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Effect of *Paeonia lactiflora* Pall. Seed Extracts and *Forsythia viridissima* Lindl. Extracts on Antioxidative System and Lipid Peroxidation in Erythrocytes of Rats Fed High-Cholesterol Diet

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Abstract

This study was conducted to investigate the antioxidative effects of *Paeonia lactiflora* Pall. (PL) seed extracts and *Forsythia viridissima* Lindl. (FVL) extracts on the antioxidative defense system and lipid peroxidation in the erythrocytes of rats fed a high-cholesterol diet. Sprague-Dawley male rats weighing 100±10 g were randomly assigned to nine experimental groups and fed 0.5% cholesterol. The HC group did not receive any supplement, while the MP1 group was supplemented with 0.1% methanol extract of PL seed, the MP2 group with 0.2% methanol extract of PL seed, the EP1 group with 0.05% ether-soluble fraction of PL seed, the EP2 group with 0.1% ether-soluble fraction of PL seed, the MS1 group with 0.05% methanol extract of FVL, the MS2 group with 0.1% methanol extract of FVL, the ES1 group with 0.025% ethyl acetate-soluble fraction of FVL, and the ES2 group with 0.05% ethyl acetate-soluble fraction of FVL. The experimental diets were fed *ad libitum* for 3 weeks. The erythrocyte SOD activity in the EP1 and EP2 groups increased 38% and 59%, respectively, when compared with the HC group, while the erythrocyte GSHpx activity in the EP1, EP2, and ES2 groups increased 30%, 31%, and 29%, respectively, when compared with the HC group. The level of erythrocyte TBARS was significantly lower in the MP2, EP1, and EP2 groups than in the HC group, yet the level of serum TBARS was significantly lower in the all supplemented groups than in the HC group. The level of serum HDL-TBARS was significantly lower in the EP1 and EP2 groups than in the HC group, while the level of serum LDL-TBARS was significantly lower in the all the supplemented groups than in the HC group. Accordingly, the results indicated that the PL seed extracts and FVL extracts reduced oxidative damage by activating the antioxidative defense system in the erythrocytes of rats fed a high-cholesterol diet.

Key words: *Paeonia lactiflora* Pall. seed extract, *Forsythia viridissima* Lindl. extract, high-cholesterol diet, erythrocyte antioxidative system, lipid peroxidation

INTRODUCTION

The incidence of cardiovascular disease is increasing rapidly every year, reflecting current diets, changes in the living environment and aging populations (1,2). Hypercholesterolemia, diet factors, smoking, and hypertension are all known contributors to cardiovascular disease. In particular, the diet factors include the ratio of unsaturated fatty acid to saturated fatty acid in the diet lipids (3), diet cholesterol (4), and diet lipid content (5). Consequently, a high-lipid diet and high-cholesterol diet, which increases the lipid peroxide content in the tissue and serum, has been found to cause oxidative damage to the body (6-10).

In such previous studies (8-10), after increasing the lipid peroxide content, the effect of vitamin E and several antioxidants was investigated, as the quantity and activity of antioxidative enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GSHpx), and catalase (CAT), were found to be insufficient in a high-cholesterol state with oxidative stress and imbalanced antioxidation defense mechanism due to diet factors (10-12). Therefore, since an imbalance in the antioxidation defense system is apparently an important cause of cardiovascular disease (10,12), the study of antioxidation activation materials that can control this is very important, and several recent studies have focused on identifying the antioxidation functions of various materials, including the use of medicinal plants in food.

In oriental medicine, the perennial herb *Paeonia lactiflora* Pall., belonging to Ranuclaceae Paeonia, is commonly used for treating spasms, infections, stress, fever, urinary problems, and for sedation (13), while *Forsythia viridissima* Lindl, belonging to Oleaceae, is used to treat

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puffiness, gonorrhea, urinary problems, hemorrhoids, tuberculosis, itching, and as a poison antidote (14). *Paeonia lactiflora* Pall. seed is used only by present seed breeding, and is not used for herb medicine and is abolished. Despite production of *Paeonia lactiflora* Pall. seed is low and physiology vitality is excellent by high-volume mass production is available but present removes peony flower for peony root production, study about antioxidation effect in living body of *Paeonia lactiflora* Pall. seed extract is unprepared. Previous research on the physiological activity of *Paeonia lactiflora* Pall. seeds has found a high anticancer activity (15), antioxidation (16), inhibition of tyrosinase and lipoxygenase (17), and antioxidation effect from peony acupuncture (18,19) in resveratrol and its glycoside and oligomers *in vitro*.

Meanwhile, previous research on the physiological activity of *Forsythia viridissima* Lindl. has found superior antioxidation activity *in vitro* (20-22), anti-inflammatory effects (23), antimicrobial action (24), and hypotensive action (25). Consequently, in previous studies, when rats fed a high-cholesterol diet were supplied with *Paeonia lactiflora* Pall. seed extracts and *Forsythia viridissima* Lindl. extracts (26,27), there was a decrease in tissue damage and the antioxidative system of the liver tissue was strengthened. However, these studies did not examine the antioxidation function in the erythrocytes, known as an indicator of oxidation damage due to their involvement in the interior circulatory system.

Accordingly, this study investigated the antioxidative effects of different concentrations of a *Paeonia lactiflora* Pall. seed methanol extract, *Paeonia lactiflora* Pall. seed ether-soluble fraction, *Forsythia viridissima* Lindl. methanol extract, and *Forsythia viridissima* Lindl. ethyl acetate-soluble fraction on the antioxidative defense system and lipid peroxidation in the erythrocytes of rats fed a high-cholesterol diet.

MATERIALS AND METHODS

Preparation of plant materials

The *Paeonia lactiflora* Pall. (PL) seeds used in the experiment were picked in mid-August 2000 from the herb garden at the Uisong Medicinal Plant Experiment Station, Gyeongbuk, Korea, dried in the shade, and ground through a 100 mesh size. The *Forsythia viridissima* Lindl. (FVL) fruit was bought at the Yakryeong market, Deagu, Korea and the seeds crushed into a powder.

Manufacture of methanol extract, ether-soluble fraction and ethyl acetate-soluble fraction

300 g of the dried PL seeds was crushed, added to 2 L of methanol, extracted for 24 hours at room temperature, then filtrated through a paper filter (Whatman No. 2, England). After repeating the above procedures three times, the methanol extract was then pressure-reduced and concentrated using a rotary evaporator (Bü chi, Sweden) at 40°C or lower to obtain 37.5 g of extract. Next, to remove any fat, the extract was mixed twice with 1 L of an 80% methanol solution and 500 mL of *n*-hexane and the lower layer pressure-reduced and concentrated. The resulting 25.5 g of concentrated extract was then redissolved in 10% soluble methanol and fibrilated using 1 L of ether twice, resulting in 788 g of soluble ether fibrin from the upper layer.

10 kg of FVL was crushed, added to 120 L of methanol, extracted for 24 hours at room temperature, then filtrated through a paper filter (Whatman No. 2, England). After repeating the above procedures two times, the methanol extract was then pressure-reduced and concentrated using a rotary evaporator (Büchi, Sweden) at 40°C or lower to obtain 624.4 g of a methanol extract. Next, to remove any fat, the extract was mixed twice with 30 L of an 80% methanol solution and 20 L of *n*-hexane and the lower layer pressure-reduced and concentrated. The concentrated extract was then redissolved in 20% soluble methanol and fibrillated using 10 L of ethyl acetate twice, resulting in 80.1 g of soluble ethyl acetate fibrin.

Experimental animals and diet

Sprague-Dawley male rats weighing 100±10 g were purchased from Bio Genomics, Inc. (Seoul, Korea). The animals were individually housed in stainless steel cages in a room with controlled temperature (20~23°C), humidity (55~60%), and lighting (alternating 12-h periods of light and dark). All the rats were fed a pelletized commercial non-purified diet (Purina Co., Seoul, Korea) for a week after arrival. Next, the rats were randomly divided into nine groups (n=10) and fed a high-cholesterol diet (0.05%, wt/wt) for 3 weeks and fed *ad libitum*. The composition of the experimental diet are presented in Table 1. The HC group did not receive any supplement, the MP1 group was supplemented with a PL seed 0.1% methanol extract, the MP2 group with a PL seed 0.2% methanol extract, the EP1 group with a PL seed 0.05% ether-soluble fraction, the EP2 group with a PL seed 0.1% ether-soluble fraction, the MS1 group with an FVL 0.05% methanol extract, the MS2 group with an FVL 0.1% methanol extract, the ES1 group with an FVL 0.025% ethyl acetate-soluble fraction, and the ES2 group with an FVL 0.05% ethyl acetate-soluble fraction.

Preparation of erythrocytes and serum

Blood was taken from the abdominal aorta using a 22-gauge syringe and treated with a 3.8% (w/v) triso-

Table 1. Composition of experimental diets (g/kg diet)

Ingredients \ Groups	HC[1]	MP1[2]	MP2[3]	EP1[4]	EP2[5]	MS1[6]	MS2[7]	ES1[8]	ES2[9]
Corn starch[10]	480	479.9	479.8	479.95	479.9	479.95	479.90	479.98	479.95
Casein[11]	150	150	150	150	150	150	150	150	150
Sucrose[12]	150	150	150	150	150	150	150	150	150
Lard[13]	40	40	40	40	40	40	40	40	40
Soy oil[14]	75	75	75	75	75	75	75	75	75
Salt mix[15]	40	40	40	40	40	40	40	40	40
Vitamin mix[16]	10	10	10	10	10	10	10	10	10
Cholesterol[17]	5	5	5	5	5	5	5	5	5
Cellulose[18]	50	50	50	50	50	50	50	50	50
Methanol ext.[19]	-	0.1	0.2	-	-	0.05	0.1	-	-
Ether-soluble fr.[20]				0.05	0.1				
Ethyl acetate-soluble fr.[21]	-	-	-	-	-	-	-	0.025	0.05
Total (g)	1000	1000	1000	1000	1000	1000	1000	1000	1000

[1]HC: basal diet+0.5% cholesterol.
[2]MP1: basal diet+0.5% cholesterol+0.1% methanol extract of *Paeonia lactiflora* Pall. seeds.
[3]MP2: basal diet+0.5% cholesterol+0.2% methanol extract of *Paeonia lactiflora* Pall. seeds.
[4]EP1: basal diet+0.5% cholesterol+0.05% ether-soluble fraction of *Paeonia lactiflora* Pall. seeds.
[5]EP2: basal diet+0.5% cholesterol+0.1% ether-soluble fraction of *Paeonia lactiflora* Pall. seeds.
[6]MS1: basal diet+0.5% cholesterol+0.05% methanol extract of *Forsythia viridissima* Lindl.
[7]MS2: basal diet+0.5% cholesterol+0.1% methanol extract of *Forsythia viridissima* Lindl.
[8]ES1: basal diet+0.5% cholesterol+0.025% ethyl acetate-soluble fraction of *Forsythia viridissima* Lindl.
[9]ES2: basal diet+0.5% cholesterol+0.05% ethyl acetate-soluble fraction of *Forsythia viridissima* Lindl.
[10]Sam Yang Co., Seoul, Korea.
[11]Lactic Casein, 30 mesh, New Zealand Dairy Board, Welligton, N.Z.
[12]Sam Yang Co., Seoul, Korea.
[13]Cheiljedang Co., Seoul, Korea.
[14]Cheiljedang Co., Seoul, Korea.
[15]Mineral mix, AIN-76 (g/kg mixture): Calcium Phosphate, dibasic ($CaHPO_4 \cdot 2H_2O$) 500, Sodium chloride (NaCl) 74, Potassium citrate, monohydrate ($K_3C_6H_5O_7 \cdot H_2O$) 220, Potassium sulfate (K_2SO_4) 52, Magnesium oxide (MgO) 24, Manganous carbonate (45~48% Mn) 3.5, Ferric citrate (16~17% Fe) 6, Zinc carbonate (70% ZnO) 1.6, Cupric carbonate (53~55% Cu) 0.3, Potasium iodate (KIO_3) 0.01, Sodium selenite ($Na_2SeO_3 \cdot 5H_2O$) 0.01, Chromium potassium sulfate [$CrK(SO_4)_2 \cdot 12H_2O$] 0.55, filled up to 1,000 with sucrose, Harlan TEKLAD Co.
[16]Vitamin mix, AIN-76A (g/kg mixture): p-Aminobenzoic acid 11.0132, Ascorbic acid, coated (97.5%) 101.6604, Biotin 0.0441, Vitamin B_{12} (0.1% trituration in mannitol) 2.9736, Calcium pantothenate 6.6079, Choline dihydrogen citrate 349.6916, Folic acid 0.1982, Inositol 11.0132, Menadione 4.9559, Niacin 9.9119, Pyridoxine HCl 2.2026, Riboflavin 2.2026, Thiamin HCl 2.2026, Dry vitamin A palmitate (500,000 U/g) 3.9648, Dry vitamin D3 (500,000 U/g) 0.4405, Dry vitamin E acetate (500 U/g) 24.2291, Corn starch, Harlan TEKLAD Co.
[17]Sigma Chem. Co. CMC (Sodium carboxyl methyl cellulose, non-nutritivefiber), St. Louis, Missouri, USA.
[18]Harlan TEKLAD Co., Madison, Wisconsin, USA.
[19]Methanol extract of PL seed and FVL.
[20]Ether-soluble fraction of PL seeds.
[21]Ethyl acetate-soluble fraction of FVL.

dium citrate solution and heparin. The serum was then obtained by centrifugation at 1500×g for 15 minutes after leaving for 30 minutes at room temperature, while the erythrocytes were obtained by washing 3 times in a physiological saline to remove the buffer coat and fat, followed by centrifugation at 1000×g for 10 minutes at 4°C.

Measurement of antioxidative enzyme activity in erythrocytes

Measurement of superoxide dismutase (SOD) activity: To remove the hemoglobin by precipitation with chloroform ethanol, 0.4 mL of an ethanol chloroform (3:5, v/v) mixture was added to an aliquot (1 mL) of the hemolysate cooled in ice. This mixture was stirred constantly for 15 min, 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 the superoxide dismutase. The SOD activity was measured according to the method of Marklund and Marklund (28).

Measurement of glutathione peroxidase (GSHpx) activity: The hemolysate was diluted 10 times and the raw material used to measure the GSHpx activity measurement according to the method of Lawrence and Burk (29).

Measurement of oxidative damage in blood

Thiobarbituric acid reactive substances (TBARS) con-

tent in erythrocytes: The hemolysate was diluted 10 times and the raw material used to measure the lipid peroxide content, while the TBARS content was measured according to the method of Yagi (30). Material that lipid peroxide's analysis reacts with TBA. That is, measured fluorescence in excitation wave 515 nm, emission wave 553 nm extracting TBARS by *n*-butanol, and standard material used 1,1,3,3-tetra ethoxypropane this time. Reacted for 5 minutes at room temperature after add 1/12 N H_2SO_4 solution 4 mL and 10% phosphotungstic acid solution 0.05 mL for serum 0.05 mL. Then, remove supernatant liquid that a centrifuge in 1,100×g for 10 minutes and reacted again adding 1/12 N H_2SO_4 solution 2 mL and 10% phosphotungstic acid solution 0.3 mL to sludge. Next time, Reacted for 60 minutes in 95°C constant-temperature water tank adding distilled water 4 mL and 0.67% TBA solution 1 mL to sludge and removing supernatant liquid after centrifuge for 10 minutes in 1,100×g. And measured fluorescence material of *n*-butanol floor that centrifuge for 15 minutes in 1,100×g after mix adding *n*-butanol 5.0 mL cooling immediately.

Measurement of total-TBARS, HDL-TBARS, LDL-TBARS content in serum: The total-TBARS, HDL-TBARS, and LDL-TBARS content in the serum was measured according to the method of Yagi (30).

Determination of hemoglobin

The hemoglobin concentration was estimated in an aliquot of the hemolysate, that analyze SOD, GSHpx and TBARS enzyme activity of erythrocyte and did colormetric fixed quantity to 540 nm using a commercial assay kit (AM 503K, ASAN Pharmaceutical Co., Korea).

Statistical analysis

The results were assessed using ANOVA and Tukey's Honestly Significant Difference test (31). A difference was considered significant at $p<0.05$.

RESULTS

Antioxidative enzyme activity of erythrocytes

Superoxide dismutase (SOD) activity: The SOD activity levels are shown in Table 2. When compared with the HC group, the SOD activity in the MP1 (127.66±24.0) and MP2 (127.51±16.9) groups supplemented with the PL seed methanol crude extract was 27% higher, while the SOD activity in the EP1 (139.29±10.8) and EP2 (160.27±8.6) groups supplemented with the PL seed ether-soluble fraction was 39% and 59% higher, respectively. The FVL supplements produced no effect.

Glutathione peroxidase (GSHpx) activity: The GSHpx activity levels in the erythrocytes are shown in Table 3.

Table 2. Effect of *Paeonia lactiflora* Pall. seed extracts and *Forsythia viridissima* Lindl. extracts on erythrocyte superoxide dismutase (SOD) activity in rats fed high-cholesterol diet

Groups		SOD unit/min/g Hb	Groups		SOD unit/min/g Hb
PL[1] seeds	HC	100.55±14.6[3)b4)]	FVL[2]	HC	100.55±14.6[NS]
	MP1	127.66±24.0[ab]		MS1	117.71±12.8
	MP2	127.51±16.9[ab]		MS2	128.45±21.2
	EP1	139.29±10.8[a]		ES1	123.18±20.9
	EP2	160.27±8.6[a]		ES2	102.23±16.6

The experimental conditions were the same as in Tables 1.
[1)]PL: *Paeonia lactiflora* Pall.
[2)]FVL: *Forsythia viridissima* Lindl.
[3)]All values are mean±SE (n=8).
[4)]Values within a column with different superscripts are significantly different at $p<0.05$ according to Tukey's test.

Table 3. Effect of *Paeonia lactiflora* Pall. seed extracts and *Forsythia viridissima* Lindl. extracts on erythrocyte glutathione peroxidase (GSHpx) activity in rats fed high-cholesterol diet

Groups		GSHpx μmol NADPH/min/g Hb	Groups		GSHpx μmol NADPH/min/g Hb
PL[1] seeds	HC	20.24±3.2[3)b4)]	FVL[2]	HC	20.24±3.2[b]
	MP1	24.50±2.5[ab]		MS1	21.91±1.7[ab]
	MP2	24.81±3.3[ab]		MS2	23.39±7.5[ab]
	EP1	26.41±1.6[a]		ES1	24.34±5.7[ab]
	EP2	26.48±1.7[a]		ES2	26.12±1.8[a]

The experimental conditions were the same as in Tables 1.
[1)]PL: *Paeonia lactiflora* Pall.
[2)]FVL: *Forsythia viridissima* Lindl.
[3)]All values are mean±SE (n=8).
[4)]Values within a column with different superscripts are significantlydifferent at $p<0.05$ according to Tukey's test.

When compared with the HC group, the GSHpx activity in the MP1 (24.50±2.5) and MP2 (24.81±3.3) groups supplemented with the PL seed methanol crude extract was 21% and 23% higher, respectively, while the GSHpx activity in the EP1 (26.41±1.6) and EP2 (26.48±1.7) groups supplemented with the PL seed ether-soluble fraction was 39% and 59% higher, respectively.

There was no significant difference in the GSHpx activity in the groups supplemented with the FVL methanol crude extract, yet a significant ($p<0.05$) increase in the ES 2 group (26.12±1.8) supplemented with the FVL 0.05% ethyl acetate-soluble fraction.

Oxidative damage in blood

The contents thiobarbituric acid reactive substances (TBARS) in erythrocytes: The TBARS contents are shown in Fig. 1. When compared with the HC group, the TBARS contents decreased 30%, 25%, and 28% in the MP2 group supplemented with the PL seed 0.1% methanol crude extract and the EP1 (139.29±10.8) and

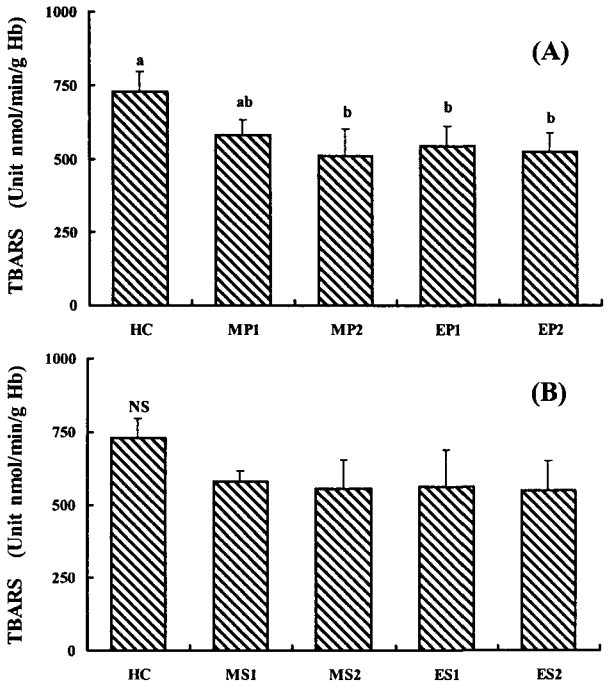

Fig. 1. Effect of *Paeonia lactiflora* Pall. seed extracts (A) and *Forsythia viridissima* Lindl. extracts (B) on erythrocyte thiobarbituric reactive substance (TBARS) contents in rats fed high-cholesterol diet.
All values are mean±SE (n=8).
Bars within different letters are significantly different at $p<0.05$ according to Tukey's test.
The experimental conditions were the same as in Table 1.

EP2 (160.27±8.6) groups supplemented with the PL seed ether-soluble fraction, respectively. There was a slight decrease in the TBARS concentration in the groups supplemented with the FVL extracts, yet not significant.

The contents of TBARS in serum : The TBARS contents in the serum, as an index of lipid peroxidation, are presented in Fig. 2 and 3. The total-TBARS level was significantly ($p<0.05$) reduced in all groups supplemented with the PL seed extracts and FVL extracts when compared with the HC group (3.71±0.3).

The HDL-TBARS level was reduced 24% and 23% in the EP1 (1.95±0.1) and EP2 (1.97±0.1) groups, respectively, supplemented with the PL seed ether-soluble fraction when compared with the HC group (2.55±0.2). The LDL-TBARS level was significantly reduced 32%, 45%, 43%, and 55% in the MP1 (0.82±0.2), MP2 (0.66±0.1), EP1 (0.68±0.2), and EP2 (0.54±0.2) groups, respectively, supplemented with the PL seed extracts when compared with the HC group (1.20±0.2), and reduced 50%, 41%, 38%, and 41% in the MS1 (0.82±0.2), MS2 (0.66±0.1), ES1 (0.68±0.2), and ES2 (0.54±0.2) groups, respectively, supplemented with the FVL seed extracts.

DISCUSSION

This study investigated the antioxidative operation of *Paeonia lactiflora* Pall. and *Forsythia viridissima* Lindl. seed extracts by examining their effect on the antioxidative defense system and lipid peroxidation in the erythrocytes of rats fed a high-cholesterol diet. Resveratrol's antioxidation effect that separate in PL seeds and lignan's antioxidaton effect that separate in FVL reported by Kim et al. (16) wished to do examine by *in vivo*.

SOD activity reduces superoxide radicals to H_2O_2, then the generated H_2O_2 becomes unpoisoned by the action of GSHpx and catalase, thereby protecting the living body from oxygen poisoning. In the present study, the erythrocyte SOD activity was significantly increased

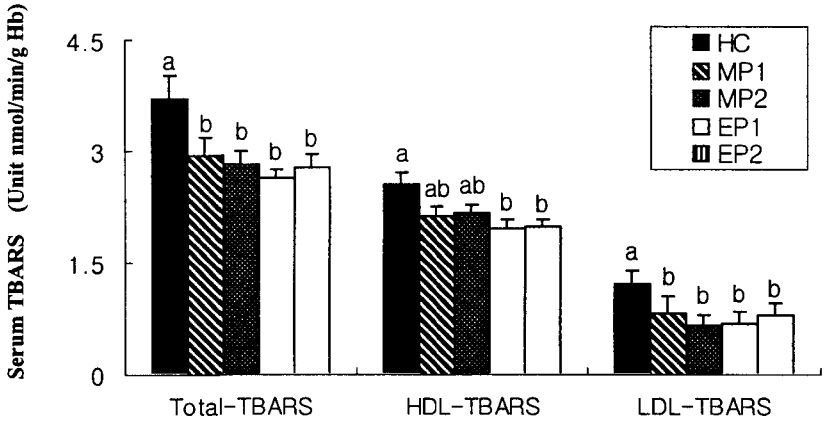

Fig. 2. Effect of *Paeonia lactiflora* Pall. seed extracts on serum thiobarbituric acid reactive substances (TBARS) contents in rats fed high-cholesterol diets.
All values are mean±SE (n=8).
Bars within different letters are significantly different at $p<0.05$ according to Tukey's test.
The experimental conditions were the same as in Table 1.

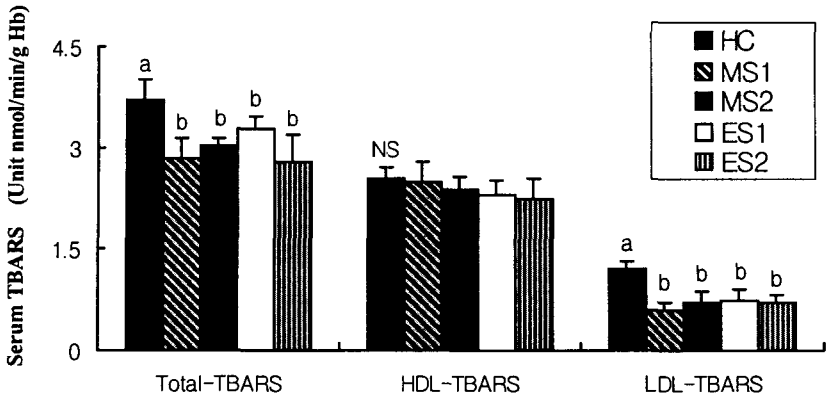

Fig. 3. Effect of *Forsythia viridissima* Lindl. extracts on serum thiobarbituric acid reactive substances (TBARS) contents in rats fed high-cholesterol diets.
All values are mean ± SE (n=8).
Bars within different letters are significantly different at $p<0.05$ according to Tukey's test.
The experimental conditions were the same as in Table 1.

in the EP1 and EP2 groups by 38% and 59%, respectively, when compared with the HC group. However, in previous research using the same extract groups no significant difference was observed in the hepatic SOD activity (26,27).

GSHpx uses a catalytic reaction to generate H_2O and oxidized glutathione (GSSG) from H_2O_2 and reduced glutathione (GSH), and generate alcohol (ROH) and H_2O from peroxide (ROOH) (32). In the present study, the erythrocyte GSHpx activity was significantly increased in the EP1 and EP2 groups supplemented with the PL seed ether-soluble fraction and the ES2 group supplemented with the FVL ethyl acetate soluble fraction when compared with the HC group. In previous research using the same extract groups, the hepatic GSHpx activity also increased in the MP2, MS2, and ES2 groups (26,27), yet rate of increase was higher in the erythrocytes than in the liver tissue. The current results are also similar to those cited in the report by Lee et al. (33), where the erythrocyte SOD and GSHpx activities were found to be higher than the hepatic SOD and GSHpx activities in rats fed a high-cholesterol diet supplemented with naringenin and a derivative diet.

TBARS is recognized as the most important radical that displays the degree of damage resulting from various toxic compounds, drugs, or physiological phenomena from disease based on increasing the stress and decreasing the antioxidative defense power in cellular tissue (34). In this study, the erythrocyte TBARS contents was significantly lower in the MP2, EP1, and EP2 groups when compared to the HC group. These results are similar to those cited in previous research (26,27) related to hepatic TBARS levels with the same extract groups. The total TBARS content in the serum, as an index of lipid peroxidation, was significantly lower in all the groups supplemented with the PL seed extracts and FVL extracts when compared with the HC group. Also, the HDL-TBARS content was significantly decreased in the EP1 and EP2 groups supplemented with the PL seed ether-soluble fraction, and the LDL-TBARS content significantly reduced in all the groups supplemented with the PL seed extracts and FVL extracts. This means that the PL seed extracts effectively controlled the LDL oxidation, which is an important step in the etiology of arteriosclerosis, as such, the level of LDL-TBARS was significantly lower in the groups supplemented with PL seed extracts and FVL extracts. Accordingly, the decrease in the erythrocyte and serum lipid peroxide levels apparently resulted from an increase in the antioxidation enzyme activity, including SOD and GSHpx, caused by the PL seed extract and FVL extract supplements.

In conclusion, the current study found that oxidative damage was reduced when increasing the erythrocyte antioxidation defense enzyme activity with PL seed extract and FVL extract supplements, especially with a PL seed ether-soluble fraction, in rats fed a high-cholesterol diet.

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