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Effects of *Morus* root bark extract and active constituents on blood lipids in hyperlipidemia rats

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Chemical compounds studied in this article:
Mulberrofuran C (PubChem CID: 157143)
Sanggenon G (PubChem CID: 44108701)
Moracin O (PubChem CID: 14539883)
Moracin P (PubChem CID: 14539884)

ABSTRACT

Objective: Chinese crude drug Mori Cortex Radicis (the root cortex of *Morus* species) has been used as a folk medicine to treat hypertension, diabetes, as well as in expectorant, diuretic agents. This investigation aims to study the anti-hyperlipidemia effects of Mori Cortex Radicis (MCR) extracts in hyperlipidemic rat models and the potential therapeutic activities of compounds isolated from the extracts.

Materials and methods: The effects of MCR on hypolipidemic parameters were investigated using Wistar rats induced by high-lipid emulsion. Sixty healthy Wistar rats were randomly divided into 6 groups: normal group, hyperlipidaemia model group, simvastatin, and high-, medium- and low-dose MCR extracts. After four weeks, body weight, total cholesterol (TC), triglycerides (TG), high and low-density lipoproteins (HDL, LDL), as well as aspartate aminotransferase (AST), alanine aminotransferase (ALT) were measured. To further investigation, four major active compounds were isolated from extracts through high performance liquid chromatography (HPLC) and their diacylglycerol acyltransferase 1 (DGAT1) inhibitory activity was evaluated.

Results: MCR dose-dependently reduced serum TC, TG, LDL-C, inhibited the activity of ALT, AST, and increased HDL-C. Furthermore, *in vitro* biochemistry tests revealed that four active isolates showed moderate inhibitory activity against DGAT1 with IC_{50} values ranging from 62.1 ± 1.2 to 99.3 ± 2.3 μM.

Conclusions: The results demonstrated that MCR could effectively ameliorate hyperlipidaemia and inhibit DGAT1 that a key enzyme closely related to hyperlipidaemia and type 2 diabetes. It may provide a new pharmacological basis for treating hyperlipidaemia and related diseases using MCR.

1. Introduction

Globesity, a term coined by WHO to warn us the phenomemon that the global epidemic of obesity, is a serious health risk that is presently rarely curable (Deitel, 2002). Excessive intake of dietary fat can be an important contributing factor to both obesity and hyperlipidemia. Obesity is closely related to several of metabolic diseases such as diabetes, liver diseases, atherosclerosis and hypertension (Hirabara et al., 2007; Kopelman, 2000). Moreover, it is well known that obesity induced by high cholesterol diets and high fat may be responsible for nonalcoholic fatty liver disease (NAFLD). The fight against such diseases mainly through three aspects: better eating habits, better exercise and drugs. It is commonly accepted that treating with drugs is a far more effective strategy for combating this chronic disease. Therefore, efforts to discover effective drugs and study the anti-obesity therapeutics have been intensified. One of potential therapeutics studied in recent years is inhibiting synthesis of triacylglycerol (TG). Although TG is essential for normal physiology, excessive TG accumulation results in obesity and related diseases. Inhibiting synthesis of TG may ameliorate obesity and other cardiovascular diseases (Peizhong et al., 2008). Diacylglycerol acyltransferase (DGAT), found in a series of physiological and pharmacological experiments, is an important enzyme in TG synthesis. DGAT acylate 1,2-diacyl-*sn*-glycerol and form the triacyl-snglycerol in the last step of synthesis of TG. Two DGAT enzymes, DGAT1 and DGAT2, have been identified and both enzymes are ubiquitously expressed. However, only DGAT1 is regarded as a key enzyme that responsible for synthesis of TG (Cases et al., 2001; Zhou et al., 2014). A mass of studies and investigations proved that pharmacological inhibition of DGAT1 may be a feasible therapeutic

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strategy for obesity and type 2 diabetes (Nakada et al., 2010).

Mori Cortex Radicis, the root bark of *Morus alba* L and other plants of genus *Morus* (Moraceae), has been traditionally used for the treatment of diabetes, hypertension, arthritis, and hyperlipidemia (Li et al., 2011; Yang et al., 2015). *Morus* is widely cultivated in China, Korea and Japan. It is well known as a rich resource of biologically active components. Chemical and pharmacological experiments have resulted in the isolation of a wide range of compounds such as prenylated flavonoids, coumarins, stilbenes, and Diels-Alder type adducts (Lee et al., 2012; Wang et al., 2015). In this study, we investigated the bioactivity-guided fraction and isolated four active compounds. In order to further clearly identify which compound is effect on dyslipidemia, we tested DGAT1 inhibitory activity of the compounds. Moreover, a series of *in vivo* anti-obesity experiments of Mori Cortex Radicis bioactive extracts were completed. The parameters of anti-obesity of Mori Cortex Radicis extracts was evaluated by using high fat emulsion induced obese rats (Umar et al., 2005). Characterization of active compounds was accomplished by spectroscopic and physico-chemical analyses and compared with literature data.

The following is abbreviation existed in this paper: Mori Cortex Radicis (MCR); nonalcoholic fatty liver disease (NAFLD); Total cholesterol (TC); Triglycerides (TG); High-density lipoproteins (HDL); Low-density lipoproteins (LDL); Aspartate aminotransferase (AST), Alanine aminotransferase (ALT); High performance liquid chromatography (HPLC); Diacylglycerol acyltransferase 1 (DGAT1); tyrosine phosphatase 1B (PTP1B); hydroxy methylglutaryl coenzyme A (HMG-CoA); Ethylene Diamine Tetraacetic Acid (EDTA); STE (Sucrose, Tris–HCl, EDTA).

2. Materials and methods

2.1. Plant materials

The root bark of *Morus alba* L was collected in Shangqiu, Henan province, People's Republic of China, and authenticated by Professor Gao Li (College of Pharmacy, Yanbian University). A voucher specimen of the plant (No. 20121006) was deposited at the College of Pharmacy, Beihua University, Jilin, People's Republic of China.

2.2. Extraction and isolation of compounds using high performance liquid chromatography (HPLC)

The dried *Morus* root bark (6.0 kg) was extracted with MeOH (6.0 L) at room temperature for 24 h and the solution was concentrated to obtain a crude extract (1.8 kg, yield: 30.0%). This extract was suspended in H_2O, partitioned successively with hexane and EtOAc. The EtOAc fraction (20.0 g) was subjected to the RP-18 column (40.0×3.5 cm^2) and using the elution of MeOH/H_2O (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 to 1:0) to afford nine fractions (Fr.1-Fr.9). Furthermore, Fr.6 (3.1 g) having DGAT inhibitory activity was investigated. Part of Fr.6 (90.0 mg) was applied to C_{18} reversed-phase HPLC column (10×250 mm^2, 10 μm) and eluted with a gradient of MeOH–H_2O (60:40–85:15) at a flow rate of 2.0 mL/min. This resulted in the isolation of four compounds, compound **1** (7.9 mg), compound **2** (24.2 mg), and compound **3** (8.6 mg), compound **4** (7.4 mg) (see Fig. 1).

2.3. Reagents and solvents

Methanol (MeOH), ethyl acetate (EtOAc), hexane, and HPLC grade, were purchased from Sinopharm Chemical Reagent Co.,Ltd. Total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL), highdensity lipoprotein cholesterol (HDL), alanine transaminase (ALT), aspartate transaminase (AST) were all purchased from Nanjing Jiancheng Bioengineering Institute. Simvastatin was the product of MSD pharmaceutical company.

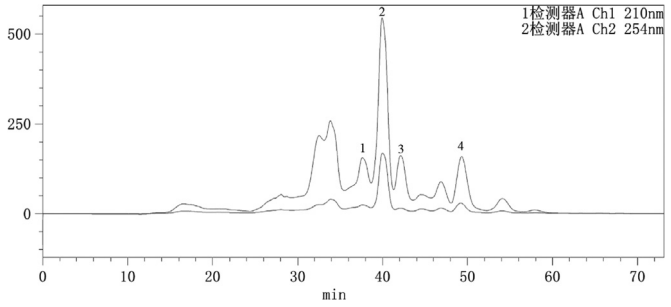

Fig. 1. HPLC spectrum of MCR extracts: 1. Mulberrofuran C; 2. Sanggenon G; 3. Moracin O; 4. Moracin P. HPLC conditions: stationary phase: Shimadzu C_{18} column (10×250 mm^2, 10 μm particle size), Shimadzu, Tokyo, Japan; mobile phase: methanol (A) and H_2O (B) in gradient (0 min, A:B=60:40, 60 min, A:B=85:15); flow rate: 2 mL/min; detective wavelength: 210 nm; temperature: 30 °C.

2.4. Equipment

Hitachi 7600 automated biochemistry analyzer (Hitachi Instruments Co., Ltd), Tecan infinite M200 microplate reader (Tecan Trading Co., Ltd), TG16-WS centrifuge (Shanghai Lu Xiangyi Centrifuge Instrument Co., Ltd), HPLC were carried out using a Shimadzu System LC-6AD pump equipped with a model SPD-20Avp UV detector (Shimadzu, Tokyo, Japan), and an Optima Pak® C_{18} column (10×250 mm^2, 10 μm particle size, Shiseido Fine Chemicals, Tokyo, Japan).

2.5. Experimental animals and ethical aspects

Wistar rats, male, weighing 180–200 g and mice ICR, male, weighting 18–20 g, were purchased by Changchun Yisi Animal Technology Co., Ltd as laboratory animals, certificate number: SCXK 2011-0004. All animals were housed under the standard laboratory conditions (12 h light-dark cycles with controlled temperature, 23 ± 1 °C), Diet and water were provided ad libitum. After the experiments, all animals were anesthetized and blood, organs were collected. The protocol for this study involving animals and their maintenance were approved by Animal Care and Use Committee of Korea Research Institute of Bioscience and Biotechnology (Approved no. KRIBBA-371-13-01).

2.6. In vivo anti-obesity effect of Morus alba L extracts

2.6.1. Animals groups and models

To introduce hyperlipidaemia models, rats were given intragastric of 10 mL/kg of high fat emulsion in the morning for 21 days. High fat emulsion was prepared as published methods (Aslan et al., 2010; Zhao et al., 2012): In brief, the mixture was initiated by the addition of 20 g of lard and 10 g of cholesterol to a 200 mL beaker, then heated and molten (Abliz et al., 2014; Zanwar et al., 2014). 1 g of propacil was mixed to use as oil phase after fully dissolved; water phase was made by adding 30 ml of distilled water and 2 g of sodium deoxycholate into another 200 mL beaker, then 10 ml of Tween-80 and 10 mL of propylene glycol were blended; next, the water phase was slowly transferred into the oil phase and then added distilled water to 100 mL to produce the high fat emulsion.

Healthy adult male Wistar rats were randomly divided into six experimental groups of ten rats: normal group, hyperlipidaemia model group, low (40 mg/kg), medium (80 mg/kg) and high dosage (160 mg/kg) of MCR extracts and Simvastatin groups (5 mg/kg). The normal group was given the same amount (10 mL/kg) of