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# Pedunculoside, a novel triterpene saponin extracted from *Ilex rotunda*, ameliorates high-fat diet induced hyperlipidemia in rats

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ABSTRACT

Pedunculoside (PE) is a novel triterpene saponin extracted from the dried barks of *Ilex rotunda* Thunb. The present study aims to explore lipid-lowering effects of PE on hyperlipidemia rat induced by high-fat diet. The rats were fed with the high-fat diet and subjected to intragastric administration of PE at doses of 30, 15, or 5 mg/kg daily for 7 weeks. The results demonstrated that treatment with PE for 7-week dramatically decreased serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) and reduced liver TC in hyperlipidemia rat induced by high-fat diet. Furthermore, the results also showed that PE modulated the expression of enzymes involved in lipid metabolism including peroxisome proliferator-activated receptor α (PPAR-α), sterol regulatory element-binding protein 1 (SREBP-1), fatty acid synthase (FAS) and stearoyl CoA desaturase-1 (SCD-1) mRNA in liver. Besides, PE-treated group decreased weights and diameters of epididymal adipose hyperlipidemia rat. Mechanism study demonstrated that PE regulated PPAR-γ, CCAAT/Enhancer-binding Protein α (C/EBPα)、and SREBP-1 expression as well as inhibited phosphorylation of AMPK in MDI (methylisobutylxanthine, dexamethasone, insulin) induced-3T3L1 cells. Molecular Docking confirmed interaction between PE with proteins involving PPAR-γ, C/EBPα and SREBP-1. In summary, these findings may support that PE is a novel lipid-lowering drug candidate.

## 1. Introduction

Hyperlipidemia is characterized by abnormal lipid levels with the increase of blood total cholesterol (TC), triglyceride (TG) and low-density lipoprotein cholesterol (LDL-C) along with the decrease of high-density lipoprotein cholesterol (HDL-C) [1,2]. Excessive absorption of lipids from the diet is considered as a major risk factor for hyperlipidemia [3–5]. Additionally, epidemiology studies demonstrate that hyperlipidemia is the most important risk factor for cardiovascular diseases (CVDs), including atherosclerosis, myocardial infarction and stroke [6,7]. Therefore, effective and safe pharmacological interventions for hyperlipidemia are urgently needed.

Inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase has been widely used for hyperlipidemia therapy via decreasing serum TC and LDL-C [8]. For instance, simvastatin is commonly used in the treatment of hyperlipidemia. However, statin therapy is limited by individual differences, potential adverse effects and drug dependence [9,10]. Thus, it is still necessary to find novel lipid-lowering agents. Plant-derived compounds provide resources for drug discovery [11,12]. Among these compounds, triterpenoids have garnered significant interest as promising lipid-lowering agents [13–15]. For instance, SREBP-1 is a major transcription factor activating the expressions of genes involved in fatty acid biosynthesis and hypertriglyceridemia [16]. Betulin, an abundant naturally derived triterpene, down-regulates fatty acid synthesis by inhibiting SREBP-1c. Additionally, studies demonstrate that triterpene from Yerba Mate tea exerts anti-hypolipidemic effects by up-regulating expression of PPAR-α [17]. These findings imply that triterpenoids are resources for the discovery of lipid-lowering drug.

Pedunculoside (PE), structurally a novel triterpene saponin as shown in Fig. 1A, is a main bioactive component isolated from Jiubiying. Jiubiying is dried bark of *Ilex rotunda* Thunb and used as

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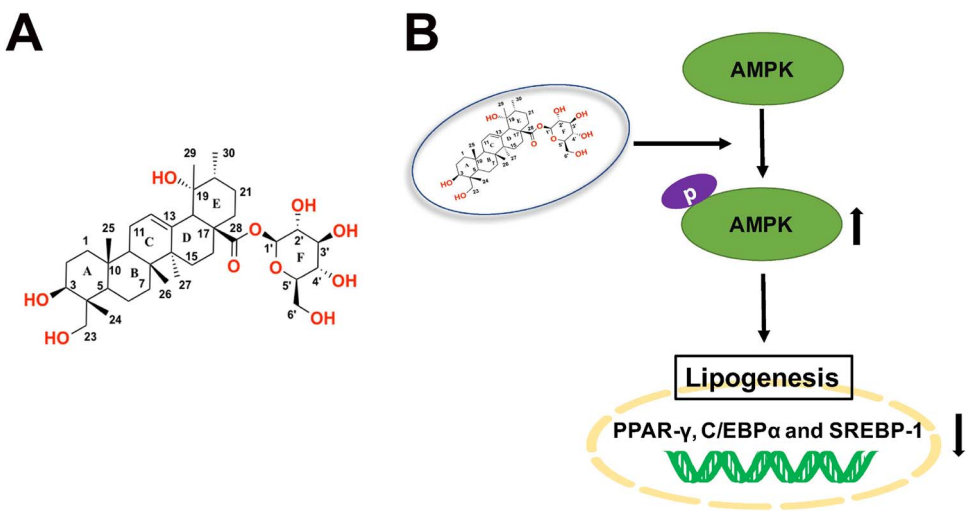


**Fig. 1.** The chemiacl structure of Pedunculoside (PE) and its proposed mechanisms.
(A) The chemical structure of PE, (B) Effects of PE on lipid mechanism by AMP-regulated protein kinase (AMPK). AMPK activation suppresses lipogenesis, leading to the decreases of gene including PPAR-γ, C/EBPα, and SREBP-1.

traditional Chinese medicine for CVDs therapy [13]. The present study aims to explore lipid-lowering property of PE. Furthermore, based on critical roles of PPAR-α and SREBP-1 in lipid metabolism, we hypothesize that PE may exert its lipid-lowering effects partly through the regulation of lipogenesis and fatty acid β-oxidation (Fig. 1B).

## 2. Materials and methods

### 2.1. Ethics statement

All animal experiments performed in this study were approved by the Institutional Animal Care and Use Committee of Yangzhou University and handled following the International Animal Ethics Committee Guidelines, ensuring minimum animal suffering.

### 2.2. Chemical, antibody, and reagents

Pedunculoside (PE) (purity > 99%) was obtained from Anshi Pharmaceutical CoLtd (Zhongshan, China). Simvastatin was purchased from Hangzhou Moshadong Pharmaceutical Co., Ltd (Hangzhou, Zhejiang, China).

Polyclonal antibodies against SREBP-1, C/EBPα, PPAR-γ, AMPK and phospho-AMPK were purchased from Wanlei Bio Technology Co., Ltd (Shenyang, China). 3-Isobutyl-1-methylxanthine and dexamethasone were purchased from Sigma-Aldrich Chemical Co (St. Louis, MO, USA). 3-[4,5-dime- thylthylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) and Dimethyl sulfoxide (DMSO) were purchased from Beyotime Biotechnology Co., Ltd (Haimen, Jiangsu, China). Insulin was purchased from Biosharp Biotechnology Co., Ltd (Hefei, China). All other chemicals reagents were all of the analytical purity and purchased from Sinopharm Chemical Reagent Co.,Ltd (Shanghai, China).

Total cholesterol (TC), triglyceride (TG), low-density lipoprotein-cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) reagents kits were purchased from JianCheng Bioengineering Institute (Nanjing, China).

### 2.3. Animals and cell lines

Forty-eight male SD rats, 5–6 weeks, weighting 150 ± 10 g, were purchased from Experimental Animal center of Nantong University (Laboratory animal certificate : SCXK(Su)20140001) and acclimatized under standard conditions for laboratory animals: a temperature of 23 ± 2 °C, a relative humidity of 50 ± 5%, and a 12 h light/dark cycle.

Mouse 3T3-L1 preadipocytes were obtained from Chinese Academy of Sciences Cell Bank of Type Culture Collection (Shanghai, China) and maintained in DMEM (HyClone, Logan, UT, USA) containing 10% fetal bovine serum (FBS) (Wisent, Quebec, Canada), 100 units/ml penicillin, and 100 μg/ml streptomycin at 37 °C in the presence of 5% $CO_2$.

### 2.4. Induction of hyperlipidemia and therapy

To generate the hyperlipidemia model, rats were fed with high-fat diet (Xietong Medical Bioengineering Co., Ltd., Nangjing, China) composed of 66.5% basal diet, 10% lard, 10% yolk powder, 10% sugar, 0.5% bile salts and 3% cholesterol. The blank control group was fed with normal food made up of 50% corn, 20% wheat bran, 15% soybean, 10% wheat flour and 5% fishmeal, and an appropriate amount of sodium chloride (g/g).

After one week adaptive feeding, forty-eight rats were randomly divided into six groups (n = 8 per group). All drugs and the vehicle were given intragastrically at 8:00 a.m. and the administrated volumes are 1 ml to avoid affecting food consumption. In the blank control group, the rats were fed with normal diet and administered with 0.5% sodium carboxymethylcellulose (CMC-Na) solution. In model group, the rats were fed with the high-fat diet and administered with 0.5% CMC-Na solution. In the positive control group, the rats were fed with the high-fat diet and administered with simvastatin (Sim) at a dose of 10 mg/kg/d. In the test-drug group, the rats were fed with the high-fat diet and administered with PE at doses of 30, 15, or 5 mg/kg/d. The experimental period was 7 weeks.

Dynamic serum lipid monitoring was performed from 4th to 7th weeks. Blood was collected from the retro-orbital venous plexus under light ether anesthesia using a glass capillary tube after overnight fasting (only food but not water was withheld overnight). At the end of the experimental period, all rats fasted prior to conducting the experiment. Blood samples were collected from the retro-orbital plexus and centrifuged at 3000*g* for 15 min using Heraeus sepatech centrifuge (Eppendorf) to separate serum for the measurement of serum lipid parameters.

### 2.5. Analyses of serum lipid profiles

The concentrations of TC, TG, HDL-C, and LDL-C in serum were determined using commercial kits according to the manufacturer's instructions (JianCheng Bioengineering Institute, Nanjing, China).