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Original Article

Cholesterol-lowering effect of *Aralia elata* (Miq.) *Seem* via the activation of SREBP-2 and the LDL receptor

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Abstract

Background: Hyperlipidemia causes arteriosclerosis, a risk factor for coronary heart disease. Prevention of hyperlipidemia by improving dietary habits has recently attracted attention. In this regard, we investigated whether *Aralia elata* (Miq.) *Seem* (AE) extract inhibits hepatic cholesterol accumulation and modulate the cellular signaling pathway.

Methods: To determine AE's cholesterol regulating mechanism, we measured cholesterol level, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity and cholesterol regulating-related gene expression in HepG2 cells and in high-fat diet (HFD)-induced mice using ELISA and RT-PCR assay.

Results: The AE extract reduced cholesterol levels and HMG-CoA reductase activity in hepatocellular carcinoma HepG2 cells. In addition, it also reduced the plasma cholesterol concentrations in HFD-induced mice. Furthermore, the AE extract increased the gene expression of the LDL-receptor (LDL-R); sterol-regulatory-element binding protein-2 (SREBP-2); ATP-binding cassette, sub-family A, member 1 (ABCA1); and scavenger receptor class B member 1 (SR-B1) in a dose-dependent manner. However, the AE extract did not affect the gene expression of acetyl-coenzyme A acetyltransferase (ACAT) in either the HepG2 cells or mice.

Conclusion: We demonstrated that the AE extract activated genes related to cholesterol metabolism, such as SREBP-2 and LDL-R, which resulted in hypocholesterolemic activities.

Keywords: *Aralia elata* (Miq.) *Seem*; Cholesterol; HMG-CoA; LDL-R; SREBP-2

1. Introduction

Cholesterol is an essential nutrient that is present in cell membranes and regulates hormone production, and lipid digestion and transport. However, a high blood-cholesterol concentration caused by an excess supply of cholesterol from the diet or low-density lipoprotein cholesterol (LDL) receptor inactivation is a major risk factor for cardiovascular disease. Thus, the maintenance of a normal plasma cholesterol concentration is an important way to prevent cardiovascular disease.[1,2]

The enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase is a rate-limiting enzyme involved in hepatic cholesterol biosynthesis. Regulation of HMG-CoA reductase occurs via two mechanisms, transcriptional and post-translational regulation.[3] First, sterol-regulatory-element binding protein 2 (SREBP-2) transcriptionally regulates HMG-CoA reductase by binding to and activating the promoter region of the HMG-CoA reductase gene.[4] Second, AMP-activated protein kinase (AMPK) regulates HMG-CoA reductase through a post-transcriptional mechanism via the phosphorylation of HMG-CoA reductase, which leads to a decrease in the enzyme's efficiency.[5]

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

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Numerous drugs that lower cholesterol levels have been used to treat hypercholesterolemia.[6] However, the undesirable side effects of these compounds have caused concerns about their therapeutic use.[7] Therefore, recently there has been great interest in using bioactive compounds derived from plants for their cholesterol-lowering effects, because of their low toxicity and reduced side effects.[8–10]

Aralia elata (Miq.) *Seem* (AE), is a member of the *Araliaceae* family and is widely distributed in east-Asia countries.[11] AE's barks and root cortexes have been used as traditional medicine against diabetes, gastric ulcers, hepatitis, rheumatoid arthritis, and other cytotoxic and inflammatory conditions.[12–14]

An ethanol extract of AE was found to be effective for improving hyperglycemia and insulin resistance.[15,16] In addition to this, it was reported that a saponin from AE significantly decreased total cholesterol levels.[17] However, the mechanism by which the AE extract lowers lipid levels has not yet been delineated. The objective of this study was to investigate the effect of the dietary supplement AE on cholesterol synthesis in hepatocytes and high-fat-induced mice, in addition to the mechanisms by which it acts.

2. Methods

2.1. Samples and reagents

AE extract, obtained by using 70% ethanol, was purchased from the Plant Extract Bank (Jeju, Korea). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin–streptomycin (PS) were obtained from Invitrogen (Carlsbad, CA, USA). A Cell Titer-Glo® assay kit was obtained from Promega (Madison, WI, USA). All other chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA), unless specified otherwise.

2.2. Cell culture

The human hepatocellular carcinoma cell line, HepG2, was obtained from the Korean Cell Line Bank (Seoul, Korea). HepG2 cells were cultured in DMEM supplemented with 10% FBS and 1% PS in an incubator with 5% CO_2 at 37 °C.

2.3. Cell viability

HepG2 cells seeded in 24-well plates were treated with AE. The AE extract was dissolved in dimethyl sulfoxide (DMSO) (Sigma Aldrich, St. Louis, MO, USA) and then diluted in phosphate-buffered saline (PBS) (Invitrogen, Carlsbad, CA, USA) to obtain final concentrations of 10, 50, and 100 μg/ml. Cells were treated with the extract for 24 h, and the cell viability was measured using the Cell Titer-Glo® assay. The viability was expressed as the percentage of live cells in each well.

2.4. Animals

Six-week-old male C57BL/6 mice (23 ± 0.5 g; OrientBio, Seong-Nam, Korea) were kept in a humidity-controlled room under a 12-h light–dark cycle, with food and water available ad libitum for 1 week. The mice were then divided randomly into four groups of five animals each. One group of C57BL/6 mice was fed standard rodent chow (Harlan Teklad Mouse/Rat Diet 7002, Envigo, Cambridgeshire, UK). The other groups were fed a high-fat diet (HFD) that contained 60% fat, 14% protein, and 26% carbohydrate. Another two groups of mice were administrated either 100 or 300 mg/kg of AE extract by oral gavages for 4 weeks. The final group was administered an equal volume of PBS. Dietary intake and body weight were measured weekly. At the end of 4 weeks, all mice were fasted overnight and anesthetized with CO_2. Blood samples were collected from abdominal aortas with heparinized syringes and centrifuged at 3000 g for 15 min. Organs were extracted, rinsed with saline solution and then weighed. Serum and tissue samples were stored at −70 °C until further analysis. The study was approved by the Institutional Animal Care and Use Committee of the National Academy of Agricultural Science (NAAS-201411), and all procedures were conducted in accordance with the Animal Experiments Guidelines of the National Academy of Agricultural Science.

2.5. Determination of HMG-CoA reductase activity

HMG-CoA reductase activity was determined using a HMG-CoA reductase activity assay kit (Abcam, Cambridge, UK). The enzymatic reaction was performed in the presence or absence of the AE extract for 15 min at 37 °C in a 96-well plate. The inhibition potential of HMG-CoA reductase was expressed by detecting the optical density (OD) change of NADPH at 340 nm using a microplate reader (Molecular Devices, Sunnyvale, CA, USA). Pravastatin was used as a positive control.

2.6. Cholesterol quantification

HepG2 cells and liver tissues were treated with chloroform/isopropanol (2:1) for 30 min at room temperature, and then the lipid-extracted solvent was transferred to test tubes. The organic solvent was removed using a vacuum centrifuge, and the lipids were re-suspended in 95% ethanol. The intracellular cholesterol level was quantified with a cholesterol/cholesteryl ester kit (Abcam, Cambridge, UK) according to the manufacturer's instructions. After lipid extraction, cells were lysed with RIPA buffer and centrifuged at 13,000× *g* for 10 min to collect the supernatant for the measurement of cellular protein concentrations. Lipid levels were normalized to the total cellular protein concentration as determined using the bicinchoninic acid (BCA) protein assay (GenDEPOT, Katy, TX, USA).

2.7. Real-time reverse transcription–polymerase chain reaction (RT–PCR) analyses

The total RNA was extracted from HepG2 cells or liver tissue using an RNeasy Plus Mini kit (Qiagen, Stanford, VA, USA) according to the manufacturer's instructions, and cDNA

was synthesized from the total RNA. Quantitative PCR was performed with a Rotor-Gene Q Real-time Thermal Cycler (Qiagen, Stanford, VA, USA). The PCR was carried out using 2X SYBR Green PCR kit (Qiagen, Stanford, VA, USA). All results were normalized to the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

2.8. Statistical analysis

Statistical analyses were performed with SPSS version 12.0 (SPSS Inc., Chicago, IL, USA). Data are represented as the mean ± standard error of the mean (SEM) from three independent experiments, unless stated otherwise. Statistical analyses were carried out using Student's *t*-test, and $p < 0.05$ was considered significant.

3. Results

3.1. Effect of AE extract on cell viability

The cytotoxicity of the AE extract at various concentrations on HepG2 cells was determined via measuring the intracellular level of ATP. The AE extract had no cytotoxic effects on HepG2 cells at all concentrations tested. Therefore, concentrations of 10–100 μg/ml of AE extract were selected for all subsequent experiments (data not shown).

3.2. Effect of AE extract on cholesterol quantification

The total cholesterol levels in the cells incubated with 10, 50, and 100 μg/ml AE extract were significantly lower, at 2%, 13%, and 35%, respectively. The AE extract significantly inhibited the synthesis of free cholesterol in a dose-dependent manner. In addition, the AE extract also significantly inhibited the synthesis of the cholesteryl ester to 3–54% of the control (data not shown).

3.3. Effect of AE extract on HMG-CoA reductase activity

We examined whether the AE extract inhibited the activity of HMG-CoA reductase. As shown in Fig. 1a, the AE extract inhibited HMG-CoA reductase in a concentration-dependent manner; however, this was not the case for the HMG-CoA reductase mRNA expression (Fig. 1b). Provastatin, a well-established cholesterol-lowering drug, was used as a positive control and reduced the enzyme activity to 61% of the control.

3.4. Effect of AE extract on gene expression related to cholesterol metabolism

Relative mRNA expression levels of cholesterol-metabolism-related genes were determined using quantitative PCR. As shown in Fig. 1c, mRNA expression levels of cholesterol-metabolism-related genes such as SREBP-2; LDL-R; ATP-binding cassette, sub-family A, member 1 (ABCA1), and scavenger receptor class B member 1(SR-B1) significantly increased in the AE-treated HepG2 cells. However, the gene expression levels of acetyl-coenzyme A acetyltransferases (ACAT)-1 and 2 did not change. Together, these data strongly suggest an improvement in cholesterol metabolism with AE treatment.

3.5. Effect of AE extract on hepatic cholesterol concentrations and gene expression related to cholesterol metabolism in HFD-fed mice

AE-treated mice (300 mg/kg) were showed significant differences in liver weight and total cholesterol concentrations when compared to the controls (Fig. 2a). Furthermore, the free cholesterol concentration was also lower in groups that were administered the AE extract; however, levels of the cholesteryl ester were not significantly altered.

The HFD and AE groups experienced an increase in serum levels of total cholesterol (TC) and LDL cholesterol and decrease in HDL cholesterol as compared with the NC group (Supplementary Table 1). However, administration of AE for 4 weeks significantly decreased TG, TC and LDL cholesterol levels. HDL cholesterol was also significantly increased in AE-treated mice (300 mg/kg) groups as compared with the HFD group. The levels of serum ALT and AST as hepatic toxicity marker were significantly higher in the HFD groups, but were not reduced by AE administration.

In addition, SREBP-2 and its target gene expression in the liver were evaluated to investigate the impact of the AE extract on cholesterol metabolism. As shown in Fig. 2b, the expressions of SREBP-2, LDL-R, ABCA1, and SR-B1 genes were increased significantly, by 34%, 16%, 28%, and 27%, respectively, in the AE-treated group (300 mg/kg). However, HMG-CoA reductase mRNA expression was not significantly changed in the AE-treated group, similar to the pattern of HepG2 cells (Supplementary Fig. 1).

4. Discussion

The pharmacological activities of natural compounds have been scientifically proven, and their applications in the prevention of chronic diseases, including cardiovascular disease, have been suggested.[18,19] In the present study, *Aralia elata* extract showed hypocholesterolemic effects and ameliorated cardiovascular disease.

Cholesterol biosynthesis and adjustments of its actions occur in the liver. When the cholesterol concentration is lowered, LDL-R expression increases, and as a result, the cholesterol is taken into the cells via LDL-cholesterol and LDL-R binding. Conversely, when the cholesterol concentration increases, LDL-R expression is suppressed and less cholesterol is removed from the blood.[20] The expression of LDL-R is regulated by the SREBP owing to the presence of SRE-1 on the LDL-R gene. Therefore, when the sterol concentration in the cell is low, the SREBP is cleaved and enters the nucleus, where it is then coupled to the SRE-1 on the promoter of SREBP-target genes, one of which is LDL-R. This coupling activates gene transcription of the enzymes involved in the synthesis of cholesterol.[21,22] LDL-R, SREBP-2, and HMG-CoA reductase genes are all responsible for

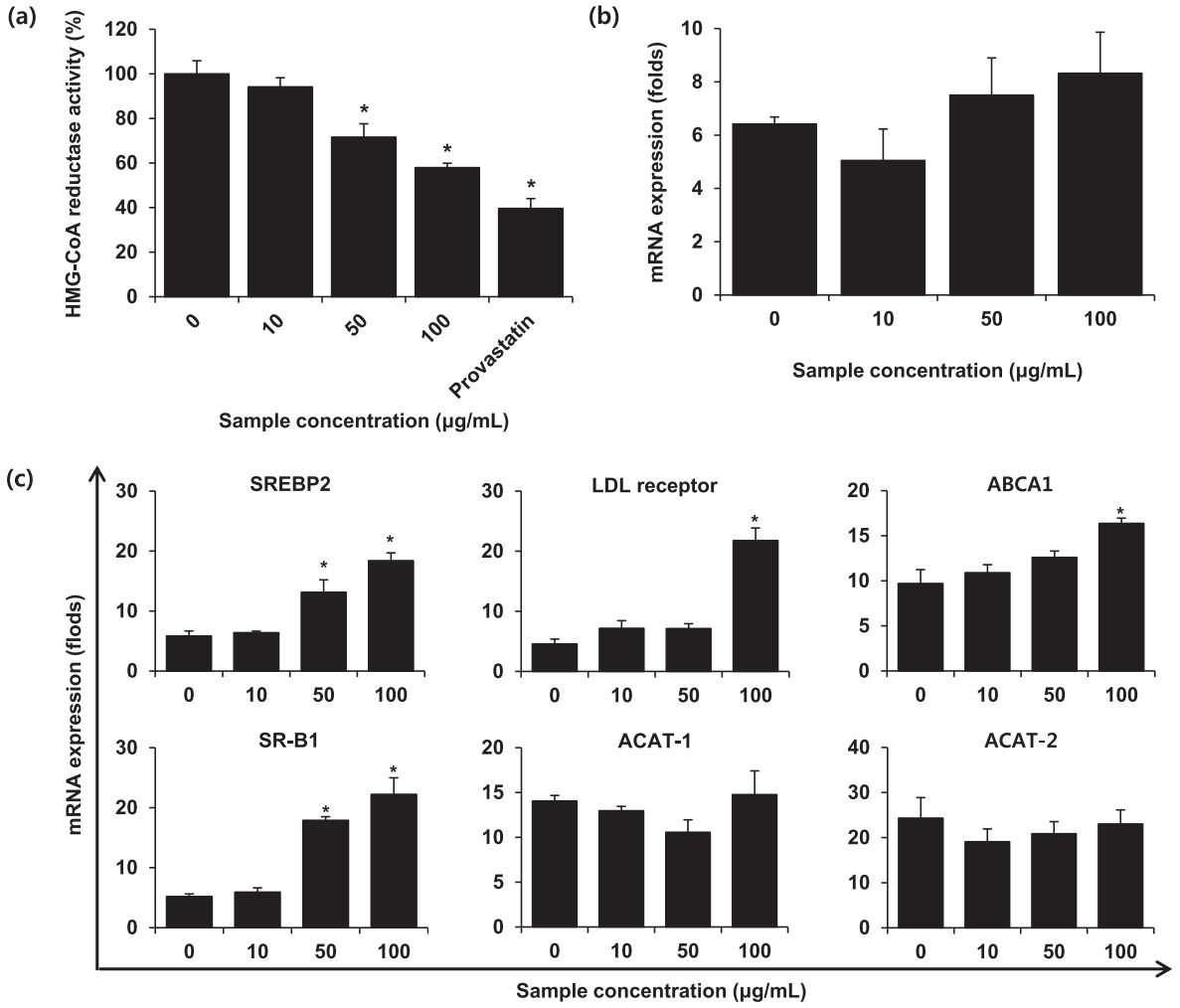

Fig. 1. Effect of *Aralia elata* (Miq.) *Seem* (AE) extract on (a) 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity, (b) HMG-CoA reductase mRNA expression, and (c) cholesterol-metabolism-related gene expression. HepG2 cells were treated with AE extract (10, 50, and 100 μg/ml) for 24 h. The HMG-CoA reductase activity was measured using an ELISA kit. The gene expression levels were quantified using quantitative PCR. Bars represent the mean ± standard error of the mean of three experiments carried out in triplicate. *$p < 0.05$ vs. control.

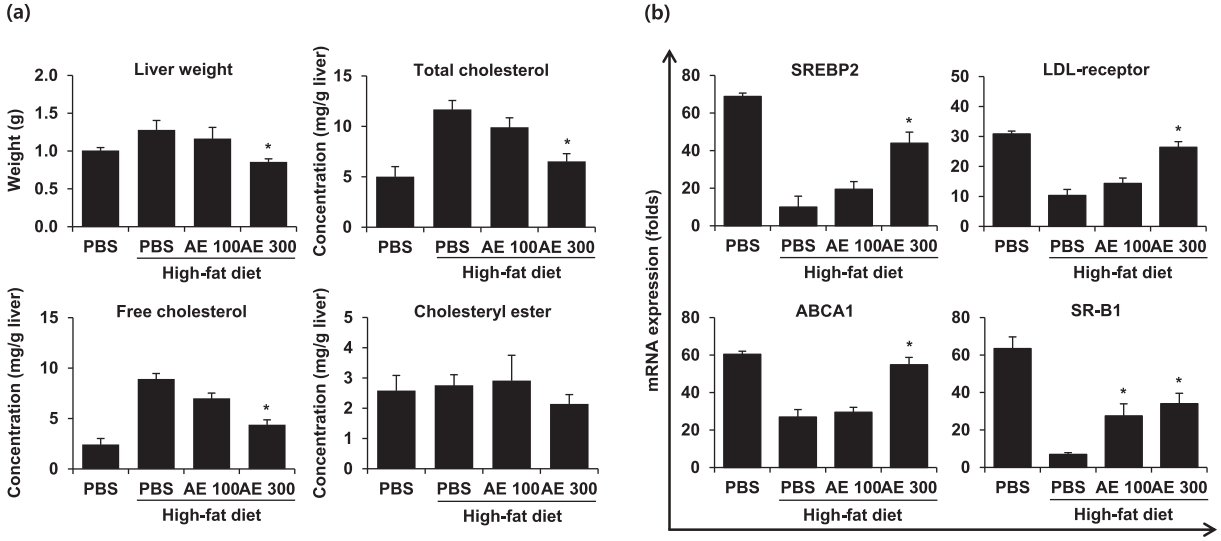

Fig. 2. Effect of *Aralia elata* (Miq.) *Seem* (AE) extract on (a) cholesterol quantity and (b) the gene expression of cholesterol metabolism in C57BL/6J mice livers. The liver weight and cholesterol, cholesterol ester, and free cholesterol in the liver were quantified after 4 weeks of AE extract administration. The results were normalized to the liver weight. The gene expressions in the livers were quantified using quantitative PCR. Bars represent the mean ± standard error of the mean (n = 5). *$p < 0.05$ vs. high-fat diet control.

cholesterol biosynthesis; therefore, it is important to regulate their expression.[23–25] HMG-CoA reductase is involved in the biosynthesis of mevalonate from HMG-CoA, and acts as a key enzyme in the cholesterol biosynthetic pathway. Our results show that AE extracts markedly suppressed hepatic HMG-CoA reductase activity, and did not significantly decrease the HMG-CoA reductase mRNA in HepG2 cells and mice liver (Fig. 1a and b and Supplementary Fig. 1). Similar results have been reported previously by Lopez et al.[30] This is due primarily to a marked decrease in the rate of translation of HMG-CoA reductase mRNA caused by feeding cholesterol.[31] Our results suggest that the enzyme is modulated post-transcriptionally by cholesterol, although it is unclear whether this control involves the formation or degradation of the enzyme protein as has been suggested by previous reports.

LDL-R can mediate the lowering of plasma cholesterol by enhancing the uptake of LDL cholesterol from the circulation into the liver. It has been reported that when the cholesterol content of the cell decreases, SREBPs, which reside in an inactive form in the cytoplasm, are cleaved by proteases and activated as transcription factors. The active SREBPs then migrate to the nucleus of the cell and bind to the genes that are directly or indirectly involved in cholesterol biosynthesis, such as HMG-CoA reductase and LDL-R, thereby modulating the expression or transcription of these genes.[26,27] As shown in Figs. 1c and 2b, the mRNA levels of both SREBP-2 and LDL-R were significantly increased in the AE-extract group in comparison with the control group. These findings support the results shown in Fig. 2a, indicating that the reduction in total cholesterol could be explained by the attenuation of HMG-CoA reductase activity, which stimulates the conversion of HMG-CoA into mevalonic acid. The decrease in the levels of LDL-cholesterol in the plasma could be supported by an increase in the levels of LDL-R mRNA, which is associated with the uptake of LDL-cholesterol into the liver. It is well known that uptake of LDL-cholesterol via LDLR provides feedback inhibition of HMGCR by inhibiting SREBP-2 activation.[32] That could explain how decreased hepatic uptake of LDL-cholesterol due to decreased LDLR in HFD-fed mice causes inhibition of SREBP-2 maturation and consequent transcriptional activation of HMGCR. In this study, we also observed similar results in HFD-fed mice. However, in the AE-treated group, we confirmed increase of SREBP-2 expression and decrease of HMGCR activity by increase of LDLR (Fig. 2b.). Consistently, the administration of AE decreased serum TC and LDL cholesterol in HFD-fed mice, suggesting that hypocholesterolemic effects of AE could be due to the inhibition of cholesterol biosynthesis by reduction of HMGCR activity and increase of SREBP-2 expression.

Next, we confirmed the effect of the AE extract on the expression of the ABCA1 and SR-B1 genes that promote reverse cholesterol transport (RCT),[28] which is a complex process that ensures the efflux of cholesterol from peripheral cells, as well as its transport back to the liver for its metabolism and biliary excretion. ABCA1 is involved in the control of high-density lipoprotein (HDL)- and apolipoprotein AI (apoAI)-mediated cholesterol efflux from macrophages. It also plays a major role in translocating cholesterol from intracellular cholesterol pools into the extracellular space and liver.[4] SR-B1 can induce cholesterol efflux by enabling HDL to bind to both the liver and cholesterol-rich domains in the plasma membrane.[29] As shown in Figs. 1b and 2b, in this study, the expression of ABCA-1 and SR-B1 mRNA significantly increased in the AE-treated group when compared with those in the control group. Therefore, the AE extract could affect the regulation of ABCA-1 and SR-B1 mRNA, resulting in the transport of accumulated cholesterol from the plasma into the liver for excretion.

In conclusion, we have demonstrated that the AE extract regulates the SREBP-2, LDL-R, ABCA1, and SR-B1 genes via multiple biological mechanisms, which result in a reduction in hepatic cholesterol levels. In light of these results, AE-extract consumption may bestow metabolic benefits on the regulation of cholesterol levels. However, in order to determine and confirm the exact effects of the AE extract on cholesterol metabolism, further human studies are necessary.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jcma.2017.06.007

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