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RESEARCH NOTE

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Effect of *Elsholtzia splendens* Extracts on the Blood Lipid Profile and Hepatotoxicity of the Mice

Eun Jeong Choi and Gun-Hee Kim*

Plant Resources Research Institute, Duksung Women's University, Seoul 132-714, Korea

Abstract Effects of extracts obtained from the flowers of *Elsholtzia splendens* on the serum lipid profile and hepatotoxicity in mice were investigated. Female ICR mice were given *E. splendens* ethanolic extract (ESEs) orally at a dose of 10 or 50 mg/kg BW for 50 days. Significant dose-dependent decreases in triglyceride and low-density lipoprotein (LDL)-cholesterol of serum were observed. In addition, ESEs prolonged the lag-time of LDL oxidation *in vitro*. In the serum of ICE mice given ESEs orally at 10 and 50 mg/kg BW, the serum levels of aspartate aminotransferase (AST) and lactic dehydrogenase (LDH) increased significantly, while total protein, albumin, creatinine, alanine aminotransferase (ALT), and total bilirubin did not change. Therefore, ESEs may be beneficial to human health, although it has some hepatotoxicity.

Keywords: *Elsholtzia splendens, in vivo*, hepatotoxicity, lipid profile, low-density lipoprotein (LDL)-oxidation

Introduction

Recently, a great deal of attention has focused on the biological properties of traditional herbal preparations and their beneficial effects on health (1-4). Traditional herbs, which have chemical ingredients with pharmacological and toxicological effects, are interesting alternatives to the use of supplements. Many researchers have investigated the practical benefits of traditional herbs and their modes of action.

Elsholtzia splendens is an ingredient in traditional medicines in northeast Asia and belongs to a subclass of the family Labiatae (5,6). Besides, *Elsholtzia* genus includes *E. ciliate, E. saxatilis, E. angustifolia,* and *E. splendens* Nakai etc. *E. splendens* and *E. ciliate* are used mainly in folk remedies for diarrhea, as expectorants and for their diuretic effects (7,8). Although it is well known in Chinese traditional medicine, *E. splendens* and most other such traditional herbs are used despite the fact that no studies have investigated their biological or physio-logical effects.

Several researchers have demonstrated that some species of *Elsholtzia* have physiological effects *in vitro* (6,7). *Elsholtzia blanda* can reduce infarct size during acute myocardial infarction by inhibiting myocardial apoptosis *in vivo* (9,10), and *E. splendens* Nakai extracts have been reported to have anti-inflammatory activity (11). Our research group has also identified useful biological activities of *E. splendens*, such as its antioxidant, anti-inflammatory, and antitumor actions *in vitro* (12,13). We found that *E. splendens* may be used as a food material and has the potential to relieve and prevent disease, such as cancers and the symptoms of rheumatoid arthritis.

Until recently, however, there have been no reports on the effect of *E. splendens* ethanolic extract (ESEs) *in vivo*. Therefore, we evaluated the effect of ESE on the cytotoxicity and serum lipid profile of female ICR mice *in vivo*.

*Corresponding author: Tel: +82-2-901-8496; Fax: +82-2-901-8661
E-mail: ghkim@duksung.ac.kr
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Materials and Methods

Preparation of *Elsholtzia splendens* extract The flowers of *E. splendens* were collected from a home garden during efflorescence in the fall (from September to October). Ethanol extracts from *E. splendens* were prepared as followed; briefly, flowers of *E. splendens* were freeze-dried and crushed. Then after, freeze-dried materials were extracted with 80% ethanol for 30 min at room temperature (5 g of dried materials per 500 mL solution). The yield (w/w) of the dehydrated powder among the primary net dry weight plant was about 1.6%.

Animal care and serum analysis Female ICR mice (23-25 g; Central Lab. Animal Inc., Seoul, Korea) were housed 5 to a polypropylene cage (24±2°C, 40-50% relative humidity) under controlled lighting (12-hr light/dark cycle). Mice were fed an AIN 93M diet (Dyets, Bethlehem, PA, USA) and allowed free access to water. After an adaptation period, mice were divided randomly into 3 treatment groups. Extract of *E. splendens* (ESEs) was suspended in water and administered orally to 2 of the 3 groups at 10 and 50 mg/kg BW for 50 day, respectively. Mice in the remaining (control) group were given the vehicle alone as orally administration. Animal care in this study conformed to *the Guide for the Care and Use of Laboratory Animals*, published by the U.S. National Institutes of Health (14). At the end of experiment, mice were rapidly anesthetized using ether at 6 hr after final administration of ESEs. After blood was taken from the heart by heart puncture, serum was obtained by centrifuging the blood at 600×g for 15 min. Triglycerides, total cholesterol, high-density lipoprotein (HDL)- and low-density lipoprotein (LDL)-cholesterol contents were determined by Advia 1650 chemistry Analyzer (Siemens Medical Solutions Diagnostics, Norwood, MA, USA) and using appropriate kits (Bayer AG, Barmen, Germany). In addition, total cholesterol/HDL cholesterol ratio, an index of the atherogenic profile, was also calculated.

Serum α-tocopherol content The content of α-tocopherol

was analyzed by high performance liquid chromatography (HPLC) according to the method of previously our study (15). To quantify α-tocopherol contents in serum, sample was deproteinized with ethanol containing 0.01% ascorbic acid. Extraction was carried out twice with 1 mL of *n*-hexane. The aqueous and organic phases were separated by centrifugation and the upper (organic) phase was dried under nitrogen gas. The dried residue was re-dissolved in 150 μL of methanol and injected into the HPLC system. HPLC analysis was performed using a system consisting of a Hewlett Packard 1084B liquid chromatography, and variable wavelength detector adjusted at 290 nm (Hewlett Packard, Houston, TX, USA). The mobile phase (methanol/water, 95 : 5, v/v) was processed at a flow rate of 1.0 mL/min. Sample was analyzed on a 4.6×250 mm RP C-18 Nova Pak column (5 μm) (Millipore, Schwalbach, Germany) and HPLC-grade α-tocopherol (Sigma-Aldrich, St. Louis, MO, USA) was used as standards.

LDL oxidation *in vitro* LDL oxidation was determined according to the method of Puhl *et al.* (16). Human LDL (Sigma-Aldrich) was diluted in phosphate-buffered saline (PBS) to 200 mg of protein/L and dialyzed overnight against PBS at 4°C to remove the EDTA. LDL (100 μg of protein/mL) was oxidized in PBS (pH 7.4) with 5 μM copper in the presence or absence of ESEs at 5 and 20 μg/mL. The oxidation of LDL was followed continuously by measuring the formation of conjugated dienes at absorbance 234 nm for 4 hr using spectrophotometer (DU 600; Beckman Coulter, Fullerton, CA, USA).

Statistical analyses All values are expressed as means± SD. Data were analyzed by unpaired Student's *t*-test or one-way analysis of variance followed by Duncan's multiple range comparison test (SigmaStat, Jandel, San Rafael, CA, USA). For all comparisons, differences were considered statistically significant at $p<0.05$.

Results and Discussion

The study was aimed to examine the effects of *E. splendens* extracts (ESEs) on the lipid profiles and hepatotoxicity *in vivo*. In the present study, no statistically significant differences were observed in food intake and body weight gain between the control group and those of group treated with ESEs (data not shown).

Generally, the estimation of lipid profile has provided an enormous scientific evidence base and also has shown the possibility as lipid-altering compounds focused on LDL-cholesterol that have been shown to reduce coronary heart disease (17). In the serum of ICR mice given ESEs orally at 10 or 50 mg/kg BW for 50 days, triglyceride and LDL-cholesterol levels decreased significantly in a dose-dependent manner ($p<0.05$, Table 1). LDL-cholesterol decreased by 27.7 and 57.0% at 10 and 50 mg/kg BW ESEs, respectively. Conversely, HDL-cholesterol increased by 18.5% with 50 mg/kg BW of ESEs, although the difference was not significant. Similar pattern was observed in atherogenic index (total cholesterol/HDL-cholesterol ratio), which is considered as important measures of atherosclerotic milieu (18).

In addition, ESEs improved the blood lipid profile of mice by decreasing LDL. It has been suggested that LDL-cholesterol mediates inflammation and the pathogenesis of diseases associated with oxidative stress, such as atherogenesis and atherosclerosis (19,20). In addition, the oxidation of LDL *in vitro* was determined by measuring the formation of the conjugated diene (Fig. 1). The time lag in conjugated diene production, which indicates the resistance of LDL to oxidation, was prolonged when LDL was incubated with ESEs. In the presence of 5 and 20 μg ESEs/mL, the time lag was extended to 55 and 60 min, respectively, whereas LDL as a control extended the time lag by 40 min. Therefore, ESEs delayed LDL oxidation in dose-dependent manner. Various antioxidant molecules, such as vitamins and phytochemicals, significantly attenuate the development of atherosclerosis (21-24). Atherosclerosis is a chronic inflammatory disease of the arterial wall, and suppressed LDL oxidation may be a powerful mechanism affecting this.

In our study, the α-tocopherol levels increased significantly in the serum of mice given ESEs orally at 50 mg/kg BW (Fig. 2). Compared to the control value, the α-tocopherol level increased by 9.3 and 14.3%, respectively, at 10 and

Table 1. Effects of *Elsholtzia splendens* ethanol extracts (ESEs) on lipid profile and biochemical parameters in serum of ICR mice[1)]

mg/dL or units/mL Serum	Control	ESEs 10 mg/kg BW	ESEs 50 mg/kg BW
Total cholesterol	105.0±10.9	121.7±25.6	124.4±23.2
Triglyceride	122.0±22.5^{a}	88.7±16.1^{ab}	28.0±4.0^{b}
HDL-Cholesterol	50.1±4.9^{a}	56.6±8.1^{a}	62.0±9.4^{b}
LDL-Cholesterol	8.5±0.7^{a}	6.2±0.3^{b}	3.7±0.7^{c}
Atherogenic index	2.10±0.07	2.13±0.17	1.98±0.09
Total protein	4.89±0.23	5.07±0.19	4.73±0.39
Albumin	3.07±0.21	3.22±0.14	2.97±0.37
Creatinine	0.44±0.06	0.45±0.04	0.45±0.03
Total bilirubin	0.10±0.03	0.11±0.03	0.09±0.02
Aspartate amino transferase (AST)	105.0±18.0^{a}	139.0±7.1^{ab}	152.0±5.7^{b}
Alanin amino transferase (ALT)	27.7±6.2	34.3±7.0	35.3±5.8
Lactate dehydrogenase (LDH)	462.9±96.1^{a}	901.3±35.9^{b}	1102.7±160.8^{b}

[1)]Atherogenic index is calculated as total cholesterol/HDL-cholesterol. Values are mean±SD ($n=6$). The alphabetic letters represent the statistical significance at $p<0.05$.

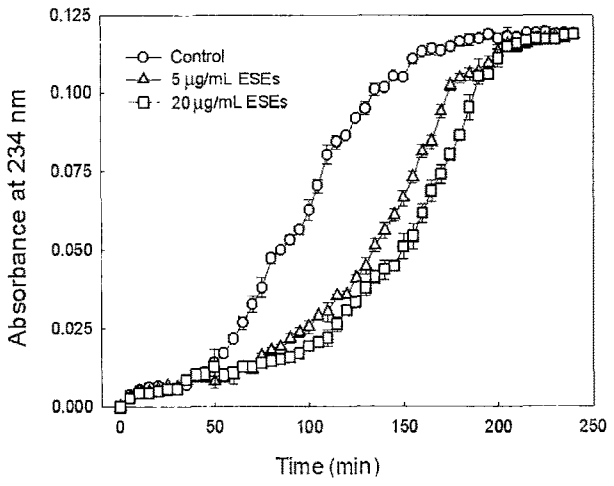

Fig. 1. Effects of *Elsholtzia splendens* ethanol extracts (ESEs) at 5 and 20 μg/mL on the generation of conjugated diene in LDL fraction. Values shown are a representative experiment.

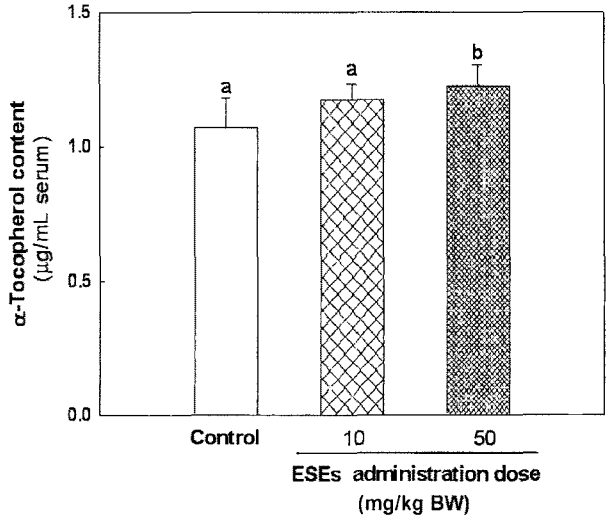

Fig. 2. Effects of *Elsholtzia splendens* ethanol extracts (ESEs) on α-tocopherol contents in serum of ICR mice. Values are mean±SD (n=6). The alphabetic letters represent the statistical significance at $p<0.05$.

50 mg/kg BW ESEs. These results suggest that ESEs contains various bioactive molecules including antioxidants that contribute to health. Until now, active ingredients in ESEs have not yet been identified.

We found significant increases in the serum aspartate aminotransferase (AST) and lactic dehydrogenase (LDH) levels of ICR mice given ESEs, while the alanine aminotransferase (ALT) level increased slightly, but not significantly (Table 1). The changes in the serum levels of AST, ALT, and LDH may have resulted from leakage of these enzymes from the liver into the circulation, and may indicate liver damage and altered liver function (25). Although ESEs increased the levels of some indicators of liver damage, the serum levels of total protein, albumin, creatinine, and total bilirubin did not change compared to control values.

The public in general and some health care providers regard natural herbal extracts as safe, although there are no experimental data for that confidence. In fact, most natural herbal extracts have the potential to cause serious adverse effects. Some are used despite being toxic, depending on whether they are classified as nutritional supplements or pharmaceutical drugs such as chemotherapy agents. Toxicity is as much of an issue for ESEs as it is for the other natural herb extracts. The present study found that ESEs might not be safe and free of some side effects, although this depends on the dosages and period. Moreover, there is much evidence for the beneficial physiological effects of ESEs.

Our results suggest that ESEs is beneficial to human health via a decrease in LDL-cholesterol and delayed oxidation, suggesting that ESEs also has increased anti-oxidant molecule such as α-tocopherol. Therefore, the use of ESEs is thought to depend on whether they are used as food supplements or drugs, and the consumption condition must also be considered. In the future, it will be of interest to identify the active ingredients in extracts of *E. splendens*. Experiments targeting the active ingredients in ESEs are required to confirm and extend previous observations.

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References

1. Chang R. Bioactive polysaccharides from traditional Chinese medicine herbs as anticancer adjuvants. J. Altern. Complem. Med. 8: 559-565 (2002)
2. Lee KH. Research and future trends in the pharmaceutical development of medicinal herbs from Chinese medicine. Public Health Nutr. 3: 515-522 (2000)
3. Arab L. Epidemiologic challenges in the study of the efficacy and safety of medicinal herbs. Public Health Nutr. 3: 453-457 (2000)
4. Oh JH, Kim EO, Lee SK, Woo MH, Choi SW. Antioxidant activities of the ethanol extract of *hamcho* (*Salicornia herbacea* L.) cake prepared by enzymatic treatment. Food. Sci. Biotechnol. 16: 90-98 (2007)
5. Heo J. *Dongeubogam* (Oriental Medicine). Namsandang, Seoul, Korea. p.1173 (2000)
6. Ahn DK. Illustrated Book of Korean Medicinal Herbs. Kyo-Hak Publishing Co., Seoul, Korea. p.35 (1998)
7. Lee SY, Chung MS, Kim MK, Baek HH, Lee MS. Volatile compounds of *Elsholtzia splendens*. Korean J. Food Sci. Technol. 37: 339-344 (2005)
8. Jeong JH, Lim HB. The chemical composition and biological activities of volatile flavor components of *Elsholtzia splendens*. Anal. Sci. Technol. 18: 500-510 (2005)
9. Ling H, Lou Y. Total flavones from *Elsholtzia blanda* reduce infarct size during acute myocardial ischemia by inhibiting myocardial apoptosis in rats. J. Ethnopharmacol. 101: 169-175 (2005)
10. Ling H, Lou Y, Wu H, Lou H. Total flavones from *Elsholtzia blanda* reduce infarct size and improve heart function during acute myocardial infarction by inhibiting myocardial apoptosis in canines. Acta Cardiol. 60: 295-301 (2005)
11. Kim DW, Son KH, Chang HW, Bae K, Kang SS, Kim HP. Anti-inflammatory activity of *Elsholtzia splendens*. Arch. Pharm. Res. 26: 232-236 (2003)
12. Choi EJ, Kang EJ, Lee YS, Kim GH. Pro-apoptotic activity of *Elsholtzia splendens* extract in human breast cancer MDA-MB-453 cells (abstract no. 59). In: Abstracts: 7th International Conference and Exhibition on Nutraceuticals and Functional Foods (Worldnutra

2006). Nov. 5-8, Reno, NV, USA. NRC Institute for Nutrasciences and Health (NRC-INH) (2006)
13. Choi EJ, Lee YS, Kim GH. Antioxidative characteristics of extracts from aromatic herb *Elsholtzia splendens*. Food. Sci. Biotechnol. 16: 489-492 (2007)
14. Guide for the Care and Use of Laboratory Animals. Institute of Laboratory Animal Resources, Commission on Life Sciences and National Research Council, National Academy Press, Washington DC, USA (1996)
15. Choi EJ. The prooxidant, rather than antioxidant, acts of daidzein *in vivo* and *in vitro*: Daidzein suppresses glutathione metabolism. Eur. J. Pharmacol. 542: 162-169 (2007)
16. Puhl H, Waeg G, Esterbauer H. Methods to determine oxidation of low-density lipoproteins. Method Enzymol. 233: 425-441 (1994)
17. Tiyyagura SR, Smith DA. Standard lipid profile. Clin. Lab. Med. 26: 707-732 (2006)
18. Wranicz JK, Cygankiewicz I, Rosiak M, Kula P, Kula K, Zareba W. The relationship between sex hormones and lipid profile in men with coronary artery disease. Int. J. Cardiol. 101: 105-110 (2005)
19. Bosutti A, Grassi G, Zanetti M, Aleksova A, Zecchin M, Sinagra G, Biolo G, Guarnieri G. Relation between the plasma levels of LDL-cholesterol and the expression of the early marker of inflammation long pentraxin PTX3 and the stress response gene p66(ShcA) in pacemaker-implanted patients. Clin. Exp. Med. 7: 16-23 (2007)
20. Ros E. Intestinal absorption of triglyceride and cholesterol. Dietary and pharmacological inhibition to reduce cardiovascular risk. Atherosclerosis 151: 357-379 (2000)
21. Korantzopoulos P, Kokkoris S. The antioxidant effects of statins may extend beyond atherosclerosis: Potential benefits for atrial fibrillation and heart failure. Atherosclerosis 175: 187 (2004)
22. Meagher E, Rader DJ. Antioxidant therapy and atherosclerosis: Animal and human studies. Trends Cardiovas. Med. 11: 162-165 (2001)
22. Lau BH. Suppression of LDL oxidation by garlic. J. Nutr. 131: 985S-988S (2001)
23. Park EJ, Park NS, Park HR, Jin BR, Lee SM. Fruiting body extracts of *Paecilomyces tenuipes* Ameliorate lipid and antioxidant metabolism in rats fed a high fat-cholesterol diet. Food. Sci. Biotechnol. 15: 710-714 (2006)
24. Yousef MI, Awad TI, Mohamed EH. Deltamethrin-induced oxidative damage and biochemical alterations in rat and its attenuation by Vitamin E. Toxicology 227: 240-247 (2006)