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# The antihypercholesterolemic effect of columbamine from *Rhizoma Coptidis* in HFHC-diet induced hamsters through HNF-4α/FTF-mediated CYP7A1 activation

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ABSTRACT

The aim of this study was to investigate the antihypercholesterolemic activity and potential molecular mechanism of columbamine (COL) that was prepared by extraction from *Rhizoma Coptidis* in hamsters and HepG2 cells. The results displayed that the COL from *Rhizoma Coptidis* was a safe natural compound with a $LD_{50}$ 0f 1524.6 mg/kg and no detectable toxic symptoms during the observation of chronic toxicity. COL dose-dependently reversed the abnormal lipid levels induced by HFHC diet. Specifically, COL(M) and COL(H) significantly reduced the blood lipid levels(TC, TG and LDL-c) and enhanced the fecal contents of TBA by 21.8% and 25.1% respectively in hamsters. COL up-regulated the genes of CYP8B1, CYP7A1 and LDLR in mRNA and protein level, and down-regulated those of HMGCR to a different degree. Especially, CYP7A1 were significantly up-regulated by COL in hamsters ($p < 0.01$). Further analysis indicated that COL obviously activated the mRNA and protein expression of the transcription factors FTF, HNF-4α, and inhibited those of SHP. Promoter luciferase assay showed that COL induced the expression of FTF and HNF-4α, further transactivating CYP7A1, which accelerated the conversion of liver cholesterol to bile acids. It concluded that the COL showed high lipid-lowering activities through indirectly transactivating CYP7A1 by upregulating FTF and HNF-4α, and directly activating CYP7A1 catalytic activity by strongly interacting with receptor and ligand, therefore promoting cholesterol catabolism and accelerating the excretion of bile acids.



## 1. Introduction

Long-term HFHC diet can cause hyperlipidemia or hypercholesterolemia, both of them are primary risk factors contributing to the development of cardiovascular diseases, which is associated with higher levels of total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-c) and lower levels of high-density lipoprotein cholesterol (HDL-c) [1,2]. Increasing evidences suggest that controlling the level of TC can interfere with the progression of atherosclerosis and reduce cardiovascular events [3,4].

To control the cholesterol homeostasis in hypercholesterolemia animals, the most common-used ways involved in regulating expressions of key genes or the activity of key enzymes in cholesterol metabolism. Such as 3-hydroxy-3-methyl glutaryl coenzyme A reductase (HMGCR) is the key enzyme involved in cholesterol biosynthesis [5,6], low-

*Abbreviations:* COL, columbamine; TC, total cholesterol; TG, triglyceride; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; TBA, total bile acids; RC, *Rhizoma Coptidis*; HPLC, high-performance liquid chromatography; HMGCR, 3-hydroxy-3-methyl glutaryl coenzyme A reductase; LDLR, low-density lipoprotein receptor; ASBT, apical sodium-dependent bile salt transporter; APOA4, apolipoprotein A4; CYP8B1, sterol 12-hydroxylase; CYP27A1, mitochondrial sterol 27-hydroxylase; CYP7A1, cholesterol 7α-hydroxylase; FXR, farnesoid X receptor; SREBP2, sterol regulatory element-binding protein 2; SHP, atypical nuclear small heterodimer partner; HNF-4α, hepatocyte nuclear factor 4-alpha; FTF, fetoprotein transcription factor; HC, high cholesterol; HFHC, high fat and high cholesterol; Sim, simvastatin; NC, normal control group; COL(L), columbamine at low dosage; COL(M), columbamine at medium dosage; COL(H), columbamine at high dosage.

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density lipoprotein receptor (LDLR) plays a vital role in the endocytosis of cholesterol [7,8], apolipoprotein A4(APOA4) can promoted the transportation of cholesterol from extrahepatic tissues to the liver [9], and so on.

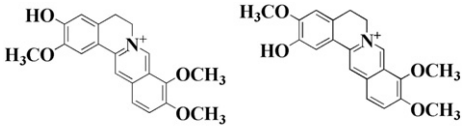
Especially, Bile acid synthesis plays a critical role in the maintenance of mammalian cholesterol homeostasis [10]. The main bile acid biosynthetic pathway is initiated by cholesterol 7α-hydroxylase (CYP7A1), a P450 isozyme of the CYP7A family. CYP7A1 is depressed by bile acid feedback and is activated by dietary factors such as cholesterol in rodents [11]. Recently, many studies have focused on the nuclear factors that may mediate the effect of bile acids on the gene expression of CYP7A1. The CYP7A1 gene may be regulated in several ways. One mediator is atypical nuclear small heterodimer partner (SHP), an atypical, orphan member of the nuclear receptor family that lacks a DNA-binding domain. SHP in turn binds to fetoprotein transcription factor (FTF) and hepatocyte nuclear factor 4-alpha (HNF-4α) which are inactivated resulting in a loss of activation of the CYP7A1 gene [12]. Additional reports associated those transcription factors action with CYP7A1 of the enterohepatic BA recycling system and with pancreatic cholesterol esterase, all of which pointing to an important systemic role in cholesterol metabolism [12,13].

Currently, statins was widely used for the treatment of cardiovascular diseases, but these drugs have showed badly side effects [14]. Hence, intense research efforts have concentrated on the progression of natural hypolipidemic drugs. *Rhizoma Coptidis* has been used for more than two thousand years by Chinese medicinal physicians for their antihyperglycemic and hypolipidemic effects [15]. It is demonstrated that the main active compounds in *Rhizoma Coptidis* are alkaloids, including berberine (BBR), coptisine (COP), palmatine (PAL), jatrorrhizine (JAT), columbamine (COL), and et al. [16]. The previous study suggested that BBR was defined as a promising lipid-lowering drug mainly by up-regulating LDLR expression through improving mRNA stability [17]; COP decreased cholesterol biosynthesis by down-regulating mRNA and expression of HMGCR [18]; PAL could decrease cholesterol by improving cholesterol transport through up-regulating LDLR mRNA and protein expression and decreasing re-uptake of cholesterol and bile acids in intestinal tract through inhibiting down-regulating the expression of ASBT [19]; JAT boosted up the conversion of cholesterol into bile acids to decline the accumulation of cholesterol in blood [20]. Specifically, among them, COL owns a structure similar with JAT (Fig. 1). Therefore, we inferred that COL possibly has cholesterol-lowering efficacy. Although COL exhibited anti-proliferative effects on metastatic human osteosarcoma U2OS cells with low toxicity [21], there are few reports on whether COL possessed antihypercholesterolemic effect in the body up to now. Therefore, in this study it was conducted to investigate hypocholesterolemic activity and its underlying mechanism of COL.

## 2. Material and methods

### 2.1. Materials

References columbamine (COL) and simvastatin (Sim) were supplied by Chengdu Must Bio-technology Co., LTD (Chengdu, China). COL (>98.0% by HPLC) used in animal and cell experiments was extracted from RC by an established procedure in our lab, identified by melting points, $^{1}H$ and $^{13}C$ NMR, and the data were listed in the Supplementary material 1 and stored in −80 °C before used. Cholesterol, methylthiotetrazole (MTT) and dimethyl sulfoxide (DMSO) were obtained from Sigma Chemicals (St Louis, MO). Commercial kits for analysis of TC, TG, LDL-c and HDL-c were supplied by Huili Biotechnology Co., Ltd. The antibodies were purchased from Proteintech group, Inc. (Wuhan, China).




Jatrorrhizine Columbamine

**Fig. 1.** The structure of JAT and COL.

### 2.2. Animals

The Kunming mice of both genders (20 ± 2 g) for acute toxicity (SYXK-YU 2007–0003) and the Sprague Dawley (SD) rats of both genders (200 ± 20 g) for sub-chronic toxicity (SYXK-YU 2007–0005) were approved by the Laboratory Animal Center of the Chongqing Medical University. Male Syrian golden hamsters (100 ± 5 g) for the research of cholesterol metabolism were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. (SCXK 2012–0001). Subsequently, all animals were kept in an air-conditioned room with a 12 h light and dark cycle with free access to food and water one week to accommodate the environment before experiments. The handling and procedures were conducted according to the national institution's guidelines for the use and care of laboratory animals.

### 2.3. Safety assessment of COL

#### 2.3.1. Acute toxicity

In accordance with previous method, Kunming mice were randomly divided into 8 groups with 10 mice per group (half of males and females) to test the acute toxicity of coptisine [22]. COL dissolved in distilled water was administered orally at the eight doses (400, 600, 900, 1350, 2025, 3037.5, 4556.3 and 6834.4 mg/kg). Cumulative mortality within one week after the treatment was used for the calculation of LD50 value.

#### 2.3.2. Sub-chronic toxicity

Forty SD rats were divided into the control and COL groups (half genders each one). The study was used the human equivalent dose (HED) with the body surface area (BSA) normalization method to calculate animal dosage [23]. Preliminary test to determine range of sub-lethal doses of coptisine on SD rats (154 mg/kg·day) was carried out using the maximum HED of coptisine (25 mg/kg) for an adult (60 kg) as a reference [24]. A vehicle-control group formed by 10 rats consumed the same volume of 0.9% saline. All animals were observed once daily for general appearance and behavior, twice daily for the clinical signs of toxicity and mortality. Besides, their body weight was recorded every 10 days.

At the end of treatment period, the hematological parameters were collected including alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), total protein (TP), albumin (ALB), globulin (G), albumin/globulin ratio (A/G), total bilirubin (TBIL), blood urea nitrogen (BUN), creatinine (CREA),potassium (K), sodium (Na) and chloride (Cl). Moreover, the hearts, livers, spleens, lungs, kidneys, stomachs and brains of the rats were removed, weighed and portions fixed for pathological examination.

### 2.4. Serum and feces lipid metabolism analysis in hamsters

Syrian golden hamsters were divided into six diet regimen groups: a control diet consisting of normal food; a HFHC diet in combination with simvastatin(1.2 mg/kg·day);a HFHC diet a HFHC diet in combination with one of three COL dosages (23.35, 46.7 or 70.05 mg/kg·day), and each group contained 10 hamsters. The food for the HFHC was prepared by mixing pellets from the normal diet with 10% lard, 10% egg yolk powder and 1% cholesterol [17]. The diet intake and body weight of each animal were recorded throughout the course of the experiment. Feces of each hamster were collected at the 3rd day prior to termination of the study for the analysis of cholesterol and bile acids. At the end of the period, the feces of each animal were collected for the analysis of