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Antioxidative Activity of Korean Wild Leaf Vegetables: *Pleurospermum Kamtschaticum, Aderophora Remotiflor* and *Aster Glheni**

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ABSTRACT

The purpose of this study was to evaluate the antioxidative potential of Korean wild leaf vegetables *in vivo* as well as *in vitro*. The antioxidative activities of *Pleurospermum kamtschaticum*, *Aderophora remotiflor* and *Aster glheni* were evaluated as a reference for *Spinacia oleracea*. Fifty rats were fed either a control diet or one of several vegetable diets for 4 weeks. The level of thiobarbituric acid reactive substance (TBARS) and the activity of catalase and superoxide dismutase (SOD) in the liver were compared. The levels of total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and triglyceride (TG) in plasma were also compared. Also, the contents of β-carotene, vitamin C, vitamin E and total phenolic compound, including flavonoid and thiobarbituric acid (TBA) value using linoleic acid model system, were measured in Korean wild leaf vegetables. The TBARS values of *Aderophora remotiflor* and *Aster glheni* tended to be lower than that of *Spinacia oleracea*. The SOD activity of *Aster glheni* was significantly higher than that of *Spinacia oleracea* and reached 265% that of the control animals, whereas there was no difference between the control animals and the vegetable diet animals in terms of catalase activity. Compared to the control animals, TG and TC levels were significantly lower and showed a lower arthriosclerotic index. TBA values of *Pleurospermum Kamschaticum* and *Aderophora remotiflor* were only 18% of control value. TBA value of *Spinacia oleracea* was 41% of control value. These results suggest that *Aderophora remotiflor* and *Aster glheni* could have antioxidative potency *in vivo* as well as *in vitro* and potential value for functional food to improve the plasma lipid profile. Flavonoid and phenolic compounds could be the major contributing factor in the antioxidative potential of *Aderophora remotiflor* and *Aster glheni*.

KEY WORDS: antioxidant, antioxidative activity, *Pleurospermum kamtschaticum*, *Aderophora remotiflor* and *Aster glheni*.

INTRODUCTION

Many chronic diseases such as coronary heart disease, cancer and inflammatory and neurological disorders have been associated with the action of free radicals.[1-5] Free radicals such as the superoxide anion, the hydroxyl radical, hydrogen peroxide (H_2O_2) and singlet oxygen can be generated *in vivo* by exogenous factors such as imbalances in the diet, smoke, pollutants and other toxins, or from endogenous sources such as lipid peroxidation, inflammation, secondary lesions and biochemical reactions.[1,2,6] The long-term presence of these oxidizing species will eventually detrimentally affect the human body.[5] Hence, a great deal of effort has been dedicated to research on molecules with antioxidant and free radical scavenging activities.

There are two primary defense systems against free radicals:an enzymatic system and non-enzymatic system. In the enzymatic antioxidant system, catalase and superoxide dismutase (SOD) are important and in the non-enzymatic antioxidant system, antioxidant vitamins like vitamin E, vitamin C and β-carotene[6-9] as well as flavonoids and other plant phenolics[10-12] may have similar effects.

There have been numerous studies on the antioxidant capacity of plants that indicate the presence of important antioxidants such as antioxidant vitamins, flavonoids and phenolics in plants.[13] Nurucchwi (*Pleurospermum kamtschaticum*), Moshidae (*Aderophora remotiflor*) and Sumssukbujengee (*Aster glheni*) have been used as wild vegetables in Korea. These wild vegetables possess antiphlogistic, stomachic, analgesic, and antimutagenic effects.[14-16] Therefore, as part of our continuing studies on the biological activity of natural products, we evaluated the antioxidative activity of *Pleurospermum kamtschaticum*, *Aderophora remotiflor* and *Aster glheni in vivo* as well as *in vitro*.

MATERIALS AND METHODS

1. Animals and diets

A total of 50 male Sprague-Dawley rats (Samyang Exp. Animals Co.) of 120 - 140 g were divided into 5 groups

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of 10 rats each with similar body weights: control, *Spinacia oleracea*, *Pleurospermum kamtschaticum*, *Aderophora remotiflor* and *Aster glheni* group. *Adenophora remotiflor* and *Spinacia oleracea* were purchased at a local market in Seoul, *Pleurostermum kamtschaticum* in Chuncheon and *Aster glheni* in Ullung Island, Korea. Vegetables were freeze-dried and powdered. Animals received 10% vegetable diets or control diet for 4 weeks. The control diet was a vitaminfree cascin-based semisynthetic diet that met AIN-93 recommendations.[17] The nutritional composition of the 10% vegetable diets was manipulated to get a composition similar to that of the control diet. Thus, all experimental diets contained 20% protein, 5% fat, 65% carbohydrates, 5% fiber and 3850 kcal/kg by weight. Prior to initiating the respective vegetable diets, rats were given ad libitum access to the control diet for 1 week to allow them to adapt to the diet and feeding schedule and to bring all the rats to a similar metabolic status.

2. Sample collection and analysis

At the end of week 4, animals were anesthetized with ether and sacrificed by decapitation. Immediately following decapitation, blood was collected in heparinized tubes and centrifuged to separate the plasma. Organs were rapidly blotted dry and weighed. Plasma and tissues were stored at −70℃ until analyzed. Plasma total cholesterol (TC) was analyzed with a commercial kit based on enzymatic method (Youngdong Pharmaceutical Co., Korea). High-density lipoprotein-cholesterol (HDL-C) was analyzed using a commercial kit. The analysis was based on the same analytical method used for total cholesterol, after the precipitation of low density lipoprotein, very low density lipoprotein and chylomicron with polyethyleneglycol (International Reagent Co., Japan). Arteriosclerotic index was calculated as (TC − HDL-C)/HDL-C. Triglyceride (TG) was analyzed using a commercial kit based on Trinder method (Youngdong Pharmaceutical Co., Korea).

Vitamin C was measured by 2,4-dinitrophenylhydrazine colorimetric procedure.[18] β-Carotene was measured by the HPLC method.[19,20] Vitamin E was measured using colorimetric method using α, α-dipyridyl.[21] Flavonoid was measured using Kang *et al.* method.[22] Total phenol was measured by colorimetric method modified by Chung *et al.*[23] Thiobarbituric acid (TBA) was measured using linoleic acid model system modified by Kim *et al.*[24] Liver thiobarbituric acid reactive substance (TBARS) was measured by colorimetric method modified by Buckingham.[25] A spectrophotometric method for the determination of catalase activity was used.[26] The activity of superoxide dismutase (SOD) was determined by the Flohe method.[27]

For statistical analysis, all data were first evaluated by analysis of variance. For those F values that were significant, the least significant difference test was performed. A p value < 0.001 was considered to be statistically significant.

RESULTS

The effect of a Korean wild leaf vegetable diet on body weight and feed efficiency ratio (FER) is shown in Table 1. The body weight gain of *Aster glheni* was significantly lower than that of the control animals. The FER of *Pleurospermum kamtschaticum* was significantly higher and the FER of *Aster glheni* was lower than that of the control animals. Table 2 shows the effect of the Korean wild leaf vegetable diet on organ weights. There were no differences in organ weights between control and vegetable diet animals except *Aster glheni*, regardless of the different kinds of vegetables. The organ weights of *Aster glheni* were significantly lower than the organ weights of the control animals. As shown in Table 3, there were no differences between the control animals and vegetable diet animals in the values of TBARS. However, the SOD activity of *Aster glheni* was significantly higher than that of the control and *Spinacia oleracea* animals. The catalase activities of *Pleurospermum kamtschaticum* and *Aderophora remotiflor* were significantly lower than that of the *Spinacia oleracea*. Table 4 shows the effects of the Korean wild leaf vegetable diet on plasma lipid profile. Compared to control animals, the levels of TG, TC and HDL-C of *Aster glheni* were significantly lower. Also, the levels of TC and HDL-C in the control and *Spinacia oleracea* animals tended to be higher than those in the Korean wild leaf vegetable diet animals. The arteriosclerotic index of *Aderophora remotiflor* and *Aster glheni* tended to be lower than that of the controls, though there were no significant differences between the controls and vegetable diet animals in arterio-

Table 1. The effect of Korean wild leaf vegetable diet on body weight gain (g) and feed efficiency ratio (FER)[1,2]

	Control	*Spinacia oleracea*	*Pleurospermum kamtschaticum*	*Aderophora remotiflor*	*Aster glheni*
BW	280 ± 7.00[a]	272 ± 4.11[a]	278 ± 5.83[a]	275 ± 3.80[a]	198 ± 6.13[b]
FER	0.37 ± 0.09[ab]	0.35 ± 0.01[a]	0.43 ± 0.02[c]	0.40 ± 0.01[bc]	0.26 ± 0.02[d]

1) Values are mean SEM, n = 10

2) Within a given row, those values with different superscripts are significantly different ($p < .05$)

Table 2. The effect of Korean wild leaf vegetable diet on organ weights (g)[1)2)]

	Control	*Spinacia oleracea*	*Pleurospermum kamtschaticum*	*Aderophora remotiflor*	*Aster glheni*
Liver	8.81 ± 10.34[a]	8.37 ± 0.19[a]	8.51 ± 0.26[a]	8.43 ± 0.18[a]	5.75 ± 0.20[b]
Heart	0.89 ± 0.03[a]	0.88 ± 0.01[a]	0.87 ± 0.03[a]	0.91 ± 0.02[a]	0.66 ± 0.02[b]
Kidney	1.06 ± 0.03[a]	1.04 ± 0.03[a]	1.01 ± 0.02[a]	1.01 ± 0.02[a]	0.78 ± 0.03[b]
Spleen	0.82 ± 0.05[a]	0.72 ± 0.08[a]	0.81 ± 0.03[a]	0.72 ± 0.04[a]	0.49 ± 0.02[b]

1) Values are mean SEM, n = 10
2) Within a given row, those values with different superscripts are significantly different ($p < .05$)

Table 3. The effect of Korean wild leaf vegetable diet on TBARS[1)] and antioxidative enzyme activities of liver[2)3)]

	Control	*Spinacia oleracea*	*Pleurospermum kamtschaticum*	*Aderophora remotiflor*	*Aster glheni*
TBARS[3)]	7.73 ± 0.83[a]	7.41 ± 0.99[a]	7.29 ± 0.89[a]	5.85 ± 0.55[a]	5.85 ± 0.40[a]
SOD[4)]	17.3 ± 0.38[a]	23.2 ± 0.39[c]	30.2 ± 1.07[ab]	26.7 ± 1.27[a]	45.7 ± 2.51[b]
Catalase[5)]	5857 ± 81.0[ab]	5963 ± 74.4[a]	5345 ± 149[b]	5359 ± 493[b]	5656 ± 124[ab]

1) TBARS: thiobarbituric acid reactive substance, nmol/g liver
2) Values are mean SEM, n = 10
3) Within a given row, those values with different superscripts are significantly different ($p < .05$)
4) SOD: superoxide dismutase, SOD activities are expressed as units per minute per mg protein (1 unit inhibits the rate of reduction of cytochrome C by 50% in a coupled system with xanthine and xanthine oxidase at pH 7.8 and 25℃ in a 3 ml reaction volume)
5) Catalase activities are expressed as nmole formaldehyde utilized per mg protein

Table 4. The effect of Korean wild leaf vegetable diet on plasma lipid profile[1)2)]

	Control	*Spinacia oleracea*	*Pleurospermum kamtschaticum*	*Aderophora remotiflor*	*Aster glheni*
Triglyceride (mg/dl)	67.7 ± 4.76[ab]	76.3 ± 7.74[ab]	81.3 ± 7.25[a]	72.9 ± 5.96[ab]	60.3 ± 2.62[b]
Total cholesterol (mg/dl)	54.8 ± 4.01[ab]	58.5 ± 3.43[a]	43.5 ± 2.72[bc]	31.0 ± 3.50[cd]	28.9 ± 4.30[d]
HDL-cholesterol (mg/dl)	17.1 ± 2.08[ab]	16.13 ± 2.09[a]	14.9 ± 2.64[abc]	12.1 ± 1.08[bc]	11.6 ± 1.69[c]
Arteriosclerotic index[3)]	2.47 ± 0.41[a]	2.47 ± 0.40[a]	2.73 ± 0.69[a]	1.61 ± 0.27[a]	1.96 ± 0.59[a]

1) Values are mean SEM, n = 10
2) Within a given row, those values with different superscripts are significantly different ($p < .05$)
3) Arteriosclerotic index were calculated as (TC − HDL-C)/HDL-C

Table 5. Antioxidant contents in Korean wild leaf vegetables[1)2)]

	Spinacia oleracea	*Pleurospermum kamtschaticum*	*Aderophora remotiflor*	*Aster glheni*
β-carotene (g/100 g)	39486 ± 350[a]	18593 ± 351[b]	10183 ± 232[c]	3938 ± 9.28[d]
Vitamin C (mg/100 g)	17.4 ± 0.87[c]	95.6 ± 5.21[a]	60.8 ± 0.90[b]	17.4 ± 2.30[c]
Vitamin E (mg/100 g)	12.6 ± 0.46[a]	8.52 ± 0.05[b]	12.5 ± 0.10[a]	8.12 ± 0.55[b]
Flavonoid (mg/100 g)	749 ± 97.0[d]	5155 ± 418.4[a]	3123 ± 55.1[c]	3787 ± 85.4[b]
Total phenolic compounds (mg/100 g)	8870 ± 420[d]	19920 ± 120[a]	29850 ± 1910[a]	24540 ± 780[b]

1) Values are mean SEM, n = 10
2) Within a given row, those values with different superscripts are significantly different ($p < .05$)

sclerotic index. Table 5 shows the content of antioxidants in Korean wild leaf vegetables. On a freeze-dried weight basis, the β-carotene contents of Korean wild leaf vegetables were significantly lower than that of the *Spinacia oleracea*. Vitamin E contents of *Pleurospermum kamtschaticum* and *Aster glheni* were also signigicantly lower than that of the *Spinacia oleracea*. Vitamin C contents of *Pleurospermum kamschaticum* and *Aderophora remotiflor* were significantly higher than that of the *Spinacia oleracea*. Moreover, the contents of flavonoid and total phenolic compounds of Korean wild leaf vegetables were significantly higher than those of *Spinacia oleracea*. As shown in Table 6, all the vegetables tested had marked antioxidative activity when compared with the controls. TBA values of *Pleurospermum Kamschaticum* and *Aderophora remotiflor* were only 18% of control value. TBA value of *Spinacia oleracea* was 41% of control value.

DISCUSSION

The value of spinach (*Spinacia oleracea*) was used as reference value to compare the relative antioxidative potency of wild leaf vegetables because among cultivated vegetables, spinach is commonly accepted to be an excellent source of antioxidant nutrients.[18)28)] For body weight and FER, the values of the *Aster glheni* animals were lower

Table 6. Change of thiobarbituric acid (TBA) value of linoleic acid substrates during storage at 40℃[1)2)]

	Control	BHA	*Spinacia oleracea*	*Pleurospermum kamtschaticum*	*Aderophora remotiflor*	*Aster glheni*
1 day	0.294 ± 0.026[a]	0.003 ± 0.001[d]	0.096 ± 0.028[b]	0.049 ± 0.006[c]	0.044 ± 0.001[c]	0.068 ± 0.028[bc]
2 day	0.501 ± 0.104[a]	0.005 ± 0.001[d]	0.200 ± 0.038[b]	0.098 ± 0.029[c]	0.097 ± 0.015[c]	0.152 ± 0.025[bc]
3 day	0.750 ± 0.118[a]	0.006 ± 0.001[d]	0.320 ± 0.044[b]	0.154 ± 0.036[b]	0.150 ± 0.036[b]	0.247 ± 0.050[b]
4 day	1.050 ± 0.160[a]	0.006 ± 0.001[d]	0.405 ± 0.087[b]	0.190 ± 0.053[c]	0.193 ± 0.053[c]	0.337 ± 0.055[bc]
5 day	1.204 ± 0.151[a]	0.009 ± 0.001[d]	0.477 ± 0.091[b]	0.221 ± 0.054[c]	0.220 ± 0.030[c]	0.420 ± 0.089[b]
6 day	1.323 ± 0.171[a]	0.011 ± 0.003[d]	0.537 ± 0.071[b]	0.242 ± 0.046[c]	0.240 ± 0.027[c]	0.485 ± 0.085[b]

1) Values are mean SEM, n = 10
2) Within a given row, those values with different superscripts are significantly different ($p < .05$)

than that of other vegetable diet animals. The organ weights of *Aster glheni* were significantly lower than that of other group animals. Hence, it was supposed that diet taste and intake of *Aster glheni* were different from those of other vegetables.

The potent antioxidative activities of the vegetables were observed *in vivo* and *in vitro*. In this study, there were no differences between the control animals and vegetable diet animals in the values of TBARS. Although as reported by Park *et al.*,[28] liver TBARS concentrations were significantly decreased in animals fed the Korean native plant diet, the TBARS levels of *Aderophora remotiflor* and *Aster glheni* tended to lower than that of *Spinacia oleracea* in the present study. However, the SOD activities of *Pleurospermum kamtschaticum* and *Aster glheni* were higher than those of control animals and *Spinacia olercea* animals. Moreover, the SOD activity of *Aster glheni* was significantly higher than that of the *Spinacia oleracea* and reached 265% that of the control animals. But no difference was seen among all groups in terms of catalase activity. The strong antioxidant potency of the wild leaf vegetables might be a result of either antioxidant vitamin or non-antioxidant vitamin compounds, phenolic compounds including flavonoid or both. Numerous *in vitro* studies have shown these phenolics to possess strong radical scavenging activity at least equal in potency to other important dietary antioxidants, such as vitamin C and vitamin A.[30-31] Flavonoids inhibit lipid peroxidation or inhibit the formation of lipid peroxide[32-35] and the antioxidant capacities of some flavonoids were found to be several times stronger on the basis of molar concentration than vitamin E and vitamin C.[36-37] Much evidence demonstrated a multitude of interaction among the antioxidants.[38-40] Vitamin A, C and E showed additive antioxidative effects when used together.[39] Dietary flavonoids represent an important source of antioxidants and showed the sparing effect on vitamin E and β-carotene.[41] Furthermore, flavonoids increase the activity of antioxidant enzymes such as SOD and catalase.[42] The flavonoid and phenolic compound content of *Aster glheni* was significantly higher than that of *Spinacia oleracea*, whereas the vitamin E and β-carotene content of *Aster glheni* was lower than that of *Spinacia oleracea*. Therefore, the lower value of TBARS in *Aster glheni* versus *Spinacia oleracea* may be a result of higher SOD activity and higher flavonoid and phenolic compound content in *Aster glheni*, despite lower vitamin E and β-carotene content. Although there was no difference in SOD activity between *Aderophora remotiflor* and *Spinacia oleracea*, vitamin C, the flavonoid and phenolic compound content of *Aderophora remotiflor* was significantly higher than that of *Spinacia oleracea*. Furthermore, the TBA value of *Adenophora remotiflor* reached 80% that of BHA, a strong synthetic antioxidant, while the antioxidative activity of *Spinacia oleracea* and *Aster glheni* reached 40% that of BHA. In accounting for these results, it could be considered that the TBARS value of *Aderophora remotiflor*, like *Aster glheni*, was lower than that of *Spinacia oleracea*.

The higher antioxidative activity may effect the blood lipid profile, which has been associated with free radical mediated events. Flavonoid decreasing the level of plasma total cholesterol[43-46] and the hypocholesterolemic effect was also reported in other polyphenol-containing foods.[45-47] Compared to Spinacia oleracea, the levels of TG, TC and the asteriosclerotic index of *Aderophora remotiflor* and *Aster glheni*, given the higher flavonoid and phenolic compound content and the higher antioxidative activity, were lower in this study. Therefore, flavonoid and phenolic compounds could be the major contributing factor in the antioxidaive potential of *Aderophora remotiflor* and *Aster glheni*, in spite of the lower vitamin E and β-carotene content compared to *Spinacia oleracea*.

These results suggest that *Aderophora remotiflor* and *Aster glheni* could have antioxidative potency *in vivo* as well as *in vitro* and potential value as a functional food to improve the plasma lipid profile.

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