Research Article

©*ISISnet Publishers*

Bioscience Research, 9(2): 59-67, 2012
Available online at **www.isisn.org**
Print ISSN: 1811-9506
Online ISSN: 2218-3973

Effect of silymarin on liver phoshpatidate phosphohydrolase in hyperlipidemic rats

E. Heidarian[1*] and M. Rafieian-Kopaei[2]

[1]Clinical Biochemistry Research Center, Shahrekord University of Medical Sciences, Shahrekord, **Iran**.
[2]Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, **Iran**.
***Corresponding author**

Previous studies have shown that silymarin, a flavonoid antioxidant, possesses a hypolipidemic effect. The aim of this study was to evaluate the effect of silymarin on liver phoshpatidate phosphohydrolase, total plasma cholesterol, plasma lipoproteins, liver and plasma triglyceride, plasma malondialdehyde, and plasma antioxidant in rats fed with high cholesterol diet enriched with fat. Male rats were fed by standard pellet diet (group I), standard diet accompanied with silymarin (group II), lipogenic diet (containing saturated fat, cholesterol and ethanol) plus silymarin (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. Group II showed a significant reduction (20%) ($p < 0.001$) in the liver PAP activity compared to group I. The atherogenic ratio of total cholesterol to high density lipoprotein cholesterol, liver triglyceride, plasma total cholesterol, and triglyceride levels significantly decreased ($p < 0.05$) due to silymarin treatment in group III compared to group IV. Significant reduction in plasma malondialdehyde ($p < 0.05$) and significant elevation ($p < 0.05$) in plasma antioxidant power observed in group III compared to group IV. These results clearly suggested that silymarin can be effective to reducing liver phosphatidate phosphohydrolase activity and liver triglyceride. Furthermore, silymarin had blood lipid-reducing and beneficial antioxidant effects in hyperlipidemic diets.

Key words: Hyperlipidemia, liver triglyceride, plasma lipids, phosphatidate phosphohydrolase, silymarin.

Silymarin (SM), extracted mainly from the seeds of milk thistle (*Silybum marianum* L.), is usually composed of a mixture of six major isomeric flavonolignans (silychristin, silydianin, silybin A, silybin B, isosilybin A, and isosilybin B) (Wen *et al*., 2008). Extracts of milk thistle have been used for centuries as herbal remedies for liver and biliary tract diseases (Gazák *et al*., 2007; Basiglio *et al*., 2009). Previous animal studies and clinical trials have shown the safety of SM in humans and animals (Dixit et al. 2007).

Phosphatidate phosphohydrolase (PAP, EC 3.1.3.4) is a major enzyme in controlling the synthesis of triacylglycerol and glycerophospholipids (Carman and Han, 2006). PAP catalyzes the dephosphorylation of phosphatidic acid to yield inorganic phosphate (Pi) and 1 ,2 diacylglycerol. Then, the diacylglycerol serves as a precursor for the biosynthesis of major glycerol-phospholipids in animal cells (Fleming and Yeaman, 1995; Carman and Han, 2006). Additionally, triglyceride (TG) plays as an important storage molecule in living organisms and allows organisms to survive periods of food deprivation. In human diseases, the control 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 hepatocytes, two different types 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) is a regulatory enzyme in TG and phospholipids

biosynthesis. PAP_1 is located in cytosol and microsomal fractions. PAP_1 requires Mg^{2+} for its activity (Carman and Han, 2006). The second form is PAP_2 and it does not require Mg^{2+} for its activity. PAP_2 is present in plasma membrane which is primarily involved in lipid signaling pathways by modulating the second messengers of diacylglycerol and phosphatidic acid (Carman and Han, 2006).

Hypercholesterolemia is considered a risk factor for coronary heart diseases. Previous studies have suggested that polyphenolic compounds found in fruits, vegetables, and plants reduce coronary heart disease mortality (Maron, 2004). Milk thistle (*Silybum marianum* L.) is a plant rich in phenolic compounds. There is positive evidence for hypolipidemic effects of SM (Metwally et al. 2009). The consumption of SM leads to the reduction of cholesterol in liver and plasma very low density lipoprotein (VLDL). Also, SM decreases VLDL cholesterol / HDL (high density lipoprotein) cholesterol ratio in rats fed on high-cholesterol diet or on high-sucrose diet. Nevertheless, the previous studies on SM focus less on enzymes involving in triglyceride metabolism, especially PAP enzyme, in details. Therefore, the aim of this study was to determine the effects of dietary supplementation with SM on the liver PAP, liver triglyceride content, plasma lipids, plasma antioxidant capacity, and plasma malondialdehyde (MDA) levels in rats fed with high cholesterol diet enriched with fat.

MATERIALS AND METHODS

Chemicals

Phosphatidic acid (sodium salt), dithiothreitol (DTT), 2,4,6-tripyridyl-s-triazine (TPTZ), silymarin, and phenylmethylsulfunyl fluoride (PMSF) were purchased from Sigma (Sigma Chemical Co., USA). 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.

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. Animals were randomly divided into 4 experimental groups (n = 6/ group) as below:

Group I, normal control rats which received standard pellet chow (Pars dam, Iran). This group received 0.5 ml distillated water by gavage to produce equal injection shock similar to other groups.

Group II, animal rats fed with a standard pellet chow plus 25mg/kg body weight /day SM dissolved in 1% arabic gum (Metwally *et al*., 2009) for 45 days.

Groups III and IV, the rats fed with a lipogenic diet containing standard pellet chow supplemented with 0.5% cholic acid, 20% saturated fat, and 2% cholesterol for two wk to produce hyperlipidemia. Additionally, group III and group IV drank water containing 3% ethanol (Yanardag *et al*., 2005). After 2 weeks of lipogenic diet feeding, group III orally received 25mg/kg body weight /day SM suspended in 1% arabic gum accompanied with lipogenic regime for 45 days. The rats in group IV were maintained on lipogenic diet (hyperlipidemic control group) throughout the experiment and received 0.5 ml distillated water by gavage to produce equal injection shock similar to other groups. On d 60 of the experiment, fasted animals were 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, high density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C) levels were determined by enzymatic method (Pars Azmun kit, Iran) using atuoanalyzer (BT 3000, France). Very low density lipoprotein cholesterol (VLDL-C) was calculated with Fridewald formula (1972). Liver triglyceride was extracted from liver tissue using a chloroform–methanol mixture by Folch-altered method 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 inorganic phosphate from it in order to assess the liver PAP activity and the liver 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 °C for 5 min (Heidarian *et al*., 2011). The homogenate was centrifuged at 4500 rpm at 4 °C for 10 min and then, the supernatant was kept for the enzyme assay.

Determination of liver PAP activity

PAP activity was measured by the method of Yanagita *et al*. (2003) with slight modification. In brief, PAP activity was assayed in the assay buffer containing 50 mM Tris–HCl (pH 7.4), 1 mM of phosphatidate, 1.25 mM magnesium chloride, and 50 to 100 µg of liver enzyme solution in a final assay volume of 0.2 mL. The mixture was incubated for 15 min at 37°C. The reaction was stopped by the addition of 0.8 mL of a solution containing 0.13% sodium dodecyl sulfate, 1.25% ascorbic acid, 0.32% ammonium molybdate-4H2O, and 0.75 N H2SO4. Then, the liberated inorganic phosphate was measured. The phosphomolybdate color was developed at 45°C for 20 min, and the absorbance was measured at 820 nm. Non-enzymatic phosphate release was determined by inactivating the enzymes through boiling for 1 min without substrate. The enzyme activity was expressed as phosphate nanomoles in one minute per milligram of protein. All assays were linear in relation to the incubation time and the protein concentrations used in them. Protein concentrations were determined by the method of Bradford (1976) calibrated with bovine serum albumin.

Measurement of plasma malondialdehyde (MDA)

Lipid peroxidation in the plasma was estimated by the calculation of MDA levels using thiobarbituric acid according to the method of Ohkawa *et al*. (1979). The plasma samples were incubated for 1 hour at 95°C with thiobarbituric acid. After the reaction of MDA with thiobarbituric acid, the reaction color product was measured spectrophotometrically at 532 nm using a Unico 1200 UV–visible spectrophotometer (USA). 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 (1996). 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.

Statistical analysis

Results are expressed as mean ± S.D. The data were analyzed by SPSS software (version 11.5). One-way analysis of variance (ANOVA) was carried out, and the statistical comparisons among the groups were performed with Tukey's test. Differences were considered significant at capacity < 0.05 level.

RESULTS

Effect of SM on the liver triglyceride, cholesterol, and PAP activity

Table 1 demonstrates the effect of SM on liver triglyceride, cholesterol**,** and PAP activity in experimental groups. In group IV, high cholesterol diet caused significant ($p < 0.001$) accumulation of cholesterol and TG in the liver in comparison with groups I and III. In group II, SM significantly decreased (57%) ($p = 0.001$) the content of TG in the liver compared to group I. No significant change was observed in liver cholesterol between groups I and II ($p > 0.05$). Also, in group III SM caused almost threefold decrease (66%) ($p < 0.001$) in the content of liver TG and led to a noticeable reduction (41%) ($p < 0.001$) of hepatic cholesterol content compared to group IV.

Group II showed a significant reduction (20%) ($p < 0.001$) 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 groups II and III. Also, there was a noticeable reduction ($p < 0.05$) in PAP activity between groups III and IV compared to group I.

Effect of SM on plasma lipoproteins

Table 2 shows the values of lipid parameters found after feeding rats on lipogenic diet in experimental groups. The levels of plasma TG, TC, VLDL-C, and LDL-C in group IV (consuming lipogenic diet)

Table 1: Effect of SM on the liver TG, cholesterol, and PAP activity in experimental groups.

Groups	PAP activity (nmolPi/min/mg protein)	Liver triglyceride (mg/g tissue)	Liver cholesterol (mg/g tissue)
Group I (control)	14.24 ± 0.61	4.32 ± 0.41	1.58 ± 0.21
Group II	11.38 ± 0.46*	1.82 ± 0.35*#	1.26 ± 0.24#
Group III	10.92 ± 0.68*	5.48 ± 0.65#	4.84 ± 0.70*#
Group IV	10.46 ± 0.83*	16.35 ± 1.74*	8.20 ± 0.92*

The data were expressed as mean ± S.D.; n = 6 in each group. Normal control (I); control supplemented with silymarin (II); hyperlipidemic rats treated with silymarin (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 SM on TC, TG, LDL-C, HDL-C, VLDL-C levels and atherogenic index in rats fed by high cholesterol diet.

Parameters	Group I	Group II	Group III	Group IV
TC (mg/dl)	58.66 ± 4.88	51.33 ± 5.25	79.97 ± 4.75**	121.16 ± 9.55 #
TG (mg/dl)	54.37 ± 7.23	43.01 ± 3.69*	66.08 ± 5.78**	91.83 ± 7.96#
HDL-C (mg/dl)	58.50 ± 5.01	48.22 ± 4.95*	53.23 ± 3.68**	40.84 ± 4.72#
LDL-C (mg/dl)	18.55 ± 2.05	13.05 ± 1.51*	34.27 ± 3.08**	47.30 ± 4.09#
VLDL-C (mg/dl)	10.58 ± 1.61	8.81 ± 0.98	13.13 ± 1.15**	18.36 ± 1.58#
TC/HDL-C (units)	1.03 ± 0.13	1.04 ± 0.11	1.51 ± 0.13**	2.67 ± 0.22#
LDL/HDL-C (units)	0.31 ± 0.20	0.26 ± 0.27	0.67 ± 0.11**	1.16 ± 0.13#

The data are expressed as mean ± S.D.; n = 6 in each group. Normal control (I); control supplemented with SM (II); hyperlipidemic rats treated with SM (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 (lipogenic regime).
# $p < 0.001$ compared with the corresponding value for groups I and II.

significantly increased ($p < 0.001$) compared to other groups. The level of HDL-cholesterol significantly increased ($p < 0.001$) in group III compared to group IV. In group III, the plasma level of cholesterol significantly decreased ($p < 0.001$) in comparison with group IV. The plasma level of TG in groups II and III (groups supplemented with SM) was significantly lower ($p < 0.05$) than groups I and IV, respectively. In group II the plasma levels of HDL-C and LDL-C significantly reduced ($p < 0.05$) compared to group I. VLDL-C was declined in group II in contrast with group I (control group) but it was not significant ($p > 0.05$). The level of VLDL- C in group III showed a remarkable reduction ($p < 0.001$) compared with group IV. Group IV showed a noticeable ($p < 0.001$) elevation in atherogenic index of plasma (TC/HDL-C and LDL/ HDL-C) with respect to other groups. A significant reduction ($p < 0.001$) was observed for atherogenic index of plasma in group III compared with group IV. No significant change was seen for atherogenic index of plasma in group II compared with group I ($p > 0.05$).

Effects of SM on the plasma level of MDA

Rats fed on lipogenic diet without tratment (group IV) showed a significant increase ($p < 0.001$) in plasma MDA compared to group I fed on normal diet (Fig. 1). In group III (hyperlipidemic rats treated with SM) the consumption of SM led to an important reduction ($p < 0.05$) of plasma MDA compared to group IV. No significant change was observed in plasma MDA level between groups I and II ($p > 0.05$).

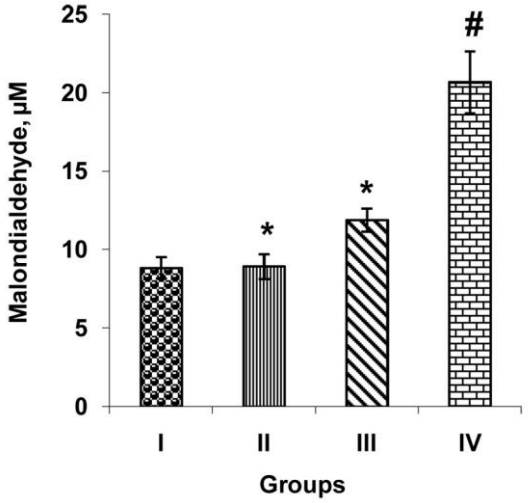

Figure 1. Plasma malondialdehyde level in normal diet (I); normal diet supplemented with silymarin (II); hyperlipidemic rats treated with silymarin (III); hyperlipidemic rats without treatment (IV) groups. The data are expressed as mean ± S.D, n = 6 in each group.

# $p < 0.001$ compared with the corresponding value for normal control animals.

***p** < 0.001 compared with the corresponding value for hyperlipidemic rats without treatment.

Effect of SM on the plasma level of antioxidant power

Fig. 2 shows, the plasma level antioxidant power (FRAP) in each experimental animal group. In groups II and III (rats administrated with SM) the plasma antioxidant power significantly increased ($p < 0.001$) as compared to groups I and IV, respectively. There was a noticeable reduction ($p < 0.001$) in plasma level antioxidant power in group IV (hyperlipidemic animals) in comparison to group I. No significant change was observed in plasma level antioxidant power between groups II and III ($p > 0.05$).

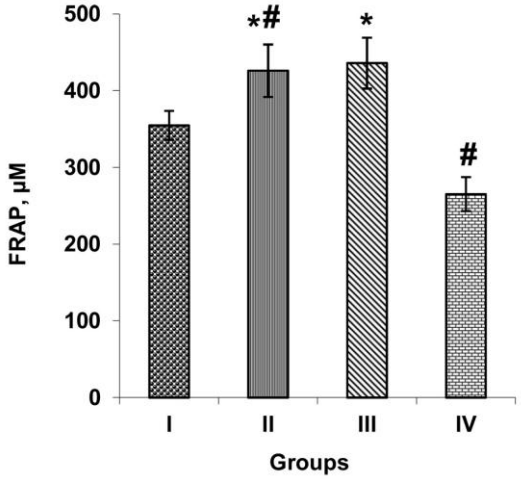

Figure 2. Plasma level antioxidant power (FRAP) in normal diet (I); normal diet supplemented with silymarin (II); hyperlipidemic rats treated with silymarin (III); hyperlipidemic rats without treatment (IV) groups. The data are expressed as mean ± S.D, n = 6 in each group.

# $p < 0.001$ compared with the corresponding value for normal control animals.

***p** < 0.001 compared with the corresponding value for hyperlipidemic rats without treatment.

DISCUSSION

Elevated serum lipid and lipoprotein levels, especially hypercholesterolemia, increase the risk of cardiovascular diseases, fatty liver, carcinogenesis, and atherosclerosis (Gould et al. 2007). Nowadays, many new synthetic oral antihyperlipidemic drugs are available but they have adverse side effects such as myopathy, increase in hepatic aminotransferases, and rhabdomyolysis condition (Anfossi *et al*., 2004). In recent years, many studies have been conducted on using of pure flavonoids owing to their biological properties for treating many important common diseases especially obesity and its complications (Šobolová *et al*., 2006; Andersen *et al*., 2010). In previous studies, SM is introduced as lipid-lowering therapeutic agent (Šobolová *et al*., 2006; Metwally et al. 2009) but, to the best of our knowledge, there has been no study investigating the effect of SM on PAP in hypercholesterolemic animals or humans. In this study, the ingestion of the lipogenic diet led to elevation of the plasma levels of

cholesterol, VLDL, LDL, and liver TG and cholesterol in group IV(hyperlipidemic animals) whereas, in groups II and III (rats treated with SM) the plasma levels of cholesterol, VLDL, LDL, liver TG, and liver cholesterol decreased compared to groups I and IV, respectively. The same results reported by other investigators (Škottová *et al*., 2003; Turley, 2004; Metwally et al. 2009). It has been demonstrated that the consumption of diet rich in fatty acids and cholesterol leads to an accumulation of cholesterol and TG in nonadipose tissues such as the liver. Also, cholesterol represses the activity of HMG-CoA reductase and blocks cholesterol synthesis in the liver. In addition, cholesterol leads to elevated hepatic VLDL secretion and suppression of the synthesis and activity of LDL receptors. Therefore, VLDL remnants and LDL circulate for prolonged period and this cause a rise in total plasma cholesterol (Goldstein and Brown, 1984; Turley, 2004). Moreover, previous studies have shown that the polyphenolic fraction of SM positively modifies lipoprotein profile in plasma. In addition, SM counteracts the development of fatty liver in rats fed on lipogenic diet (Škottová et al. 2003). Additionally, it has been reported that feeding rats by SM leads to significant decrease of total plasma and liver cholesterol through the inhibition of intestinal cholesterol absorption (Škottová *et al*., 2003; Šobolová et al. 2006). Therefore, in this study the significant reduction of LDL and VLDL cholesterol after SM treatment could be due to a decrease in VLDL formation and secretion from the liver. Moreover, the SM supplementation with lipogenic diet caused reduction of plasma LDL-cholesterol and atherogenic index. These results suggest that SM can be applicable to reduce the coronary heart diseases in hyperlipidemic conditions. Additionally, in this research the liver cholesterol reduction was, at least in part, through SM inhibition of intestinal cholesterol absorption which previously reported (Škottová et al. 2003; Šobolová et al. 2006). In our study, rats fed on high cholesterol diet had lower HDL cholesterol concentration than the groups fed by standard laboratory diet. The reduction of HDL cholesterol in several species has been reported following cholesterol feeding (Sorci-Thomas et al. 1989; Rudel, 1997). In this study, the increase in plasma level of HDL fraction in treated hyperlipidemic rats (group III) was another positive change observed after SM treatment. Studies in nonhuman primates indicate that cholesterol-enriched diets result in the reduction of plasma HDL concentration through accelerated apoA-I clearance from the plasma (Sorci-Thomas et al. 1989). Also, there is evidence that plant polyphenolics can increase the liver secretion of apoA-I (Theriault et al. 2000). Therefore, in this study, the elevation of HDL fraction in rats treated with SM can occur due to SM polyphenolic property on apoA-I metabolism.

The published works hitherto do not assess the effect of SM on PAP activity in hyperlipidemic rats. Also, there is no reported data on lipid-lowering mechanism(s) of SM on PAP enzyme. Recently, the effect of garlic reported on the liver PAP activity in normal and hyperlipidemic rats (Heidarian et al. 2011). The consumption of garlic, as a medicinal plant, led to the reduction of liver PAP activity and liver TG content. In present work, our data have shown that the SM supplementation results in higher reduction of PAP activity in group II than group I (control). Therefore, reduction in liver PAP activity, owing to SM, subsequently led to declining of plasma and liver TG which is useful to reduce the risk of cardiovascular diseases, fatty liver, and atherosclerosis. On the other hand, in animals fed with high cholesterol diet (groups III and IV) PAP activity decreased compared to control group whereas, the liver TG and cholesterol concentrations increased in this groups (Table 1). It has been reported that excessive intake of fatty acids caused 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 demonstrated 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 causes the inactivation of PAP (Elabbadi et al. 2005). Therefore, in this study, the decrease of PAP activity (Table 1) in groups fed with high cholesterol diet enriched with fat (groups III and IV) seems to be caused by the accumulation of TG, fatty acids or acyl-CoA esters in the liver. Nevertheless, the reduction of PAP activity in groups fed with high lipid regime (Groups III and IV) can probably act, at least in part, as a defense mechanism of liver for reducing the

production of endogenous liver TG. Therefore, serum and liver TG will decrease and possibly, reduce the risk of liver damage especially fatty liver and cirrhosis. Besides, the elevated liver TG and cholesterol in group III significantly reduced as opposed to group IV (Table 1) through supplementation with SM. Therefore, the SM can reduce the liver content of TG and cholesterol. Overall, SM can be useful in lowering and treating fatty liver in hyperlipidemic regime.

In this research, we did not assess the effects of SM on the other enzymes involving in the lipid metabolism, especially glucose-6-phosphate dehydrogenase (G6PDH) and malic enzyme. These enzymes produce NADPH employed for fatty acid and cholesterol biosyntheses. We propose that future studies can focus on other possible mechanisms of the triglyceride-lowering action of the SM or the bioactive components of milk thistle on the mentioned enzymes.

Oxidative stress of plasma lipoproteins, erythrocytes, and several tissues such as liver, heart and aorta have been demonstrated in experimental animals fed on high cholesterol diet (Küskü-Kiraz et al. 2010). Also, increased oxidative stress parameters have been reported in hypercholesterolemic individuals (Ondrejovičová et al. 2010). The level of MDA is considered as a biomarker of lipid peroxidation. It has been demonstrated that the phenolic content of plant materials is related to their antioxidant activity (Juan and Chou, 2009). SM is well-known for its potent antioxidant and chemoprotectant effects on liver through its flavonolignans. It is easily absorbed from gastrointestinal tract and has strong free radical scavenging properties. Also, silymarin increases the activity of the antioxidant enzymes, such as superoxide dismutase (SOD), catalase, and inhibits lipid peroxidation (Post-White *et al*., 2007). In this research, SM supplementation caused significant decreases in plasma lipid peroxidation together with an increase in plasma antioxidant power (Figs. 1and 2). There are data suggesting the ability of phenolics to modulate and positively affect lipoprotein metabolism (Škottová et al. 2003). The ability of SM to protect a cell membrane against toxic materials is attributed mainly to its antioxidant potential to eliminate reactive oxygen species (ROS), chain-breaking activity and a reduction in ROS production (Gazák et al. 2007; Basiglio et al. 2009). Therefore, on the basis of our results, SM can probably play, at least in part, an anti-atherogenic role by lowering lipids oxidation in hyperlipidemic diets.

CONCLUSIONS

These results clearly suggested that silymarin can be effective in reducing liver phosphatidate phosphohydrolase activity and liver triglyceride. Also, silymarin has blood lipid-reducing, anti- fatty liver, and antioxidant effects in hyperlipidemic regimes, probably due to its polyphenolic properties.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ACKNOWLEDGEMENTS

This study was funded by Shahrekord University of Medical Sciences (grant number 812), Shahrekord, Iran.

REFERENCES

Andersen C, Rayalam S, Della-Fera MA, Baile CA, 2010. Phytochemicals and adipogenesis. Biofactors. 36**:** 415-422.

Anfossi G, Massucco P, Bonomo K, Trovati M, 2004. Prescription of statins to dyslipidemic patients affected by liver diseases: a subtle balance between risks and benefits. Nutr. Metab. Cardiovasc. Dis. 14**:** 215-424.

Basiglio CL, Sánchez Pozzi EJ, Mottino AD, Roma MG, 2009. Differential effects of silymarin and its active component silibinin on plasma membrane stability and hepatocellular lysis. Chem. Biol. Interact. 179**:** 297-303.

Benzie IF, Strain JJ, 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal. Biochem. 239**:** 70-76.

Bradford MM, 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72**:** 248-254.

Carman GM, Han GS, 2006. Roles of phosphatidate phosphatase enzymes in lipid metabolism. Trends Biochem. Sci. 31**:** 694-699.

Dixit N, Baboota S, Kohli K, Ahmad S, Ali J, 2007. Silymarin: A review of pharmacological aspects and bioavailability enhancement approaches.

Indian J Pharmacol. 39**:** 172-179.
Elabbadi N, Day CP, Gamouh A, Zyad A, Yeaman SJ, 2005. Relationship between the inhibition of phosphatidic acid phosphohydrolase-1 by oleate and oleoyl-CoA ester and its apparent translocation. Biochimie 87**:** 437-443.
Fleming IN, Yeaman SJ, 1995. Purification and characterization of N-ethylmaleimide-insensitive phosphatidic acid phosphohydrolase (PAP2) from rat liver. Biochem. J. 308 (Pt 3)**:** 983-989.
Friedewald WT, Levy RI, 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem. 18**:** 499-502.
Gazák R, Walterová D, Kren V, 2007. Silybin and silymarin-new and emerging applications in medicine. Curr. Med. Chem. 14**:** 315-338.
Goldstein JL, Brown MS, 1984. Progress in understanding the LDL receptor and HMG-CoA reductase, two membrane proteins that regulate the plasma cholesterol. J. Lipid Res. 25**:** 1450-1461.
Gould AL, Davies GM, Alemao E, Yin DD, Cook JR (2007). Cholesterol reduction yields clinical benefits: meta-analysis including recent trials. Clin. Ther. 29**:** 778-794.
Heidarian E, Haghighi B, 2008. Enzymological characteristic of plasma membrane phosphatidate phosphohydrolase (PAP_2) from rat liver. Iran. J. Sci. Technol. A 32: 117-122.
Heidarian E, Jafari-Dehkordi E, Seidkhani-Nahal A, 2011. Effect of garlic on liver phosphatidate phosphohydrolase and plasma lipid levels in hyperlipidemic rats. Food Chem. Toxicol. 49**:** 1110-1114.
Juan MY, Chou CC, 2009. Enhancement of antioxidant activity, total phenolic and flavonoid content of black soybeans by solid state fermentation with Bacillus subtilis BCRC 14715. Food Microbiol. 27**:** 586-591.
Küskü-Kiraz Z, Mehmetçik G, Dogru-Abbasoglu S, Uysal M, 2010. Artichoke leaf extract reduces oxidative stress and lipoprotein dyshomeostasis in rats fed on high cholesterol diet. Phytother. Res. 24**:** 565-570.
Maron DJ, 2004. Flavonoids for reduction of atherosclerotic risk. Curr. Atheroscler. Rep. 6**:** 73-78.
Metwally M, El-Gellal A, El-Sawaisi S, 2009. Effects of silymarin on lipid metabolism in rats. World Appl. Sci. J. 6**:** 1634-1637.
Norman SR, Preparation of lipid extracts. In: John MI. (Ed.), Methods of Enzymology. Academic Press, London; 1969. Vol. 14.
Ohkawa H, Ohishi N, Yagi K, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95**:** 351-358.
Ondrejovičová I, Muchová J, Mišľanová C, Nagyová Z, Duračková Z, 2010. Hypercholesterolemia, oxidative stress and gender dependence in children. Prague Med. Rep. 111**:** 300-312.
Petersen KF, Shulman GI, 2006. Etiology of insulin resistance. Am. J. Med. 119**:** S10-S16.
Post-White J, Ladas EJ, Kelly KM, 2007. Advances in the use of milk thistle (Silybum marianum). Integr. Cancer Ther. 6**:** 104-109.
Reue K, Phan J, 2006. Metabolic consequences of lipodystrophy in mouse models. Curr. Opin. Clin. Nutr. Metab. Care 9: 436-441.
Rudel LL, 1997. Genetic factors influence the atherogenic response of lipoproteins to dietary fat and cholesterol in nonhuman primates. J. Am. Coll. Nutr. 16**:** 306-312.
Skottová N, Večeřa R, Urbánek K, Vána P, Walterová D, Cvak L, 2003. Effects of polyphenolic fraction of silymarin on lipoprotein profile in rats fed cholesterol-rich diets. Pharmacol. Res. 47**:** 17-26.
Sobolová L, Skottová N, Večeřa R, Urbánek K, 2006. Effect of silymarin and its polyphenolic fraction on cholesterol absorption in rats. Pharmacol. Res. 53**:** 104-112.
Sorci-Thomas M, Prack MM, Dashti N, Johnson F, Rudel LL, Williams DL, 1989. Differential effects of dietary fat on the tissue-specific expression of the apolipoprotein A-I gene: relationship to plasma concentration of high density lipoproteins. J. Lipid Res. 30**:** 1397-1403.
Theriault A, Wang Q, Van Iderstine SC, Chen B, Franke AA, Adeli K, 2000. Modulation of hepatic lipoprotein synthesis and secretion by taxifolin, a plant flavonoid. J. Lipid Res. 41**:** 1969-1979.
Turley SD, 2004. Cholesterol metabolism and therapeutic targets: rationale for targeting multiple metabolic pathways. Clin. Cardiol. 27**:** III16-21.

van Herpen NA, Schrauwen-Hinderling VB, 2008. Lipid accumulation in non-adipose tissue and lipotoxicity. Physiol. Behav. 94**:** 231-241.

Wen Z, Dumas TE, Schrieber SJ, Hawke RL, Fried MW, Smith PC, 2008. Pharmacokinetics and metabolic profile of free, conjugated, and total silymarin flavonolignans in human plasma after oral administration of milk thistle extract. Drug Metab. Dispos. 36:65-72.

Yanagita T, Han SY, Wang YM, Tsuruta Y, Anno T, 2003. Cycloalliin, a cyclic sulfur imino acid, reduces serum triacylglycerol in rats. Nutrition 19**:** 140-143.

Yanardag R, Peksel A, Yesilyaprak B, Doger MM, Arisan-Atac I, 2005. Effects of a combination of niacin and chromium (III)-chloride on the skin and lungs of hyperlipemic rats. Biol. Trace Elem. Res. 103**:** 249-260.