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The effects of *A. senticosus* supplementation on serum lipid profiles, biomarkers of oxidative stress, and lymphocyte DNA damage in postmenopausal women

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ABSTRACT

This study examined the effects of *Acanthopanax senticosus* supplementation on serum lipid profiles, biomarkers of oxidative stress, and lymphocyte DNA damage in postmenopausal women. Forty postmenopausal women, ages 40–65, were randomly divided into two groups: (1) control group (calcium) and (2) treatment group (calcium plus *A. senticosus*). Both groups were treated for 6 months. Blood samples were obtained before and after supplementation at 6 months. The following blood parameters were measured: serum total cholesterol, triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), serum malondialdehyde (MDA), ccdd protein-carbonyl (PC) levels, the degree of lymphocyte DNA damage by comet assay, total ferric reducing antioxidant power (FRAP), uric acid, and total bilirubin in serum. The treatment group had significant decreases ($p < 0.001$) in serum LDL (127.54 ± 29.79 mg/dL vs 110.33 ± 22.26 mg/dL) and the LDL/HDL ratio (2.40 ± 0.65 vs 2.11 ± 0.58) after *A. senticosus* supplementation. Serum MDA concentrations decreased by 2.2% in the control group and by 12.61% in the treatment group after 6 months of intervention; however, the reductions were not significant in either group. Protein-carbonyl levels and lymphocyte DNA damage decreased significantly ($p < 0.001$ and $p < 0.05$, respectively) after 6 months of *A. senticosus* supplementation. These results suggest that *A. senticosus* supplementation may have beneficial effects against oxidative stress and improve serum lipid profiles without subsequent side effects.

Aging is the progressive intrinsic deterioration in physiological status that culminates in death [1]. Although the underlying mechanisms of aging are still unclear, the free radical theory of aging is widely accepted. Reactive oxygen species (ROS) are constantly generated in vivo and cause oxidative damage to biological molecules such as lipids, proteins, and DNA [2].

Recently, there has been increasing interest in the antioxidant activity of phytochemicals present in the diet. Antioxidants are believed to play a very important role in the body's defense system against ROS. The dietary supplementation of antioxidants provides protection against cardiovascular disease, several common types of cancer, as well as other chronic diseases [3,4]. Several studies have shown that the consumption of antioxidant-rich foods decreases levels of oxidative damage in humans [5].

Acanthopanax senticosus (*A. senticosus*) is a common Asian herb known as "Siberian Ginseng" or "*Eleutherococcus senticosus*" that is used as an adaptogenic medicine [6]. The major active components of *A. senticosus* are eleutherosides, chiisanosides, isofraxidin, acanthosides, daucosterine, β-sitosterol, sesamine, and savinine [7]. Crude A. *senticosus* extracts have been used as popular health supplements to treat stress-induced physiological changes [8,9] as well as various allergic conditions [10], inflammation [11], cancer [12], chronic bronchitis, hypertension, ischemic heart disease, and gastric ulcers, in Asia. However, no comprehensive study has evaluated their health benefits and side effects in humans. Therefore, the possible health benefits of A. *senticosus* supplementation were undertaken in postmenopausal women. In the present paper we report the effects of A. *senticosus* supplementation on serum lipid profiles, biomarkers of oxidative stress and lymphocyte DNA damage as well as hepatochemical safety in postmenopausal women.

Materials and methods

Plant materials and extraction. Acanthopanax senticosus leaves (2006 product) were purchased from Yang-Gu in Kangwondo, Korea. The leaves had a moisture content of <10% by weight, and were air-dried at 60 °C for 36 h. The supernatant was filtered with filter paper (Whatman No. 3, England), and the concentrated

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Table 1
General characteristics of the study subjects

General characteristics	Control group	Treatment group
Age (years)	48.9 ± 4.4	49.6 ± 3.8
Height (cm)	154.9 ± 4.1	154.1 ± 5.3
Weight (kg)	59 ± 5.0	57.2 ± 6.9
BMI (kg/m2)	24.6 ± 2.0	24.1 ± 2.7

Values are means ± SD.

extracts were freeze-dried for 72 h. This process produced 8.15 g (yield: 16.3%) of brown powder.

Human subjects. Healthy postmenopausal women were recruited from Kyunghee Medical Center. The inclusion criteria were as follows: (1) spontaneous menopausal women under age 65; (2) no consumption of hormone replacement drugs, tamoxifen, or steroid drugs during the previous 2 months; (3) no history of liver or renal diseases; (4) non-alcohol consuming and non-smoking. A total of forty postmenopausal women were included in the study and were divided into two groups: (1) control group ($n = 16$): calcium intake (500 mg per day); (2) treatment group ($n = 24$): calcium plus *A. senticosus* [500 mg calcium/day plus 2 capsules of *A. senticosus* (500 mg) three times daily]. All the women were treated for 6 months. The subjects underwent a clinical interview and physical examination, and general characteristics are presented in Table 1. No significant differences were observed between the two groups. In addition, dietary patterns were assessed by food frequency questionnaire and did not differ between the two groups (data not shown). All study procedures were reviewed and approved by the Institutional Review Board of Kyung Hee University. All the participants were informed regarding the aims and methods of the study and gave their informed consent.

Sample collection. Blood samples were obtained before and after supplementation at 6 months. Fresh blood samples were collected after a 12 h fast into tubes without anticoagulant and into EDTA-containing tubes. The serum was immediately separated and stored at −80 °C until lipid profile and antioxidant activity analyses were performed. Lymphocytes were isolated by density gradient sedimentation (Histopaque 1077) and were frozen to −80 °C in a mixture containing FBS and DMSO prior to storage in liquid nitrogen.

Blood lipid profiles. Fasting venous blood samples were collected to estimate total serum cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and serum triglyceride levels using an autoanalyzer (Vital Scientific, Netherlands).

GOT, GPT, and creatinine. Hepatochemical safety parameters (GOT, GPT, and creatinine) were analyzed by a Selectra E autoanalyzer (Vital scientific, Netherlands).

Ferric reducing ability of plasma (FRAP) assay. FRAP analysis was used to determine antioxidant power in the serum and was achieved by applying the Benzie method [13]. This method assays the transition of ferric ions to ferrous ions through a colored ferrous–tripyridyltriazine complex, which is measured using a spectrophotometer. The FRAP reagent consisted of 300 mM sodium acetate buffer (pH 3.6), TPTZ reagent, and 20 mM FeCl. The TPTZ reagent was prepared by dissolving 40 mM HCL in 10 mM TPTZ [2,4,6,-tri(2-pyridil)-*S*-triazine, MW 312.34]. Then, 25 mL of acetate buffer, 2.5 mL of TPTZ reagent, and 2.5 mL of FeCl solution were combined and incubated in a 37 °C water bath for 10–15 min. All reagents and samples were chelex-treated (2 g/L) in deionized Milli-Q water. To prepare a (+/−)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid standard, Trolox was dissolved in MeOH to obtain a 10 mM Trolox solution, which was then diluted to a 1 mM solution with 9 mL of water and stored at −80 °C. Next, 270 μl of freshly prepared FRAP reagent was warmed to 37 °C and added to 30 μl of diluted sample. Finally, spectrophometric readings were taken at 593 nm using Softmax software.

Malondialdehyde (MDA) determination. Serum malondialdehyde is used as a marker of lipid peroxidation and was measured using a commercially available kit (Zeptometrix). Fifty microliters of serum sample or standard were mixed with 50 μl of SDS solution; then, 1.25 mL of TBA/buffer reagent was forcefully added down the side of each tube. This reaction mix was incubated at 95 °C for 60 min. After incubation, the mixture was cooled to room temperature in an ice bath for 10 min. The absorbance values of standards and samples were read at 532 nm by a spectrophotometer.

Protein-carbonyl assay. Oxidative protein damage was assessed by determining the concentration of carbonyl groups based on the reaction with dinitrophenylhydrazine (DNP) using a commercially available kit (Biocell). Briefly, proteins were precipitated by the addition of 28% trichloroacetic acid and were redissolved in DNP. The absorbance values of samples were read at 450 nm and the results are given as nmol/mg.

Uric acid and total bilirubin. Here, sample analysis was performed using a Selectra E autoanalyzer (Vital scientific, Netherlands). Uric acid (enzymatic photometric test) was determined by using (*N*-ethyl-*N*-(hydroxyl-3-sulfopropyl)-*m*-toluidin (TOOS) and total bilirubin was analyzed using the DCA method.

Comet assay. The comet assay was employed to assess lymphocyte DNA damage and conducted under alkaline conditions using the method of Singh et al. [14] with modifications. Here, lymphocytes were mixed with 0.5% low melting agarose (LMA) and were applied to precoated slides containing 1% normal melting agarose (NMA). An additional layer of LMP was then placed on the slides. After the agarose gel had solidified, the slides were placed in a lysis solution [100 mM EDTA, 2.5 M NaCl, 10 mM Tris–HCl, 1% *N*-lauroylsarcosine (pH 10), 1% Triton X-100, and 10% dimethyl sulfoxide (DMSO)] for at least 1 h. They were then immersed in a freshly prepared electrophoresis buffer (300 mM NaOH; 1 mM EDTA, pH 13) inside a horizontal gel electrophoresis tank. After a 40 min DNA unwinding period, electrophoresis was conducted at 25 V (300 mA) for 20 min. Following electrophoresis, the slides were washed three times with neutralization buffer (0.4 M Tris/HCl, pH 7.5) for 5 min, and were then washed with ethanol for another 5 min. Each slide was stained with ethidium bromide (20 μg/μl) and viewed using a fluorescence microscope. Tail length (TL, μm) and tail moment (TM, the product of multiplying the DNA percentage in the tail with the tail length) were used as measures of DNA damage. At least 50 cells were scored from duplicate slides.

Statistical analysis. All data are presented as means ± SD. The statistical analysis was performed with the SPSS statistical program (version 12.0 software, SPSS Chicago, IL, USA) using paired *t*-tests, and p values of <0.05 were considered significant.

Results

Blood lipid profiles

Table 2 presents the serum lipid profiles of the control and treatment groups before and after intervention. There were no significant changes in the lipid profiles of the control group after 6 months of intervention. And in the treatment group, there were no significant changes in serum cholesterol, triglycerides, and HDL levels during the intervention period; however, serum LDL (127.54 ± 29.79 mg/dL vs 110.33 ± 22.26 mg/dL) and the LDL/HDL ratio (2.40 ± 0.65 vs 2.11 ± 0.58) decreased significantly ($p < 0.001$) after 6 months of supplementation with *A. senticosus*.

Table 2
Serum lipid profiles of subjects

Lipid parameter	Control group		Treatment group	
	0 month	6 months	0 month	6 months
Total cholesterol (mg/dL)	204.25 ± 32.21	213.25 ± 29.57	194.42 ± 33.64	197.88 ± 27.83
Triglyceride (mg/dL)	131.38 ± 57.86	127.69 ± 49.02	115.75 ± 71.35	127.54 ± 29.79
LDL cholesterol (mg/dL)	127.44 ± 35.51	116.50 ± 19.01	127.54 ± 29.79	110.33 ± 22.26*
HDL cholesterol (mg/dL)	55.63 ± 11.94	54.75 ± 13.23	54.75 ± 10.78	54.42 ± 11.91
LDL/HDL ratio	2.42 ± 0.90	2.26 ± 0.71	2.40 ± 0.65	2.11 ± 0.58*

Values are means ± SD.
* $p < 0.001$ (paired t-test).

Table 3
GOT, GPT, and creatinine levels of the control and treatment groups

	Control group		Treatment Group	
	0 month	6 months	0 month	6 months
GOT (IU/L)	20.81 ± 4.28	19.53 ± 7.16	22.32 ± 5.60	24.33 ± 10.14
GPT (IU/L)	20.56 ± 8.05	19.17 ± 5.47	22.84 ± 8.77	21.67 ± 10.82
Creatinine (mg/dL)	0.75 ± 0.12	0.74 ± 0.10	0.71 ± 0.10	0.70 ± 0.11

Values are means ± SD.

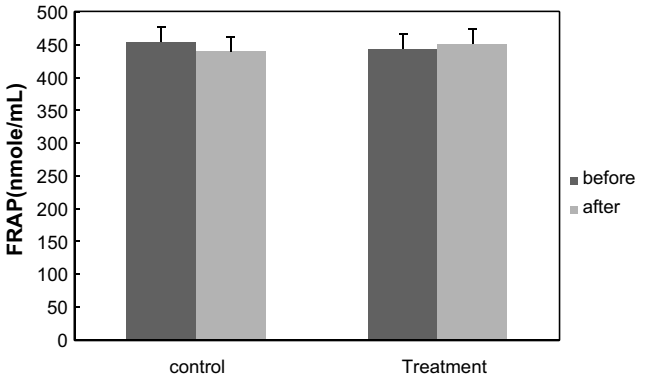

Fig. 1. FRAP values in serum of the control and treatment groups. The values are expressed as means ± SD. Statistical analysis was performed by a Student's t-test.

GOT, GPT, and creatinine levels

There were no changes in GOT, GPT, and creatinine levels after intervention for either group (Table 3). This data suggests that *A. senticosus* supplementation has no side effects.

FRAP values

The FRAP assay, which measures ferric reducing ability, indicates the antioxidant capabilities of serum by examining the reduction of Fe^{3+} to Fe^{2+} at a low pH, generating a colored ferrous–tripyridytriazine complex [13].

The changes in total antioxidant activity after intervention were insignificant in both groups (Fig. 1). In the control group, FRAP values decreased by approximately 3.2%. However, in the treatment group, FRAP values had a tendency to increase, and an approximate 1.81% increase was found after 6 months of *A. senticosus* supplementation.

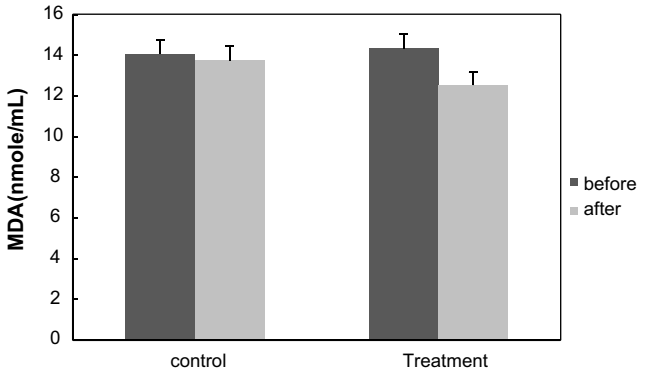

Fig. 2. MDA concentrations in serum of the control and treatment groups. The values are expressed as means ± SD. Statistical analysis was performed by a Student's t-test.

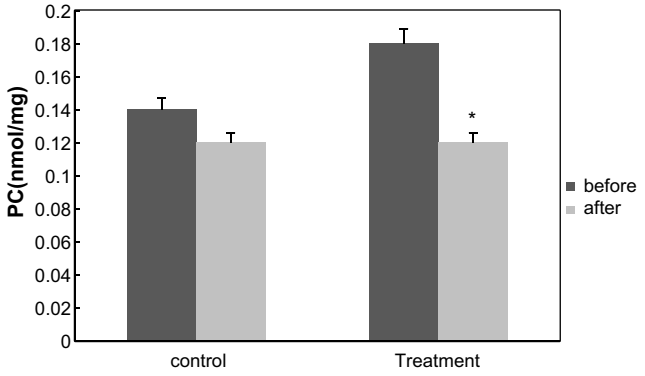

Fig. 3. PC concentrations in serum of the control and treatment groups. The values are expressed as means ± SD. Statistical analysis was performed by a Student's t-test (* $p < 0.001$).

MDA concentrations

Fig. 2 presents the malondialdehyde concentrations of both groups before and after intervention. MDA concentrations decreased by 2.2% in the control group and by 12.61% in the treatment group after 6 months of intervention; however there was no significant difference in either group. Increasing the number of participating subjects could result in significantly different MDA concentrations for the treatment group.

Protein-carbonyl levels

Protein oxidation was determined by the protein-carbonyl content of serum samples and is shown in Fig. 3. For the treatment group, carbonyl content significantly decreased from 0.18 ± 0.10 to 0.12 ± 0.05 nmol/mg with *A. senticosus* supplementation. In contrast, the protein-carbonyl level of the control group did not change significantly after intervention.

The protein-carbonyl level is the most general indicator and by far the most commonly used marker of protein oxidation in serum. Carbonyls are relatively difficult to induce as compared to methionine sulphoxide and cysteinyl derivatives, and may therefore indicate more severe oxidative stress. In general, elevated protein-carbonyl levels are not only a sign of oxidative stress, but also of disease-derived protein dysfunction [15–18].

Table 4
Serum uric acid and total bilirubin levels of the control and treatment groups

	Control group		Treatment group	
	0 month	6 months	0 month	6 months
Uric acid (mg/dL)	4.58 ± 0.90	4.25 ± 0.41	4.27 ± 0.52	4.19 ± 0.25
Total bilirubin (mg/dL)	0.67 ± 0.16	0.66 ± 0.15	0.79 ± 0.30	0.74 ± 0.25

Values are means ± SD.

Table 5
The degree of lymphocyte DNA damage (expressed as % tail DNA, TM, and TL) assessed by comet assay

	Control group		Treatment group	
	0 month	6 months	0 month	6 months
Tail DNA (%)	17.44 ± 4.94	16.61 ± 4.46	19.00 ± 4.54	16.75 ± 4.71*
Tail moment	9.21 ± 3.28	9.30 ± 3.74	11.09 ± 4.42	9.58 ± 4.15*
Tail length (μm)	66.17 ± 15.68	65.74 ± 13.31	73.09 ± 19.96	67.70 ± 19.4*

Values are means ± SD.
* $p < 0.05$ (paired t-test).

Uric acid and total bilirubin levels

Table 4 presents the uric acid and total bilirubin levels of the groups before and after intervention. There were no significant differences in either group after intervention.

Uric acid and total bilirubin, which serves as a water soluble antioxidant, the biggest contributors to serum FRAP values [19]. In this study, uric acid and total bilirubin levels decreased after *A. senticosus* supplementation. It indicated that the increase of FRAP values may influence other antioxidant substances in *A. senticosus*.

Comet assay

The comet assay was conducted to detect single or double strand DNA breaks. Broken DNA fragments result in an increased migration rate in electrophoresis and form a diffuse DNA area, which resembles a comet tail after staining. Table 5 presents the DNA damage of lymphocytes for both groups. After 6 months of *A. senticosus* supplementation, the treatment group showed a significant improvement in lymphocyte DNA damage as indicated by percent of tail DNA (19.00 ± 4.54 vs 16.75 ± 4.71%), tail moment (11.09 ± 4.42 vs 9.58 ± 4.15), and tail length (73.09 ± 19.96 vs 67.70 ± 19.4 μm). However, there was no significant difference observed in the control group.

Discussion

Dietary antioxidant supplementation can be part of a protective strategy to minimize oxidative damage in the elderly. The phenolic compounds in *A. senticosus*, such as isofraxidin and eleutherosides B and E from the stem bark [20]; eleutheroside E2 and isomaltol 3-*O*-α-D-glucopyranoside from the roots [21]; chiisanoside, chiisanogenin, and hyperin from the leaves [22]; as well as protocatechuic acid, syringin, chorogenic acid, caffeic acid, liriodendrin, and isofraxidin in ethanol extract of whole *A. senticosus* [23], have a protective effect against oxidative damage. One study recently evaluated the antioxidant activities of *A. senticosus* in rats [24,25]. It seems that partial improvements in serum lipid profiles would occur via roles played by the herb's functional components [26]. The present study showed that subjects had significant decreases in serum LDL levels and LDL/HDL ratios after *A. senticosus* supplementation. In a previous study from our laboratory, we evaluated the effects of *A. senticosus* supplementation on serum lipid profiles in rats. These data also showed that LDL and the LDL/HDL ratio significantly decreased after *A. senticosus* supplementation.

The MDA assay is commonly used to evaluate lipid peroxidation in serum due to its simplicity; however, several substances have interfering effects on this assay [27]. According to a previous report, serum MDA levels were correlated with other oxidative stress parameters such as protein-carbonyl levels and lymphocyte DNA damage [28]. In our study, serum MDA concentrations did not change significantly after *A. senticosus* supplementation; however, the treatment group had greater reductions in MDA concentration than the control group.

Since free radicals can react not only with lipids, but also with proteins and DNA, the protein-carbonyl assay is proposed to have some advantages over the MDA assay. Products of oxidative protein modification are generally more stable and sensitive [29]. In this study, there were significant decreases in serum protein-carbonyl levels after 6 months of *A. senticosus* supplementation.

DNA damage is one of the more reliable markers to detect oxidative stress [30,31]. In this study, *A. senticosus* supplementation significantly decreased lymphocyte DNA damage according to comet assay results. A recent report by Lee and coworkers [32] showed that water extracts of a commercially dried *A. senticosus* tea had a protective effect against oxidative DNA damage.

In conclusion, we evaluated serum lipid profiles, FRAP, uric acid, total bilirubin, MDA, PC, and lymphocyte DNA damage in control and *A. senticosus*-supplemented groups of postmenopausal women before and after intervention. The results suggest that *A. senticosus* supplementation may have beneficial effects against protein oxidation and lymphocyte DNA damage as well as improve serum lipid profiles by significantly reducing LDL cholesterol levels and the LDL/HDL ratio without subsequent side effects.

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