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Li-Gan-Shi-Liu-Ba-Wei-San improves non-alcoholic fatty liver disease through enhancing lipid oxidation and alleviating oxidation stress

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ABSTRACT

Ethnopharmacological relevance: Mongolian medicine is an important constituent of traditional Chinese medicine. Its representative prescription, Li-Gan-Shi-Liu-Ba-Wei-San (LGSLBWS), is widely used for long-term treatment of chronic liver disease and nonalcoholic fatty liver disease (NAFLD).
Aim of the study: This study explored the effects and mechanism of LGSLBWS on NAFLD.
Materials and methods: NAFLD rat model was established with high-fat diet. The effects of LGSLBWS on lipid metabolism, liver function, and hepatic morphology were observed in NAFLD rats. Superoxide dismutase (SOD) and malondialdehyde (MDA) contents in the liver, as well as the expression levels of peroxisome proliferator-activated receptor (PPAR)α, PPARβ, inhibitor of nuclear factor κB α(IκBα), and inducible nitric oxide synthase (iNOS) were all detected. Finally, the effects of LGSLBWS on fatty acid oxidation, PPARα, PPARβ, IκBα, and iNOS were determined in HepG2 cells.
Results: LGSLBWS significantly reduced the fat deposition in the liver and the serum aspartate aminotransferase levels in NAFLD rats. Serum triglyceride and free fatty acid levels were reduced by LGSLBWS. Total cholesterol and triglyceride contents in the liver were also downregulated. SOD and MDA levels were increased and decreased by LGSLBWS, respectively. LGSLBWS can significantly promote fatty acid oxidation of HepG2 cells. Upregulation of PPARα, PPARβ, and IκBα and downregulation of iNOS by LGSLBWS were both observed in the NAFLD model and HepG2 cells.
Conclusions: LGSLBWS can significantly improve NAFLD by enhancing fatty acid oxidation and alleviating oxidative stress.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) has become a global health problem with its incidence increasing annually. NAFLD has become the primary cause of chronic liver disease and abnormal liver function not only in Europe and the United States but also in Asia. The total prevalence rate of NAFLD has reached as high as 15–30% (Wang et al., 2014). The main pathology of NAFLD is hepatic fatty degeneration, which should be excluded from excessive drinking, viral infection, and congenital and autoimmune liver diseases (Choi et al., 2014). Approximately 25% of NAFLD cases may develop into cirrhosis, and some of which may progress to liver failure or cancer (Wong et al., 2015). Although the risk of NAFLD has gained increasing attention, its pathogenesis is not entirely clear. “Two-hit” theory is widely accepted to explain the pathogenesis of NAFLD. The “first hit” represents fat deposition in liver cells caused by insulin resistance and lipid metabolism disorder, leading to hepatocellular fat degeneration. The “second hit” indicates fatty hepatitis caused by oxidative stress and cytokines (Stojsavljevic et al., 2014). However, effective and safe treatments are rare because of the uncertain etiology of NAFLD.

Traditional Chinese medicine has demonstrated significant clinical efficacy in NAFLD treatment (Yin et al., 2014). Li-Gan-Shi-Liu-Ba-Wei-San (LGSLBWS) is an outstanding type of Mongolian medicine comprising eight types of Chinese herbal medicine, namely, pomegranate, cinnamon, cardamom, piper longum, safflower, amomum tsao-ko, dried ginger, and nutmeg (Wu, 1984; Bai, 1986). From the perspective of Chinese medicine, LGSLBWS

Abbreviations: ALT, alanine aminotransferase; AST, aspartate transaminase; DMSO, Dimethyl Sulphoxide; FFA, free fatty acid; HDL-C, high density lipoprotein cholesterol; IκBα, inhibitor of nuclear factor κB α; iNOS, inducible nitric oxide synthase; KRP, Krebs-Ringer phosphate; LDL-C, low density lipoprotein cholesterol; LGSLBWS, Li-Gan-Shi-Liu-Ba-Wei-San; MDA, malondialdehyde; MTT, 3-(4,5-D imethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide; NAFLD, nonalcoholic fatty liver disease; NO, nitric oxide; PPAR, peroxisome proliferator-activated receptor; SOD, superoxide dismutase; TC, total cholesterol; TG, triglyceride

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has served the functions of “dispelling cold, regulating'qi,' and ‘smoothing’ the liver.” However, its specific molecular biological mechanism has not been deeply studied. In the present study, the effects and mechanism of LGSLBWS on NAFLD were explored in animal and cell experiments based on “two-hit” theory, and the results can provide experimental evidence for clinical applications.

2. Materials and methods

2.1. LGSLBWS Preparation

LGSLBWS was prepared according to the quality standard of Chinese Medical Encyclopedia (Bai, 1986) and provided by Preparation Center of Mongolian Medicine, Chinese and Mongolian Medicine Hospital of Inner Mongolia. The voucher specimen was deposited at the herbarium of the center (voucher number seen in Table 1). Its components (scientific name followed by Chinese name)included Punica granatum L.-Shiliu, Cinnamomum cassia Presl-Rougui, Alpinia blepharocalyx K.Schum. -Doukou, Piper longum L.-Bibo, Carthamus tinctorius L.-Honghua, Amomum tsao-ko Crevost & Lemarié-Caoguo, Zingiber officinale Roscoe -Ganjiang and Myristica fragrans Houtt.-Roudoukou. The detail of all components were shown in Table 1.

2.2. Establishment of NAFLD animal model

SD rats were randomly divided into control and NAFLD groups after being fed for 1 week. The rats in the control group were fed with standard basic feed, whereas those in the NAFLD group were fed with high-fat emulsion (1000 g of high-fat emulsion, 100 g of lard, 20 g of cholesterol, 5 g of cholate, 50 g of sucrose, and 825 g of water). In clinic, the normal dose of LGSLBWS is 9 g/d for adult (Wu, 1984). Afer calculation using conventional method (Shi, 1987), the rats in the NAFLD group were randomly divided into model control group, “LGSLBWS1” group (LGSLBWS administrated at 1.5 g/kg daily), and “LGSLBWS2” group (LGSLBWS administrated at 0.75 g/kg daily), and each group consisted of eight rats. The rats in the normal control group were provided with isometric distilled water, whereas the rats in the NAFLD model group were treated with isometric distilled water after intragastric administration of fat emulsion 2 weeks later. The rats in the LGSLBWS groups were treated with corresponding drugs based on the above doses, once a day for 4 weeks. After the last drug administration, the rats were fasted for 16 h, and serum samples were separated for detection. This animal study was performed in accordance with the “Regulation for the Administration of Affairs Concerning Experimental Animals” promulgated by the State Council of China in 1988 and was approved by the Ethics Committee of the Third Military Medical University.

2.3. Cell culture

HepG2 cells were purchased from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy and Science (Shanghai, China) and cultured using the methods recommended by ATCC. Ten percent of fetal bovine serum was added into the culture medium, and the sample was placed in an incubation box (37 °C, 5% CO_2). Trypsin was used to digest the cells after 85% to 95% of them were adhered and fused. The cells were then transferred onto a six-well plate. The culture medium was changed to 10% fetal calf serum after cell adherence. Subsequently, LGSLBWS was added to the medium. A final concentration of 10% PBS was used as negative control. Cells processed by drugs for 48 h were used to detect fatty acid oxidation, as well as RNA and protein extraction.

2.4. Detection of lipid metabolism and oxidative stress

Serum triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), aspartate transaminase (AST), alanine aminotransferase (ALT), and free fatty acid (FFA) contents were detected by an automatic biochemistry analyzer. The rats were executed, and their livers were immediately removed. Following gross anatomical observation, up to 1 g of liver tissue was used to prepare 10% liver tissue homogenate with cold saline. Supernatant was then separated after centrifugation. TC and TG concentrations were also detected using the automatic biochemical analyzer. Additionally, superoxide dismutase (SOD) and malondialdehyde (MDA) contents were determined in accordance with the manufacturer’s instructions.

2.5. Morphological observation of liver tissues

The rats were fasted for 16 h after the last drug administration, and their livers were immediately removed. Fresh liver tissues were used for fixation along with 4% paraformaldehyde, paraffin embedding, and H&E staining. The pathological morphology of the liver tissues was observed under a microscope.

2.6. Real-time PCR

Total RNA was extracted, and the total RNA concentration was detected. Up to 0.97 μg of RNA was used for reverse transcription in accordance with the instructions indicated on the PrimeScript™ RT reagent kit (TaKaRa). Real-time PCR was performed following the instructions in the SYBR® Green Real-time PCR Master Mix kit (Toyobo). The primers were designed using Primer Premier 5.0. Upstream primer of peroxisome proliferator-activated receptor (PPAR)α: 5′-AAGGTCAAGGCCCGGGTCATAC-3′, downstream primer of PPARα: 5′-CTGGGATAGCCTTGGCAAATT-3′, upstream primer of PPARβ: 5′-TA TCCGCTTTGGACGGATGCC-3′, downstream primer of PPARβ: 5′-AGAGAA GGCCTTCAGGTCGGC-3′, upstream primer of GAPDH: 5′-ACCCATCACCATCT TCCAGGAG-3′, and

Table 1
Components of Li-Gan-Shi-Liu-Ba-Wei-San.

Chinese name	Scientific name	Part used	Voucher number	Family	Weight (g)
Shiliu	Punica granatum L.	Peel	MYSL-20070915	Punicaceae	250
Rougui	Cinnamomum cassia Presl	Peel	MYRG-20061223	Ephedraceae	50
Doukou	Alpinia blepharocalyx K.Schum.	Fruit	MYDK-2006126	Zingiberaceae	50
Bibo	Piper longum L.	Fruit	MYBB-20070509	Piperaceae	150
Honghua	Carthamus tinctorius L.	Flower	MYHH-20071112	Asteraceae	50
Caoguo	Amomum tsao-ko Crevost & Lemarié	Fruit	MYCG-20070319	Zingiberaceae	50
Ganjiang	Zingiber officinale Roscoe	Root	MYGJ-20061107	Zingiberaceae	200
Roudoukou	Myristica fragrans Houtt.	Fruit	MYRK-20061010	Myristicaceae	50