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Original Article

Ethyl acetate extract of sappanwood alleviates experimental atherosclerosis in rats through changes in FGF21 and SREBP-2 expression

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Abstract: Sappanwood extract shows promising effects against atherosclerosis. The fibroblast growth factor 21 (FGF21) and sterol regulatory element-binding protein 2 (SREBP2) are involved in atherosclerosis development. This study aimed to examine whether sappanwood ethyl acetate extract (SEAE) alleviates experimental atherosclerosis in rats through FGF21/SREBP-2 signaling. Rats were randomized to six groups (n=10/group): blank control, model, simvastatin (positive control, 4.2 mg/kg/d), and SEAE high-, medium-, and low-dose (2.30, 1.15, and 0.575 g/kg/d, respectively). The high-fat- and vitamin D3-induced rodent model of atherosclerosis was created (except in the blank control group). Aorta and liver underwent histopathologic examination. SREPB-2 and FGF21 expression levels were examined by real-time RT-PCR and western blot. Compared with the blank control group, the model group showed aortic and hepatic histopathology compatible with the development of atherosclerosis due to a high-fat diet. In addition, total cholesterol, triglycerides, and low-density lipoprotein cholesterol (LDL-C) were elevated (all $P<0.05$). SREBP2 expression was high, and FGF21 expression was low (both $P<0.05$). Compared with the model group, SEAE alleviated the changes in liver and aorta by histopathology and decreased total cholesterol, triglycerides, and LDL-C (all $P<0.05$), especially in the medium-, and high-dose groups. In addition, medium-dose SEAE increased FGF21 levels (mRNA: +296%; protein: +69%; $P<0.05$) and decreased SREBP2 levels (mRNA: -44%; protein: -77%; $P<0.05$). Simvastatin, as the positive control, had similar effects to those of SEAE. In conclusion, SEAE improves lipid metabolism and alleviates atherosclerosis through changes in FGF21 and SREBP-2 expression levels.

Keywords: Sappanwood, extract, atherosclerosis, fibroblast growth factor 21, sterol regulatory element-binding protein 2, lipoproteins

Introduction

Atherosclerosis is the major cause of coronary artery disease (CAD) and cardiovascular diseases (CVDs) in general. It is a complex process involving the accumulation of lipids in the intima of major arteries, ultimately leading to stenosis and thrombus formation if the fibrous cap covering the necrotic core is ruptured. Inflammation triggers the recruitment of macrophages that scavenge modified lipoproteins (like oxidized low-density lipoproteins (LDL) or acetylated LDL), leading to the formation of foam cells within the intima. With lesion progression, the foam cells die and form the necrotic core rich in inflammatory and thrombotic factors. The fibrous cap is a dynamic element under the regulation of a number of factors involved in collagen secretion and proteolysis and is directly associated with the risk of thrombus [1-3].

Since blood cholesterol levels, especially LDL cholesterol (LDL-C) levels are directly associated with the development of atherosclerosis, the main treatment strategy is currently to decrease the blood cholesterol levels using drugs like statins [4, 5]. In addition, statins also have pleiotropic effects such as decreasing inflammation, macrophage recruitment, and the expression of proteolytic enzymes, which play additional roles in preventing the progression of atherosclerosis and the appearance of CVD [6-8]. Hence, any drug that modulates the vari-

ous factors involved in the complex process of atherosclerosis could be used to prevent it [4, 5].

Traditional medicines have been playing important roles in human health for centuries. Today, those medicines can be used as complementary approaches or in patients unwilling to use conventional medical therapies or with intolerance/allergies to them [9-11]. A number of plant-based compounds have been shown to be effective against atherosclerosis [12, 13]. Among them, sappanwood extract shows promising effects [14]. Protosappanin A, a compound extracted from sappanwood, has been shown to possess anti-hyperlipidemia and anti-inflammatory effects in rabbits through the NF-κB pathway [15]. In models of osteoarthritis, sappanwood extracts have been shown to decrease the expression of matrix metalloproteinases (MMP) by suppressing interleukin (IL)-1β [16]. Shin et al. [14] showed that sappanwood extract could be used to manage endothelial dysfunction, which is the first step of atherosclerosis development.

The fibroblast growth factor 21 (FGF21) is an important hormone secreted by the liver and plays an important role in energy and lipoprotein metabolism [17]. It increases lipid oxidation, ketogenesis, and resistance to growth hormones. It is also involved in the mechanisms of drugs activating peroxisome proliferator-activated receptors (PPARs) to decrease blood glucose levels and sensitize the tissues to insulin [18]. FGF21 expression is strongly associated with dyslipidemia and CVD [17]. A lack of FGF21 expression leads to accelerated atherosclerosis [17]. The beneficial effects of adiponectin on atherosclerosis are also mediated by FGF21 [18]. FGF21 mimetics have been suggested for the treatment of atherosclerosis [17]. Those effects are inhibited by the hepatic overexpression of the sterol regulatory element-binding protein 2 (SREBP2) [18]. In addition, SREBP2 is involved in the endothelial susceptibility to the development of atherosclerosis [19] and participates in hypercholesterolemia [20].

The effects of sappanwood extract on the expression of FGF21 and SREBP2, and on atherosclerosis are unknown. Therefore, the present study aimed to examine whether sappanwood ethyl acetate extract (SEAE) alleviates experimental atherosclerosis in rats through changes in the FGF21/SREBP-2 signaling. The results could provide new clues for improving lipid metabolism and preventing atherosclerosis.

Materials and methods

Ethyl acetate extract of sappanwood

Crude sappanwood produced in Yunnan was purchased from Heilongjiang Medicinal Material Corporation. Ethyl acetate extraction was performed by the Pharmacy College of Heilongjiang University of Chinese Medicine. In detail, sappanwood crude powder (40-mesh) was soaked in 75% ethanol for 4 h and subjected to reflux extraction at 85°C for 2 h. After filtration, the process was repeated twice in order to extract the active ingredients fully. The refluxes were mixed, concentrated by a rotary evaporator, dried in a water bath, and oven-dried to a constant weight. The dry powder was suspended in ddH_2O and extracted with ethyl acetate to obtain the SEAE.

Animals

Sixty specific pathogen-free Sprague-Dawley male rats (8 weeks of age, 200-220 g) were purchased from the Laboratory Animal Center of Heilongjiang University of Chinese Medicine (certificate No. SYXK2016004 (Heilongjiang)). The rats were fed chow food and water ad libitum and were caged in a controlled environment (12 h light/dark cycle; temperature, 20-25°C; humidity, 55±15%). The rats were adapted for 1 week, provided with normal feed of fishmeal 6%, bran 24.5%, corn 24.5%, bean cake 12%, barley 21%, and other ingredients 12%. All animal experiments were approved by the Animal Care and Use Committee of Heilongjiang University of Chinese Medicine.

High-fat- and vitamin D3-induced rodent model of atherosclerosis

The rats were randomized to six groups (n=10/group; n=10 for the blank control group and n=50 total for the model groups), according to a random number table. The high-fat- and vitamin D3-induced rodent model of atherosclerosis was created as previously described [21]. After 1 week of adaptive feeding, the blank control group (n=10) was intraperitoneally injected with saline for 3 consecutive days and given

Table 1. Primers for real-time PCR

Primer	5'→3'	Size (bp)	GenBank No.
SREBP-2	F: GCCCTGGAAGTGACTGAGAG	167	NM_001033694
	R: GAAGTAGTGCCGCTGACATTG		
FGF21	F: GTCTCCTGCTGCCTGTCTTC	128	NM_130752
	R: GGTGTCCTGGTCGTCATCTG		
GAPDH	F: AAGTTCAACGGCACAGTCAAG	189	NM_017008
	R: ATACTCAGCACCAGCATCACC		

Note: F: forward; R: reverse.

normal feed (corn 24.5%, bran 24.5%, barley 21%, bean cake 12%, fish meal 6%, and other ingredients 12%) for 12 weeks. The model group (n=50) was intraperitoneally injected with vitamin D3 (700,000 IU/kg/d) for 3 days, and was given high-fat feed (0.1% propylthiouracil, 1% cholesterol, 5% lard, 0.5% sodium cholate, and 93.4% normal feed) for 12 weeks. After 12 weeks, two model rats were randomly selected and sacrificed to confirm model success using abdominal aortas.

Intervention

After a 12-week period using this model, the blank control and model groups were orally administered with sodium carboxymethyl cellulose solution (0.5%, 10 ml/kg/d). The SEAE in the high-, medium-, and low-dose groups were given orally as crude doses of 2.30, 1.15, and 0.575 g/kg/d, respectively. The simvastatin group was orally administered simvastatin at 4.2 mg/kg/d, prepared by grinding simvastatin tablets and dissolving them into 0.5% sodium carboxymethyl cellulose solution, thereby making a simvastatin suspension of 0.42 mg/ml. Treatment for all groups lasted 28 consecutive days.

Blood and tissue harvesting

After drug administration, rats were food- and water-fasted for 12 h, and anesthetized by intraperitoneal injection of 20% urethane solution. Anesthetized rats were fixed on an operating table. The anesthetic dose was adjusted according to each rat's condition. After local disinfection with iodine tincture, an abdominal incision was made. Subcutaneous connective tissues were isolated, and the abdominal aorta was exposed. Blood was sampled from the aorta, kept at room temperature for 1 h, and centrifuged at 4°C and 3000 rpm for 15 min; the supernatant was aspirated into a PE tube and stored at -20°C. Triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and LDL-C levels were measured using an automaticbiochemical analyzer (AU5800, Beckman Coulter). After sacrifice, the abdominal aorta and liver were sampled, fixed in formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Real-time RT-PCR

The samples were placed in a mortar (precooled using liquid nitrogen) and ground. Total RNA extraction was performed using the TRIzol reagent (Invitrogen Inc., Carlsbad, CA, USA), according to the manufacturer's instructions. cDNA synthesis was performed using the Golden 1st cDNA Synthesis Kit (HaiGene, Cat. No. D0401A). Specific mRNA quantification was performed by real-time PCR using the 2× SYBR Green qPCR Mix (HaiGene, Cat. No. A2201) in a Bio-Rad Mini-Opticon 2 Real-Time PCR System (Bio-Rad, Hercules, CA, USA). The gene-specific primers are presented in **Table 1**. All reactions involved initial denaturation at 95°C for 15 min, followed by 40 cycles of 95°C for 10 s and 60°C for 30 s. $2^{-\Delta\Delta Ct}$ was calculated to represent the relative mRNA expression of the target gene. GAPDH was used as an internal control.

Western blot

The samples were placed in a mortar (precooled using liquid nitrogen) and ground. The samples were lysed on ice using the RIPA buffer (100 µl per 0.5×10^6 cells; HaiGene, Cat. No. C2501), 1 µl of benzonase nuclease (HaiGene, Cat. No. C2001), and 1 µl of protease inhibitor (1×) (Sigma, Cat. No. P8340). After 30 min, the lysate was centrifuged at room temperature and 13,000 rpm for 10 min, and the supernatant was taken for western blotting. Proteins were quantified using a BCA Protein Assay Kit (HaiGene, Cat. No. C3001). For western blot assay, equal amounts of proteins were separated by 10% SDS-PAGE and transferred to nitrocellulose (NC) membranes (Pall Life Sciences, Ann Arbor, MI, USA, Cat. No. 66485). The membranes were blocked by 5% skim milk powder for 1 h at room temperature. Proteins were detected by western blot using the following primary antibodies: SREBP-2 (1:1000; Proteintech Group Inc., Chicago, IL, USA, Cat. No. 14508-1-AP), FGF21 (1:200; Boster Bioen-

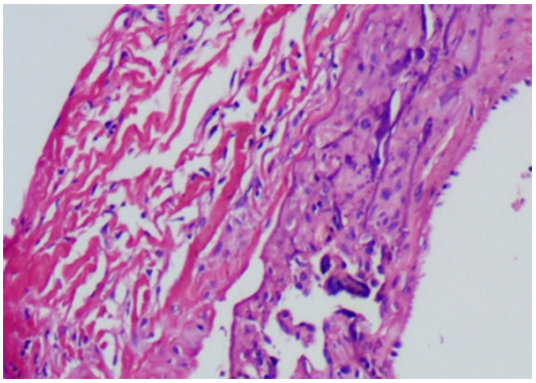

Figure 1. Successful atherosclerosis (AS) modeling. Hematoxylin and eosin (H&E) staining of the abdominal aorta (magnification: ×400).

gineering Co., Wuhan, China, PB1118), and β-actin (1:2000; GenScript, Piscataway, NJ, USA). Following overnight incubation with the primary antibody, each blot was washed four times with TBS with 0.05% Tween 20 (TBST) buffer. The blots were incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody (1:5000, GenScript, Piscataway, NJ, USA, A00098) and HRP-conjugated goat anti-mouse secondary antibody (1:5000; GenScript, Piscataway, NJ, USA, A00160). Proteins were detected using an enhanced chemiluminescence reagent (HaiGene, M2301). Band intensity was quantified using the Image-Pro software (Media Cybernetics, Inc., Rockville, MD, USA).

Statistical analysis

All statistical analyses were conducted using SPSS 22.0 (IBM, Armonk, NY, USA). Data were expressed as means ± standard deviations (SD) and analyzed using a one-way analysis of variance (ANOVA) with the SNK-q test for post hoc analysis. A *P*-value <0.05 was considered significant.

Results

General condition of the rats

After 12 weeks of modeling, two model rats were randomly selected and sacrificed. It could be seen that the wall of the abdominal aorta was irregularly thickened, with blurred boundaries between the different layers. The intima was also thickened, accompanied by significant endothelial denudation, nuclear enlargement, and areas of endothelial discontinuity. In addition, the medial smooth muscle cells were disorderly arranged. Breakage of elastic fibers, calcium deposition, and atherosclerotic plaques could also be seen. The results suggested the successful establishment of the AS model (**Figure 1**).

No rat died during the 12-week modeling period. After drug administration, three rats died, one from the high-dose group and two from the simvastatin group. Autopsy showed that the two rats in the simvastatin group died from drugs entering the trachea during administration, whereas the rat in the high-dose group died of unknown cause. The overall death rate was 5.0%.

After modeling, rats showed rapid weight gain. Compared with the blank control, the rats in the five other groups showed rapid weight growth, dark hair, and reduced activity. After drug administration, the rats in the medium-dose, high-dose, and simvastatin groups were improved, showing restored hair luster, increased activity, and more sensitive response to environmental stimulation compared with the model group.

Effects of SEAE on serum TG, TC, LDL-C, and HDL-C in the high-fat- and vitamin D3-induced rodent model of atherosclerosis

The model group showed higher TG, TC, and LDL-C compared with the blank control group (all $P<0.05$), without difference regarding HDL-C (**Table 2**). The medium- and high-dose SEAE groups and the simvastatin group showed lower TG, TC, and LDL-C compared with the model group (all $P<0.05$). There were no differences among the model group and all three SEAE groups regarding HDL-C (all $P>0.05$).

All statistical analyses were conducted using SPSS 22.0 (IBM, Armonk, NY, USA). Data were expressed as means ± standard deviation (SD) and analyzed using a one-way analysis of variance (ANOVA) with the SNK-q test for post hoc analysis.

Effects of SEAE on abdominal aorta histopathology in the high-fat- and vitamin D3-induced rodent model of atherosclerosis

The rats in the blank control showed normal morphology of the intima, tunica media, and adventitia of the abdominal aorta. The bound-

Table 2. Effects of SEAE on serum TG, TC, LDL-C, and HDL-C in high-fat- and vitamin D3-induced rodent model of atherosclerosis

Group	n	TG (mmol/L)	TC (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)
Blank control	10	0.48±0.21	1.82±0.32	0.69±0.32	0.87±0.30
AS Model	8	1.25±0.65*	3.77±1.05**	2.73±1.18*	0.91±0.40
Low-dose SEAE	9	0.74±0.34	3.26±0.98	1.55±0.68$^{\#}$	0.89±0.43
Medium-dose SEAE	9	0.65±0.32$^{\#,\Delta}$	2.42±0.54$^{\#,\Delta}$	1.56±0.28$^{\#,\Delta}$	1.01±0.28
High-dose SEAE	9	0.58±0.37$^{\#,\Delta}$	2.09±0.55$^{\#,\Delta}$	1.32±0.71$^{\#,\Delta}$	0.99±0.34
Simvastatin	8	0.65±0.31$^{\#}$	2.19±0.51$^{\#}$	1.18±0.79*	1.32±0.51

SEAE: sappanwood ethyl acetate extract; TG: triglycerides; TC: total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol. Data are shown as means ± standard deviations (SD). $^{*}P<0.05$ *vs.* blank control; $^{\#}P<0.05$ *vs.* AS model; $^{\Delta}P<0.05$ *vs.* Simvastatin; $^{**}P<0.01$ *vs.* blank.

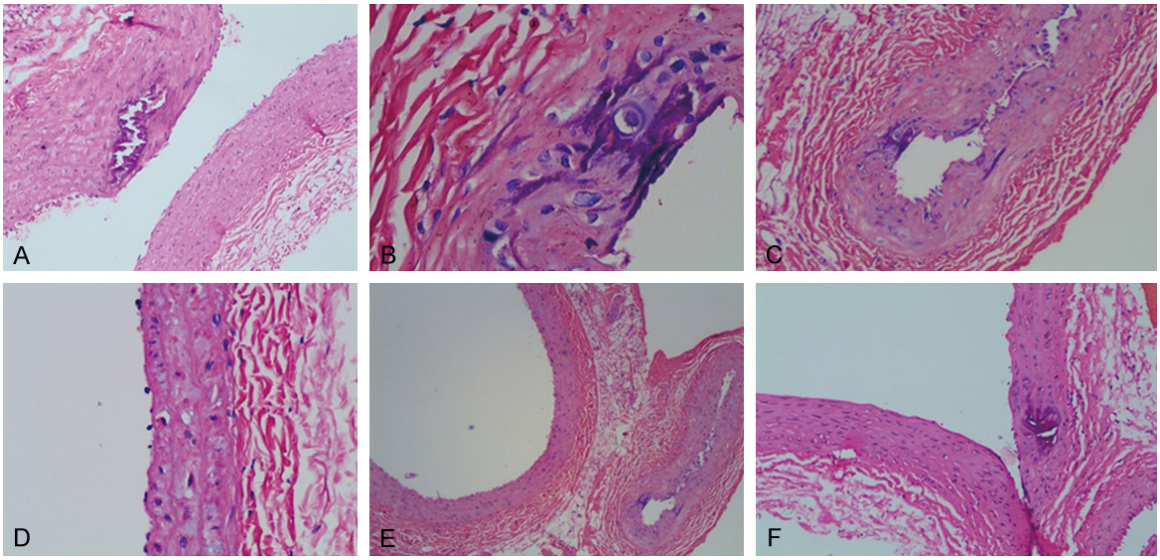

Figure 2. Effect of sappanwood ethyl acetate extract (SEAE) on abdominal aorta pathology in high-fat- and vitamin D3-induced atherosclerosis (AS) rat model. (A) Blank control: the rats were intraperitoneally injected with saline for 3 consecutive days and given normal feed for 12 weeks, and then orally administered sodium carboxymethyl cellulose solution (0.5%; 10 ml/kg/d) for 28 days. (B) AS model: the rats were intraperitoneally injected with vitamin D3 (700,000 IU/kg/d) for 3 days, and given high-fat feed for 12 weeks, and then orally administered sodium carboxymethyl cellulose solution (0.5%; 10 ml/kg/d) for 28 days. (C) Low-dose, (D) Medium-dose, (E) High-dose SEAE: the rats were intraperitoneally injected with vitamin D3 (700,000 IU/kg/d) for 3 days and given high-fat feed for 12 weeks, and then given oral SEAE with crude doses of 2.30 g/kg/d, 1.15 g/kg/d, and 0.575 g/kg/d, respectively, for 28 days. (F) Simvastatin: the rats were intraperitoneally injected with vitamin D3 (700,000 IU/kg/d) for 3 days and given high-fat feed for 12 weeks, and then orally administered simvastatin at 4.2 mg/kg/d for 28 days. Abdominal aortic pathology was determined by H&E staining (magnification: ×400).

aries were clear, the structure was intact, the epithelium was continuous, and no plaques or lipid depositions were observed. Spindle-shaped vascular smooth muscle cells were regularly arranged along the medial membrane. Loose connective tissue could be seen in the adventitia (**Figure 2**).

The rats in the model group showed thickened intima, accompanied by endothelial denudation, disintegration, and discontinuity. The medial smooth muscle cells showed swelling, migration, proliferation, and irregular arranging. The elastic fibers were broken, foam cells, and monocytes were aggregated, and atheromatous plaques were formed in combination with calcium salt deposition (**Figure 2**).

Rats in the SEAE low-dose group showed a disordered structure of the intima, tunica media, and adventitia of the abdominal aorta. Endothelial cells were irregularly arranged with unsmooth surface, swelling, and denudation. The medial smooth muscle cells showed disorder

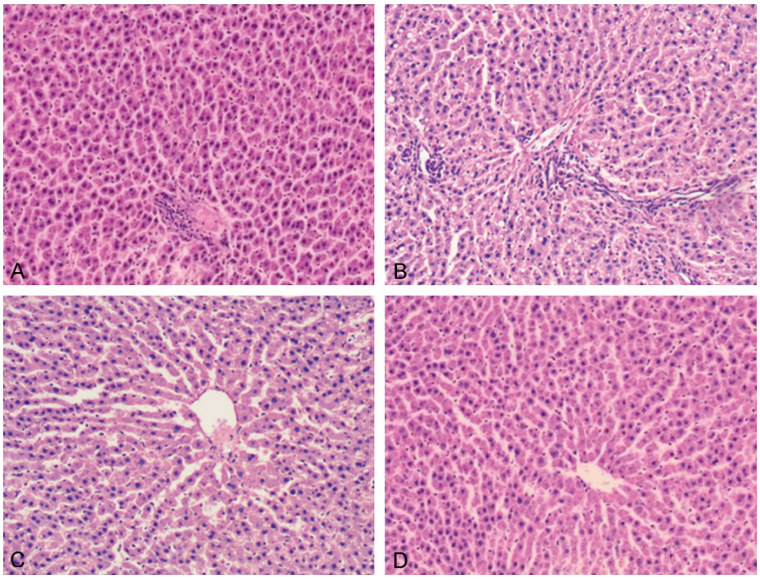

Figure 3. Effect of sappanwood ethyl acetate extract (SEAE) on liver pathology in high-fat- and vitamin D3-induced atherosclerosis (AS) rat model. Liver pathology was determined by H&E staining (magnification: ×100). A. Blank control; B. AS model; C. Medium-dose SEAE; D. Simvastatin.

and hyperplasia. Loose connective tissues could be seen in the adventitia (**Figure 2**).

The rats in the medium-dose group showed intact intima, tunica media, and adventitia of the aorta and the boundaries were clear. Nevertheless, the arrangement of the endothelial cells was still irregular, and slight endothelial denudation could be seen. The medial smooth muscle cells were disordered, accompanied by hyperplasia. The adventitia had loose connective tissues (**Figure 2**).

The rats in the high-dose group showed intact aorta intima, tunica media, and adventitia, with clear boundaries. The arrangement of the endothelial cells was still irregular and accompanied by slight denudation. The arrangement of the medial smooth muscle cells was also irregular, and disorder and hyperplasia could be seen. The adventitia had loose connective tissue (**Figure 2**).

As for the simvastatin group, the structure of the aortic intima, tunica media, and adventitia were generally complete. The endothelial cells were slightly irregular with very insignificant denudation. The medial smooth muscle cells displayed irregular arrangement, accompanied by disorder and hyperplasia. The adventitia had loose connective tissue (**Figure 2**).

Effects of SEAE on liver histopathology in the high-fat- and vitamin D3-induced rodent model of atherosclerosis

In the blank control group, no abnormal structures were seen. The central vein was in the center of the liver, and hepatocytes radiated from the central vein. The hepatic cells were clear, without steatosis. The nuclei were located in the center of the cells, and the cytoplasm was abundant (**Figure 3**).

In the model group, hepatic cells were swollen, accompanied by multiple lipid droplets, and the nuclei were irregular, forming lipid-like vacuoles. The interstitium showed focal degeneration or dispersed infiltration of inflammatory cells (**Figure 3**).

In the medium-dose group, the hepatocytes were mildly swelled, no lipid-like vacuoles were formed, and slight infiltration of inflammatory cells could be seen (**Figure 3**).

In the simvastatin group, cell swelling was improved and was accompanied by water-like degeneration. There were a few balloon-like changes, and several inflammatory cells and proliferating fibroblasts could be seen in the stroma (**Figure 3**).

Effects of SEAE on mRNA and protein expression levels of SREBP-2 and FGF21 in the high-fat- and vitamin D3-induced rodent model of atherosclerosis

qRT-PCR analysis showed that the model group had higher SREBP-2 mRNA levels and lower FGF21 mRNA levels compared with the blank control group (+406%, $P<0.05$; -86%, $P<0.05$; respectively). Compared with the model group, the SEAE medium-dose group and the simvastatin group showed lower SREBP-2 mRNA levels (-44%, $P<0.05$; -50%, $P<0.05$; respectively) and higher FGF21 mRNA levels (+296%, $P<0.05$; +270%, $P<0.05$; respectively). There were no

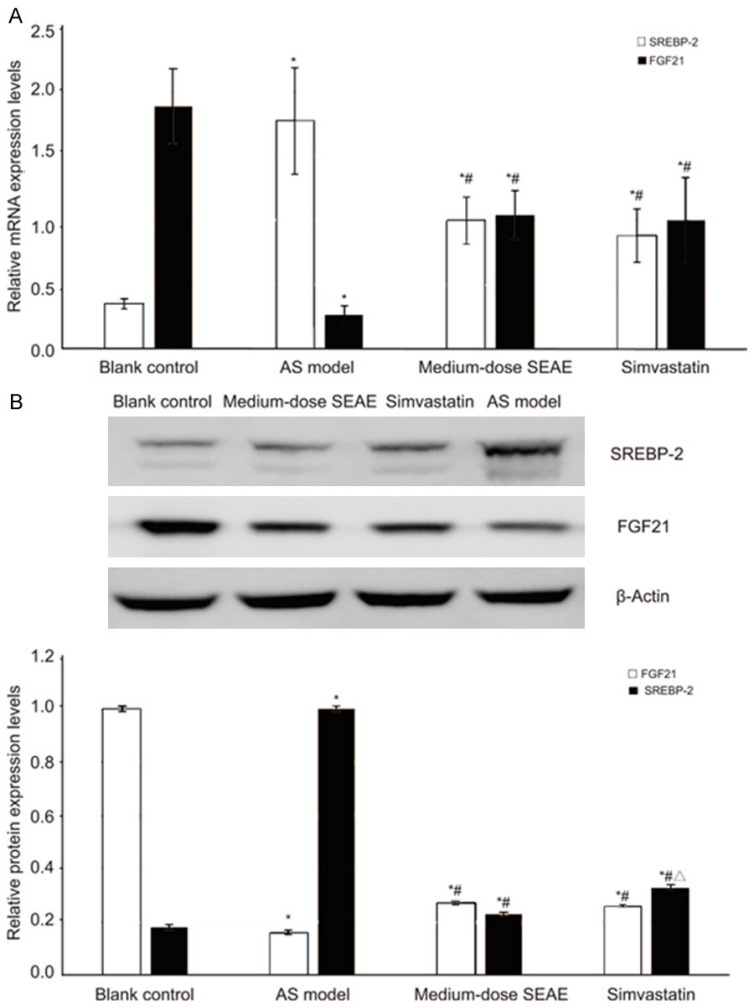

Figure 4. Effects of sappanwood ethyl acetate extract (SEAE) on mRNA and protein expression levels of SREBP-2 and FGF21 in the high-fat- and vitamin D3-induced atherosclerosis (AS) rat model. A. mRNA expression levels of SREBP-2 and FGF21 were determined by real-time RT-PCR. GAPDH was used as an internal control. B. Protein expression levels of SREBP-2 and FGF21 were determined by western blot. β-actin was used as an internal control. Data are shown as means ± standard deviations (SD) (n=3 each group). $^{*}P<0.05$ *vs.* blank control; $^{\#}P<0.05$ *vs.* AS model; $^{\Delta}P<0.05$ *vs.* simvastatin.

differences between the SEAE medium-dose group and the simvastatin group (**Figure 4**).

All statistical analyses were conducted using SPSS 22.0 (IBM, Armonk, NY, USA). Data were expressed as means ± standard deviations (SD) and analyzed using a one-way analysis of variance (ANOVA) with the SNK-q test for post hoc analysis. A *P*-value <0.05 was considered significant.

Western blot showed that the model group had higher SREBP-2 protein levels and lower FGF21 protein levels compared with the blank control group (+456%, *P*<0.05; -84%, *P*<0.05; respectively). Compared with the model group, the SEAE medium-dose group and the simvastatin group showed lower SREBP-2 protein levels (-77%, *P*<0.05; -67%, *P*<0.05; respectively) and higher FGF21 protein levels (+69%, *P*<0.05; +63%, *P*<0.05; respectively). The SREBP2 protein levels were higher in the simvastatin group than in the SEAE medium-dose group (**Figure 4**).

All statistical analyses were conducted using SPSS 22.0 (IBM, Armonk, NY, USA). Data were expressed as means ± standard deviations (SD) and analyzed using a one-way analysis of variance (ANOVA) with the SNK-q test for post hoc analysis. Rank sum test was used for measurement data that does not obey a normal distribution. A *P*-value <0.05 was considered significant.

Discussion

SEAE shows promising effects against atherosclerosis [14-16]. FGF21 and SREBP2 are involved in the development of atherosclerosis [17-19]. Nevertheless, the effects of SEAE on the expression of FGF21 and SREBP2 and on atherosclerosis were unknown. The results suggest that the atherosclerosis model was successfully induced and that SEAS alleviated the changes in aortic and liver histology induced by the high-fat and vitamin D3 modeling, and these changes were similar to those inducedby simvastatin. Limited literature is available about these effects of sappanwood. Nevertheless, previous studies showed that SEAE limited liver injury in rats with iron overload [22] or induced by carbon tetrachloride [23]. Another study in rabbits showed that the main active compound of SEAE alleviated aortic atherosclerosis [15].

Of course, blood cholesterol, especially LDL-C, is the strongest factor for atherosclerosis progression [24, 25]. The effects of statins on

blood cholesterol levels and atherosclerosis development are well known [4, 5]. In the present study, the extents of the decreases in TC, TG, and LDL-C by SEAE were similar to those of simvastatin, suggesting that the anti-atherosclerosis effect of SEAE could be due, at least in part, to decreased blood lipid levels, as supported by a recent study in rabbits [15].

Multiple mechanisms could be involved in the effects of SEAE on atherosclerosis. A study in rabbits showed that the main active compound of SEAE (protosappanin A) alleviated aortic atherosclerosis through the inactivation of NF-κB signaling [15]. NF-κB signaling is involved in atherosclerosis development, mainly at the level of inflammation and activation of immune cells [26, 27]. Another compound from sappanwood, brazilin, has been shown to inhibit the inducible nitric oxide (NO) synthase (iNOS), which is a member of the NO synthase (NOS) family [28]. NO is required for normal endothelial function but is toxic and pro-inflammatory to the endothelium at the high concentrations that can be reached by iNOS activity [28]. Hence, alleviation of endothelial inflammation and improvement in endothelial function could be a mechanism through which SEAE exerts its beneficial effects on aortic histology. Accordingly, it has been suggested that SEAE could be used to manage endothelial dysfunction [14] and oxidative stress [29]. In cell models of osteoarthritis, SEAE has been shown to decrease the expression of MMPs by suppressing IL-1β [16]. MMPs are involved in a number of diseases, including the weakening of the fibrous cap of atherosclerotic lesions, increasing the risk of rupture and thrombotic events [30]. In addition, IL-1β itself is involved in atherosclerosis development, and its inhibition could be a therapeutic basis [31].

FGF21 and SREBP2 are two important actors in atherosclerosis. Indeed, FGF21 expression is strongly associated with dyslipidemia and CVD [17], and the lack of FGF21 expression also leads to accelerated atherosclerosis [17]. On the other hand, SREBP2 is involved in the endothelial susceptibility to atherosclerosis [19] and participates in hypercholesterolemia [20]. In the present study, FGF21 expression was inhibited, and SREBP2 expression was enhanced in the atherosclerosis model group. On the other hand, these changes were reversed by SEAE, in the same way as simvastatin did. Based on the inhibition effect of SREBP2 on FGF21 and on the involvement of FGF21 in adiponectin and PPARs signaling [18], it can be hypothesized that SEAE modulates a whole network of signaling pathways involved in atherosclerosis, but additional studies are necessary to determine those pathways.

The present study has limitations. Indeed, only two mRNAs/proteins were examined, and a more comprehensive panel of mRNAs/proteins involved in atherosclerosis, inflammation, and oxidative stress is necessary to understand the effects of SEAE on atherosclerosis. In addition, only the effects of SEAE on aorta and liver histopathology and blood lipids were examined. Future studies should also examine inflammation, immune cell recruitment, and oxidative stress in response to SEAE, as well as the possible toxicity. A previous study showed that SEAE had no acute or subacute toxicity in female and male rats [32], but this needs confirmation.

In conclusion, the results suggest that SEAE improves lipid metabolism and prevents atherosclerosis through changes in FGF21 and SREBP2 mRNA and protein expression levels. The effects of SEAE are similar to those of simvastatin.

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Disclosure of conflict of interest

None.

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