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The Effect of Supplementation of Persimmon Leaf Extract on Lipid and Antioxidant Metabolism in Rats Fed a High-cholesterol Diet*

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Fruits and vegetables reportedly have a protective effect against hyperlipidemia and oxidative disease. Accordingly, this study aimed to investigate the lipid-lowering effect and antioxidative capacity of persimmon leaf extract (PLE) in rats fed a high-cholesterol diet. Male rats were fed a high-cholesterol (1% wt/wt) or high-cholesterol diet supplemented with Lovastatin (0.02% wt/wt) or PLE (0.2% wt/wt) for 5 weeks. The concentration of plasma total cholesterol was significantly lower in the PLE group than in the lovastatin group. However, the concentration of plasma HDL-cholesterol and the ratio of HDL-cholesterol/total-cholesterol (%) were significantly higher in the PLE group than in the control group. The PLE supplement also significantly lowered the contents of hepatic cholesterol and triglyceride. In comparing fecal sterol contents, the PLE group saw a significant increase of both neutral and acidic sterol compared to the other groups. The PLE supplement significantly lowered plasma GOT and GPT activity, which are indices of hepatic toxicity. Plasma TBARS concentration was significantly lower in the PLE group than in the control group, while hepatic TBARS level was not significantly different between the groups. In a comparison of hepatic antioxidant parameters, SOD, catalase and GSH-Px activity were significantly higher in the PLE group than in the control group. However, the PLE supplement significantly lowered antioxidant enzyme activity in the erythrocyte. Furthermore, these results suggest that supplementation of PLE promoted the excretion of fecal sterols, thereby leading to decreased absorption of dietary cholesterol. In addition, PLE may play an important role in regulating antioxidative capacities by altering SOD and CAT activity.

Key words : Persimmon leaf, Lipid-lowering, Antioxidant metabolism, High-cholesterol diet

INTRODUCTION

Hypercholesterolemia is considered a major risk factor in the progression of coronary athrosclerosis and is associated with an increase in the incidence of myocardial ischemia and cardiac events.[1,2] Plasma cholesterol concentration can be regulated by the biosynthesis of cholesterol, removal of cholesterol from the circulatory system, absorption of dietary cholesterol, and excretion of cholesterol via bile and feces. Recently, there was an extensive investigation of the factors influencing plasma lipids.

A large number of plants, which have been used as food and medicine in Asia, often based on the traditional prescriptions in literature, have been supplying spices, beverages and medicines to the other parts of the world.[3] Diets rich in fruits and vegetables are associated with a lower risk of coronary heart disease and cancer. Fruits and vegetables are considered to be the main source of dietary antioxidants. Although vitamin E, vitamin C and carotenoids have received the most attention as anti-carcinogens and as defenses against degenerative diseases of aging, the nutritional role of flavonoid antioxidants in fruits and vegetables has generally been overlooked. Although little is known about their absorption and excretion in humans and very little is known about their bioavailability4), tannins and catechins would appear to have direct antioxidant effects by scavenging free radicals as phenolic bioflavonoids.[5]

The fruits of the persimmon (*Diospyros kaki Thunb*; Ebenaceae) have been used for the treatment of apoplexy, hematemesis, chilblan and burns. Persimmon leaves containing vitamin C, caffeine, tannin, amino acids and flavonoids are commonly used as a tea in Asia.[6-8] Recently, condensed tannin, an astringent component in green persimmon fruits, was found to prolong the life span of stroke-prone spontaneously hypertensive rats and to inhibit the angiotensin converting enzyme.[9,10] Tannin has been shown to lower serum and hepatic cholesterol concentrations and increase fecal sterol excretion in rats with hypercholesterolemia.[11]

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The objective of this study was to investigate the effects of persimmon leaf extract on lipid lowering and to evaluate its effect on antioxidant actions in rats fed a high-cholesterol diet.

MATERIALS AND METHODS

1. Animals and diets

Fifty male Sprague-Dawley rats weighing between 50 and 55 g were purchased from the Korea Research Institute of Chemical Technology (Daejon, Korea). The animals were individually housed in stainless steel cages in a room with controlled temperature (24℃) and lighting (alternating 12-hour periods of light and dark). The rats were fed a palletized commercial chow diet for 10 days after arrival. Next, the rats were randomly divided into three groups (n = 10) and fed a high-cholesterol diet (1%, wt/wt). Either lovastatin (0.02%) or Persimmon leaf extract (0.2%) was supplemented with the experimental diet for 6 weeks. The composition of the experimental diet, as shown in Table 1, was based on the AIN-76 semisynthetic diet.[12)] The animals were given food and distilled water ad libitum throughout the experimental period. Food consumption and weight gain were measured daily and weekly. Feces collected during the last 3 days using metabolic cages were used to determine the fecal sterol. At the end of the experimental period, the rats were anesthetized with Ketamine following a 12-hour fast. Blood samples were collected from the inferior vena cava in heparin-coated tubes to determine the plasma lipid profile. The livers were removed and rinsed with physiological saline. All samples were stored at -70℃ until analyzed.

Table 1. Composition of Experimental Diets (%)

Composition	Control	Lovastatin	PLE
		n=10	
Casein	20.0	20.0	20.0
D,L-methionine	0.3	0.3	0.3
Corn starch	15.0	15.0	15.0
Sucrose	49.0	48.98	48.0
Cellulose powder	5.0	5.0	5.0
Corn oil	5.0	5.0	5.0
AIN-76 mineral mixture[1]	3.5	3.5	3.5
AIN-76 vitamin mixture[2]	1.0	1.0	1.0
Choline bitartrate	0.2	0.2	0.2
Cholesterol	1.0	1.0	1.0
Lovastin	-	0.02	-
PLE[3]	-	-	0.2
Total	100	100	100

[1]AIN-76 mineral mixture (Harlan Teklad Co. USA)
[2]AIN-76A vitamin mixture (Harlan Teklad Co. USA)
[3]PLE : Persimmon leaf extract

2. Extraction of persimmon leaf

The persimmon leaf was obtained from an herbal medicine market (Gongju, Korea). The dried persimmon leaf (2.6 Kg) was then homogenized to a fine powder. Seven hundred g of powdered material was boiled in 3.5 L of distilled water for 1 hr. The water extract was filtered through Whatman No. 1 filter paper and reextracted using the same method. These water extracts were combined and evaporated under a vacuum at 40℃ and dried to a powder using a freeze-dryer at -50℃. Six hundred and fifty fine g of powder was obtained from 2.6 Kg of the dried persimmon leaves.

3. Plasma and hepatic lipids

Plasma cholesterol and HDL-cholesterol concentrations were determined using a commercial kit (Sigma) based on a modification of the cholesterol oxidase method of Allain et al.[13)] HDL-fractions were separated using a Sigma kit based on the heparin-manganese precipitation procedure.[14)] Plasma triglyceride concentrations were measured enzymatically using a kit from Sigma Chemical Co., a modification of the lipase-glycerol phosphate oxidase method.[15)] Hepatic lipids were extracted using the procedure developed by Folch et al.[16)] Dried lipid residues were dissolved in 1 mL of ethanol for cholesterol and triglyceride assays. Triton X-100 and a sodium cholate solution (in distilled H_2O) were added to 200 L of the dissolved lipid solution to produce final concentrations of 5 g/L and 3 mmol/L, respectively. Hepatic cholesterol and triglycerides were analyzed with the same enzymatic kit as used in the plasma analysis.

4. HMG-CoA reductase and ACAT activities

The microsomes were prepared according to the method developed by Hulcher and Oleson[17)] with a slight modification. Two grams of liver tissue were homogenized in 4 mL of an ice-cold buffer (pH 7.0) containing 0.1 M of triethanolamine, 0.02 M of EDTA, and 2 mM of dithiothreitol, pH 7.0. The homogenates were centrifuged for 10 min at 10,000 g and then at 12,000 g at 4℃. Next, the supernatants were ultracentrifuged twice at 100,000 g for 60 min at 4℃. The resulting microsomal pellets were then redissolved in 1 mL of a homogenation buffer for protein determination[18)] and finally analyzed for HMG-CoA reductase and ACAT activity.

HMG-CoA reductase activity was determined using the method described by Shapiro et al.[19)] with a slight modification, involving the use of freshly prepared hepatic microsomes. An incubation mixture (120 L) containing microsomes (100~150 g) and 500 nmol of NADPH (dissolved in a reaction buffer containing 0.1 M of triethanolamine and 10 mM of EDTA) were preincubated at 37℃ for 5 min. Next, 50 nmol of

[^{14}C]-HMG-CoA (specific activity; 2.1420 GBq/mmol; NEMTM Life Science Products, Inc., Boston, MA) was added and the incubation was continued for 15 min at 37℃.

The reaction was terminated by the addition 30 L of 6 M of HCl. The resultant reaction mixture was incubated at 37℃ for a further 15 min to convert the mevalonate into mevalonolactone. The incubation mixture was centrifuged at 10,000 g for 5 min, and the supernatant was spotted on a Silica Gel 60 F254 TLC plate with a mevalonolactone standard. The plate was developed in benzene/acetone (1 : 1, v/v), and air-dried. Finally, the Rf 0.3~0.6 region was removed by scraping using a clean razor blade, and ^{14}C radioactivity was determined using a liquid scintillation counter (Packard Tricarb 1600TR; Packard Instrument Company, Meriden, CT). The results were expressed as pmole mevalonate synthesized per min per mg protein.

ACAT (Acyl-CoA : Cholesterol Acyltransferase) activity was determined using freshly prepared hepatic microsomes as developed by Erickson et al.[20] and modified by Gillies et al.[21] To prepare the cholesterol substrate, 6 mg of cholesterol and 600 mg of Tyloxapol (Triton WR-1339, Sigma) were each dissolved in 6 mL of acetone, mixed well, and completely dried in N_2 gas. The dried substrate was then redissolved in 20 mL of distilled water to a final concentration of 300 g of cholesterol/mL. Next, reaction mixtures containing 20 L of a cholesterol solution (6 g of cholesterol), 20 L of a 1 M of potassium-phosphate buffer (pH 7.4), 5 L of 0.6 mM bovine serum albumin, 50~100 g of the microsomal fraction, and distilled water (up to 180 L) were preincubated at 37 ℃ for 30 min. The reaction was then initiated by adding 5 nmol of [^{14}C]-Oleoyl CoA (specific activity; 2.0202 GBq/mmol; NEMTM Life Science Products, Inc.) to a final volume of 200 L. The reaction time was 30 min at 37 ℃. The reaction was stopped by the addition 500 L of isopropanol/heptane (4 : 1, v/v), 300 L of heptane, and 200 L of 0.1 M potassium phosphate (pH 7.4). Then, the reaction mixture was allowed to stand at room temperature for 2 min. Finally, an aliquot (200 L) of the supernatant was subjected to scintillation counting. ACAT activity was expressed as pmole cholesteryl oleate synthesized per min per mg protein.

5. Fecal sterols

Fecal neutral sterols were determined using a simplified micro-method developed by Czubayko et al.[22] Gas-liquid chromatography was carried out with a Hewlett-Packard gas chromatograph (Model 5809; Palo Alto, CA) equipped with a hydrogen flame-ionization detector and SacTM-5 capillary column (30 m 0.25 mm i.d., 0.25 m film; Supelco Inc., Bellefonate, PA, USA). Helium was used as the carrier gas. The temperatures were set at 230℃ for the column and 280 ℃ for the injector/detector temperature. 5-cholestane (Supelco Inc.) was used as the internal standard. The daily neutral sterol excretion was calculated based on the amount of cholesterol, coprostanol, and coprostanone in each sample. Fecal bile acid was extracted with t-butanol and quantified enzymatically with 3α-hydroxysteroid dehydrogenase.[23]

6. Preparation of erythrocyte and hepatic antioxidant enzyme source

After centrifugation at 1000 ×g for 15 mim at 4℃, plasma and buffy coat were carefully removed. The separated cells were washed three times by resuspending in 0.9% NaCl solution and repeating the centrifugation. The washed cells were lysed in an equal volume of water and mixed thoroughly. Hemoglobin concentration was estimated in aliquot of this hemolysate, using a commercial assay kit (No. 525-A, Sigma, Chemical Co.). Appropriately, diluted hemolysate was then prepared from the erythrocyte suspension by the addition of distilled water for the estimation of catalase (CAT) and glutathione peroxidase (GSH-Px) activity.

Futhermore, to remove hemoglobin by precipitation with chloroform : ethanol, 0.4 mL of ethanol : choloroform (3 : 5, v/v) mixture was added to an aliquiot (1 mL) of the hemolysate cooled in ice.[24] This mixture was stirred constantly for 15 min and then diluted with 0.2 mL of water. After centrifugation for 10 min at 1600 g, the pale yellow supernatant was separated from the protein precipitate and used to assay for superoxide dismutase (SOD).

The preparation of the enzyme source fraction in the hepatic tissue was as follows. One gram of hepatic tissue was homogenized in a five-fold weight of a 0.25 M sucrose buffer and centrifuged at 600 g for 10 min to discard any cell debris. Then, the supernatant was centrifuged at 10,000 g for 20 min to remove the mitochondria pellet.

Finally, the supernatant was further ultracentrifuged at 105,000 g for 60 min to obtain the cytosol supernatant. The amount of protein in the mitochondrial and cytosolic fractions was measured according to the method of Bradford[18] using bovine serum albumin as the standard.

7. Antioxidant Enzyme Activities

SOD activity was spectrophotometrically measured using a modification of the method of Marklund and Marklund.[25] Briefly, SOD was detected based on its ability to inhibit the superoxide-mediated reduction. One unit was determined as the amount of enzyme that inhibited the oxidation of pyrogallol by 50%. The activity was expressed as unit/g Hb and that of tissue

as unit/mg protein. CAT activity was measured using the Aebis[26)] method with a slight modification, in which the disappearance of hydrogen peroxide was monitored spectrophotometrically at 240 nm for 5 min. A molar extinction coefficient of 0.041 $mM^{-1}cm^{-1}$ was used to determine CAT activity. The activity was defined as decreased H_2O_2 nmol/min/g Hb and that of tissue as nmol/min/mg protein.

8. GSH-Px activities

GSH-Px activity was measured using Paglia and Valentines[13)] method with a slight modification. The reaction mixture contained 1 mM glutathione, 0.2 mM NADPH, and 0.24 unit of glutathione reductase in 0.1 M of Tris-HCl (pH 7.2) buffer. The reaction was initiated by adding 0.25 mM H_2O_2 and the absorbance was measured at 340 nm for 5 min. A molar extinction coefficient of 6.22 $mM^{-1}cm^{-1}$ was used to determine the activity. The activity was expressed as the oxidized NADPH nmol/min/g Hb and that of tissue as nmol/min/mg protein.

9. Lipid Peroxidation

As a marker of lipid peroxidation production, plasma and hepatic thiobarbituic acid reactive substances (TBARS) concentrations were measured using the method of Ohkawa et al.[27)] Two hundred L of the plasma and hepatic homogenate (20%, w/v) was mixed with 200 L of 8.1% (w/v) sodium dodecyl sulfate, 1.5 mL of 20% (w/v) acetic acid (pH 3.5) and 1.5 mL of 0.8% (w/v) TBA. The reaction mixture was heated at 95℃ for 60 min. After cooling the mixture, hepatic mixture was added to 1.0 mL of distilled H_2O and 5.0 mL of a butanol : pyridine (15 : 1) solution. This reaction mixture was centrifuged at 800 g for 15 min and the resulting colored layer was measured at 532 nm using 1,1,3,3,- tetraethoxypropane (Sigma Chemical Co.) as the standard.

10. Statistical analysis

All data is presented as the mean±SE. The data was evaluated by one-way ANOVA using an SPSS program, and the differences between the means assessed using Duncans multiple-range test. Statistical significance was considered at $p<0.05$.

Table 2. Effects of Lovastatin and PLE Supplementation on Weight Gains, Food Intake and Organ Weights in High Cholesterol Fed-Rats

	Control	Lovastatin	PLE
Weignt Gain(g/day)	7.93±0.29[a]	7.84±0.30[a]	7.10±0.21[a]
Food Intakes(g/day)	24.21±0.54[a]	23.76±0.61[a]	24.76±0.21[a]
Organ Weights(g)			
Liver	16.45±0.68[a]	17.23±0.79[a]	16.90±0.69[a]
Heart	1.43±0.01[a]	1.36±0.01[a]	1.34±0.01[a]
Kidney	3.11±0.01[a]	2.89±0.01[a]	3.09±0.14[a]

Values are mean±S.E [ab]Means in same raw not sharing a common superscript different among groups($p<0.05$)

RESULT

1. Food intake, weight gain, and organ weights

There was no significant difference in food intake, weight gain or organ weights among the groups (Table 2). Accordingly, these were not seemingly affected by lovastatin or PLE supplements.

2. Plasma and hepatic lipids

The supplementation of PLE significantly lowered plasma total cholesterol and triglyceride concentration compared to the other groups (Table 3). However, the supplementation of lovastatin and PLE significantly increased HDL-cholesterol concentration compared to the other groups. The supplementation of PLE significantly increased HDL-C/total-C ratios compared to the other groups. The atherogenic index was significantly lower in the lovastatin and PLE groups than in the control groups. In addition, the supplementation of lovastatin and PLE significantly lowered the hepatic triglyceride level compared to the control group.

Table 3. Effects of Lovastatin and PLE Supplementation on Plasma and Hepatic Lipids in High Cholesterol-Fed Diet

	Control	Lovastatin	PLE
Plasma			
TC1(mg/dl)	82.1±1.82[a]	77.4±1.84[a]	63.2±3.43[b]
TG(mg/dl)	55.5±2.14[b]	69.0±4.12[a]	32.3±1.67[c]
HDL-C(mg/dl)	22.4±0.76[c]	23.4±0.42[b]	27.8±0.96[a]
HDL-C/TC(%)	27.3±0.82[b]	30.2±0.55[b]	44.0±1.49[a]
Athero. Index2	2.7±0.28[a]	2.3±0.27[b]	1.8±0.45[c]
Liver			
TC(mg/g)	74.71±0.93[a]	63.53±2.34[b]	72.51±2.31[a]
TG(mg/g)	93.99±8.01[a]	71.31±4.85[b]	70.00±2.70[b]

Values are mean±S.E. [ab]Means in the same row not sharing a common superscript are significantly different ($p<0.05$) between the groups.
[1]TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol.
[2]Athero. Index (atherogenic index) : (total cholesterol-HDL-cholesterol)/HDL-cholesterol.

3. Hepatic HMG-CoA reductase and ACAT activity

Hepatic HMG-CoA reductase activity was significantly lower in the lovastatin group than in the other groups. However, hepatic ACAT activity did not differ between the groups (Table 4).

4. Fecal sterols

The daily excretion of fecal sterols is shown in Figure 1. The effect of the PLE supplementation did result in

Table 4. Effects of Lovastatin and PLE Supplementation on hepatic HMG-CoA reductas and ACAT activities in High Cholesterol Fed-Rats

	Control	Lovastain	PLE
		pmol/min/mg/protein	
HMG-CoA reductase activities	59.30±3.33[a]	49.54±1.46[b]	64.56±2.90[a]
ACAT activities	111.57±3.09[a]	106.66±2.23[a]	106.71±5.38[a]

Values are mean±S.E.[ab]Means in the same row not sharing a common superscript are significantly different ($p<0.05$) between the groups.

certain changes in the fecal sterol under the cholesterol-fed condition. The PLE supplement induced an increase in the excretion of neutral, acidic and total fecal sterol compared to the other groups.

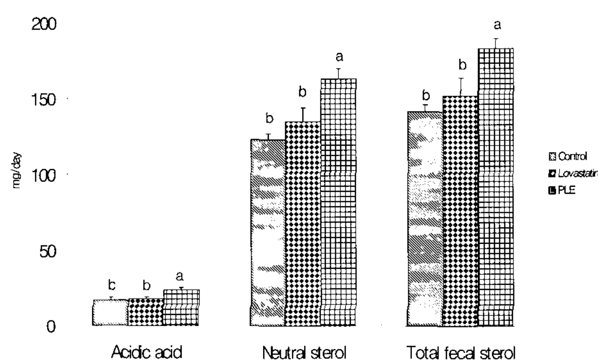

Fig 1. Effect of Lovastatin and PLE Supplemention on Excretion of Fecal sterols

Values are mean±S.E. [ab]Means in the same colum sharing a common superscript are not significantly different between the groups by Duncan's multiple test at $p<0.05$.

5. Plasma and hepatic TBARS level

Plasma TBARS level was significantly lower in the lovastatin and PLE groups than in the control group, whereas there was no difference in hepatic TBARS level between the groups (Table 5).

Table 5. Effects of Lovastatin and PLE Supplementation on plasma and hepatic TBARS levels

	Control	Lovastatin	PLE
Plasma TBARS(nmol/ml)	2.31±0.01[a]	1.93±0.01[b]	1.40±0.01[c]
Liver TBARS(nmol/g tissue)	27.11±1.44[a]	27.41±0.90[a]	28.92±1.01[a]

Values are mean±S.E. [ab]Means in the same row not sharing a common superscript are significantly different ($p<0.05$) between the groups.

6. GOT and GPT activity in plasma

The supplementation of PLE significantly lowered plasma GOT activity compared to the other groups. However, there was no difference in GPT activity between the groups (Table 6).

Table 6. Effects of Lovastatin and PLE Supplementation on Plasma GOT and GPT activities in High Cholesterol Fed-Rats

	Control	Lovastatin	PLE
		Kamen/ml	
GOT	84.36±3.61[a]	89.57±2.94[a]	73.17±2.43[b]
GPT	18.79±2.29[a]	20.45±2.76[a]	16.92±1.07[a]

Values are mean±S.E. [ab]Means in the same row not sharing a common superscript are significantly different ($p<0.05$) between the groups.

7. Erythrocyte and hepatic antioxidant enzyme activity

The antioxidant enzyme activity of erythrocyte and liver are shown in Table 7.

Hepatic SOD and GSH-Px activity were higher in the PLE compared to the other groups. However, there was no difference in CAT activity between the groups.

Table 7. Effects of Lovastatin and PLE Supplementation on Antioxidant enzyme activies in liver and erythrocyte in High Cholesterol-Fed Rats

	Control	Lovastatin	PLE
Erythrocyte			
SOD(unit/mg Hb)	290.34±11.63[a]	278.33±11.12[a]	164.63±16.80[b]
CAT(μmol/min/mg Hb)	411.84±20.89[a]	404.07±35.95[a]	373.01±34.81[a]
GSH-Px(nmol/min/mg Hb)	19.34±2.22[a]	14.19±0.96[b]	11.53±0.84[b]
Liver			
SOD(unit/mg protein)	0.21±0.01[b]	0.27±0.01[a]	0.30±0.01[a]
CAT(μmol/min/mg protein)	2.42±0.01[a]	2.87±0.17[a]	2.58±0.16[a]
GSH-Px(nmol/min/mg protein)	11.41±1.05[b]	11.20±0.99[b]	14.09±0.60[a]

Values are mean±S.E. [ab]Means in the same row not sharing a common superscript are significantly different ($p<0.05$) between the groups.

DISCUSSION

It has been established that phenolic substances in the diet can promote antioxidant activity and have a preventive effect on atherosclerosis.[28-31] The natural sources of these compounds are vegetables and fruits.

The present study investigated the effects of dietary persimmon leaf extract (PLE) to determine their possible role in a high-cholesterol-fed condition. The results suggest that the plasma lipid-lowering and antioxidative effects of PLE supplements were very potent in high cholesterol-fed rats. Most studies on tannic acid in the persimmon have focused on its peel and fruit. There have been few studies of the biological activity of the persimmon leaf. Gorinstein and Kulasek[32] reported that two diets, fortified with 7% whole dry persimmon and

7% phenol-free dry persimmon, given to cholesterol-fed rats improved plasma lipid levels. However, an antioxidant effect was only seen in the diet supplemented with whole persimmon. According to the results, PLE lowered the plasma TC, TG and hepatic cholesterol while increasing fecal sterols. PLE supplementation also lowered the level of hepatic triglyceride, just as lovastatin did. In the present study, the increase in fecal sterols in the PLE group indicates that there seemed to be a reduction in cholesterol absorption by the supplementation of PLE in high cholesterol-fed rats. There was no significant difference in HMG-CoA reductase activity between the PLE and control groups. This indicates that cholesterol biosynthesis was not affected by the supplementation of PLE.

Antioxidant enzymes are present in biological systems and cooperate with antioxidants to defend against oxidative stress-induced cellular damage. Erythrocyte and the liver are thought to be good systems for studying the relationship between antioxidants and oxidative stress. The presence of hemoglobin, with peroxidative activity, potentially catalyzes the oxidation of intracellular macromolecules. Thus, an efficient antioxidant defense system, including enzymes and antioxidants, is crucial to enable erythrocyte to prevent oxidative damage. The liver, the major organ responsible for drug detoxification, can generate free radicals through microsomal cytochrome p-450-catalyzed reactions.

Superoxide dismutase (SOD) is involved in quenching active oxygen species and in the formation of H_2O_2. H_2O_2 is decomposed to H_2O by catalase and GSH peroxidase (GSH-Px). The dietary PLE did have a significant affect on hepatic and erythrocyte antioxidant enzyme activity, SOD, GSH-Px. SOD converts superoxide radicals into hydrogen peroxide, which is then converted to water by both CAT and GSH-Px. As a result, these enzymes can prevent damage by detoxifying reactive oxygen species. Lipid peroxidation is an autocatalytic process, which is a common consequence of cell death. This process may cause peroxidative tissue damage in inflammation, cancer, toxicity of xenobiotics and aging.[33] The level of plasma TBARS in the PLE-treated animals was significantly lower than in the control group, thus indicating a decreased rate of lipid peroxidation. The overall potential of an antioxidant defense was greater in the PLE-supplemented group than in the control group. Besides, SOD activity was significantly lower in the PLE group than in the control group. These results can be supported by the fact that the cell could be protected from lipid peroxidation by intracellular antioxidant processes and cellular SOD, GSH-Px.

Even though flavonoids are generally regarded as good antioxidants, most results are based on *in vitro* studies and very little is known about their actual *in vivo* capacity to function as antioxidants.[4] These results suggest that the free-radical scavenging activity of the SOD is only effective when it is accompanied by an increase in the activity of catalase and/or GSH-Px. The reason is that SOD generates hydrogen peroxide as a metabolite, which is more toxic than oxygen radicals in cells and needs to be scavenged by catalase and/or GSH-Px.[11] As such, a concomitant increase in catalase and/or GSH-Px activity is essential if a beneficial effect from the high SOD activity is to be expected.[11]

Ahn et al.[34] reported that the strong radical scavenging activity of persimmon seed extract and grape seed extract *in vitro* may result, at least in part, from a high tannin content, and that the decrease of lipid peroxidation in persimmon seed extract-administered rats was due to an increase in antioxidant enzyme activity.

A concomitant decrease in antiperoxidative enzyme activity, namely SOD and GSH-Px, was observed in the PLE group, and both enzymes play an important role in scavenging toxic intermediates of incomplete oxidation in the erythrocyte. As a decrease in antioxidant enzyme activity, in general, was most pronounced for the stronger antioxidants tested[35], it can be hypothesized that the antioxidant enzyme in erythrocyte is down-regulated by the supplementation of PLE in response to an improved antioxidant status of the erythrocyte due to the presence of antioxidants in PLE. However, it was up-regulated in the liver although the reason is unknown.

In conclusion, it is worth mentioning that PLE seemed to exhibit an inhibitory effect on cholesterol absorption since it enhanced the fecal neutral sterol. However, more studies are needed, with various animal models and using functional compound(s) present in PLE, to elucidate the lipid-lowering action of PLE and the differential effects of PLE in the antioxidant enzyme activity of the erythrocyte and liver.

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