Pharmacological Research 99 (2015) 74–81

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Dihydromyricetin improves glucose and lipid metabolism and exerts anti-inflammatory effects in nonalcoholic fatty liver disease: A randomized controlled trial

Shihui Chen[a,c], Xiaolan Zhao[b], Jing Wan[a], Li Ran[a], Yu Qin[a], Xiaofang Wang[b], Yanxiang Gao[a], Furong Shu[a], Yong Zhang[a], Peng Liu[a], Qianyong Zhang[a], Jundong Zhu[a,*], Mantian Mi[a,*]

[a] Research Center of Nutrition and Food Safety, The Third Military Medical University, Chongqing Key Laboratory of Nutrition and Food Safety, Chongqing 400038, PR China
[b] Health Care Center of Southwest Hospital (the First Affiliated Hospital of the Third Military Medical University), Chongqing 400038, PR China
[c] Center of Preventive Treatment of Disease, Foshan Hospital of Traditional Chinese Medicine, Foshan 528000, PR China

ARTICLE INFO

Article history:
Received 5 January 2015
Received in revised form 21 May 2015
Accepted 21 May 2015
Available online 30 May 2015

Chemical compounds studied in this article:
Dihydromyricetin (PubChem CID: 161557)
Ampelopsin (PubChem CID: 161557)

Keywords:
Dihydromyricetin
Nonalcoholic fatty liver disease
Insulin resistance
Cytokeratin-18 fragment
Fibroblast growth factor 21

ABSTRACT

Ampelopsis grossedentata, a medicinal and edible plant, has been widely used in China for hundreds of years, and dihydromyricetin is the main active ingredient responsible for its various biological actions. We investigated the effects of dihydromyricetin on glucose and lipid metabolism, inflammatory mediators and several biomarkers in nonalcoholic fatty liver disease. In a double-blind clinical trial, sixty adult non-alcoholic fatty liver disease patients were randomly assigned to receive either two dihydromyricetin or two placebo capsules (150 mg) twice daily for three months. The serum levels of alanine, aspartate aminotransferase, γ-glutamyl transpeptidase, glucose, low-density lipoprotein-cholesterol and apolipoprotein B, and the homeostasis model assessment of insulin resistance (HOMA-IR) index were significantly decreased in the dihydromyricetin group compared with the placebo group. In the dihydromyricetin group, the serum levels of tumor necrosis factor-alpha, cytokeratin-18 fragment and fibroblast growth factor 21 were decreased, whereas the levels of serum adiponectin were increased at the end of the study. We conclude that dihydromyricetin supplementation improves glucose and lipid metabolism as well as various biochemical parameters in patients with nonalcoholic fatty liver disease, and the therapeutic effects of dihydromyricetin are likely attributable to improved insulin resistance and decreases in the serum levels of tumor necrosis factor-alpha, cytokeratin-18, and fibroblast growth factor 21.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the most frequent chronic liver diseases worldwide. It represents a wide spectrum of hepatic disorders, ranging from simple hepatic steatosis (SHS) to nonalcoholic steatohepatitis (NASH), liver cirrhosis and sometimes even hepatocellular carcinoma [1]. The prevalence of NAFLD is markedly increasing among obese adults and children around the world [2,3]. NAFLD is estimated to affect 10–24% of the general population in various countries [4]. In China, the community prevalence of NAFLD is approximately 15% [5]. Currently, weight reduction *via* dietary calorie restriction, physical activity and other lifestyle modifications is the only proven effective therapeutic strategy for NAFLD [6]. Given the poor compliance with weight reduction exhibited by NAFLD patients, pharmacological interventions have become an important strategy for the treatment of NAFLD.

Although the precise mechanisms underlying NAFLD are not yet fully understood, the "multiple hit" hypothesis is a widely accepted concept for the pathogenesis of NAFLD [7]. The first hit

Abbreviations: ANCOVA, one-factor analysis of covariance; ALT, alanine aminotransferase; ALP, alkaline phosphatase; APN, adiponectin; Apo, apolipoprotein; AST, aspartate aminotransferase; BMI, body mass index; CVs, coefficient variations; CK-18, cytokeratin 18; DHM, dihydromyricetin; ELISA, enzyme-linked immunosorbent assay; FGF21, fibroblast growth factor 21; GGT, γ-glutamyl transpeptidase; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; IQR, interquartile range; IR, insulin resistance; LDL-C, low-density lipoprotein cholesterol; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; SHS, simple hepatic steatosis; TC, total cholesterol; TG, triglycerides; TNF-α, tumor necrosis factor alpha.

* Corresponding authors. Tel.: +86 23 68752305; fax: +86 023 68752305.
E-mail addresses: zjdnfs@126.com (J. Zhu), mimantian@hotmail.com (M. Mi).

http://dx.doi.org/10.1016/j.phrs.2015.05.009
1043-6618/

is linked to the accumulation of hepatic fat, which is mainly due to insulin resistance (IR) [8]. The subsequent hits include oxidative stress, mitochondrial dysfunction, lipid peroxidation, and the release of inflammatory mediators [9]. Despite the lack of currently approved therapeutic drugs for NAFLD, insulin sensitizers, antioxidants, and anti-inflammatory agents have been demonstrated to be beneficial for the treatment of NAFLD. The efficacy of these drugs suggests that dietary flavonoids, which exert anti-oxidative and anti-inflammatory activity, may also constitute promising treatment options for NAFLD.

Ampelopsis grossedentata is a medicinal and edible plant that is widely distributed in southern China. Its tender stem and leaves have been consumed as a medicinal tea (Tengcha) for the prevention and treatment of the common cold, sore throat, and icteric viral hepatitis for hundreds of years. Dihydromyricetin(DHM), also called ampelopsin, is the most abundant flavonoid in *A. grossedentata*; the contents of DHM in its leaves exceeds 30% [10]. DHM has been reported to exert a number of biological and pharmacological actions, including anti-oxidative, anti-inflammatory, hepatoprotective, lipid and blood glucose regulatory, and anticancer effects [11,12]. Li found that the intragastric administration of *A. grossedentata* reduces the serum levels of total cholesterol (TC) and triglycerides (TG) and increases the serum high-density lipoprotein cholesterol (HDL-C) levels in hyperlipidemia rats, indicating that ampelopsin exerts a hypocholesterolemic effect [13]. Chen et al. [14] also found that Tengcha flavones and DHM decrease the serum TC, TG and LDL-C contents, increase the HDL-C levels and ameliorate the denaturation of liver cells. Thus, Tengcha flavones and DHM may prevent the harmful effects of hyperlipemia on the liver. Similarly, DHM is also an effective treatment for high blood glucose levels. Qin et al. [15] showed that the oral administration of 0.25 g/kg/d and 0.125 g/kg/d DHM can reduce the blood glucose levels in alloxan-induced diabetic or hyperglycemic mice. Moreover, our previous studies showed that DHM can improve physical performance under simulated high altitude conditions by preserving mitochondrial function in skeletal muscle [16] and ameliorate insulin resistance in skeletal muscle by regulating autophagy both *in vitro* and *in vivo* [17].

Because the pathogenesis of NAFLD includes insulin resistance, oxidative stress, mitochondrial dysfunction and inflammation in the liver, the anti-oxidative, hepatoprotective, lipid and blood glucose regulatory and anti-inflammatory properties of DHM and based on the findings of our previous studies, we hypothesized that DHM may be a potential therapeutic agent for patients with NAFLD. To clarify this hypothesis, a double-blind, randomized, placebo-controlled clinical trial was conducted to determine whether supplementation with DHM improves hepatic steatosis and the biochemical parameters of NAFLD patients. We also sought to elucidate some of the mechanisms underlying the action of DHM.

2. Methods

2.1. Study design

This study was a randomized, double-blind, placebo-controlled trial. When an eligible patient was recruited into the study, an investigator who was not involved in the trial used Excel's random number generator to generate a number that would determine whether a patient would be treated with placebo or DHM. If the last digit was an even number, the patient was assigned to the control group, whereas if the last digit was an odd number, the patient would be included in the DHM group. Both the researchers and patients were blinded to the randomization and will be un-blinded at the end of the study.

2.2. Subjects

Community-dwelling subjects that were diagnosed with NAFLD through a B-mode ultrasound at the Southwest First Affiliated Hospital of the Third Military Medical University (Chongqing, China) were enrolled for three months of treatment. The study was conducted from October 2012 to February 2013. All of the patients were aged 20–60 years, had a body mass index (BMI) greater than 20 but less than 30, a fasting blood-glucose level <7.8 mmol/L, and no weight gain or loss over the last three months, and did not receive any medical therapy. The exclusion criteria included the following: (1) excessive alcohol consumption (more than 140 g/week for men and 70 g/week for women); (2) a history of viral hepatitis, autoimmune hepatitis or other liver diseases; (3) taking any medicine that would influence glucose and lipid metabolism over the last six months; (4) liver or kidney dysfunction; or (5) malignant tumors. All of the participants provided written informed consent. This study was approved by the Medical Ethical Committee of the Third Military Medical University. All of the procedures complied with the Helsinki Declaration and current ethical guidelines. The clinical trial registration number is ChiCTR-TRC-12002377.

2.3. Materials

DHM powder was purchased from Zelang Medical Technological Co., Ltd. (Nanjing, China; CAS No. 27200-12-0) and was purified (purity ≥ 98%) from natural products by high-performance liquid chromatography (HPLC). The placebo and DHM capsules were identically packaged. The total content of DHM was 150 mg/capsule. The DHM capsules also contained pullulan and maltodextrin, whereas the placebo capsules only contained pullulan and maltodextrin.

The toxicity of DHM is very low, and the maximum tolerated dose in rats is 5–10 g/kg [18,19]. DHM, which was administered to mice by oral gavage at doses of 160 mg/kg, 320 mg/kg and 480 mg/kg, corresponding to 10-, 20- and 30-fold increases compared with the human dose, prevented alcohol-induced liver glutathione depletion and the malonaldehyde content increase, decreased the triacylglycerol level, and mitigated steatosis of the liver [18]. Based on the data from these previous studies, the dose of DHM used in this study was 600 mg/d, which was calculated using the body surface area normalization method, as described previously [20].

2.4. Interventions

The effect of DHM on patients with NAFLD has not yet been examined, but previous studies have found that the consumption of dietary flavonoids for two weeks to 12 months can improve the HOMA, LDL-C, HDL-C, diastolic blood pressure and other indexes in type 2 diabetes and cardiovascular diseases [21]. We hypothesized that the consumption of DHM for three months can improve glucose and lipid metabolism in NAFLD patients. Thus, the total duration of this trial was 12 weeks. All of the patients were administered two 150 mg capsules (placebo capsules or DHM capsules) twice daily. All of the subjects were advised to maintain their habitual diet and lifestyle during the intervention period. The participants were followed-up with a telephone call to obtain relevant information each month.

2.5. Experimental determinations

The patients' age, gender, race, health habits, and medical history were obtained using a questionnaire. Each participant underwent a clinical examination at baseline and of the end of intervention, and this examination included anthropometric