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Silybum marianum oil attenuates hepatic steatosis and oxidative stress in high fat diet-fed mice

Shu Yun Zhu[a,b,*,1], Ning Jiang[c,1], Jing Yang[a], Jie Tu[d], Yue Zhou[a], Xiang Xiao[a], Ying Dong[a]

[a] School of Food and Biological Engineering, Jiangsu University, Zhenjiang 212013, China
[b] Jiangsu Hengshun Group Co., Ltd., Zhenjiang 212000, China
[c] Institute of Vegetables, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China
[d] College of Biotechnology, Jiangsu University of Science and Technology, Zhenjiang 212018, China

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ABSTRACT

In the present study, the effects of *Silybum marianum* oil (SMO) on hepatic steatosis and oxidative stress were investigated during the development of nonalcoholic fatty liver disease (NAFLD) in high fat diet (HFD)-fed mice. The results showed that body weight, fat mass, and serum biochemical parameters such as triglyceride, free fatty acid, glucose and insulin were reduced by SMO treatment. Meanwhile, SMO decreased the histological injury of liver and the levels of hepatic triglyceride, cholesterol and free fatty acid in HFD-fed mice. SMO administration elevated the activities of superoxide dismutase (SOD) and catalase (CAT) and reduced the level of malondialdehyde (MDA) in the liver. Enzyme linked immunosorbent assay showed that SMO significantly decreased the levels of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) in HFD mice. Furthermore, the mRNA levels of sterol regulatory element binding protein 1c (SREBP-1c), fatty acid synthase (FAS) and liver X receptor α (LXRα) were lower, but peroxisome proliferator-activated receptor α (PPARα) was higher in mice treated with SMO compared with the HFD group. The results indicated that SMO could play a certain protective role against HFD-induced NAFLD, and the protective effects might be associated with attenuating lipid accumulation, oxidative stress and inflammation, improving lipid metabolism.

1. Introduction

Obesity is a metabolic state associated with hepatic abnormalities and increased risk of chronic diseases such as type 2 diabetes, cardiovascular diseases, and so on. Obesity is described as a major risk factor for nonalcoholic fatty liver disease (NAFLD) [1]. NAFLD is characterized by abnormal lipid deposition in the liver without alcohol consumption and is the most common and prevalent liver disease worldwide [2]. NAFLD is a continuum of hepatic injuries, which progress from simple hepatic steatosis to nonalcoholic steatohepatitis, fibrosis, and cirrhosis and also increase the risk of metabolic disorders [3,4]. Because of the high prevalence and its subsequent risk, NAFLD has be regarded as health and social problems which should be well treated [5].

The pathogenesis of NAFLD is complex, and the underlying mechanisms remain largely unknown. Various genetic and environmental factors (e.g., lipid peroxidation, insulin resistance, oxidative stress, inflammation, mitochondrial dysfunction and cellular apoptosis) contribute to the occurrence of NAFLD [3–7]. Nowadays, there are no effective pharmacological treatments for NAFLD. The methods for treating NAFLD involve rational diet, exercise, and pharmaceutical therapy (e.g., metformin, statins and fibrates). However, these drugs have some adverse effects or contraindications, and no consensus exists on the most effective drug therapies [4,5,8]. Thus, the beneficial effects of dietary supplements on NAFLD are gaining increasing attention. Recently, the effects of natural bioactive substances, including antioxidants, lipid-lowering agents, anti-inflammatory compounds, and insulin sensitizers, have been evaluated in many studies [8].

Silybum marianum L. Gaernt in family Asteraceae is one of the important hepatoprotective plant. The main active substance in *S. marianum* is silymarin, which is composed of silybin, isosilybin, silydianin, and silychristine [9]. *S. marianum* oil (SMO), a by-product of silymarin production, is rich in essential fatty acids, phospholipids, sterols, and vitamin E [10]. Most of the beneficial effects of oils that contain essential fatty acids, vitamin E, squalene, and phenolic compounds have been shown to be due to their antioxidant activity and their capacity to prevent lipid oxidation [11]. Furthermore, it has been found that fish oil, olive oil, canola oil, rice bran oil and other natural products and

* Corresponding author at: Shu Yun Zhu, School of Food and Biological Engineering, Jiangsu University, 301 Xuefu Road, Zhenjiang, Jiangsu 212013, China.
E-mail address: shyzhu@ujs.edu.cn (S.Y. Zhu).
[1] Contributed equally to this work and should be considered co-first authors.

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vegetable oil have the function of preventing or treating NAFLD [12–15]. Some studies have indicated that SMO has antioxidant effects *in vitro* and *in vivo*, and it can reduce plasma cholesterol and triglyceride levels, counteract the damaging effects of CCl_4-induced injury in hepatocytes [16–18]. We previously demonstrated that SMO had a protective effect in D-gal-induced aging mice. It could attenuate lipid peroxidation, modulate the expression of apoptosis-related factors, and alleviate mitochondrial damage [19]. The pathogenesis of NAFLD is closely related to obesity, oxidative stress, mitochondrial damage and apoptosis, so we hypothesized that SMO might improve NAFLD. However, the protective effects and underlying mechanisms of SMO on NAFLD have not been explored. The main objective of the present study was to evaluate the potential protective effects of SMO during the development of NAFLD in a mouse model of long-term HFD-induced obesity. We further investigated the potential mechanisms against NAFLD.

2. Materials and methods

2.1. Animal treatment

ICR mice (male, 2-month-old) were purchased and housed in an animal house at the Laboratory Animal Research Center of Jiangsu University, Zhenjiang, China. The license number of the mice was SCXK (SU) 2013-0011. During the entire experimental period, all the animals were allowed free access to diet and water. After acclimation for a week, the mice were randomized into 4 groups ($n = 10$/group). Control: mice were fed normal diet based on a commercial diet (Xietong Organism, Jiangsu,China) in conjunction with distilled water through intragastric administration for 8 weeks; HFD: mice were fed high fat diet (w/w, 70.5% normal diet + 10% lard + 10% yolk powder + 8% sucrose + 1.5% cholesterol) in conjunction with distilled water through intragastric administration for 8 weeks; SMO 5 mL/kg: mice were fed high fat diet for 8 weeks and intragastric administered with 5 mL/kg SMO every day; SMO 10 mL/kg: mice were fed high fat diet for 8 weeks and intragastric administered with 10 mL/kg SMO every day; SMO was extracted from *S. marianum* seed kernels by AY Mantianxue Food Manufacturing Co. LTD (Anyang, China). The chemical compositions of SMO were analyzed in our previous study [19]. All the experimental procedures were approved by the Laboratory Animal Management Committee of Jiangsu University and adhered to guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. At the end of the experiment, mice were fasted overnight, and then blood and liver samples were collected for biochemical and molecular biological determinations.

2.2. Liver morphological analysis

Small pieces of mice liver (5 × 5 mm) were fixed in Bouin solution, dehydrated in ethanol, cleared in toluene and embedded in paraffin. The liver sections were deparaffinized and processed routinely for hematoxylin-eosin (H&E) and then analyzed with a light microscopy (Olympus, Japan).

2.3. Lipid profiles in serum and liver

The serum were assayed for triglyceride (TG), total cholesterol (TC), high-density lipoproteins cholesterol (HDL-C), low-density lipoproteins cholesterol (LDL-C) levels using an Olympus AU2700 Clinical Chemistry Analyzer (Olympus Inc., Japan). The contents of free fatty acid (FFA) and TG, TC in liver were measured by commercial kits (Nanjing Jiancheng Institute of Biological Engineering, China) according the manufacturer's instructions.

2.4. Biochemical measurement

The levels of glucose in serum were assayed using an Olympus AU2700 Clinical Chemistry Analyzer (Olympus Inc., Japan). The levels of insulin (INS), tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were measured using enzyme-linked immunosorbent assay (ELISA) kits (Shanghai Enzyme-linked Biotechnology Co., Ltd., China) according the manufacturer's instructions. Homeostasis model assessment of insulin resistance index (HOMA-IR) was calculated as HOMA-IR = serum glucose (mmol/L) × serum insulin (mIU/L)/22.5.

2.5. Oxidative stress evaluation

Superoxide dismutase (SOD) and catalase (CAT) activities and malondialdehyde (MDA) levels were measured using commercial assay kits (Nanjing Jiancheng Institute of Biological Engineering, China) according the manufacturer's instructions.

2.6. RNA extraction and real-time PCR

Total RNA was extracted from liver tissues using Trizol reagent (Takara, Japan) following the manufacturer's directions, and cDNA was synthesized using a PrimeScript™ RT reagent Kit with gDNA Eraser (Takara, Japan) according to the manufacturer's instructions. For RT-PCR, each reaction was prepared according to the manufacturer's instructions using Go Taq qPCR Master Mix kit (Promega, USA). The reaction conditions were as follows: initial denaturation at 95 °C for 10 min followed by 40 cycles at 95 °C for 15 s, 60 °C for 30 s, 72 °C for 30 s, and final extension at 72 °C for 10 min. β-actin was used as internal reference during the whole procedure. The relative expression of mRNA was calculated using the $2^{-\Delta\Delta Ct}$ method [20]. The primer sequences used were shown in Table 1.

2.7. Statistical analysis

All the results were expressed as mean ± SD. Statistical analysis was performed using SPSS software package, version 17.0. The values were analyzed by one-way analysis of variance (ANOVA), followed by Duncan's multiple range test. $p < 0.05$ was considered to indicate statistical significance.

Table 1
Primer sequences used for RT-PCR.

mRNA	Forward Primer (5'-3')	Reverse Primer (5'-3')
PPARα	GGCGTTTCCTGAGACCCT	ATGTTGGATGGATGTGGC
LXRα	CTGATTCTGCAACGGAGTTGT	GACGAAGCTCTGTCGGCTC
SREBP-1c	TCTGGAGACATCGCAAACAAG	TGGTAGACAACAGCCGCATC
FAS	TGCACAGAAGGGAAGGAGTACATG	ACAGCCAGGAGAATCGCAGTAGA
β-actin	GATCTGGCACCACACCTTC	ATCTTTTCACGGTTGGCCTT