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# Equisetum sylvaticum base reduces atherosclerosis risk factors in rats fed a high-fat diet

### Cheng He Lin<sup>1</sup>, Hua Li Xu<sup>2</sup>, Wei Lun Sun<sup>2</sup>, Feng Li<sup>2</sup> and Guang Zhu Lin<sup>1</sup>

<sup>1</sup>Department of Nuclear Medicine, The First Affiliated Hospital of Jilin University, Changchun 130021, China; <sup>2</sup>School of Pharmacy of Jilin University, Changchun 130021, China.

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#### Abstract

We identify an Equisetum sylvaticum alkaloid (ESA) derived from E. hyemale, which has robust antihyperlipidemic effects in rats fed a high-fat diet. ESA was isolated from E. hyemale and identified by IR, <sup>13</sup>C NMR and <sup>1</sup>H NMR. Rats were induced to hyperlipidemia and subjected to ESA treatment. In hyperlipidemic model, fed with a high-fat diet, the blood levels of TC, TG and LDL-C were increased. The administration of ESA (20 or 40 mg/kg) to those rats significantly improved the HDL-C level and reduced the levels of TC, TG, LDL-C. The atherosclerosis index and atherosclerosis risk of these rats were significantly reduced by ESA. In addition, the administration of ESA in rats increased the activity of SOD and decreased the level of MDA. These results reveal the antihyperlipidemic and anti-oxidative effects of ESA in vivo.

# Introduction

Cardiovascular disease (CVD) is one of the leading causes of premature death and disability in both Western and Eastern countries. Atherosclerosis constitutes the single most important contributor to CVD (Kitamura et al., 2011). It is well accepted that lipid abnormalities (McCrindle et al., 2007), oxidant stress (Ding et al., 2007), and chronic inflammation play important roles in the initiation of atherosclerosis and subsequent CVD. Lipid abnormalities are defined as abnormal increases of serum cholesterol, triglycerides, and low-density lipoprotein cholesterol (LDL-C) combined with a low level of high-density lipoprotein cholesterol (HDL-C) in serum. This disproportion is considered a high-risk factor for atherosclerosis and CVD (Vaidya et al., 2011).

Currently, the most commonly used lipid lowering drugs are statins and fibric acid derivatives. However, they cause complications either in monotherapy or in combination therapy. The major adverse effect of statins is myopathy, up to rhabdomyolysis with ensuing acute renal insufficiency. Fibric acid derivatives bind to

peroxisome proliferator-activated nuclear receptor alpha and cause a number of adverse effects, including liver enzyme elevations, gastrointestinal side effects and rhabdomyolysis. The combination of statins with fibric acid derivatives may cause serious adverse effects and should be avoided. In eastern counties, natural products have been used to modulate lipid metabolism and are believed to be active in controlling hyperlipidemia and associated pathologies with mild adverse effects.

Equisetum hyemale is an important herb of Chinese traditional medicine. It has been used as a traditional herbal medicine to treat various diseases such as hypertension, inflammatory diseases, acute stroke, bleeding and cancer in China (Li et al., 2012). E. hyemale inhibits lipid-rich food induced elevation of triglycer-ides and cholesterol in rats, with low toxicity (Xu et al., 1993). We isolated and identified E. sylvaticum alkaloid (ESA), which was the first alkaloid identified in E. sylvaticum. As E. hyemale has lipid lowering effects, the objectives of this study were to determine whether ESA can reduce atherosclerosis risk factors in rats fed a high-fat diet.



## **Methods and Materials**

*ESA base isolation: E. sylvaticum* was collected from Weidong, China, and identified by Prof. Jinming Zhang (Department of Pharmacognosy, School of Pharmacy, Jilin University). Dry *E. sylvaticum* leaves (3.5 kg) were extracted with 95% ethanol. The extract was filtered and concentrated to yield a red-brown material (510 g). The red-brown material was further extracted with ligroin and the aqueous phase was extracted with ethyl ether. The ethyl ether extract (18 g) was adsorbed on a silica gel column (100 g). The silica gel column was eluted stepwise with 500 mL of cyclohexane-ligroin (4:1). A compound (purity 98%) obtained by elution was purified by recrystallization and further identified by infrared absorption (5MX-FT, Nicolet, USA) and NMR (ARX-300, Bruker, USA) to be ESA.

*Hyperlipidemia induction using high-fat diet:* Male Wistar rats (180–220 g) were obtained from the Experimental Animal Center of Jilin University. All experiments were approved by the laboratory animal ethical committee of Jilin University and followed national guidelines for the care and use of animals (No. SCXK-(Jilin University) 2009-0356). The animals were housed in an air-conditioned environment with a daily photoperiod 12 hours light/dark cycle and received tap water *ad libitum.* Animals were kept for 10 days to allow acclimation to the animal facility before starting the experiments. The high-fat diet consisted of a mixture of 90% standard rat chow, 2% cholesterol, 0.2% methylthiourea cilum, 0.3% sodium cholate, 7.5% pork fat and 0.2% cholic acid. All rats had free access to water.

Antihyperlipidemic effects of the ESA: Rats were fed a standard diet for 1 week before the experiment. Sixty rats were randomly divided into six groups with 10 rats in each group: Normal group (fed with normal diet), model group (fed with high-fat diet), ESA groups (fed with high-fat diet and ESA at 10, 20 and 40 mg/kg body weight) and lovastatin group (fed with high-fat diet and lovastatin at 2.5 mg/kg body weight) as a positive

control. ESA and lovastatin were administrated orally once per day for 28 days. Normal and model groups were administrated the same volume of distilled water. Body weight and food intake were recorded twice weekly. After 28 days, the rats were fasted for 12 hours and anesthetized with chloral hydrate (350 mg/kg, i.p).

Laboratory evaluation and biochemical assays: After 28 days of treatment, rats were fasted for 12 hours. Blood samples were collected from the abdominal aorta. The samples were stored at room temperature for 1 hour to allow complete clotting, centrifuged to obtain the serum, and stored at -80°C until analysis. Total cholesterol (TC), triglycerides (TG), LDL-C and HDL-C were measured using commercial kits according to the manufacturers' instructions and an automatic biochemistry analyzer (Hitachi 7600, Japan). The atherogenic index (AI) and coronary risk index (CRI) were calculated as follows: AI = (TC-HDL-C)/HDL-C and CRI = TC/HDL-C (Dobiasova and Frohlich, 2001).

*Statistics:* All data are expressed as mean  $\pm$  SD. Statistical comparisons between different groups were performed by ANOVA test with SPSS 11.5 software. p<0.05 was considered significant.

#### Results

The white amorphous powder, which showed IR absorptions at v (cm<sup>-1</sup>), 2960 (m, -CH3), 2920 (m), 2850 (m, -CH2-), 1738 (m), 1462 (m, -CH=C-), 1050 (w, -C-O-C-), was identified as ESA (Figure 1). The molecular formula [C16H32NO]+, N, N, 4-tri-methyl-2-methoxyl-5-octyl-2, 5-dihydropyrrole, was determined by <sup>13</sup>C NMR spectrum (Figures 2). The chemical structure is shown in Figure 3. It cannot be found in the Chemical Abstract Service database.

Four weeks of high-fat diet resulted in significantly increased TC, TG, LDL-C, CRI and AI and significantly decreased HDL-C levels (p<0.05) (Table I), as compared



Figure 1: Infrared spectrum of ESA

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Figure 2: <sup>13</sup>C NMR spectrum of ESA



Figure 3: Chemical structure of ESA

#### to the normal rats. Animals in the ESA 20 and 40 mg/

kg groups exhibited significantly lower plasma TG, TC and LDL-C levels (p<0.05), resulting in lower CRI and AI than in the model group (p<0.05). There were no marked differences in the levels of plasma LDL-C and HDL-C between the high-fat model group and the 10 mg/kg ESA group, but the TC, TG and CRI in the ESA 10 mg/kg group were lower than in the high-fat model group (p<0.05, Table I).

The activity of SOD in the high-fat diet model group decreased in the serum and liver by 17.0 and 20%, respectively (Figure 4), whereas the levels of MDA in



Figure 4: Effects of ESA on the activities of SOD and the levels of MDA in plasma (A and B) and liver (C and D) of rats fed a high-fat diet. Model indicates high-fat diet group; 10, 20 and 40 mg/kg indicate ESA level. Bars represent the mean  $\pm$  SD from 10 animals in each group. <sup>a</sup>p<0.05, compared to the model group; <sup>b</sup>p<0.05, compared to the control group

Table I									
Effects of ESA on blood lipid metabolism in hyperlipidemia rats									
Group	Dose (mg/kg)	TG (mmol/L)	TC (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)	CRI	AI		
Control	_	$0.4 \pm 0.2$	$2.5 \pm 0.4$	$3.6 \pm 1.1$	$0.9 \pm 0.3$	$2.9 \pm 1.0$	$4.2 \pm 0.9$		
Model	_	$0.7 \pm 0.2^{a}$	$8.3 \pm 2.0^{a}$	$5.6 \pm 1.9^{a}$	$0.6 \pm 0.2^{a}$	$14.7 \pm 4.2^{a}$	$15.4 \pm 3.8^{a}$		
Lovastatin	2.5	$0.4 \pm 0.2^{\circ}$	$6.1 \pm 0.8^{b}$	$3.7 \pm 1.0^{**}$	$0.8 \pm 0.2^{b}$	$7.5 \pm 2.2^{\circ}$	$4.6 \pm 1.7^{\circ}$		
	10	$0.6 \pm 0.1^{b}$	$6.0 \pm 1.1^{b}$	$5.0 \pm 2.1$	$0.6 \pm 0.3$	$10.0 \pm 2.6^{b}$	$8.4 \pm 2.0^{b}$		
ESA	20	$0.5 \pm 0.1^{b}$	$5.5 \pm 1.3^{b}$	$4.2 \pm 0.8^{b}$	$0.8 \pm 0.2^{b}$	$7.0 \pm 2.2^{\circ}$	$5.4 \pm 1.3^{\circ}$		
	40	$0.4 \pm 0.1^{\circ}$	$3.3 \pm 0.7^{\circ}$	$3.7 \pm 0.8^{\circ}$	$0.8 \pm 0.2^{b}$	$4.1 \pm 1.2^{\circ}$	$4.6 \pm 1.3^{\circ}$		
$^{a}p<0.05$ compared with control, $^{b}p<0.01$ , $^{c}p<0.005$ compared with model; Data are mean ± SD; n = 10									

the serum and liver increased 47.9 and 46.0%, respectively. The rats in the 20 and 40 mg/kg ESA groups exhibited a significant increase in SOD activity in the serum and liver, while the levels of MDA were significantly decreased in these rats. The rats in the 10 mg/kg ESA group did not experience any significant change of MDA and SOD activity in their serum or liver (Figure 4).

#### Discussion

High-fat diet induced hyperlipidemia in rats is an important model for evaluating treatments for the reduction of serum lipid and cholesterol disorders. The rise in serum cholesterol and circulating lipids levels results in preliminary damage of the endothelium of arteries, and affects the immunological system of these rats (Tobert, 2003). Using this model of hyperlipidemia, lovastatin significantly decreased serum LDL levels, LDL/HDL ratio and TG levels, indicating that the model was successfully set up to evaluate the lipid lowering effects of ESA. When ESA was administered at dosages of 20 and 40 mg/kg/day, it significantly decreased serum TG levels and LDL/HDL ratio, significantly increased HDL, and markedly lowered cholesterol and LDL-C levels. Thus, this herb derived compound produces significant normalization of dyslipidemia when given at a relatively high dosage. The administration of 10 mg/kg/day ESA did not increase HDL-C or decrease LDL-C, though serum total cholesterol and TG levels were decreased in compareson to baseline levels of the high-fat diet rats. The mechanisms of ESA's effects may be related to inhibition of HMG-CoA reductase, increasing cholesterol fecal excretion, inducing changes in gene expression involved in cholesterol homeostasis and local effects on cholesterol absorption. Lovastatin decreases serum cholesterol by directly inhibiting HMG-CoA reductase (Tobert, 2003; Filippatos, 2012). In the present study, the statin we used did not reduce the serum cholesterol level to the normal baseline. Likewise, the ESA dosages used here did not reduce the serum cholesterol and TG levels to normal values. The widely used antiatherosclerosis nature products such as Salvia miltiorrhiza and Ginkgo biloba achieve their

pharmacological effects by their anti-oxidative activity, which protects the integrity of endothelium and reduces the inflammatory reaction (Wu et al., 2012; Zeng et al., 2012). We have found that *E. sylvaticum* might modulate the lipids levels in the atherosclerotic model. More studies are needed to clarify the direct target of ESA.

Oxidative stress is the critical pathogenetic factor for atherosclerosis and other cardiovascular diseases (Mani et al., 2013). It is known that free radicals mediate various signaling pathways that are involved in vascular inflammation and lipid oxidation in atherogenesis (Yancey et al., 2003; Haas and Mooradian, 2011). Oxidative stress is defined as the disturbed balance between cellular levels of free radicals and antioxidant defenses (Hadi et al., 2013). Excessive free radicals attack all types of biomolecules including lipids molecules. The oxidation of low-density lipoproteins results in the destruction of cellular components and the integrity of the endothelia of the blood vessels, initiating the processes of atherogenesis (Vijayakumar et al., 2004; Asdonk et al., 2012). However, a sufficient level of anti-oxidants can counteract the effects of oxidative stress. One of the most important anti-oxidant enzymes is SOD, which converts superoxide to hydrogen peroxide (Zelko et al., 2002). Serum MDA is a marker of lipid peroxidation that reflects the level of lipid oxidation in animals (Singh et al., 2003). In the present study, a high-fat diet markedly increased serum and liver MDA levels, indicating the increase of lipid oxidation in the rats. At the same time, SOD activity decreased in the liver and serum. Similar to the statins, ESA reduced oxidative stress by reducing MDA and increasing the level of SOD activity both in the serum and the liver, the key organ for the metabolism of cholesterol and lipids, indicating that the protective effects of ESA in hyperlipidemia rats are related to reducing oxidative stress.

In summary, ESA derived from *E. hyemale* is effective in the amelioration of oxidative stress and lipid profile in rats fed a high-fat diet. These results imply that ESA may contribute to the prevention of atherogenesis and decreasing the incidence of CVD.

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Author Info Guang Zhu Lin (Principal contact) e-mail: zlg9012@163.com