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In vivo Antioxidant Effects of *Aralia elata Seemann* Ethanol Extract Administered with Benzo(α)pyrene

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Abstract

This study has examined the effects of *Aralia elata Seemann* ethanol extract on antioxidant enzyme systems in rats along with benzo(α)pyrene (B(α)P) administration. The ethanol extract of *Aralia elata Seemann* (50 mg/kg body wt.) was fed to rats for 4 weeks by stomach tubing. The extract administration increased antioxidant activities of glutathione sulfur transferase (GST) comparing to the control. Also total superoxide dismutase (SOD) and Cu, Zn-SOD activities were stimulated. Catalase activities were increased by 50% with the extract feeding compared to the control. Combined administration of B(α)P and the extract increased GST activity in B(α)P group. Although total SOD activity was decreased, Cu,Zn-SOD was greatly increased from 0.10 unit to 0.18 unit and catalase activity also was increased compared to the group of B(α)P. GST activity in CLE group was 1.32 unit, increased by 33% comparing to the group CL of 0.99 unit. Cu,Zn-SOD and catalase activities in the group fed high fat and ethanol extracts were increased by 25% and 39%, respectively comparing to the group of high fat. In addition, total SOD was decreased but, Cu,Zn-SOD activity was increased from 0.09 unit to 0.18 unit. Catalase activity was 76.05 unit in the group of B(α)P and extract comparing to 65.26 units in B(α)P group. Serum α-tocopherol of rat was markedly increased by the extract. Administration of B(α)P reduced α-tocopherol levels in the serum, on the other hand, lard in the diet increased α-tocopherol levels in the serum. The above results indicate that *Aralia* bud exerts antioxidant functions *in vivo* against B(α)P. Further research may be necessary for the identification of the biologically active material.

Key words: antioxidant, *Aralia elata Seeman*, SOD, GST, catalase, α-tocopherol

INTRODUCTION

Aralia elata Seemann, one of the most popular edible mountain vegetables, is classified as a shrub belonging to a species of *Araliaceae* and mostly grows in the mountain nationwide.

For a long time in Korean traditional medicine, barks and roots have been used in treating hypoglycemia, ulcer, diabetes, gastritis, colitis, schizophrenia, etc.

Quercitrin, a bioactive constituent of *Aralia elata Seemann*, has been suggested to have an effect on heart stimulation and contraction, fungal and virus infection, diabetic cataracts, activation of natural killer cells and pneumonia (1).

Essential antioxidant nutrients such as retinoids (2,3), tocopherols (4,5), ascorbic acid (6), zinc (7), selenium (8-10), flavones and phenolic compounds from diet particularly plant sources may act directly on the oxidative defense systems.

These naturally occurring or synthetic antioxidants have been proved to be effective in blocking the initiation and promotion of chemical carcinogenesis such as dimethylhydrazine, aflatoxin, B(α)P in many experimental animal models (11-14). B(α)P, a strong carcinogenic and mutagenic substance, is also produced as an environmental contaminant by gasoline, tar, coal and cigarette smoke. Dietary antioxidants were shown to modulate the cytochrome P-450 action in mice liver to form less activated metabolites of carcinogens such as B(α)P(15,16).

In this experiment, the antioxidative and detoxification effect of *Aralia elata Seemann* ethanol extract in rat has been investigated particularly in conjunction with the activation of antioxidant enzymes including glutathione sulfur transferase (GST), catalase, total-superoxide dismutase (SOD), Cu,Zn-SOD and α-tocopherol.

MATERIALS AND METHODS

Experimental animals and diet

Male Sprague Dawley rats weighing approximately 100 g were randomly divided and were maintained in a 12 : 12 dark and light cycled room. Before the start of the experiment animals were fed chow diet for a week and then divided into groups of high fat diet and benzo(α)pyrene administration. Diet groups and the experimental design of animals are shown in Table 1. Specimen of *Aralia elata Seeman* was obtained locally in Kangwon province, washed, lyophilized, ground and ethanol-extracted with a rotary evaporator (Eyela, Japan). *Aralia elata Seeman* ethanol extract (50 mg/kg body wt) was fed daily to rat for four weeks through stomach tube.

Experimental diet composition

Animals were allowed to have free access to diet (*ad libitum*) and provided powered mixed diet based on the AIN-76 formula including AIN-76 mineral and vitamin mixtures. High fat diet

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Table 1. Experimental design of animals

Group	Diet	Group	Diet
C	Basal diet	CL	Basal diet+High fat[3]
CE	Basal diet+Ethanol extract[1]	CLE	Basal diet+High fat+Ethanol extract
CB	Basal diet+B(α)P[2]	CLB	Basal diet+High fat+B(α)P
CBE	Basal diet+B(α)P+Ethanol Extract	CLBE	Basal diet+High fat+B(α)P+Ethanol extract

[1]Rats were fed ethanol extract (50 mg/kg body weight/day) during the whole experimental period.
[2]Rat were administered B(α)P (50 μg/kg) twice at 7th and 14th day of extract feeding.
[3]High fat included 10% of lard, 1% of cholesterol and 0.25% of sodium cholate in the diet.

contained 0.25% of sodium cholate, 10% of lard and 1% of cholesterol.

B(α)P, a liver carcinogen, was administered at 50 μg/kg body weight twice at 7th and 14th day since extract feeding.

Preparation of tissue specimen for antioxidant enzyme assay

Animals were fasted for 12 hours and weighed before they were sacrificed by ether anesthesia and cervical dislocation. Blood was taken by cardiac puncture, organs including liver and kidney were taken, weighed and rinsed by physiological saline. Liver sample of 1 g was homogenized with 0.25 M sucrose buffer and centrifuged at 600 g (Microspin 24 S, Sorvall instruments, USA) for 15 minutes, pellets were discarded and supernatants were obtained and kept frozen at -70°C for enzyme assay.

GST assay

Assay of cytosolic GST was performed by the method of Habig et al. (17). Cocktails including 2935 μl of 0.1 M phosphate buffer, 30 μl of 0.1 M glutathione, 25 μl of 0.12 M 2-4CNDB (1-chloro-2,4-dinitrobenzene SIGMA c6396) with 10 μl of sample were measured at 340 nm, 20 second-interval for 3 minutes and 25°C. Enzyme unit was expressed as 2-4-dinitro benzene-glutathione produced/minute/mg protein.

Catalase assay

Catalase assay was followed by Aebi (18). Cocktails including 50 μl of 1M Tris-HCl, 50 μl of 5 mM EDTA (pH 8.0), 1500 μl of H_2O_2, 1430 μl of H_2O with 20 μl of sample were measured at 240 nm, at 20 second-interval for 3 minutes. Enzyme unit was expressed as the amount of enzyme to react with H_2O_2/minute/mg protein.

Superoxide dismutase (SOD) assay

Cytosolic SOD was measured by the method of Cropo et al. (19). Mixture of 2100 μl of 50 mM potassium phosphate (pH 7.8), 300 μl of 0.5 mM xanthine, 100 μl of 1% deoxychloride, 100 μl of 2 mM potassium cyanide, 300 μl of 0.1 mM ferric cytochrome C and 20 μl of xanthine oxidase were added to 20 μl of cytosolic sample, measured at 550 nm for 3 minutes by spectrophotometer (Beckman DU series-70).

Assay of serum α-tocopherol

Serum 100 μl and α-tocopheryl acetate (50 μg/ml ethyl alcohol) 100 μl were vortex mixed for 30 seconds, 200 μl n-hexane was added and mixed for 10 minutes, centrifuged at 1500 rpm for 5 minutes, nitrogen-evaporated and dried at 40°C and then injected with 100 μl ethanol.

Waters HPLC mounted with microBondapak C_{18} utilized mobile phase of methanol and H_2O (97 : 3) and detected at 292 nm with the flow speed of 1 ml/minute.

For the quantitation of α-tocopherol, α-tocopherol(Sigma, USA) was used as a standard and α-tocopheryl acetate as an internal standard. Response factor was calculated from weight ratio and corresponding peak area ratio.

Statistical analysis

Data from individual experiment were expressed as the means±standard error of means (SEM). Statistical analysis was performed by SAS (statistical analysis system) program and Duncan's multiple range test at $p<0.05$.

RESULTS AND DISCUSSION

Extracts administration increased antioxidant activities of GST comparing to the control (Table 2). Combined administration of B(α)P and the extracts increased GST activity in B(α)P groups. Catalase activities were also increased by 50% in the extracts feeding compared to the control. Total SOD and Cu,Zn-SOD activities were also shown to be stimulated (Table 3). Although the total SOD activity decreased from 0.219 to 0.189 with the administration of B(α)P, Cu,Zn-SOD greatly increased from 0.100 unit to 0.184 unit. The catalase activity was also increased compared to the group of B(α)P.

GST activity in group CLE was 1.319 units, increased by 33% compared to the group CL of 0.983 unit (Table 4). Cu,Zn-SOD and catalase activities of the group fed high fat and ethanol

Table 2. Specific activities of GST and catalase in the liver of rats fed *Aralia elata Seemann* ethanol extract with B(α)P administration

Group[1]	GST (unit/mg protein)	Catalase (unit/mg protein)
C	1.313±0.076[2][b3]**	59.831±4.286
CE	1.705±0.073[a]	90.663±4.352
CB	1.401±0.031[b]	71.242±5.506
CBE	1.431±0.027[b]	81.019±4.265

[1]C: Control group
CE: *Aralia elata Seemann* ethanol extract fed group
CB: B(α)P treated group
CBE: B(α)P and *Aralia elata Seemann* ethanol extract administered group
[2]Mean±S.E.M.(standard error of mean)
[3]Values within the same column with different alphabets are significantly different among groups by Duncan's multiple range test.
**$p<0.01$

extracts combined were increased by 25% and 39%, respectively compared to the group of high fat (Table 5). In addition, total SOD decreased but, Cu,Zn-SOD activity increased from 0.090 unit to 0.191 unit. Catalase activity was 76.054 unit in the group of CLBE, a 16.5% increase, compared to 65.259 unit of the group CLB. As shown in Table 3 and 5, B(α)P lowered the activity of total SOD even in the presence of *Aralia* extract, which was not case with Cu,Zn-SOD.

Serum α-tocopherol of rat was markedly increased by the extracts. Serum α-tocopherol contents of group CE was 0.938 μg/ml, an increase of 72%, compared to the control of 0.544 μg/ml (Table 6). In B(α)P administered groups, α-tocopherol contents of group CBE was 0.890 μg/ml, an increase of 68%, compared to the group CB of 0.531 μg/ml.

Table 7 shows the contents of serum α-tocopherol in rats fed high fat and the ethanol extracts. The contents of α-tocopherol in group CLE was 0.948 μg/ml, an increase of 74% and 47%, respectively, compared to the control of 0.544 μg/ml and group CL of 0.644 μg/ml.

Administration of B(α)P reduced α-tocopherol levels in the serum as shown both in Table 6 and 7. On the otherhand, lard in the diet, increased α-tocopherol levels in the serum. Although α-tocopherol of group CLB was reduced by 16% compared to that of group CL, it increased by 35% by extract feeding in the group of CLBE compared to the group CLB of 0.547 μg/ml. The results from this study show that α-tocopherol, a potent antioxidant, may play a role in inhibiting the effects of B(α)P in possible carcinogenic action or damage in the liver.

Many evidences above suggest that *Aralia elata* possess medicinal functions including anti-inflammatory and anti-ulcer and antioxidant activities. Stimulation of antioxidant enzymes such as GST, SOD and catalase in the liver of rats in addition to the increase of α-tocopherol in the serum observed in this study may lead to the protection of cell components against peroxide or carcinogenic damages.

Similar results that the stimulation of antioxidant activities

Table 3. Specific activities of SOD in the liver of rats fed *Aralia elata Seemann* ethanol extract with B(α)P administration

Group[1)]	Total SOD (unit/mg protein)	Cu,Zn-SOD (unit/mg protein)
C	0.219±0.011[2)]	0.125±0.008[bc3)**]
CE	0.234±0.008	0.148±0.021[ab]
CB	0.209±0.013	0.100±0.004[c]
CBE	0.189±0.014	0.184±0.001[a]

[1)]Refer to Table 2.
[2)]Mean±S.E.M.(standard error of mean)
[3)]Values within the same column with different alphabets are significantly different among groups by Duncan's multiple range test.
**p<0.01

Table 4. Specific activities of glutathione sulfur transeferase and catalase in the liver of rats fed high fat and *Aralia elata Seemann* ethanol extract

Group[1)]	GST (unit/mg protein)	Catalase (unit/mg protein)
C	1.313±0.076[2)a3)**]	96.449±4.416
CL	0.983±0.031[c]	148.005±31.450
CLE	1.319±0.056[a]	87.075±4.352
CLB	1.118±0.023b[c]	65.259±6.729
CLBE	1.208±0.056[ab]	76.054±5.363

[1)]C: Control group
CL: High fat group
CLE: High fat and *Aralia elata Seemann* ethanol extract fed group
CLB: High fat and B(α)P administered group
CLBE: High fat, B(α)P and *Aralia elata Seemann* ethanol extract administered group
[2)]Mean±S.E.M.(standard error of mean)
[3)]Values within the same column with different alphabets are significantly different among groups by Duncan's multiple range test.
**p<0.01

Table 5. Specific activities of superoxide dismutase in the liver of rats fed high fat and *Aralia elata Seemann* ethanol extract

Group[1)]	Total SOD (unit/mg protein)	Cu,Zn-SOD (unit/mg protein)
C	0.219±0.011[2)]	0.125±0.008[b3)**]
CL	0.213±0.019	0.119±0.004[bc]
CLE	0.221±0.006	0.145±0.006[b]
CLB	0.213±0.014	0.090±0.006[c]
CLBE	0.208±0.022	0.191±0.025[a]

[1)]Refer to Table 4.
[2)]Mean±S.E.M.(standard error of mean)
[3)]Values within the same column with different alphabets are significantly different among groups by Duncan's multiple range test.
**p<0.01

Table 6. Contents of serum α-tocopherol in rats fed ethanol extract of *Araria elata Seemann* with B(α)P administration

(unit: μg/ml)

Group[1)]	α-Tocopherol
C	0.544±0.077[2)b3)*]
CE	0.938±0.098[b]
CB	0.531±0.080[a]
CBE	0.890±0.139[ab]

[1)]Refer to Table 2.
[2)]Mean±S.E.M.(standard error of mean)
[3)]Values within the same column with different alphabets are significantly different among groups by Duncan's multiple range test.
*p<0.05

Table 7. Contents of serum α-tocopherol in rats fed high fat and ethanol extract of *Aralia elata Seemann* (unit: μg/ml)

Group[1)]	α-Tocopherol
C	0.544±0.077[2)b3)*]
CL	0.644±0.047[ab]
CLE	0.948±0.146[a]
CLB	0.547±0.028[b]
CLBE	0.748±0.130[ab]

[1)]Refer to Table 4.
[2)]Mean±S.E.M.(standard error of mean)
[3)]Values within the same column with different alphabets are significantly different among groups by Duncan's multiple range test.
*p<0.05

by *Aralia* extract have been observed. More than 50% of inhibition on cytokine-induced neutrophil chemoattractant induction was shown to be exhibited by the dichloromethane fraction of *Aralia continentalis* with 0.05 mg/ml as a final concentration (20).

The levels of plasma lactic acid, glucagon, insulin and liver glycogen in exercised rats were not changed by oral administration of *Araliaceae* saponin extracts. On the other hand, plasma glucose levels in resting rats were decreased by saponin extracts of *Aralia* (21).

According to Hernandez et al. (22), the extract of *Aralia elata* root revealed antiulcer and antisecretory characteristics. The structures of saponins, known to be a potent inhibitor of ethanol absorption, were determined by Yoshikawa et al. (23,24) extracted from the bark and root of *Aralia elata*. The existence of antioxidant activity in *Aralia* extract by way of measuring superoxide generation in human organism was also reported (25).

Antioxidants are known to alleviate oxidative stress by scavenging free radicals, modulation of the action of procarcinogens or by the induction of the antioxidant defense system enzymes. Neoplasia in lung and fore-stomach of mice induced by B(α)P were shown to be diminished by dietary antioxidants which may interrupt the process and interaction of carcinogens with DNA and macromolecules of the cell (11,13).

Detoxification through conjugation with glutathione to form thioethers occurs by a step via the GST catalyzed or spontaneous detoxification process (26-32). Hence, GST seems directly involved in the protection of DNA from oxidative damages such as free-radicals. GST also plays an important role in the termination of the lipid peroxidation chain reaction (33-36).

The results that the stimulation of antioxidant enzyme activities and the increase of α-tocopherol levels shown in this study may provide an evidence of the importance of plant dietary sources in the protection of cell components against chemically induced carcinogenic damages. Therefore, a long-term dietary manipulation containing antioxidant food components could reduce risk of liver cancer and cancers of other kinds as well.

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