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Full Length Research Paper

Lipid-lowering effect of artichoke on liver phosphatidate phosphohydrolase and plasma lipids in hyperlipidemic rats

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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 (PAP), plasma lipid levels, plasma malondialdehyde (MDA), and plasma antioxidant in hyperlipidemic rats. Male rats were fed by standard pellet diet (Group 1), standard diet supplemented with 10% artichoke (Group 2), lipogenic diet (containing sunflower oil, cholesterol and ethanol) plus 10% artichoke (Group 3) and only lipogenic diet (Group 4). On day 60 of the experiment, liver PAP activity, liver triglyceride (TG), plasma lipids, plasma MDA, and plasma antioxidant levels were measured. PAP activity, liver TG, the ratio of total cholesterol (TC) to high density lipoprotein (HDL) cholesterol, plasma TC and TG levels were significantly decreased due to artichoke treatment in Groups 2 and 3 compared to Groups 1 and 4, respectively. Significant reduction in plasma MDA and significant elevation in plasma antioxidant power observed in Groups 2 and 3 compared to Groups 1 and 4, respectively. The results clearly indicated that artichoke can be useful for the reduction of PAP activity and liver TG. Also, artichoke has beneficial effects in the controlling of hyperlipidemia, abnormalities in lipid profiles and oxidative stress in hyperlipidemic regimes.

Key words: Artichoke, hyperlipidemia, 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 (Gould et al., 2007; Laker, 2006; McKenney, 2001). Many studies have been conducted on plant flavonoids that might be beneficial in reducing the risk of obesity and its complications (Andersen et al., 2010; Mulvihill and Huff, 2010). In this respect, artichoke (*Cynara scolymus* L.) is introduced as new lipid-lowering therapeutic agent (Joy and Haber, 2007; Küskü-Kiraz et al., 2010). 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 (Joy and Haber, 2007; Shimoda et al., 2003). Also, artichoke is full of natural bioactive components, that is, 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 (LDL) *in vitro* experiments (Juzyszyn et al., 2008; Wang et al., 2003; Zapolska-Downar et al., 2002). 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 (Carman and Han, 2006). The produced diacylglycerol serves as a precursor for the biosynthesis of major glycerolipids in animal cells (Carman and Han, 2006; Fleming and Yeaman, 1995). In addition, triglyceride (TG)

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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 (Petersen and Shulman, 2006; Reue and Phan, 2006). In rat liver, two distinct forms of PAP have been reported based on N-ethylmaleimide (NEM) sensitivity (Carman and Han, 2006; Heidarian and Haghighi, 2008). The NEM-sensitive form (PAP_1), located in cytosol and microsomal fraction, requires mmagnesium ion (Mg^{2+}) for its activity and is a regulatory enzyme in TG and phospholipids biosynthesis (Carman and Han, 2006). 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 (Brindley, 2004; Sciorra and Morris, 2002).

Most of the previous studies on artichoke have shown that artichoke has cholesterol and TG lowering effects (Joy and Haber, 2007; Shimoda et al., 2003). A study has shown the inhibition effect of artichoke on HMG-CoA reductase in the cholesterol biosynthesis pathway (Gebhardt, 1998). Nevertheless, most of the previous studies on artichoke focus less on enzyme involving in TG 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 TG content, plasma antioxidant, and malondialdehyde (MDA) levels in hyperlipidemic rats.

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 shown. Group 1, normal control rats which received standard pellet chow; Group 2, animal rats fed with a standard pellet chow supplemented with 10% artichoke; Groups 3 and 4, the rats fed with a lipogenic diet containing standard pellet chow supplemented with 0.5% cholic acid, 20% sunflower oil, and 2% cholesterol for 2 weeks to produce hyperlipidemia. Additionally, Groups 3 and 4 drank water containing 3% ethanol (Yanardag et al., 2005). In Group 3, after 2 weeks, 10% artichoke was added into lipogenic regime for 45 days, whereas the rats in Group 4 were maintained on lipogenic diet (hyperlipidemic control group). On the 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 (2000 *g*) for 10 min and were stored at -80°C 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 . Liver TG was extracted from liver tissue by Folch-altered method which invented by Norman (1969).

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 Pi from it to assess the liver PAP activity and the liver content TG. 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°C for 5 min (Haghighi and Honarjou, 1987). The homogenate was centrifuged at 4500 rpm at 4°C 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 (Haghighi and Honarjou, 1987). 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 (1976).

Measurement of malondialdehyde

The plasma MDA level was determined using TBA according to the method of Ohkawa et al. (1979). The plasma samples were incubated for 1 hour at 95°C with TBA, after the reaction of MDA with TBA, 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

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

Group	PAP activity (nmolPi/min/mg protein)	Liver TG (mg/g tissue)
1 (control)	9.41 ± 0.39	3.80 ± 0.39
2	8.52 ± 0.90*#	2.64 ± 0.35*
3	6.46 ± 0.61*	5.16 ± 0.10*#
4	6.73 ± 0.27*	7.38 ± 0.52*

The data were expressed as mean ± S.D; **n=** 6 in each group; Group 1, normal control; Group 2, control supplemented with10% artichoke; Group 3, hyperlipidemic rats treated with 10% artichoke; Group 4, hyperlipidemic rats without treatment * P < 0.05 compared with the corresponding value for group 1 (normal control animals); #P < 0.001 compared with the corresponding value for group 4 (hyperlipidemic animals).

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

Group	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDLC (mg/dl)	VLDL (mg/dl)	Atherogenic index (unit)	
						TC/HDL-C	LDL/HDL-C
1	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
2	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*
3	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**
4	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; Group 1, normal control; Group 2, control supplemented with 10% artichoke; Group 3, hyperlipidemic rats treated with 10% artichoke; Group 4, hyperlipidemic rats without treatment * P < 0.001 compared with the corresponding value for Group 1 (normal control animals); ** P < 0.001 compared with the corresponding value for Group 4 (lipogenic regime); **#** P < 0.001 compared with the corresponding value for Groups 1 and 2.

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 (1996). In this method, the complex between iron (II) ion (Fe^{2+}) and TPTZ gives a blue color with absorbance at 593 nm. Ferrous sulfate heptahydrate ($FeSO_4.7H_2O$) was used as a standard of FRAP assay at a concentration range between 100 to 1000 µM.

Statistical analysis

All data were expressed as mean ± standard deviation (S.D). The data were analyzed by statistical package for the social sciences (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 TG and PAP activity in experimental groups. Group 2 showed a significant reduction (P < 0.05) in the liver PAP activity compared to Group 1. No significant change (P > 0.05) was observed in liver PAP activity of Group 4 compared to Group 3. Also, there was a noticeable reduction (P < 0.05) in PAP activity between Groups 3 and 4 compared to Group 1. Group 4 showed a significant increase (P < 0.001) in the liver TG in comparison with Groups 1 and 3. The liver TG declined (P < 0.05) in Groups 2 and 3 compared with Groups 1 and 4, 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 4 (consuming lipogenic diet) were significantly increased (P < 0.05) compared to other groups. The plasma HDL-C in Group 3 had no significant change (P > 0.05) compared with Group 4. In Groups 2 and 3, the plasma level of cholesterol significantly decreased (P < 0.001) in comparison with Groups 1 and 4, respectively. On the other hand, the plasma level of TG in Group 3 (consuming oil and cholesterol diet supplemented with10% artichoke) significantly decreased (P < 0.001) compared to Group 4. In Group 2 the plasma levels of HDL-C and LDL-C significantly decreased (P < 0.001) compared to Group 1. VLDL-C in Group 3 showed an important reduction (P < 0.001) compared with Group 4. There was a significant (P < 0.001) elevation in atherogenic index (TC/HDL-C and LDL/ HDL-C) of Group 4 with respect to Group 1 while, a significant reduction (P < 0.001) was observed in groups 2 and 3 compared with

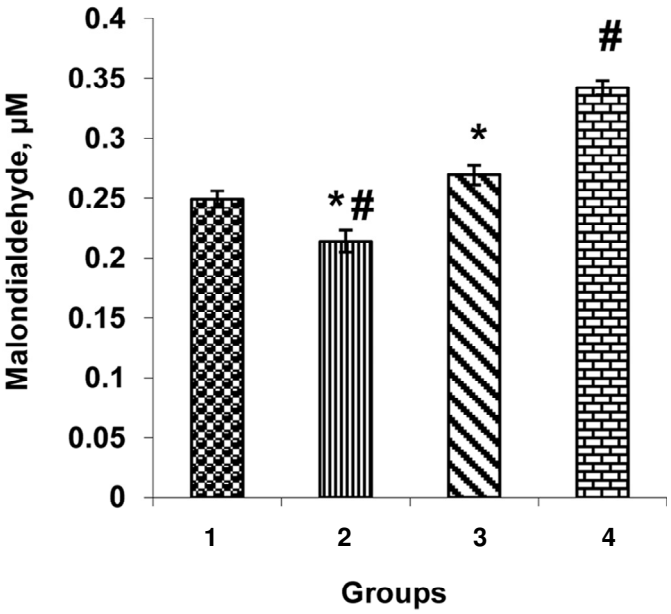

Figure 1. Plasma MDA levels in Groups 1 to 4. Group 1, normal diet; Group 2, normal diet supplemented with 10% artichoke; Group 3, hyperlipidemic rats treated with 10% artichoke; Group 4, hyperlipidemic rats without treatment. The data are expressed as mean ± S.D; n=6 in each group; #P < 0.001 compared with the corresponding value for normal control animals; *P < 0.001 compared with the corresponding value for hyperlipidemic rats without treatment.

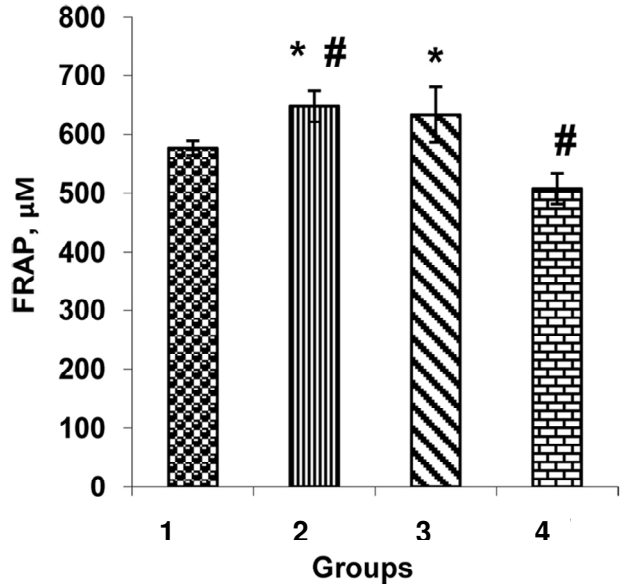

Figure 2. Plasma antioxidant capacity (FRAP) in Groups 1 to 4. Group 1, normal diet; Group 2, normal diet supplemented with 10% artichoke; Group 3, hyperlipidemic rats treated with 10% artichoke; Group 4, hyperlipidemic rats without treatment . The data are expressed as mean ± S.D; n=6 in each group; # 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.

Groups 1 and 4, respectively.

Effect of artichoke on the plasma level of MDA

Figure 1 shows that plasma MDA was significantly increased (P < 0.05) in Group 4 after the consumption of lipogenic diet when compared with other groups. On the other hand, in Group 2 the consumption of artichoke led to a significant (P < 0.001) reduction of plasma MDA in comparison with Group 1 (control). Also, in Group 3 a significant (P < 0.001) reduction of plasma MDA was seen as compared with Group 4.

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 plasma FRAP values of Group 2 compared to the Groups 1 and 4. Also, a significant reduction (P < 0.001) was observed in the plasma FRAP values between Groups 3 and 4.

DISCUSSION

Hyperlipidemia with serum elevated concentrations of cholesterol and triacylglycerol is considered to be the cause of cardiovascular disease (Frishman, 1998). Treatment of hyperlipidemia needs diet control, exercise, and using lipid-lowering compounds such as drugs and diet (Stone, 1996). 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 (Miller, 2001). 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 (Heidarian et al., 2011). 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 have been reported by other investigators (Küskü-Kiraz et al., 2010; Shimoda et al., 2003). 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 (Gebhardt, 1998). Moreover, chlorogenic acid is another bioactive component of artichoke that reported as lipid-lowering agent in the artichoke (Joy and Haber, 2007; Wider et al., 2009). 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 (Table 1) and liver TG in Group 2 than Group 1 (control). Although, the reduction of the plasma TG in Group 2 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 3 and 4) 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 (van Herpen and Schrauwen-Hinderling, 2008). 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 (Elabbadi et al., 2005). Therefore, the reduction of PAP activity in this study in groups fed with high lipid regime (Groups 3 and 4) 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 2 and 3) 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 3 and 4) compared to the Group 1 (normal control). The elevated liver fat in Group 3 was significantly reduced as opposed Group 4 (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 hyper-lipidemic regime. Moreover, the artichoke supplemen-tation 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 nicotinamide adenine dinucleotide phosphate (NADPH) employed for fatty acid and cholesterol syntheses. We suggest that future studies focus on other possible mechanisms of the TG 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 (Jemai et al., 2008; Küskü-Kiraz et al., 2010; Sudhahar et al., 2007). Increased oxidative stress parameters have been detected in hypercholesterolemic individuals (Ondrejovičová et al., 2010). The level of MDA is considered as a biomarker of lipid peroxidation (Lykkesfeldt, 2007). In the present study, artichoke supplementation caused significant decreases in plasma lipid peroxidation together with elevation of plasma antioxidant power (Figure 1 and 2). In this respect, there are published reports concordant with our results (Juzyszyn et al., 2010; Küskü-Kiraz et al., 2010). 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 (Brown and Rice-Evans, 1998; Pérez-Garcia et al., 2000). Both caffeoyl-quinic acids and flavonoids present in artichoke are considered to be responsible for its anti-atherogenic actions through their antioxidant capacity (Wang et al., 2003). The antioxidant barriers of the artichoke extract's constituents rely on the inhibition of ROS generation, ROS neutralization, or the induction of endogenous antioxidants (Jiménez-Escrig et al., 2003; Juzyszyn et al., 2008; Pérez-Garcia et al., 2000). 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 TG, oxidative stress, plasma cholesterol, and TG 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.

Abbreviations

PAP, Phosphatidate phosphohydrolase; **TG**, triglyceride; **ROS**, reactive oxygen species; **LDL**, low density lipoproteins; **HDL**, high density lipoprotein; **Pi**, inorganic phosphate; **NEM**, N-ethylmaleimide; **PAP_1**, **Mg^{2+}**,

mmagnesium ion; **MDA**, malondialdehyde; **DTT**, dithiothreitol; **TPTZ**, 2,4,6-tripyridyl-s-triazine; **PMSF**, phenylmethylsulfunyl fluoride; **EDTA**, ethylenediaminetetra acetic acid; **EGTA**, ethyleneglycol-bis (beta-aminoethyl ether)-N,N,N',N'-tetraacetic acid; **TBA**, 2-thiobarbituric acid; **$FeCl_3.6H_2O$**, ferric chloride; **TC**, total cholesterol; **HDL-C**, high density lipoprotein cholesterol; **LDL-C**, low density lipoprotein cholesterol; **VLDL-C**, very low density lipoprotein cholesterol; **TEP**, 1,1,3,3-tetraethoxypropane; **Fe^{2+}**, iron (II) ion; **$FeSO_4.7H_2O$**, ferrous sulfate heptahydrate; **S.D**, standard deviation; **SPSS**, statistical package for the social sciences; **ANOVA**, analysis of variance; **G6PDH**, glucose-6-phosphatedehydrogenase; **NADP**, Nicotinamide adenine dinucleotide phosphate.

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