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Original Article

Typha angustifolia extract reduces diet-induced hyperlipidemia in rats

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ABSTRACT

Objective: To observe the lipid-lowering effect of *Typha angustifolia* extract (TAE) in Sprague Dawley rats.
Methods: The rats were randomly divided into six groups, including the hypercholesterolemic diet (HYD) group. Ad libitum food was given to the animals for 60 d to establish dyslipidemia models. The rats were received an ig administration of 1 mL medicine daily. After 120 d the animals were sacrificed by cervical dislocation; Blood was collected to measure total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), nitric oxide (NO), and endothelin (ET) plasma concentration; Livers were collected to measure ApoE mRNA and protein expression; Morphologic changes of aorta ventralis tissue were also observed.
Results: Compared with HYD group, TAE had the ability of reducing TC, TG, LDL-C, NO, and ET ($P < 0.01$), thereby increasing ApoE mRNA and protein expression of the liver ($P < 0.01$).
Conclusion: These results suggested that TAE was capable of effectively decreasing the circulating lipids levels and enhancing the protective effects of artery.

1. Introduction

Atherosclerosis is frequently associated with hypercholesterolemia, a chronic disease and a leading cause of death worldwide. *Typha angustifolia* L., which is a traditional Chinese herbal medicine (Feng et al., 2012; Zhang, 1986), has been used for thousands of years in traditional Chinese medicine and has been widely used in indigenous medicine for the treatment of atherosclerotic plaque, hemorrhagic diseases, diabetes, and inflammation diseases (Feng et al., 2012; Yin & Yu, 1992).

Flavonoids extracted from *T. angustifolia* is the main content and extract of *T. angustifolia*, which can potentially reduce atherosclerotic plaque formation in experimental animal models (Zhao et al., 1990), inhibit the proliferation of aortic smooth muscle cells in culture (Zhao, Zhang, Xu, & Wang, 1983), protect aortic endothelial cell from injury by fibrin, raise tissue plasmingen (tPA) activity (Zhao et al., 1990), activate mononuclear cells, and promote regression of cholesterol granuloma of rats (Ren, 1984)

T. angustifolia extract (TAE) significantly reduces prothrombin time (PT), activates partial PT, and recalcification time, and directly activates factor XII in the coagulation cascade (Ohkura, Tamura, Tanaka, Matsuda, & Atsumi, 2011). It is capable of increasing body tolerance to acute hypoxia and promoting adaptation to highlands (Peng, 1990).

TAE is capable of increasing insulin sensitivity by enhancing the peroxisome proliferator-activated receptor (PPAR), mRNA expression of PPARα and PPARγ, and increasing glucose intake in the 3T3-L1 adipocytes as well as decreasing the efflux of free fatty acid from the cells (He et al., 2006), and improving insulin-induced glucose uptake via the β-arrestin-2-mediated signaling in C2C12 myotubes (Feng et al., 2012; He et al., 2006). TAE inhibits the IL-6 mRNA expression and IL-6 protein secretion via nuclear factor-kappaB pathway in C2C12 skeletal muscle cells, which may be one of its mechanisms in relieving inflammation conditions. The above-mentioned studies indicated that there were several mechanisms in the bioactivities of *T. angustifolia*. Its anti-atherosclerotic effects resulted from various biologic effects. Therefore, we hypothesized that TAE may be able to treat or prevent hyperlipidemia. To test this hypothesis, we evaluated the effect of TAE in rats with hyperlipidemia fed with a control diet or hypercholesterolemic diet on circulating lipid levels, liver ApoE mRNA, and protein expression.

2. Materials and methods

2.1. Materials

T. angustifolia were purchased from National Institute of China Three Gorges University for Control of Pharmaceutical and Biological Products (Yichang, China); The purity of these standards

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was higher than 99% by normalization method of peak areas determined by High Performance Liquid Chromatography-Diode Array Detection (HPLC-DAD). Ultrapure water for HPLC analysis was prepared by a Milli-Q water purification system (Millipore, Milford, MA, USA); Other reagents were of analytical grade. Flavonoids were the main content of *T. angustifolia* extract with yield of 1.8% flavone; Isorhamnetin-3-*O*-neohesperidoside (87%) and typhaneoside (7%) were the main components of flavones (Fig. 1). The identified constituents were well standardized and qualified by National Institute of China Three Gorges University for Control of Pharmaceutical and Biological Products. Other constituents in *T. angustifolia* were vanillic acid, succinic acid, sucrose, minerals etc.

2.2. Animals

A total of 60 adult SD rats (30 male rats and 30 female rats, Animal Certification No: SCXK ER 2016-0002), weighing (180 ± 15) g were housed in individual cages. The animals were kept at an environmental temperature of (23 ± 3) °C in a 12 h light/dark controlled room. All animal procedures were performed according to the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of China Three Gorges University (Permit Number: 11-3789), China. All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering in an ethically proper way by guidelines as set by the World Health Organization.

2.3. Animal groups and administration

T. angustifolia extract was dissolved with ultrapure water solution. Before beginning the experiment, the rats were submitted during seven days to an adaptive period of conditioning with standard diet. The animals were randomly divided into six groups ($n = 10$) as follows: control group, hypercholesterolemic diet group (HYD), hypercholesterolemic diet + 6 mg/kg TAE group (HYD + 6 mg/kg TAE), hypercholesterolemic diet + 120 mg/kg TAE group (HYD + 120 mg/kg TAE), hypercholesterolemic diet + 720 mg/kg nicotinic acid group (HYD + nicotinic acid), and hypercholesterolemic diet + 5 mg/kg fluvastatin group (HYD + fluvastatin). Moderate dose (6 mg/kg) and high dose (120 mg/kg) of TAE were chosen in HYD + TAE groups according to a previous study (Ren, 1984). The rats of the control group were fed with a standard diet (corn flour 36%, wheat flour 35%, wheat bran 15%, soybean flour 10%, yeast powder 1%, salt 1%, bone powder 1%, and fish liver oil 1%). The rats of the HYD group were fed with high-fat (cholesterol 2.0%, sodium cholate 0.5%, lard 10.0%, propylthiouracil 0.2%, white sugar 5.0%, and basal feed 82.3%) diet.

2.4. Determination of plasma lipids, nitric oxide, and endouthenlin

Serum triglycerides (TG), total cholesterol (TC), LDL-cholesterol (LDL-C), and HDL-cholesterol (HDL-C) were measured enzymatically with Commercial Kits (Jiancheng Bioengineering Inc, Nanjing, China). Nitric oxide (NO) and endothenlin (ET) were determined by routine enzymatic methods using ELISA Kits (Kehua Bioengineering Inc, Shanghai, China).

2.5. Aortaventralis histomorphological examination

The isolated fresh aortaventralis tissues were fixed with paraformaldehyde for 48 h, and serially sectioned into 4 μm paraffin slices. The paraffin slices were applied to hematoxylin-eosin staining according to previous protocol (Altorjay et al., 2007). Images were captured using the Olympus Micro Image analysis software (version 4.0, Olympus Optical, Tokyo, Japan).

2.6. Real-time fluorescent quantitative RT-PCR for ApoE mRNA expression

The quantity of mRNA was assessed by the intactness of 28S and 18S bands and the lack of degradation on agarose-gel electrophoresis. Total cellular RNA was extracted from aortaventralis using the TRIzol Reagent (TaKaRa, Kyoto, Japan) according to the manufacturer's protocol. The synthesis of the cDNA was performed with the Reverse Transcription System (TaKaRa) using 500 ng of RNA. All samples were run in duplicate on 96-well optical PCR plates in a final reaction volume of 20 μL. The PCR parameters were 1 cycle at 95 °C for 30 min, and 40 cycles at 95 °C for 5 s, and 62 °C for 21 s. The ApoE specific gene primers and the internal

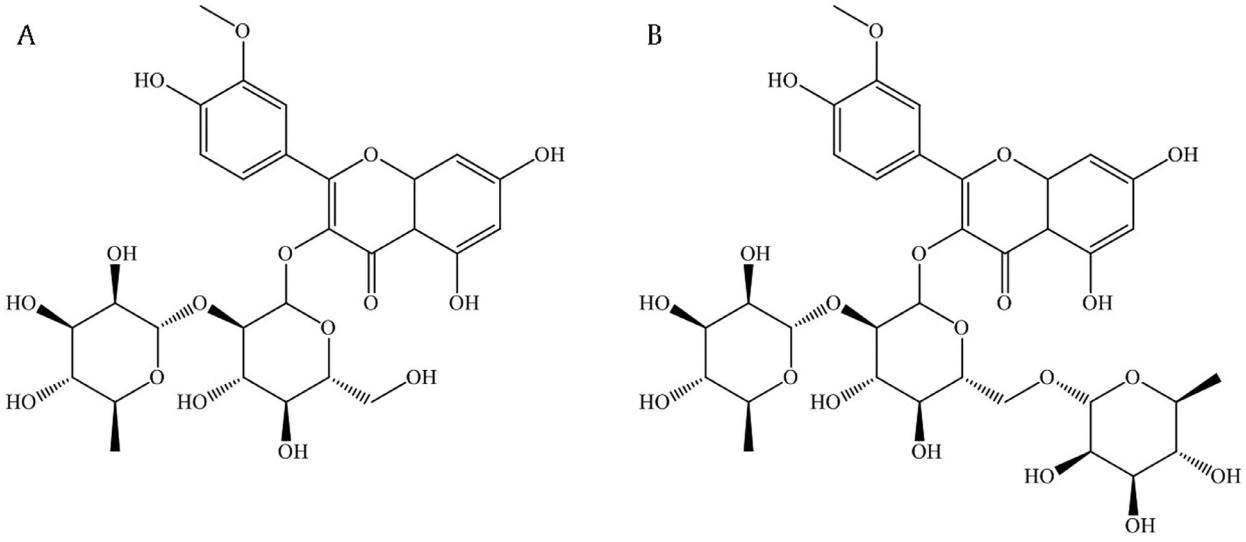

Fig. 1. Chemical structures of two active components in *T. angustifolia* extract. A: isorhamnetin-3-*O*-neohesperidoside (chemical formula: $C_{28}H_{32}O_{16}$, molecular weight: 624.54); B: typhaneoside (chemical formula: $C_{34}H_{42}O_2$, molecular weight 770.68).

control gene primers (β-actin) were as follows (ApoE: Forward: 5′-TGTGAGTGCTATCCGTGACG-3′; Reverse: 5′-TATCTGCTGGGTCTGCTCCT-3′. β-actin: Forward: 5′-GTAAAGACCTCTATGCCA CA-3′ Reverse: 5′-GGACTCATCGTACTCCTGCT-3′).

2.7. Western blot for ApoE protein expression

Liver tissue samples were prepared by homogenization in RIPA buffer (Sigma, St. Louis, MO, USA) as described previously (Liu et al., 2011; Papackova, Dankova, Palenickova, Kazdova, & Cahova, 2012). Lysates were quantified using a Micro BCA assay reagent kit (Thermo Scientific, USA) as described previously (McDonald et al., 2010). Aliquots (40 µg each) were resolved by SDS-PAGE, subjected to electrophoresis at 80 V for 50 min and 120 V for 1.5 h, and transferred to PVDF membranes. The membranes were then blocked in Trans-Blot SD Semi-Dry Cell Accessories (Bio-rad) for 20 min, and then incubated overnight at 4 °C with rabbit-rat ApoE (1:1000 dilution, Santa Cruz Biotechnology, Inc., USA) primary antibody, incubated for 1 h at room temperature with HRP-labeled goat anti-rabbit IgG (1:10 000 dilution, Cell Signaling Technology, Inc, Boston, USA). Images were captured using the ImageQuant TL imaging analysis system (ImageQuant Las4000mini, General Electric Company, Stockholm, Sweden), and analyzed using Quantity One 4. 6.2 Software (Bio-Rad, California, USA). ApoE expression level was then normalized against the level of the house keeping gene, b-tubulin. Each Western blot assay was repeated for two or three times.

2.8. Statistical analysis

Data were reported as mean ± SEM. Statistical significance was analyzed using one-way analysis of variance (ANOVA) followed by comparisons using the Scheffe's multi-comparison test for multi-group comparisons. Analyses were carried out by using the statistical package Sigma plot 12.0.

3. Results

3.1. Mesurement of plasma lipids

A significantly lower plasma TC, TG, and LDL-C in HYD + 6 mg/kg TAE group was noted when compared with HYD group. Rats treated with medium (6 mg/kg) and high (120 mg/kg) dosage of TAE presented a significant reduction of circulating TC, TG levels as compared to HYD group (Fig. 2).

High-fat diet caused the elevation of hepatic total TG and TC levels. TAE treatment decreased the hepatic total TG and TC levels. As shown in Fig. 2, hepatic TC levels were dose-dependently decreased by 19.23% and 34.61% at the doses of 6 mg/kg and 120 mg/kg, respectively. TAE also significantly inhibited the decrease in hepatic total TG levels, and exhibited 32.81% and 45.31% reduction at medium and high doses, respectively.

The results demonstrated that both medium and high doses of TAE can lower hepatic total TG and TC levels.

3.2. Mesurement of plasma NO and ET

At 8th weeks, liver tissue was collected and NO, ET levels in the liver were measured by ELISA. As shown in Table 1, blood NO and ET levels in HYD group were significantly higher than those in the control group. Compared with HYD group, 6 mg/kg TAE, 120 mg/kg TAE, nicotinic acid, and fluvastatin groups significantly decreased blood NO levels, while the 120 mg/kg TAE, nicotinic acid, and fluvastatin groups significantly decreased blood ET levels.

3.3. Aortaventralis histomorphology

Thrombus formation occurred in the aortaventralis in the HYD and HYD + nicotinic acid groups, while there was no thrombosis in the control, HYD + 6 mg/kg TAE group, HYD + nicotinic acid, and HYD + fluvastatin groups. (Fig. 3).

3.4. ApoE mRNA and protein expression in liver

Compared to the HYD group, the HYD + 6 mg/kg TAE, HYD + 120 mg/kg TAE, HYD + nicotinic acid, and HYD + fluvastatin groups significantly increased ApoE mRNA expression (Fig. 4).

Rat was treated for 180 d, lysates were examined by Western blotting analysis. ApoE protein expression in the HYD group increased as compared to the control group. Compared to

Table 1
Plasma NO and ET levels in different groups of rats (means ± SEM, $n = 10$).

Groups	NO/(µmol•mL^{-1})	ET/(pg•mL^{-1})
Control	2.2 ± 0.3	31.4 ± 2.8
HYD	14.0 ± 1.5*	50.3 ± 2.7*
HYD + 6 mg/kg TAE	11.7 ± 1.9**	48.1 ± 3.4
HYD + 120 mg/kg TAE	8.9 ± 1.8**	42.3 ± 3.6**
HYD + nicotinic acid	9.2 ± 2.1**	43.0 ± 5.7**
HYD + fluvastatin	6.0 ± 1.3**	31.7 ± 3.5**

*$P < 0.01$ *vs* control group, **$P < 0.01$ *vs* HYD group.

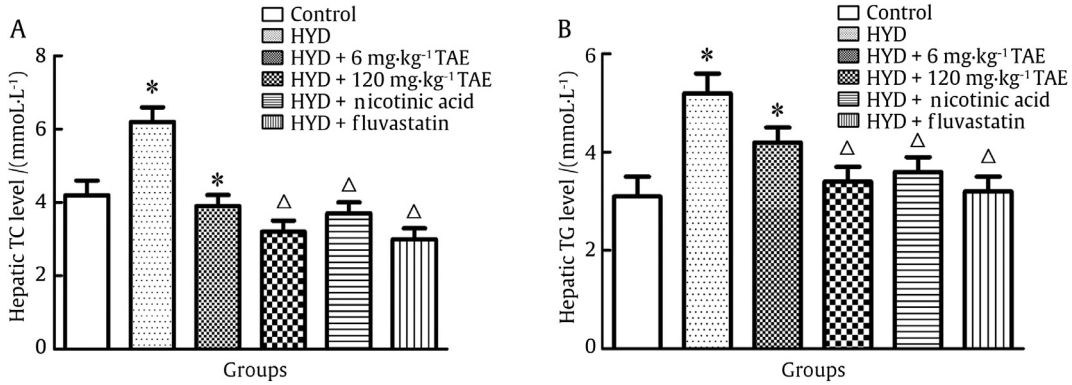

Fig. 2. Changes of hepatic total TC (A) and TG (B) levels in rats (*$P < 0.05$ *vs* control group, $^{\triangle}P < 0.05$ *vs* HYD group).

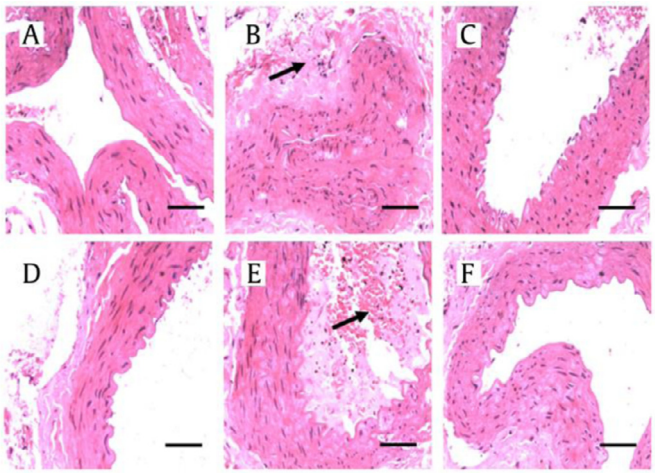

Fig. 3. Pathological changes in rat aortaventralis tissue (Scale bar, 200 μm). (A) control group, (B) HYD group, (C) HYD + 6 mg/kg TAE group, (D) HYD + 120 mg/kg TAE group, (E) HYD + nicotinic acid group, (F) HYD + fluvastatin group.

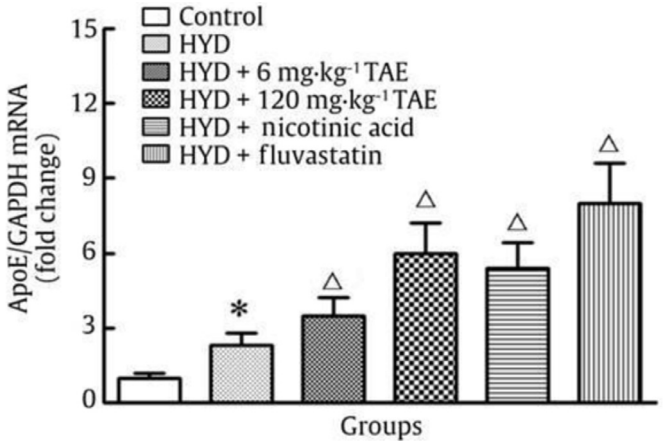

Fig. 4. ApoE mRNA expression in liver by qRT-PCR ($n = 10$). $^{*}P < 0.05$ *vs* control group, $^{\Delta}P < 0.05$ *vs* HYD group.

the HYD group, the HYD + 6 mg/kg TAE, HYD + 120 mg/kg TAE, HYD + nicotinic acid, and HYD + fluvastatin groups significantly increased ApoE protein expression. These results suggest that TAE has positive effects on ApoE protein in the liver, with the latter acting as a type of biological chaperone, ferrying cholesterol and fats around the liver (Fig. 5).

4. Discussion

Hypercholesterolemia is one of the main factors in the development of atherosclerotic cardiovascular disease (Hartman, Defelice, Tzu, Meehan, & Sanchez, 2011). Several animal studies have confirmed the hyperlipidemia properties of a cholesterol diet, which include increasing TC, TG, and LDL-C in the lipoprotein pattern, the mechanisms of which remain unclear (Naveh, Werman, Sabo, & Neeman, 2002). A previous study has shown that consumption of a high-cholesterol diet caused impaired arterial expansive remodelling in ApoE$^{-/-}$ mice with significantly altered *in vivo* haemodynamics as a consequence (Johansson et al., 2005). In our study, feeding rats with HYD resulted in a significant increase in TC, TG, and LDL-C of serum. However, reduced weight was seen in all groups except for HYD group, all of these benefits may be not due to less weight gain but treatment factor. *Typhae Pollen* total flavone, the extract from *Typhae Pollen*, is reported to enhance glucose uptake in C2C12 myotubes *in vitro*, ameliorate insulin resistance and dyslipidemia, but fail to significantly increase body weight in type 2 diabetic rats induced by high-fat diet and low-dose streptozotocin (Feng et al., 2014). Additionally, the improvement in palmitic acid-induced impairment of glucose-stimulated insulin secretion by *Typhae Pollen* total flavone in INS-1 cells was restrained by U-73,122, staurosporine, and calcium channel inhibitor nifedipine, respectively (Feng et al., 2012).

Nicotinic acid and fluvastatin have been widely prescribed in humans with regulating serum cholesterol levels for a number of years (Rizos, Agouridis, & Elisaf, 2010). Niacin has been used for more than 50 years in the treatment of cardiovascular disease (Digby, Ruparelia, & Choudhury, 2012), which was shown to reduce LDL-C and TG and markedly increase HDL-C levels (Hartman et al., 2011). Statins are competitive inhibitors of the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which are effective drugs in the treatment of hyperlipidaemia and the prevention of cardiovascular events (Jeger & Dieterle, 2012). In the present study, we found that TAE effectively decreased TC, TG, and LDL-C, and no serious side effects occurred.

Cholesterol-fed ApoE$^{-/-}$ animals also showed decreased NO-dependent vasodilation in atherosclerosis-prone regions, whereas endothelial function was intact in regions less prone to atherosclerosis in the descending thoracic aorta (Johansson et al., 2005). In addition, studies demonstrated excessive NO lead to the induction of mitochondrial respiratory chain inhibition and myocardial dysfunction (Siow, Li, Rowlands, de Winter, & Mann, 2007). TAE pretreatment can effectively inhibit plasma NO generation. Plasma NO concentration in HYD + 120 mg/kg TAE group was lower than in HYD group, which may consequently prevent angiospasm and protect vessel. *Typhae Pollen* is a main ingredient of Silsosangami

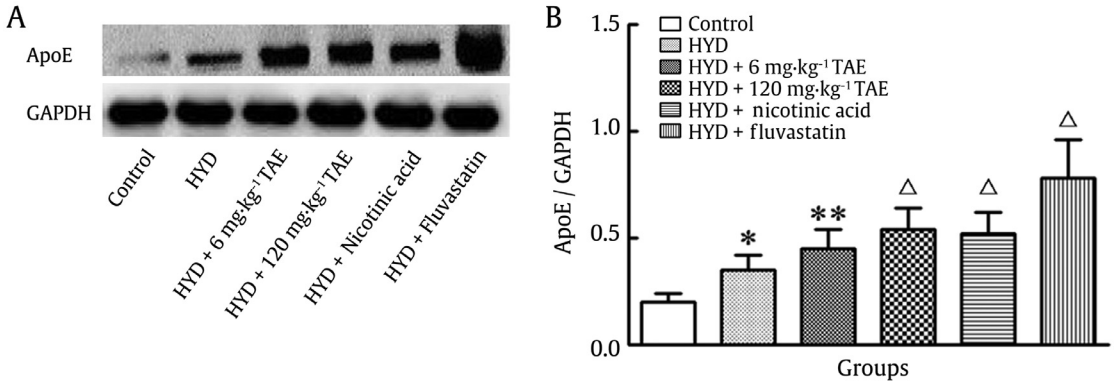

Fig. 5. Elevation of ApoE expression in liver by Western blot (A) and autoradiographs ApoE protein quantified by densitometry (B) ($n = 10$). $^{*}P < 0.05$ *vs* control group, $^{\Delta}P < 0.05$ *vs* HYD group.

(a traditional Korean formulation consisting of seven medicinal herbs) that exerts anti-inflammatory effects associated with the inhibition of neutrophil functions and of NO and PGE2 production (Park, Kim, & Lee, 2004). It also inhibits platelet coagulation and thrombotic action (Park, Ahn, Kim, Lee, & Kim, 2004). Although no lipid foam cells migrated to regions of the aorta ventralis and smooth muscle cells in the six groups of the present study, thrombus formation was identified in aorta ventralis in the HYD and HYD + nicotinic acid groups. However, no thrombus formation occurred in the TAE group, suggesting that TAE may prevent thrombosis, protect blood vessels and prevent arteriosclerosis.

Endothelin (ET) was first isolated from porcine aortic endothelial cells in 1988 (Yanagisawa et al., 1988), which has been demonstrated as one of the most potent known vasoconstrictors. ET has multiple biologic actions, and produces a sustained and long-lasting suppressor response *in vivo* (Rubino, Loesch, & Burnstock, 1999). Increased plasma ET concentrations were exhibited in HYD group in our study. TAE pretreatment can effectively inhibit plasma ET generation.

This study has examined the contribution of liver-derived ApoE expression levels on the modulation of diet-induced hypercholesterolemia using rat models in which liver expressed physiological levels of ApoE. This animal model offered some partial advantage that avoided the use of transgenic expression of ApoE from that could have varying ApoE expression levels.

5. Conclusion

A significant finding of this study is that TAE responds to diet-induced hypercholesterolemia by raising hepatic ApoE protein levels and thereby reducing plasma cholesterol levels. In addition, results of this study have provided evidence that ApoE mRNA and protein is produced by the liver in the setting of hypercholesterolemia. We also demonstrated that TAE promotes ApoE expression in regulating blood fat. TAE is therefore considered to be an effective agent for the treatment of hyperlipidemia and arteriosclerosis.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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