value for normal control animals.
***P < 0.001 compared with the corresponding value for hyperlipidemic rats without treatment.**

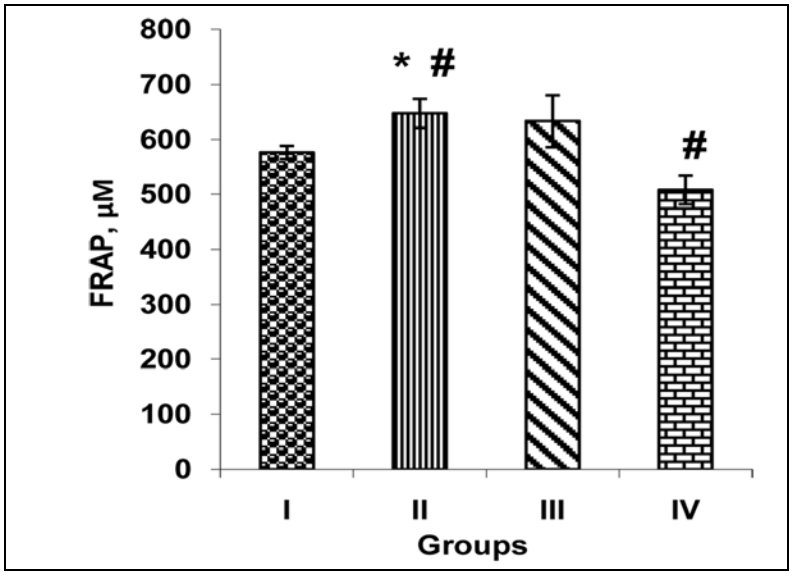

Figure 2. Plasma antioxidant capacity (FRAP) in normal diet (I); normal diet supplemented with 10% artichoke (II); hyperlipidemic rats treated with 10% artichoke (III); hyperlipidemic rats without treatment (IV) groups. The data are expressed as mean ± S.D, n = 6 in each group.
 # P < 0.05 compared with the corresponding value for normal control animals.
***P < 0.05 compared with the corresponding value for hyperlipidemic rats without treatment.**

Discussion

Hyperlipidemia with serum elevated concentrations of cholesterol and triacylglycerol is considered to be the cause of cardiovascular disease [27]. Treatment of hyperlipidemia needs diet control, exercise, and using lipid-lowering compounds such as drugs and diet [28]. Lipid-lowering drugs such as fibrates and bile acid sequestrants were used for many years. Nevertheless, the side effects of drugs led to synthesis new oral antihyperlipidemic drugs such as statins (HMG CoA reductase inhibitors).

Although the side effect of statins is relatively low but, they can cause rhabdomyolysis condition [29]. Therefore, the research for natural compounds with lipid-lowering properties and with less or no adverse effects, especially medicinal plants, is warranted. These plants contain biological active substances including antioxidant, hypoglycemic, and hypolipidemic compounds. Unfortunately, there is less information about enzymatic or lipid-lowering mechanisms for many of these medicinal plants, especially their effects on PAP enzyme. In this respect, we reported the effect of garlic on the liver PAP activity in normal and hyperlipidemic rats [30]. The supplementation of garlic, as a medicinal plant, led to reducing liver PAP enzyme and liver TG. In this study, our data have shown that artichoke supplementation in hyperlipidemic rats lead to highly effective in reducing plasma cholesterol and LDL levels as compared to the high cholesterol and control diet groups (Table 2). Also, artichoke caused significant decreases in TG and the ratio of cholesterol to HDL cholesterol in plasma of rats fed by lipemic diet. Lipid lowering effects of artichoke has been reported by other investigators [6, 8]. Studies on cultured hepatocytes suggested that artichoke inhibits the incorporation of ^{14}C-labelled acetate into the non-saponifiable lipid fraction and thus reduces the cholesterol biosynthesis. Luteolin, a flavonoid constituent of artichoke, was found to play a major role in the inhibition of cholesterol biosynthesis and reduction of serum cholesterol [19]. Moreover, chlorogenic acid is another bioactive component of artichoke that reported as lipid-lowering agent in the artichoke [7, 31]. Nevertheless, the published works do not assess the effect of artichoke on PAP activity and liver TG in hyperlipidemic rats. In our study the artichoke supplementation results in higher reduction of PAP activity (Tables 1) and liver TG in group II than group I (control). Although, the reduction of the plasma TG in group II was not significant, it was accompanied with a decline in the liver PAP activity in this group (Tables 1 and 2). On the other hand, in animals fed by lipemic regime (groups III and IV) PAP activity decreased with respect to control group whereas, their liver TG concentration increased in this study (Table 1). It has been reported that excessive intake of fatty acids results in accumulation of TG in many tissues, especially in fat tissue and non-adipose tissues such as liver [32]. In addition, it was shown that fatty acid esters lead to the inactivation of PAP. Fatty acids and their acyl-CoA esters regulate PAP by a negative allosteric interaction. The formation of PAP fatty acid (or acyl-CoA esters) complex results in the inactivation of PAP [33]. Therefore, the reduction of PAP activity in this study in groups fed with high lipid regime (groups III and IV) is due to the accumulation of TG, fatty acids or acyl-CoA esters in the liver (Table 1). Nevertheless, the reduced activity of PAP in groups fed with high lipid regime (Groups II and III) can probably act as a defense mechanism of liver for reducing the production of endogenous liver TG. Thus, serum and liver TG will decline and likely, reduce the risk of liver damage especially fatty liver and cirrhosis. Besides, in our study liver fat concentration significantly increased in animal groups fed by lipogenic regime (Groups III and IV) compared to the group I (normal control). The elevated liver fat in group III was significantly reduced as opposed group IV (Table 1) through the supplementation with artichoke. Therefore, the artichoke leaves can be able to reduce the liver content of TG by diminishing PAP activity. Overall, artichoke can be useful in lowering and the treatment of fatty liver in

hyperlipidemic regime. Moreover, the artichoke supplementation with lipogenic regime led to reduction of plasma LDL-cholesterol and atherogenic index. These results indicate that artichoke can be applicable for reducing the coronary heart diseases in hyperlipidemic conditions.

In this study, we did not evaluate the effects of artichoke on the other enzymes involving in the lipid metabolism, especially glucose-6-phosphate dehydrogenase (G6PDH) and malic enzyme. These enzymes generate NADPH employed for fatty acid and cholesterol syntheses. We suggest that future studies focus on other possible mechanisms of the triglyceride-lowering action of the artichoke or the bioactive components of artichoke on the mentioned enzymes.

Oxidative stress of plasma lipoproteins, erythrocytes and several tissues such as liver, heart and aorta have been reported in experimental animals fed on high cholesterol diet [6, 34, 35]. Increased oxidative stress parameters have been detected in hypercholesterolemic individuals [36]. The level of MDA is considered as a biomarker of lipid peroxidation [37]. In the present study, artichoke supplementation caused significant decreases in plasma lipid peroxidation together with elevation of plasma antioxidant power (Figs. 1and 2). In this respect, there are published reports concordant with our results [6, 38]. Artichoke is known to have antioxidant effect. Previous studies have reported that the antioxidant potential of artichoke is dependent on radical scavenging by its constituents such as cynarin, chlorogenic acid and flavonoids such as caffeoylquinic acids [39, 40]. Both caffeoylquinic acids and flavonoids present in artichoke are considered to be responsible for its anti-atherogenic actions through their antioxidant capacity [11].The antioxidant barriers of the artichoke extract's constituents rely on the inhibition of ROS generation, ROS neutralization, or the induction of endogenous antioxidants [10, 40, 41]. Therefore, on the basis of our results, artichoke can probably play an anti-atherogenic role by lowering lipids oxidation in hyperlipidemic diets.

Conclusion

Our findings indicate that artichoke can be useful to decrease PAP activity, liver triglyceride, oxidative stress, plasma cholesterol, and triglyceride levels in hyperlipidemic rats. Also, artichoke has beneficial effects in the control of fatty liver, plasma lipid abnormalities, hyperlipidemia, and oxidative stress in hyperlipidemic diet conditions.

Conflict of Interest

The authors declare that there is no conflict of interest.

Acknowledgements

This study was funded by Ilam University of Medical Sciences, Ilam. Iran. Also, authors great thanks to Dr. Hossein Zeinali for his help to provide and identify the artichoke from Isfahan Agricultural Research Center (Iran).

References

1. McKenney JM. Pharmacotherapy of dyslipidemia. Cardiovasc Drugs Ther. 2001;15(5):413-422.
2. Laker MF. Cardiovascular disease prevention: the new Joint British Societies' guidelines. Ann Clin Biochem. 2006;43(Pt 5):335-339.
3. Gould AL, Davies GM, Alemao E, Yin DD, Cook JR. Cholesterol reduction yields clinical benefits: meta-analysis including recent trials. Clin Ther. 2007;29(5):778-794.
4. Andersen C, Rayalam S, Della-Fera MA, Baile CA. Phytochemicals and adipogenesis. Biofactors. 2010;36(6):415-422.
5. Mulvihill EE, Huff MW. Antiatherogenic properties of flavonoids: implications for cardiovascular health. Can J Cardiol. 2010; Suppl A(26):17A-21A.
6. Küskü-Kiraz Z, Mehmetçik G, Dogru-Abbasoglu S, Uysal M. Artichoke leaf

extract reduces oxidative stress and lipoprotein dyshomeostasis in rats fed on high cholesterol diet. Phytother Res. 2010;24(4):565-570.
7. Joy JF, Haber SL. Clinical uses of artichoke leaf extract. Am J Health Syst Pharm. 2007;64(18):1904:1906-1909.
8. Shimoda H, Ninomiya K, Nishida N, Yoshino T, Morikawa T, Matsuda H, Yoshikawa M. Anti-hyperlipidemic sesquiterpenes and new sesquiterpene glycosides from the leaves of artichoke (Cynara scolymus L.): structure requirement and mode of action. Bioorg Med Chem Lett. 2003;13(2):223-228.
9. Zapolska-Downar D, Zapolski-Downar A, Naruszewicz M, Siennicka A, Krasnodębska B, Kołdziej B. Protective properties of artichoke (Cynara scolymus) against oxidative stress induced in cultured endothelial cells and monocytes. Life Sci. 2002;71(24):2897-2808.
10. Juzyszyn Z, Czerny B, Pawlik A, Droździk M. The effect of artichoke (Cynara scolymus L.) extract on ROS generation in HUVEC cells. Phytother Res. 2008;22(9):1159-1161.
11. Wang M, Simon JE, Aviles IF, He K, Zheng QY, Tadmor Y. Analysis of antioxidative phenolic compounds in artichoke (Cynara scolymus L.). J Agric Food Chem. 2003;51(3):601-608.
12. Carman GM, Han GS. Roles of phosphatidate phosphatase enzymes in lipid metabolism. Trends Biochem Sci. 2006;31(12):694-699.
13. Fleming IN, Yeaman SJ. Purification and characterization of N-ethylmaleimide-insensitive phosphatidic acid phosphohydrolase (PAP2) from rat liver. Biochem J. 1995;308(Pt 3):983-989.
14. Petersen KF, Shulman GI. Etiology of insulin resistance. Am J Med. 2006;119(5 Suppl 1):S10-16.
15. Reue K, Phan J. Metabolic consequences of lipodystrophy in mouse models. Curr Opin Clin Nutr Metab Care. 2006;9(4):436-441.
16. Heidarian E, Haghighi B. Enzymological characteristic of plasma membrane phosphatidate phosphohydrolase (PAP_2) from rat liver. Iran J Sci Technol A. 2008;32:117-122.
17. Brindley DN. Lipid phosphate phosphatases and related proteins: signaling functions in development, cell division, and cancer. J Cell Biochem. 2004;92(5):900-912.
18. Sciorra VA, Morris AJ. Roles for lipid phosphate phosphatases in regulation of cellular signaling. Biochim Biophys Acta. 2002;1582(1-3):45-51.
19. Gebhardt R. Inhibition of cholesterol biosynthesis in primary cultured rat hepatocytes by artichoke (Cynara scolymus L.) extracts. J Pharmacol Exp Ther. 1998;286(3):1122-1128.
20. Yanardag R, Peksel A, Yesilyaprak B, Doger MM, Arisan-Atac I. Effects of a combination of niacin and chromium(III)-chloride on the skin and lungs of hyperlipemic rats. Biol Trace Elem Res. 2005;103(3):249-260.
21. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6):499-502.
22. Norman SR. Preparation of lipid extracts. In: John MI. (Ed.), Methods of Enzymology. Academic Press, London; 1969;Vol. 14.
23. Haghighi B, Honarjou S. The effects of hydrazine on the phosphatidate phosphohydrolase activity in rat liver. Biochem Pharmacol. 1987;36(7):1163-1165.
24. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-254.

25. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-358.
26. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996;239(1):70-76.
27. Frishman WH. Biologic markers as predictors of cardiovascular disease. Am J Med. 1998;104(6A):18S-27S.
28. Stone NJ. Lipid management: current diet and drug treatment options. Am J Med. 1996;101(4A):4A40S-48S; discussion 48S-49S.
29. Miller CA. Update on statins and other lipid-lowering drugs. Geriatr Nurs. 2001;22(5):276-277.
30. Heidarian E, Jafari-Dehkordi E, Seidkhani-Nahal A. Effect of garlic on liver phosphatidate phosphohydrolase and plasma lipid levels in hyperlipidemic rats. Food Chem Toxicol. 2011;16:1110-1114.
31. Wider B, Pittler MH, Thompson-Coon J, Ernst E. Artichoke leaf extract for treating hypercholesterolaemia. Cochrane Database Syst Rev. 2009;7(4):CD003335.
32. van Herpen NA, Schrauwen-Hinderling VB. Lipid accumulation in non-adipose tissue and lipotoxicity. Physiol Behav. 2008;94(2):231-241.
33. Elabbadi N, Day CP, Gamouh A, Zyad A, Yeaman SJ. Relationship between the inhibition of phosphatidic acid phosphohydrolase-1 by oleate and oleoyl-CoA ester and its apparent translocation. Biochimie. 2005;87(5):437-443.
34. Jemai H, Fki I, Bouaziz M, Bouallagui Z, El Feki A, Isoda H, Sayadi S. Lipid-lowering and antioxidant effects of hydroxytyrosol and its triacetylated derivative recovered from olive tree leaves in cholesterol-fed rats. J Agric Food Chem. 2008;56(8):2630-2636.
35. Sudhahar V, Kumar SA, Varalakshmi P, Sundarapandiyan R. Mitigating role of lupeol and lupeol linoleate on hepatic lipemic-oxidative injury and lipoprotein peroxidation in experimental hypercholesterolemia. Mol Cell Biochem. 2007;295(1-2):189-198.
36. Ondrejovičová I, Muchová J, Mišľanová C, Nagyová Z, Ďuračková Z. Hypercholesterolemia, oxidative stress and gender dependence in children. Prague Med Rep. 2010;111(4):300-312.
37. Lykkesfeldt J. Malondialdehyde as biomarker of oxidative damage to lipids caused by smoking. Clin Chim Acta. 2007;380(1-2):50-58.
38. Juzyszyn Z, Czerny B, Myśliwiec Z, Pawlik A, Droździk M. The effect of artichoke (Cynara scolymus L.) extract on respiratory chain system activity in rat liver mitochondria. Phytother Res. 2010;24 Suppl 2(24):S123-128.
39. Brown JE, Rice-Evans CA. Luteolin-rich artichoke extract protects low density lipoprotein from oxidation in vitro. Free Radic Res. 1998;29(3):247-255.
40. Pérez-Garcia F, Adzet T, Canigueral S. Activity of artichoke leaf extract on reactive oxygen species in human leukocytes. Free Radic Res. 2000;33(5):661-665.
41. Jiménez-Escrig A, Dragsted LO, Daneshvar B, Pulido R, Saura-Calixto F. In vitro antioxidant activities of edible artichoke (Cynara scolymus L.) and effect on biomarkers of antioxidants in rats. J Agric Food Chem. 2003;51(18):5540-5545.