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In Vitro Antioxidant Properties of *Equisetum arvense* and Its Effects on Serum Lipid Levels in Mice Fed a High-Fat Diet

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Abstract

This study analyzed the antioxidant properties of *Equisetum arvense* and its effects on serum factor levels in mice fed a high-fat diet. The aim was to establish a new effective resource for biologically active materials. *E. arvense* stem and root extracts were obtained using deionized water at 95℃, and 70.5% ethanol at 85℃. These extracts were used to analyze the total phenolic compounds and antioxidant (ABTS, DDPH, and FRAP) activities. The effects of prepared ground samples were evaluated by feeding them to mice. *E. arvense* extracts showed strong antioxidant effects. The caffeic acid content was highest in the 70.5% ethanol extract of the vegetative stem, as determined by high-performance liquid chromatography. The blood concentrations of insulin and leptin were significantly lower in mice fed a high-fat diet supplemented with extracts of the root, reproductive stem, and vegetative stem of *E. arvense* than in mice fed only a high-fat diet. These results suggest that the polyphenolic compounds in *E. arvense* extracts exert various antioxidant effects. The stems and root of *E. arvense* can lower the blood levels of insulin and leptin, even after consumption of a high-fat diet.

Key words: *Equisetum arvense*, phenolic compound, antioxidant activity, insulin, leptin

Introduction

Eating habits have become more westernized due to improved income, and as a result, various chronic degenerative diseases have emerged as serious social problems. If left unchecked, these disorders can have adverse effects on the economy owing to the increased social costs as well as personal and national economic losses caused by personal health deterioration and disease onset. Therefore, biologically active substances that prevent chronic degenerative diseases are in demand. Although synthetic biologically active substances are widely available, natural substances are attracting increased attention among consumers based on their higher level of safety. Therefore, many studies have been conducted to extract bioactive compounds from various plants (Jung et al. 2000; Choi et al. 2003; Lee et al. 2005; Park et al. 2007; Karyagina et al. 2011; Choi et al. 2013a; Choi et al. 2013b).

Equisetum arvense is a very useful source of biologically active substances, and its young reproductive and vegetative stems are eaten as herbs during early spring in Korea (Kong MR 2013). Vegetative stems collected in the fall and dried in the shade are called *munhyeong* and are used as a diuretic or styptic in Korea. *E. arvense* is known as a “liver herb” in the American and European marketplaces and is gaining popularity as a supplement for improving liver function, hyperlipidemia, and alcohol metabolism (Kong MR 2013). The a alcohol extract of *E. arvense* contains two types of phenolic petrosin material, onitin and onitin-9-O-glucoside, and four types of flavonoids, including apigenin, luteolin, and kaempferol-3-O-glucoside (Oh et al. 2004). According to an *in vitro* experiment using HepG2 cells, onitin and luteolin have liver-protective, superoxide-scavenging, and 1,1-diphenyl-

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2-picrylhydrazyl (DPPH) free radical-scavenging activities (Oh et al. 2004). Do Monte et al. (2004) reported that *E. arvense* extracts have excellent anti-inflammatory effects. Furthermore, Revilla et al. (2002) reported that *E. arvense* extracts can regulate blood sugar in type 2 non-insulin-dependent diabetes. In Korea, some people combine extracts from the reproductive and vegetative stem of *E. arvense* with sugar to produce functional drinks at home.

This study aimed to identify the effective ingredients in the reproductive stem, vegetative stem, and root of *E. arvense* to establish a new source of biologically active materials.

Materials and Methods

1. Plant materials and extraction

Reproductive stem of *E. arvense* were collected in late March, and vegetative stem and root were collected in early May in 2013, in Suwon, Korea. They were dried thoroughly in the shade, ground in a mill, and stored at −18℃ until used. Ground samples were used for animal feed. To obtain extracts for analysis of antioxidant activity, each ground sample was extracted with deionized water at 95℃, and with 70.5% ethanol at 85℃. Extraction was performed for 3 h in a heating mantle using a reflux condenser. The extracts were evaporated in a vacuum at 45℃ and obtained in the form of a lyophilized powder.

2. Preparation of samples for antioxidant analysis

Lyophilized powder (1 g) was sonicated with 100 mL deionized water for 1 h, after which 10 mg/mL stock solution was prepared. Samples were then cooled to room temperature for 24 h. The extract was centrifuged at 8,000 rpm for 10 min, after which the supernatants were collected and stored at 4℃ prior to use within 24 h. Serial dilutions of the stock solution (1.25, 2.5, 5, 7.5, and 10 mg/mL) were prepared and tested for antioxidant activity.

3. Determination of total phenolic content (TPC)

TPC was determined using Folin-Ciocalteu (FC) reagent with gallic acid as a standard (Waterhouse AL 2002). A 20 μL aliquot of a diluted sample (stock solutions further diluted with deionized water) was added to 100 μL FC reagent and mixed well. After 5 min, 300 μL 20% sodium carbonate solution was added, and the mixture was vortexed. The samples were incubated for 2 h at room temperature in the dark. The absorbance was measured at 765 nm. The results are expressed as mg gallic acid equivalents (GAE)/g dry weight.

4. ABTS radical scavenging assay

The ABTS radical cation decolorization assay was performed in accordance with the work of Re et al. (1999) with slight modifications. ABTS solution was prepared by reacting 7 mM aqueous ABTS solution with 140 mM (2.45 mM final concentration) potassium persulfate. After storage in the dark for 12~16 h, the radical cation solution was further diluted in PBS buffer solution (pH 7.4) to an absorbance value of 0.7 (±0.02) at 734 nm and then equilibrated at 30℃. PBS buffer was used as a blank. All samples were diluted to 20~80% inhibition of the blank absorbance. Diluted samples (10 μL each) were then mixed with 1 mL ABTS solution. The decrease in absorbance was measured over 15 min, and the results are expressed as μmoL Trolox/g dry weight. Scavenging activity was calculated using the following equation:

$$\text{Inhibition (\%)} = [(AB - AA)/AB] \times 100$$

where, AB = absorbance of a blank sample at the beginning of the reaction (t = 0 min), and AA = absorbance of a test sample at the end of the reaction (t = 15 min).

5. DPPH radical scavenging assay

Radical scavenging activity was determined using the DPPH radical method proposed by Brand-Williams et al. (1995) and Sánchez-Moreno et al. (1998) with slight modifications. A solution of 60 μM DPPH in methanol was prepared daily and protected from light. Diluted samples (50 μL each) were added to 2 mL DPPH solution followed by mixing. The absorbance of the remaining DPPH radicals was determined after 30 min at 515 nm. The blank sample contained methanol alone. The results are expressed as μmoL Trolox/g dry weight. Scavenging activity was calculated using the following equation:

$$\text{Inhibition (\%)} = [(AB - AA)/AB] \times 100$$

where, AB = absorbance of a blank sample at the beginning of the reaction (t = 0 min), and AA = absorbance of a test

sample at the end of the reaction (t = 30 min).

6. Reducing power assay

The ferric ion reducing power of *E. arvense* was estimated in accordance with the procedure described by Pulido et al. (2000). FRAP reagent consisted of 25 mL 300 mM acetate buffer (pH 3.6), 2.5 mL 10 mM tripyridyltriazine solution in 40 mM HCl, and 2.5 mL 20 mM $FeCl_3 \cdot 6H_2O$ that was freshly prepared and warmed to 37℃. The FRAP reagent was then mixed with 90 μL deionized water and 30 μL sample. The final dilution of the sample in the reaction mixture was 1:34. A small aliquot of each sample was incubated at room temperature for 30 min. The absorbance of each sample was measured at 595 nm, and the results are expressed as TEAC values.

7. Polyphenol compound analysis by HPLC

The polyphenol compounds in the extracts were analyzed using the Waters 2695 Separation Module HPLC System (Waters Co., Milford, MA, USA) under the conditions shown in Table 1. The deionized water extract (50% dimethyl sulfoxide: DMSO), 70.5% ethanol extract (100% DMSO), and STD (100% DMSO) were used in the sample dissolution method. Reference materials (caffeic acid, chlorogenic acid, ferulic acid, rutin, catechin, *p*-coumaric acid, hesperidin, naringin, quercetin, and vanillic acid) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) (Chang & Rhee 1985).

Table 1. HPLC conditions for the analysis of phenolic compounds

Instrument	Conditions		
Column	Sunfire^{TM}C$_{18}$ 5.0 μm, 4.6 mm × 250 mm		
Column temp.	40℃		
Mobile phase (Gradient)	Time (min)	A[a)]	B[b)]
	0	8	92
	23	8	92
	26	15	85
	36	30	70
	40	45	55
	43	45	55
	45	8	92
	53	8	92
Detector	Waters 996 Photodiode Array Detector (280 nm)		
Flow rate	1.0 mL/min		
Injection volume	10 μL		
Run time	53 min		

[a)] Acetonitrile
[b)] 0.5% H_3PO_4/H_2O

8. Animal experiments

Experimental animals were obtained from Orient Bio Co., Ltd. Eight-week-old ICR male mice were used as a model, and there were five mice in each experimental group. Mice were adapted to solid food (PicoLab® Rodent Diet) for 1 week, randomly grouped depending on weight, and then housed with access to water and feed ad libitum for 60 days. Housing conditions were maintained as follows: room temperature 20±2℃; humidity 40~60%; and an 11±1 h light/dark cycle. The experimental period was 8 weeks. The animal procedures (approval number: SYUIACUC 2013005) were performed in accordance with the guidelines of the Adventist University Animal Experiment Ethics Committee (IACUC Institutional Animal Care and Use Committee).

Commercially available solid food for mice was ground into a powder for use as feed. The composition of the feed for the animal experimental diets is shown in Table 2. The composition of the feed for the animal experiments included 33 or 60% carbohydrates (starch + sucrose + glucose + fructose + lactose), 21% proteins, and 13 or 20% lipids for the control or high-fat diet, respectively. Vitamins and minerals comprised 1 and 3%, respectively, with 2% as fiber. The supplemented feed consisted of 80% of the standard mouse feed described above and 20% ground *E. arvense*. For each group, the total energy content was almost the same. The weights of the experimental animals' organs were determined in each group after fasting for 12 h prior to sacrifice. The animals were slightly anesthetized with ethyl ether, and their organs were removed after an abdominal incision. The removed organs were washed with cold physiological saline and weighed after connective tissues were removed. Blood was collected from the heart using a syringe. Blood samples were stored at 4℃ for ~1 h, after which the serum was separated by centrifugation at 3,000 rpm for 15 min at 5℃. Aliquots (100 μL) of serum were placed in micro tubes, and stored at −70℃ until needed.

9. Analysis of blood lipids

The serum cholesterol level was measured by the *o*-

Table 2. Composition of feed for the animal experiments (%)

Ingredient	Control	Control + HFD	HFD + 20% Rt	HFD + 20% RS	HFD + 20% VS
Carbohydrate[1)]	60	53	33	33	33
Protein	21	21	21	21	21
Lipid (corn oil)	13	20	20	20	20
Vitamin	1	1	1	1	1
Mineral mix	3	3	3	3	3
Fiber	2	2	2	2	2
E. arvense	0	0	20	20	20

[1)] Carbohydrate: starch+sucrose+glucose+fructose+lactose
HFD: High fat diet, Rt: Root, RS: Reproductive stem, VS: Vegetative stem

phthalaldehyde method of Rudel & Morris (1973) and Cho & Choi (2007). For this method, 0.1 mL sample was added to 0.3 mL 33% KOH solution and 3 mL 95% ethanol and mixed well. Serum was heated at 60℃ in a water bath for 15 min and then cooled. Then, 5 mL hexane was added and mixed with 3 mL distilled water, after which 1 mL the hexane layer was removed. The hexane layer was then concentrated and dried in nitrogen. After adding 2 mL *o*-phthalaldehyde reagent, 1 mL conc. H_2SO_4 was added as a color reagent for 10 min. The absorbance was then measured at 550 nm for 10~90 min, and the cholesterol content was quantified using the standard calibration curve. In accordance with the method of Cho & Choi (2007), 0.3 mL serum was placed in a test tube, mixed well, and left to stand for ~10 min at room temperature. The mixture was then centrifuged for ~10 min at 700 × g to separate it. Then, 3.0 mL HDL chromogenic reagent and 50 µL standard solution (100 mg/dL) were added to 50 µL supernatant or to 50 µL deionized water as a blank, mixed well, and then heated at 37℃ in a water bath for 5 min. Absorbance was measured at 555 nm, and the content of HDL cholesterol was quantified. Following the method of Cho & Choi (2007), 0.1 mL serum and 0.1 mL standard serum were placed in a test tube along with 4.0 mL each of BLF kit reagents I and II, mixed well for ~5 s, and left to stand for ~25 min at room temperature. The content of LDL cholesterol was then quantified by measuring the absorbance at 650 nm and comparing it to that of distilled water within 10 min. Again following the method of Cho & Choi (2007), the serum triglyceride (TG) level was analyzed using a TG kit reagent (Sigma Co., St. Louis, MO, USA). The TG kit reagent (1 mL) was added to 10 µL deionized water, 10 µL serum, 10 µL standard solution (300 mg/dL), and a blank and mixed well. The reaction was allowed to continue for 5 min in a 37℃ water bath. TG content was quantified by measuring the absorbance at 540 nm. For the blood insulin concentration, an enzyme-linked immunosorbent assay (ELISA) kit (Linco, USA) was used for the plasma obtained on the last day of the experiment and analyzed by analytical equipment (Molecular Devices, USA). Serum concentrations of leptin were determined by ELISA. Assays were conducted in accordance with the manufacturer's protocol (R&D Systems Inc., Minneapolis, MN, USA).

10. Statistical analysis

The mean and standard deviation were calculated for all data using the SPSS package (version 18.0). Comparison of the averages in each group was conducted by one-way ANOVA, and analysis of the significance of the differences between the averages of the groups ($p<0.05$) was conducted by Duncan's multiple range test.

Results

1. Total phenolic content (TPC)

The TPC values are shown in Table 3 and Fig. 1. The TPC ranged from 163.20±15.20 to 409.20±49.65 mg GAE/mg among the analyzed extracts. The highest TPC (409.20±49.65 mg GAE/mg) was observed for the root extract with 70.5% ethanol, while the lowest was for the vegetative stem extract with deionized water.

2. ABTS radical scavenging activity

Table 3. Total phenolic content and antioxidant activity of *E. arvense*

E. arvense	Extract concentrate (mg/ mL)	Total phenolic content (mg GAE/mg)		ABTS (μmol Trolox/mg)		DPPH (μmol Trolox/mg)		FRAP (μmol Trolox/mg)	
		Deionized water extract	70.5% ethanol extract	Deionized water extract	70.5% ethanol extract	Deionized water extract	70.5% ethanol extract	Deionized water extract	70.5% ethanol extract
Rt	1.25	36.53±13.36[1)]	40.53±11.51	317.00± 20.82	503.67± 26.03	49.33± 6.01	128.78±29.88	157.92± 5.07	232.64± 6.47
	2.5	81.20± 9.20	109.20±19.20	590.33± 38.44	669.22± 12.62	160.44± 2.55	214.33±56.30	287.64± 9.48	314.58± 2.20
	5	169.20±19.78	218.53±17.28	1,130.33± 21.86	1,395.89± 42.99	393.78±28.69	386.00±23.33	537.64±15.64	663.47±26.57
	7.5	256.53±20.57	309.87±27.68	1,607.00± 38.44	2,698.44± 74.34	626.00±17.64	729.89±14.17	717.36±21.24	1,217.36±20.08
	10	338.53±32.64	409.20±49.65	2,119.22± 29.12	3,529.56± 30.06	807.11±30.38	940.44±22.19	919.03±12.09	1,574.58±15.16
RS	1.25	17.87±10.35	20.53±10.35	285.89± 24.57	399.22± 10.18	96.00± 5.85	102.67± 2.89	160.14± 3.15	191.53± 8.01
	2.5	44.53±13.36	58.53±16.77	579.22± 27.76	693.67± 16.67	221.56±11.67	255.44±14.56	280.69± 7.92	327.08±15.83
	5	86.53±15.63	101.20±14.49	1,157.00± 17.64	1,275.89± 59.29	486.00± 1.92	629.89±30.06	529.03± 8.09	652.92± 6.01
	7.5	126.53±17.53	155.20±14.49	1,663.67± 79.37	1,837.00± 40.55	749.33±31.20	836.56±36.91	742.08±24.55	873.47±11.56
	10	170.53±16.22	203.20±11.20	2,048.11± 53.47	2,627.33±141.89	914.33±14.81	1,044.89± 0.96	884.86±23.34	1,234.31±43.91
VS	1.25	16.53±10.35	19.67±10.56	383.67± 16.67	687.00± 48.42	119.33±10.18	190.44±20.71	161.25± 2.20	291.53±10.01
	2.5	45.20±26.64	41.20±11.20	711.44± 42.99	1,259.22±108.23	206.00±15.12	296.00± 7.26	284.86±12.37	554.03±12.06
	5	76.53±13.36	85.87±15.63	1,369.22± 39.77	2,440.67±107.29	493.22± 5.85	502.11±15.12	559.58±13.64	1,069.86±24.61
	7.5	115.20±14.49	128.53±14.23	1,877.00± 20.28	3,445.11± 60.49	774.89±15.49	922.11± 7.52	811.25±17.56	1,545.97±60.72
	10	163.20±15.20	167.87±22.52	2,380.67±123.47	3,951.78± 10.18	934.89±27.84	1,021.00± 1.92	975.69±35.61	1,985.14±34.56

[1)] Values represented mean±S.D. of three parallel measurements.
Rt: Root, RS: Reproductive stem, VS: Vegetative stem

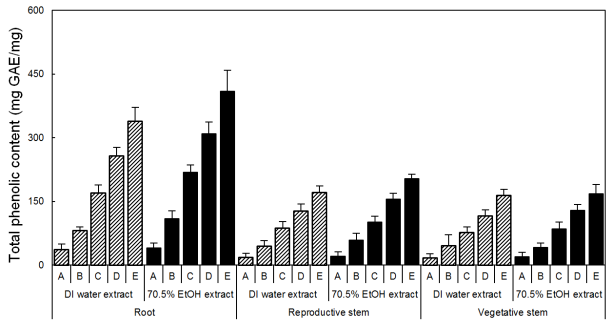

Fig. 1. Total phenolic contents of *E. arvense*. [1)] Dilution of the *E. arvense* concentrations (mg/mL) (A: 1.25, B: 2.5, C: 5.0, D: 7.5, E: 10.0)

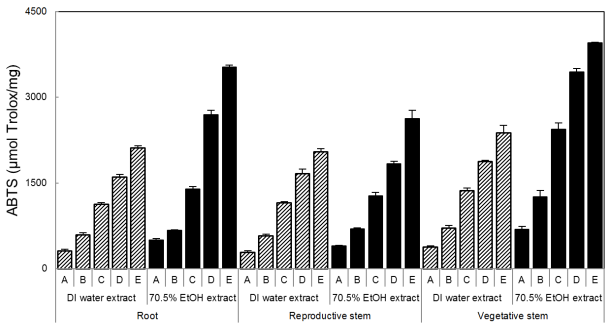

Fig. 2. ABTS scavenging activity of *E. arvense*. [1)] Dilution of the *E. arvense* concentrations (mg/mL) (A: 1.25, B: 2.5, C: 5.0, D: 7.5, E: 10.0)

ABTS scavenging activities are shown in Table 3 and Fig. 2. They values ranged from 20,148.11±53.47 to 3,951.78± 10.18 μmol Trolox/mg among the analyzed extracts. The highest ABTS scavenging activity (3,951.78±10.18 μmol Trolox/mg) was observed for the vegetative stem extract with 70.5% ethanol, whereas the lowest was for the reproductive stem extract with deionized water.

3. DPPH radical scavenging activity

DPPH scavenging activities are shown in Table 3 and Fig. 3. They ranged from 807.11±30.38 to 1,044.89±0.96 μmol Trolox/mg among the analyzed extracts. The highest DPPH scavenging activity (1,044.89±0.96 μmol Trolox/mg) was observed for the reproductive stem extract with 70.5% ethanol, whereas the lowest was for the root extract with deionized water.

4. Reducing power activity

FRAP values are shown in Table 3 and Fig. 4. They ranged from 884.86±23.34 to 1,985.14±34.56 μmol Trolox/mg among the analyzed extracts. The highest FRAP value (1,985.14± 34.56 μmol Trolox/mg) was observed for the vegetative stem

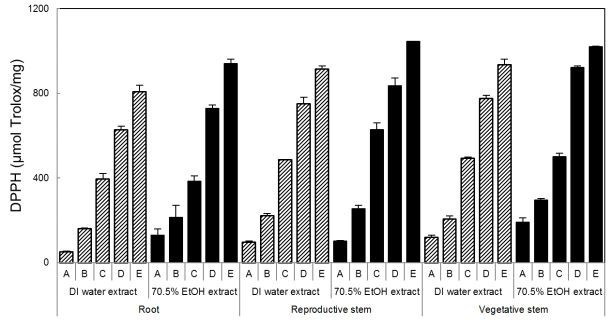

Fig. 3. DPPH scavenging activity of *E. arvense*. [1] Dilution of the *E. arvense* concentrations (mg/mL) (A: 1.25, B: 2.5, C: 5.0, D: 7.5, E: 10.0)

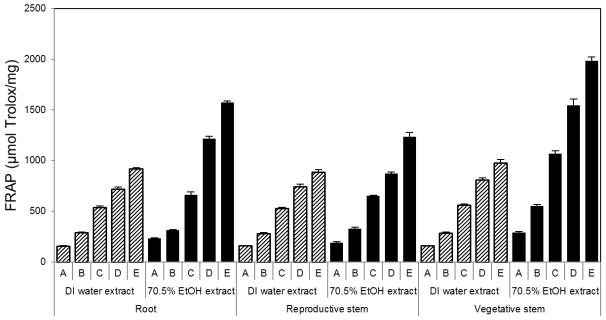

Fig. 4. FRAP value of *E. arvense*. [1] Dilution of the *E. arvense* concentrations (mg/mL) (A: 1.25, B: 2.5, C: 5.0, D: 7.5, E: 10.0)

extract with 70.5% ethanol, whereas the lowest was for the reproductive stem extract with deionized water.

5. Phenolic contents by HPLC

The phenolic compound contents of *E. arvense* are shown in Table 4. They ranged from 0.32±0.02 to 3.13±0.05 mg/g dry weight among the analyzed extracts. Caffeic acid was the most abundant phenolic compound (1.20±0.04 mg/g dry weight) for the vegetative stem extract with 70.5% ethanol, whereas rutin was the least abundant for the reproductive stem extract with 70.5% ethanol.

6. Animal experiments

The organ weights of the mice are shown in Table 5. The groups did not show significant differences in liver weight. However, the spleen and kidney weights of mice fed a

Table 4. Phenolic compound contents

Solvent	*E. arvense*	Phenolic compounds (mg/g dry weight)			
		Caffeic acid	Chlorogenic acid	Ferulic acid	Rutin
Deionized water extract	Rt	0.15±0.00[1)]	ND[2)]	0.61±0.03	ND
	RS	0.90±0.00	ND	ND	ND
	VS	0.78±0.00	0.17±0.00	ND	1.20±0.04
70.5% ethanol extract	Rt	1.00±0.01	ND	ND	ND
	RS	0.99±0.01	ND	ND	0.32±0.02
	VS	3.13±0.05	1.50±0.06	ND	2.97±0.02

[1)] Value are mean±S.D. in triplicate (n=3)
[2)] Not detected
Rt: Root, RS: Reproductive stem, VS: Vegetative stem

Table 5. Organ weights in mice

Organ	Control (n=7)	Control+HFD (n=7)	HFD+20% Rt (n=7)	HFD+20% RS (n=7)	HFD+20% VS (n=7)	Significance
Liver (g/100 g)	2.32±0.49[1)]	2.74±0.48	2.05±0.66	2.26±0.51	2.37±0.12	NS[2)]
Spleen (g/100 g)	0.18±0.02[a]	0.28±0.11[b]	0.18±0.04[a]	0.18±0.06[a]	0.19±0.03[a]	0.05[3)]
Kidney (g/100 g)	0.98±0.17[ab]	1.07±0.11[b]	0.84±0.08[a]	0.94±0.13[ab]	0.92±0.13[ab]	0.05

[1)] Mean±S.D.
[2)] NS: no significant difference at $p<0.05$ by ANOVA
[3)] Significant at $p<0.05$ by ANOVA
HFD: High fat diet, Rt: Root, RS: Reproductive stem, VS: Vegetative stem

Table 6. Comparison of blood lipid, insulin, and leptin levels in mice

Parameter	Control (n=7)	Control+HFD (n=7)	HFD+20% Rt (n=7)	HFD+20% RS (n=7)	HFD+20% VS (n=7)	Significance
TC (mg/dL)	152.71±36.39[1)]	207.71±32.50	148.00±0.00	156.50±37.48	168.00±11.64	NS[2)]
HDL-C (mg/dL)	115.71±11.34	120.00± 0.00	120.00±0.00	114.00± 8.49	120.00± 0.00	NS
LDL-C (mg/dL)	16.00± 7.57	29.00±14.79	16.00±0.00	22.00± 0.00	23.60± 2.07	NS
TG (mg/dL)	177.86±71.46	96.50±33.27	114.00±0.00	126.00±18.38	123.60±23.35	NS
HDL-C/TC	0.76	0.58	0.81	0.73	0.72	-
Insulin (ng/dL)	0.20±0.00[a]	3.25±0.21[b]	0.85±0.45[a]	0.82±0.33[a]	3.54±2.86[b]	0.05[3)]
Leptin (ng/dL)	0.20±0.00[a]	26.72±6.53[b]	5.24±2.68[ab]	8.96±2.86[ab]	9.65±2.08[ab]	0.05

[1)] Mean±S.D.
[2)] NS: no significant difference at $p<0.05$ by ANOVA
[3)] Significant at $p<0.05$ by ANOVA
HFD: High fat diet, Rt: Root, RS: Reproductive stem, VS: Vegetative stem
TC: Total cholesterol, HDL-C: Highdensity lipoprotein cholesterol, LDL-C: Lowdensity lipoprotein cholesterol, TG: Triglyceride

high-fat diet were 0.28±0.11 g/100 g body weight and 1.07± 0.11 g/100 g body weight, respectively, which were significantly higher than those of mice fed a diet containing the root, reproductive stem, and vegetative stem of *E. arvense* ($p<0.05$).

The blood lipid levels are shown in Table 6. The total cholesterol level was 207.71±32.50 mg/dL in mice fed a high-fat diet and 148.00±0.00 mg/dL in mice fed *E. arvense* root, although this difference was not significant. The levels of HDL-cholesterol were not significantly different between the groups. The LDL-cholesterol level was 29.00±14.79 mg/dL in mice fed a high-fat diet, which was higher than that of mice fed *E. arvense* root (16.00±0.00 mg/dL), although this difference was not significant.

The blood insulin and leptin levels are shown in Table 6. Compared with the other groups, the blood insulin level was significantly lower in mice fed a high-fat diet with the root (0.85±0.45 ng/dL) or reproductive stem (0.82±0.33 ng/dL) of *E. arvense* ($p<0.05$). Compared with mice fed a high-fat diet only, the blood leptin level was significantly lower in mice fed a high-fat diet containing the root (5.24±2.68 ng/dL), reproductive stem (8.96±2.86 ng/dL), or vegetative stem (9.65± 2.08 ng/dL) of *E. arvense* ($p<0.01$).

Discussion

This study analyzed the antioxidant properties of *E. arvense* and its effects on serum factor levels in mice fed a high-fat diet. Lee et al. (2005) reported high scavenging activities for *Athyrium acutipinulum* leaves and roots and for *Solidago virga-aurea* var. *gigantea* root extract based on RC_{50} values of 40.93, 35.39, and 29.08 μg/mL, respectively. DPPH is a relatively stable free radical with a dark purple color, which is lost after DPPH is reduced by antioxidants and aromatic amines and, other compounds, and this color change is widely used to identify antioxidants from natural materials (Lee et al. 2005). The RC_{50} value of the methanol extract from *Schisandra chinensis* seeds was previously reported to be 33.2 μg/mL (Jung et al. 2000), whereas the RC_{50} values of *Athyrium acutipinulum* leaves and *Solidago virga-aurea* var. *gigantea* root extract were reported to be 13.02 and 14.91 μg/mL, respectively (Lee et al. 2005). Karyagina et al. (2011) reported that the cell extract (CE) and culture medium (CM) exhibited high reducing capacities; a 50% effect was achieved by concentrations of 12 μg/mL for CE and 15 μg/mL for CM. In addition, Choi et al. (2013b) reported that the antioxidant value of *Prunus sargentii* R. seed oil is 4.63%, which is very low compared with the antioxidant value of butylated hydroxytoluene, a synthetic antioxidant. Choi et al. (2012) also reported the antioxidant values of *Acer mono* Max. and *Magnolia denudate* seed oil to be 61.85% and 67.17%, respectively. Park et al. (2007) reported antioxidant activities of 85.6% and 68.3% for flower and young leaf extracts from cherry plants respectively, indicating excellent antioxidant activity. Lee et al. (2005) reported high polyphenol (tannic acid) contents in *Cirsium nipponicum* leaf and *Athyrium acutipinulum* leaf extract (130.22±12.17 and 120.69±18.33 μg/mg, respectively), as well as high flavonoid (quercetin) contents in *Athyrium acutipinulum* leaf, *Aruncus dioicus* var. *kamtschaticus* leaf,

and *Cirsium nipponicum* leaf extract (16.75±0.43, 16.47±0.18, and 13.30±0.07 μg/mg, respectively). A previous study (Ham et al. 1997) reported that vitamin C, carotenoids, and cellulose have antimutagenic, antitumor, cancer inhibitory, antioxidative, cholesterol-lowering, and intestinal regulatory activities. Many studies have focused on medicinal plants and vegetables. It has also been reported (Ham et al. 1997) that the antioxidant activity of vegetables is due to a high polyphenolic content. Among domestic edible plants, the polyphenolic contents are highest in black tea (101.51 μg/mg), ginseng tea (28.30 μg/mg), and green tea (94.90 μg/mg), and the flavonoid contents are 16.75, 3.29, and 6.72 μg/mg in these types of tea, respectively (Choi et al. 2003).

Choi et al. (2013b) reported that liver weight was higher in mice fed *Prunus sargentii* R. seed oil. Rats are strongly affected by their diet due to a weak homeostasis, compared with humans, and the excessive intake of unsaturated fatty acids. However, a previous study (Lee et al. 2003) reported that mice with diabetes induced by streptozocin showed significant differences in liver, kidney, heart, and adipose tissue weights per 100 g compared with a control group.

Generally, the blood cholesterol level of white mice is 20~92 mg/dL (Ham et al. 1997), but Yu & Shaw (1994) reported that it can exceed 92.04 mg/dL (2.38 mmol/L) in normal white mice, depending on the breeding conditions. In a study by Choi et al. (2013b), the blood cholesterol level was significantly higher (p<0.05) in animals fed corn oil (142.50±42.13 mg/dL) and *Prunus sargentii* R. seed oil than in the control group (99.25±16.76 mg/dL). The overall blood cholesterol level in this study was higher than the values presented in previous studies (Yu & Shaw 1994; Johnson-Johnson 2001) due to the high-fat diet. A previous study (Katz & Pick 1961) also reported that cholesterol intake significantly increases serum cholesterol levels in white rats and chickens. However, the normal blood HDL cholesterol level in white mice was 68.94 mg/dL (1.78 mmol/L) in the study by Yu & Shaw (1994). The values in this study were twice as high (114.00~120.00 mg/dL).

Another previous study (Sheo HJ 2001) reported the normal blood level of LDL cholesterol in white mice to be 10.47~82.7 mg/dL. This study measured LDL cholesterol levels in mice after feeding them parts of *E. arvense* in the following order: root < reproductive stem < vegetative stem. Compared with the control group, triglyceride levels were lower in mice fed a diet containing the root, reproductive stem, and vegetative stem of *E. arvense*, although the difference was not significant. A previous study (Johnson-Johnson 2001) reported the blood triglyceride level in normal white mice to be 27~108 mg/dL, and the respective figure reported by Yu & Shaw (1994) was 78.77 mg/dL (0.9 mmol/L). In addition, Choi et al. (2013b) reported that the level of HDL-cholesterol was higher in animals fed *Prunus sargentii* R. seed oil compared to controls, while that of LDL-cholesterol was lower in this group (p<0.05).

Recently, studies attempting to identify the genetic factors involved in obesity have reported variations in the levels of leptin, leptin receptor, and carboxypeptidase E in rodents and humans (Hong et al. 2001). The results showed that leptin is highly correlated with body weight and body fat (Watson et al. 2000; Hong et al. 2001), and leptin expression and blood levels are increased by a high-fat diet (Hong et al. 2001). Hong et al. (2001) reported that, after 2 weeks on a high-fat diet, blood leptin levels increased 2.3 fold compared with the levels in the control group, and a high-fat diet increased the levels of serum leptin. This study also found that mice fed a high-fat diet showed increased serum leptin level, whereas serum leptin levels decreased in mice fed the root, reproductive stem, and vegetative stem of *E. arvense*. Previous studies (Watson et al. 2000; Hong et al. 2001) also reported that a high blood leptin level failed to inhibit fat accumulation in a high-fat diet group, because obesity itself causes leptin resistance. It was reported that the effects of leptin could be abrogated by reducing the reactivity of the central nervous system to leptin (Hong et al. 2001). Another study (Caro et al. 1996) explained that leptin resistance during obesity is due to the reduced carrying capacity of leptin. Hong et al. (2001) reported that changes in dietary composition and serum leptin content occur with the consumption of a high-fat diet, which means that the leptin hormone responds to changes in body fat. In addition, previous studies (Caro et al. 1996; Hong et al. 2001) reported a correlation between blood leptin and blood insulin levels. Havel PJ (2000) reported that the injection of insulin into humans increases blood leptin levels. Insulin, as the main regulator of leptin production, stimulates the generation of leptin in rodents and human (Hong et al. 2001). Therefore, an increase in body fat increases resistance to leptin as well as insulin, with leptin levels elevating in response (Hwang & Lee 2002).

The results obtained in this study suggest that polyphenolic compounds in the reproductive and vegetative stem and root of *E. arvense* have a variety of antioxidant effects. The stems and root can lower the levels of blood insulin and leptin even with the consumption of a high-fat diet. These components can prevent disease and are potentially very useful as a functional and medicinal herb and a novel source of physiologically active substances for humans.

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