Journal of Ethnopharmacology 208 (2017) 24–35

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Silibinin Capsules improves high fat diet-induced nonalcoholic fatty liver disease in hamsters through modifying hepatic de novo lipogenesis and fatty acid oxidation

Chun-Xue Cui[a,b,c,d], Jing-Na Deng[a,c,d], Li Yan[a,c,d], Yu-Ying Liu[a,c,d], Jing-Yu Fan[a,c,d], Hong-Na Mu[e], Hao-Yu Sun[a,b,c,d], Ying-Hong Wang[f,*], Jing-Yan Han[a,b,c,d,**]

[a] *Tasly Microcirculation Research Center, Peking University Health Science Center, Beijing 100191, China*
[b] *Department of Integration of Chinese and Western Medicine, School of Basic Medical Sciences, Peking University, Beijing 100191, China*
[c] *Key Laboratory of Microcirculation, State Administration of Traditional Chinese Medicine of the People's Republic of China, Beijing 100191, China*
[d] *Key Laboratory of Statis and Phlegm, State Administration of Traditional Chinese Medicine of the People's Republic of China, Beijing 100191, China*
[e] *Department of Pharmacology, School of Basic Medical Sciences Peking University, Beijing 100191, China*
[f] *State Key Laboratory for Bioactive Substances and Functions of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China*

ARTICLE INFO

Keywords:
NAFLD
Silibinin capsules
PPARγ
CPT–1A
AMPKα

ABSTRACT

Ethnopharmacological relevance: Silibinin Capsules (SC) is a silybin-phospholipid complex with silybin as the bioactive component. Silybin accounts for 50–70% of the seed extract of *Silybum marianum* (L.) Gaertn.. As a traditional medicine, silybin has been used for treatment of liver diseases and is known to provide a wide range of hepatoprotective effects.

Aim of the study: High fat diet (HFD)-induced nonalcoholic fatty liver disease (NAFLD) is a worldwide health problem. This study was to investigate the role of SC in NAFLD with focusing on its underlying mechanism and likely target.

Materials and methods: Male hamsters (Cricetidae) received HFD for 10 weeks to establish NAFLD model. NAFLD was assessed by biochemical assays, histology and immunohistochemistry. Proton nuclear magnetic resonance spectroscopy and western blot were conducted to gain insight into the mechanism.

Results: Hamsters fed HFD for 10 weeks developed fatty liver accompanying with increased triglyceride (TG) accumulation, enhancing de novo lipogenesis, increase in fatty acid (FA) uptake and reducing FA oxidation and TG lipolysis, as well as a decrease in the expression of phospho-adenosine monophosphate activated protein kinase α (p-AMPKα) and Sirt 1. SC treatment at 50 mg/kg silybin and 100 mg/kg silybin for 8 weeks protected hamsters from development of fatty liver, reducing de novo lipogenesis and increasing FA oxidation and p-AMPKα expression, while having no effect on FA uptake and TG lipolysis.

Conclusions: SC protected against NAFLD in hamsters by inhibition of de novo lipogenesis and promotion of FA oxidation, which was likely mediated by activation of AMPKα.

Abbreviations: SC, Silibinin Capsules; HFD, High fat diet; NAFLD, nonalcoholic fatty liver disease; TG, triglyceride; FA, fatty acid; AMPK, adenosine monophosphate activated protein kinase; NASH, nonalcoholic steatohepatitis; PPARγ, peroxisome proliferator-activated receptor γ; SREBP-1c, sterol regulatory element binding proteins 1c; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; PPARα, peroxisome proliferator-activated receptor α; CPT-1, carnitine palmitoyltransferase 1; ACADL, acyl-CoA dehydrogenase, long chain; FABP, fatty acid binding proteins; HSL, hormonesensitive lipase; ATGL, adipose triglyceride lipase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; IR, insulin resistance; TC, total cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; HPLC, high performance liquid chromatography; ELISA, enzyme-linked immunosorbent assay; ^{1}H NMR, proton nuclear magnetic resonance spectroscopy; HE, hematoxylin and eosin

* Correspondence to: State Key Laboratory for Bioactive Substances and Functions of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, No.1 Xiannongtan Street, Beijing 100050, China.
** Correspondence to: Department of Integration of Chinese and Western Medicine, School of Basic Medical Sciences, Peking University, 38 Xueyuan Road, Beijing 100191, China.
E-mail addresses: zhao837176309@163.com (C.-X. Cui), Jingnadeng12@163.com (J.-N. Deng), Lily750957@163.com (L. Yan), yuyingliu903@126.com (Y.-Y. Liu), jingyuf@sina.cn (J.-Y. Fan), hongnamu@sina.com (H.-N. Mu), Haoyu720@126.com (H.-Y. Sun), wyh@imm.ac.cn (Y.-H. Wang), hanjingyan@bjmu.edu.cn (J.-Y. Han).

http://dx.doi.org/10.1016/j.jep.2017.06.030
Received 12 November 2016; Received in revised form 26 April 2017; Accepted 18 June 2017
Available online 23 June 2017

1. Introduction

High fat diet (HFD)-induced nonalcoholic fatty liver disease (NAFLD) is currently a worldwide health problem with prevalence in 40–90% of obese population. NAFLD is a spectrum of disorder ranging from benign steatosis to nonalcoholic steatohepatitis (NASH), which can result in advanced liver fibrosis and cirrhosis (Masuoka and Chalasani, 2013; Ratziu and Poynard, 2006).

NAFLD is characterized by accumulation of fat in the hepatocytes (Mosca et al., 2016), which is caused by an imbalance of lipid metabolism (Liu et al., 2015), manifested as increased lipogenesis, disrupted fatty acid (FA) oxidation, increased FA uptake and depressed triglyceride (TG) lipolysis. The de novo lipogenesis is regulated primarily at the transcriptional level, with peroxisome proliferator-activated receptor γ (PPARγ) and sterol regulatory element binding proteins 1c (SREBP-1c) as the key transcriptional regulators (Cha and Repa, 2007; Horton et al., 2002; Souza-Mello, 2015; Vecchione et al., 2016). SREBP-1c regulates acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS), which play important roles in the metabolism of FA (Berlanga et al., 2014). Liver peroxisome proliferator-activated receptor α (PPARα) is crucial for whole-body FA homeostasis (Montagner et al., 2016). As the target gene of PPARα (Serviddio et al., 2013), carnitine palmitoyltransferase 1 (CPT-1) transfers FA from the cytosol to the mitochondria prior to beta-oxidation (Berlanga et al., 2014). In NASH rats and HFD-induced obesity mice, the activity of CPT-1 in liver was significantly decreased (Serviddio et al., 2011; Ye et al., 2016). Moreover, PPARα regulates the beta-oxidation by directly controlling expression levels of acyl-CoA dehydrogenase, long chain (ACADL) (Aoyama et al., 1998), which has been reported being decreased in HFD animal's offspring (Borengasser et al., 2011). FA uptake is governed by fatty acid binding proteins (FABP) and CD36 (Ge et al., 2010), as well as by Caveolin 1, a protein responsible for protein trafficking and formation of lipid droplets. In addition, hormonesensitive lipase (HSL) and adipose triglyceride lipase (ATGL) are two enzymes critical for hepatic lipid homeostasis, contributing to lipolysis (Reid et al., 2008), and HFD was reported to down regulate gene expression of HSL and ATGL in C57BL/6 mice (Chen et al., 2015). Finally, adenosine monophosphate activated protein kinase (AMPK) plays an important role in the homeostasis of body weight, which, along with Sirt 1, participates in regulation of energy metabolism (Hurtado-Carneiro et al., 2013).

Silybin, discovered as the first member of a new natural compounds called flavonolignans in 1959 (Biedermann et al., 2014), is the primary active ingredient in the seed extracts of the herb milk thistle (*Silybum marianum*). *Silybum marianum* originated from Southern Europe and North Africa. It was used as the drug for traumatic injuries in Germany. Notablely, Hieronymus Bock mentioned the extract of the seeds used for treating liver disorders in his book (Biedermann et al., 2014). *Silybum marianum* is officially collected in the Chinese Materia Medica with the name of ‘ shui-fei-ji’ and its seed extracts is used for the treatment of hepatitis, cirrhosis and liver intoxication caused by chemical and environmental toxins, including snakebites, insect stings, mushroom poisoning and alcohol (Abenavoli et al., 2010).

Silibinin Capsules (SC) is a silybin-phospholipid complex with silybin as the bioactive component, which is currently used in China for recovery of liver function in patients with acute and chronic hepatitis and fatty liver disease. Increasing study demonstrates the beneficial role of silybin in NAFLD patients, including attenuation of alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum lipid peroxidation, γ-GT and insulinemia (Federico et al., 2006; Loguercio et al., 2012; Stiuso et al., 2014), improvement of insulin resistance (IR) and liver histology, decrease of plasma total cholesterol (TC), TG, low density lipoprotein (LDL), and high density lipoprotein (HDL) (Huseini et al., 2006). Studies in rat showed that silybin significantly protected against HFD-induced fatty liver by restoring liver function, reducing serum fat, relative liver weight, and hepatocyte fat deposition, improving IR, and enhancing lipolysis by up-regulating ATGL expression (Grattagliano et al., 2013; Haddad et al., 2011; Yao et al., 2013, 2011). Recently, studies in cultured cells have been published to explore the mechanism underlying the role of silybin, showing that silybin reduces the up-regulation of PPARγ and SREBP-1c, increases the expression of CPT-1 in cultured steatotic hepatic cells (Vecchione et al., 2016), decreases the expression of FAS, PPARγ and SREBP-1c in HEK-293 cells (Pferschy-Wenzig et al., 2014). Nevertheless, the mechanism thereby silybin attenuates NAFLD is not fully understood, particularly not in vivo. The present study was conducted using HFD raised hamster as a model to investigate the mechanism of SC in ameliorating NAFLD, and to explore the likely target for its action.

2. Material and methods

2.1. High performance liquid chromatography

High performance liquid chromatography (HPLC) analysis was performed using an Agilent 1260 Infinity LC system (Agilent, Palo Alto, CA, USA). All chromatographic separations were carried out by the Dikma Diamonsil C18 column (particle size 5 μm, 250 × 4.6 mm ID, Beijing, China) with a mobile phase of methanol-water-glacial acetic acid (50: 50: 0.5, v/v/v). The detection wavelength was set at 288 nm and the flow rate was 1 mL/min.

2.2. SC

SC was obtained from Tasly Pharmaceutical Co. Ltd. (Tianjin, China), with one capsule containing 35 mg silybin. It was produced according to the guidelines of Good Manufacturing Practice and Good Laboratory Practice. The batch number of the SC used in this experiment was H20040299. For oral administration, the content of capsules was collected before experiment and mixed with 35 mL saline to make a suspension.

2.3. Animals and protocol

A total of 40 male hamsters (Cricetidae) with body weight ranging 80–100 g were purchased from Weitonglihua Animal Center (Beijing, China). They were acclimated to our animal care facilities for 5 days before experiment, with free access to normal chow diet and water. Hamsters were first randomly allocated to two groups, normal diet (ND) group (n=16) and HFD group (n=24). Animals in HFD group were fed on diet contained 20% lard, 0.2% cholesterol and 79.8% basic diet. After 2 weeks, the hamsters in ND group were randomly divided into two groups: Control and Control + 50 mg/kg silybin, while the animals in HFD group were randomly divided into three groups: HFD, HFD + 50 mg/kg silybin and HFD + 100 mg/kg silybin. The hamsters in Control + 50 mg/kg silybin, HFD + 50 mg/kg silybin and HFD + 100 mg/kg silybin groups received silybin orally for 8 weeks at a dose as indicated, respectively. Silybin at 50 mg/kg is a dose approximately equivalent to 300 mg per day recommended for human. The hamsters in Control and HFD groups were given the same volume of saline. Animals in all groups were allowed for free access to food and water. Body weight was measured weekly. All variables were evaluated in the end of the experiment.

2.4. Biochemical assays

After an overnight fast, animals were anesthetized with 20% urethane by intramuscular injection and blood was collected from inferior vena cava. The samples were centrifuged at 5335 g for 10 min at 4 ℃ to separate plasma. The contents of insulin, ALT, AST, LDL, and HDL in plasma were measured by enzyme-linked immunosorbent assay (ELISA) (Andygene, Richardson, USA). The concentrations of TG, TC and glucose in plasma were detected by enzymatic method