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Original Article

Protective effect of Sheng-jiang powder on nonalcoholic fatty liver disease induced by high fat diet through AMPK/mTOR signaling pathway in rats

Rui-Zhi Tan[1*], Qin Yu[2,1*], Hong-Chun Shen[1,4], Xia Zhong[1], Tao Li[3], Yu-Wei Zhang[1], Quan Gan[1], Li Wang[1]

[1]Research Center of Integrated Chinese and Western Medicine, Affiliated Traditional Medicine Hospital of Southwest Medical University, Luzhou 646000, Sichuan, China; [2]Department of Pathology, The First People's Hospital of Zigong, Zigong 643000, Sichuan, China; [3]Department of Orthopedics, The Fourth People's Hospital of Zigong, Zigong 643000, Sichuan, China; [4]Department of Nephrology, Affiliated Traditional Medicine Hospital of Southwest Medical University, Luzhou 646000, Sichuan, China. [*]Equal contributors.

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Abstract: Nonalcoholic fatty liver disease (NAFLD) has become a global public health problem, but its specific mechanism remains unclear and lacks effective therapeutic drugs. Sheng-jiang powder (SJP) has shown great potential for prevention and treatment of NAFLD in Chinese clinical reports, but the study of specific curative effects and mechanisms are still lacking. The purpose of this study was to observe the effect of SJP on high fat diet-induced rat NAFLD and to explore its underlining mechanisms. After 4 weeks of normal diet or high-fat diet, SD rats were randomly divided into the following four groups and each was given intragastric administration: Normal control group, NAFLD group, Sheng-jiang powder group and Fenofibrate group. All rats were sacrificed after 4 weeks' gavage. Blood was used to detect serum triglyceride (TG), total cholesterol (TC), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. Homogenized liver tissue was used to determine liver TG and TC contents. HE staining and oil red O staining were performed to show liver pathological changes. AMP-activated protein kinase alpha (AMPKα), p-AMPKα, mammalian target of rapamycin (mTOR) and p-mTOR protein expression in liver tissue were tested by western blot. The results showed that SJP significantly reduced the body weight, serum TG level, TG and TC content in liver of NAFLD rats, and markedly improved hepatic steatosis. SJP increased the expression of p-AMPKα protein and decreased the expression of p-mTOR protein. These results indicate that SJP can improve serum lipid and hepatic steatosis in rats with NAFLD induced by high-fat diet, and its effect may be achieved through regulating AMPK/mTOR signal pathway, inhibiting lipid synthesis and reducing hepatic lipid deposition.

Keywords: NAFLD, Sheng-jiang powder, AMPK/mTOR signal pathway, lipid synthesis

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a multifactor-induced complex clinical pathology syndrome, which is not an independent disease. According to its long-term pathological progress, it is generally divided into four types, of which type 1 (steatosis) and type 2 (lipid necrosis) are referred to as non-alcoholic fatty liver, while type 3 (steatohepatitis) and type 4 (steatohepatitis with fibrosis) are called non-alcoholic steatohepatitis [1-4]. With the increase of people having high-fat or high-sugar diet, the incidence of NAFLD is rising year by year [5]. A recent meta-analysis showed that the global prevalence of NAFLD has reached 25.24%, and the proportion of progression to fibrosis in non-alcoholic steatohepatitis is as high as 40.76% [6]. Although the early main manifestation of NAFLD is simple fatty liver, it could progress toward steatohepatitis, hepatic fibrosis, cirrhosis, and even hepatocellular carcinoma later without intervention [7-10]. Moreover, once entering the cirrhosis stage, the course of the disease will be irreversible, and liver transplantation will be required in the later stage. This undoubtedly causes serious clinical and economic burden. In addition, NAFLD can significantly increase the risk of metabolic complications such as type II diabetes mellitus [11-

14], obesity [15, 16], hyperlipidemia [17], and hypertension [6, 18]. These metabolic complications and NAFLD can promote mutually, and the metabolic conditions will deteriorate, causing low life quality and life expectancy of the patients. Due to the complicated etiology of NAFLD, its pathogenesis has not yet been fully elucidated and there are no specific drugs for the disease. Therefore, strengthening the prevention and treatment of NAFLD, seeking new ideas and new targets for treating the diseases, are very important to human life and health.

The current main treatment for NAFLD is symptomatic treatment. First, diet control [19] and proper exercise, but patients often have poor compliance, resulting in disease progression to liver fibrosis, liver cirrhosis, and even related hepatocellular carcinoma that will result in a very poor prognosis. Second, to the patients with severe hyperlipidemia, give lipid-lowering drugs, such as fibrates, but the majority of lipid-lowering drugs have severe long-term side effects, and the elimination of intrahepatic lipids is limited. The deposition of large amounts of triglyceride and other lipid components in hepatocytes is an early manifestation of NAFLD, and intervention in the early stage of the disease is an important step in preventing its progression to a more severe stage.

Compared with the current western medicine treatment of NAFLD, a large number of studies have shown that traditional Chinese medicine has a unique advantage of overall conditioning and fewer side effects. The traditional Chinese medicine compound "Sheng-jiang powder" (SJP) is a famous prescription highly praised by traditional Chinese medicine experts. It is derived from the famous Chinese medical book "Shang Han Wen Yi Tiao Bian". It was originally composed of Bombyx batryticatus, periostracum cicada, curcuma longa, rhubarb, yellow rice wine and honey. It is more often composed of four former parts now. In the prescription, Bombyx Batryticatus and periostracum cicada have the effect of detoxification, which is "Lucidity Ascending", meanwhile, curcuma longa and rhubarb can cure infectious disease, which is "Descend Turbidity", and then combination of "ascending" and "descending" effect makes the prescription have the effect of Ascending Lucidity and Descending Turbidity. Studies have shown that curcuma longa and rhubarb have the function of reducing blood fat and blood sugar [20-23]. And NAFLD is closely linked with hyperlipidemia and type II diabetes. Does this mean that SJP can play a therapeutic role in NAFLD? In recent years, it has been reported that SJP has a certain therapeutic effect on obesity-induced multiple organ injuries [24, 25], but such studies were mostly limited to simple clinical efficacy observation and did not focus on the effects of SJP on NAFLD. At present, there is still a lack of research on the treatment efficiency of SJP and related mechanisms, which greatly limits the developmental research on SJP in NAFLD. Therefore, it is necessary to strengthen the research on the exact therapeutic effect and mechanism of SJP in NAFLD. Based on these, this study selected SJP to intervene the high fat diet induced NAFLD in rats, observe the efficacy and explore its mechanism, and hope to provide some modern medical experimental evidence for SJP in treating NAFLD, and provide new ideas and new targets for clinical treatment of NAFLD.

Materials and methods

Preparation of Sheng-jiang powder and fenofibrate

Sheng-jiang powder (SJP) was derived from the famous Chinese medical book "Shang Han Wen Yi Tiao Bian", and was composed of Bombyx batryticatus, periostracum cicada, curcuma longa, rhubarb. Bombyx batryticatus (1604017), periostracum cicada (1508058), curcuma longa (1609006) and rhubarb (1601078) were purchased from Neo-green pharmaceutical technology development Co., Ltd (Sichuan, China). According to clinical evidence, the research group adjusted the SJP prescription (decoction pieces) to: Bombyx batryticatus (24 g), periostracum cicada (12 g), curcuma longa (6 g), rhubarb (24 g). According to the dose conversion relationship between rats and humans, the decoction pieces dosage for rats were converted, and the amount of decoction pieces was converted into the corresponding dose of decoction-free dosage: Bombyx batryticatus (225 mg/kg/day), periostracum cicada (104 mg/kg/d), curcuma longa (375 mg/kg/d), rhubarb (333 mg/kg/d). The corresponding dose of drug was dissolved in distilled water. Fenofibrate was purchased from Abbott Pharmaceuticals Co., Ltd. (Chicago, USA). According to previous experience [26, 27], the dose of fenofibrate was 100 mg/kg/d. Fenofibrate was mixed as suspension with sodium carboxymethyl cellulosea.

Animals and treatment

Thirty-six male Sprague-Dawley rats (100-120 g) were purchased from Chengdu Dashuo Experimental Animal Co., Ltd (Chengdu, China) and housed in regular cages and allowed free access to food and water. The animals were situated in a temperature and humidity controlled room (21.0 ± 2.0°C and 65 ± 5% humidity, respectively) and maintained on a 12-h light/12-h dark cycle. After 1 week of acclimatization, twelve rats were randomly selected as normal control group (NOR) and given basal diets (Chengdu Dashuo Experimental Animal Co., Ltd, Chengdu, China), while the remainder were given a high-fat diet (79% basal diet + 10% egg yolk + 10% lard + 1% cholesterol, Chengdu Dashuo Experimental Animal Co., Ltd, Chengdu, China) as nonalcoholic fatty liver disease group (NAFLD). After 4 weeks, 6 basal diet rats and 6 high-fat diet rats were randomly selected and anesthetized with 1% sodium pentobarbital (3-5 ml/kg) by intraperitoneal injection, then the liver tissue was harvested and stained with Oil red O and HE. The pathological changes of liver tissue were observed under microscope to determine whether the rat model of NAFLD was established successfully. After successful establishment of the model, the remaining basal diet rats were used as normal control group (NOR, basal diet, n = 6), and the remaining high-fat diet rats were randomly divided into NAFLD group (NAFLD, high-fat diet, n = 6), SJP group (SJP, high-fat diet plus SJP, n = 6), fenofibrate group (FNBT, high-fat diet plus fenofibrate, n = 6). The intervention started at the end of 4th week, SJP group was given SJP by gavage. The FNBT group was given fenofibrate, and the other two groups were given normal saline with a volume of 1 ml/100 g once per day. After continuous gavage for 4 weeks, all rats were anesthetized by intraperitoneal injection of 1% sodium pentobarbital (3-5 ml/kg), blood and liver tissues were used for follow-up measurements. All experiments were carried out according to the guidelines approved by the Animal Ethics Committee of Southwest Medical University.

Serum lipid and liver function determination

The collected blood was centrifuged and serum was collected. Serum triglyceride (TG), total cholesterol (TC), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured by an automatic biochemical analyzer.

Determination of triglyceride and total cholesterol in liver tissue

After the liver tissue was mixed with absolute ethanol (liver quality:absolute ethanol volume = 1:9) and thoroughly homogenized, the triglyceride and total cholesterol in liver tissue were measured using the triglyceride (TG) assay kit (Nanjing Jiancheng Institute of Biotechnology, A110-2 GPO-PAP Enzyme Method, Single Reagent Type, Nanjing, China) and total cholesterol (T-CHO) assay kits (Nanjing Jiancheng Institute of Biotechnology, A111-2, COD-PAP Enzyme Method, Single Reagent Type; Nanjing, China) according to manufacturer's instructions.

Hematoxylin and eosin staining

The 4% phosphate-buffered paraformaldehyde-fixed small pieces of liver were routinely processed and were cut into 3 μm serial sections. The sections were dewaxed in xylene and rehydrated in ethanol gradients and then stained with hematoxylin-eosin (H&E, Beyotime, China) for histological evaluation. Lipid in hepatocytes was dissolved and fat vacuoles appeared during processing, so hematoxylin and eosin staining can indirectly reflect the degree of lipid accumulation and hepatic steatosis through fat vacuoles. Finally, sections were imaged using a light microscope (Eclipse 80i, Nikon, Japan) at 200× magnification.

Oil Red O staining

Some of fresh liver tissues were made of frozen sections (7 um) and stained with Oil Red O (Solarbio, China) for 20 min to further reveal hepatic steatosis. Lipids were stained to red by Oil Red O, and the red lipid drops in hepatocytes directly reveal the degree of lipid accumulation and hepatic steatosis. Images were taken ditto.

Immunohistochemistry

For immunohistochemistry (IHC), the steps of dewaxing and rehydration were similar to HE staining. IHC was performed by using rabbit SP kit (SP-9001, ZSGB-BIO, China). After goat

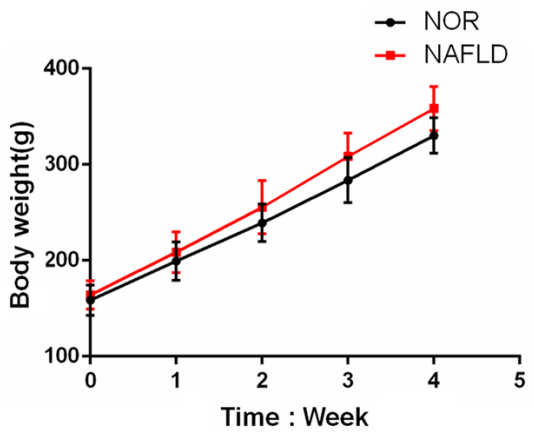

Figure 1. Changes in body weight of rats during 4 weeks of modeling.

serum blocking, the slices were incubated with rabbit anti-rat antibody against p-AMPKα (1:100, Cell Signaling Technology, Danvers, USA) at 4°C overnight and then combined with a secondary antibody (horseradish peroxidase-conjugated goat anti-rabbit antibody). At last, the antibody binding was visualized using DAB kit (ZLI-9033, ZSGB-BIO, China). The purpose of immunohistochemistry is to show the quantity and distribution of p-AMPKα protein in liver. Images were taken at 200× magnification (ditto) to present the expression of p-AMPKα in hepatocytes.

Western blotting

Fifty milligrams of liver tissue were obtained from each rat, which were ground into liver homogenate. Equal amounts of protein lysates from rat liver were resolved in SDS-PAGE gel and transferred to PVDF membranes (Milipore, USA). After 1-h blocking with 5% nonfat dried milk, antibodies against AMPKα, p-AMPKα, mTOR and p-mTOR (1:1000, Cell Signaling Technology, Danvers, USA) were added to identify AMPKα, p-AMPKα, mTOR and p-mTOR, respectively, at 4°C overnight. Subsequently, the membranes were incubated with the corresponding secondary antibody (horseradish peroxidase-conjugated secondary anti-rabbit antibodies, 1:5000; ZSGB-Bio, Beijing, China) at room temperature for 1 h. Antibody binding was visualized using ChemiDoc XRS + system (Bio-Rad, USA). Gray intensity of western blot signal band was calculated with Image J 1.47 V software (NIH, USA).

Statistical analysis

Data analysis was performed using SPSS 21.0 statistical software. The experimental results were expressed as $\bar{x} \pm s$. An independent sample t-test was used for comparison between the two groups, while one-way analysis of variance (ANOVA) was used when groups are more than 2, and LSD was used for comparison between the two groups. $P<0.05$ was considered statistically significant.

Results

High-fat diet for 4 weeks leads to massive lipid deposition in rat hepatocytes

We weighed rats and performed liver HE staining and Oil Red O staining. From the comparison of body weight at the same time point, it can be seen that the body weight of NAFLD group slightly increased, but it was not statistically significant (**Figure 1**). According to the liver HE staining, white bubbles were found in the cytoplasm of high-fat diet induced rats (**Figure 2**). The white bubbles in cytoplasm were caused by the dissolution of accumulated lipid during paraffin embedding, which indirectly represents lipid deposition levels in pathologic liver. To further confirm the lipid deposition in NAFLD group, Oil Red O staining was performed (**Figure 2**). The results demonstrated that NAFLD group showed hepatocytes with lipid accumulation.

SJP decreases body weight and serum TG levels in NAFLD rats

We weighed rats after SJP and fenofibrate intervention and found that the weight gain of rats in the NAFLD group was faster than the other three groups. Compared with the other three groups, the body weight of NAFLD group increased significantly in the 3rd and 4th week after the intervention, indicating that body weight decreased significantly after SJP and fenofibrate intervention (**Figure 3**). We also detected serum triglyceride (TG), total cholesterol (TC), aspartate transaminase (AST) and alanine aminotransferase (ALT) levels of rats after gavage. Compared with the NOR group, the serum TG levels in the NAFLD group increased significantly, and the TG levels decreased significantly after intervention with SJP and fenofibrate (**Figure 4A**). There were no

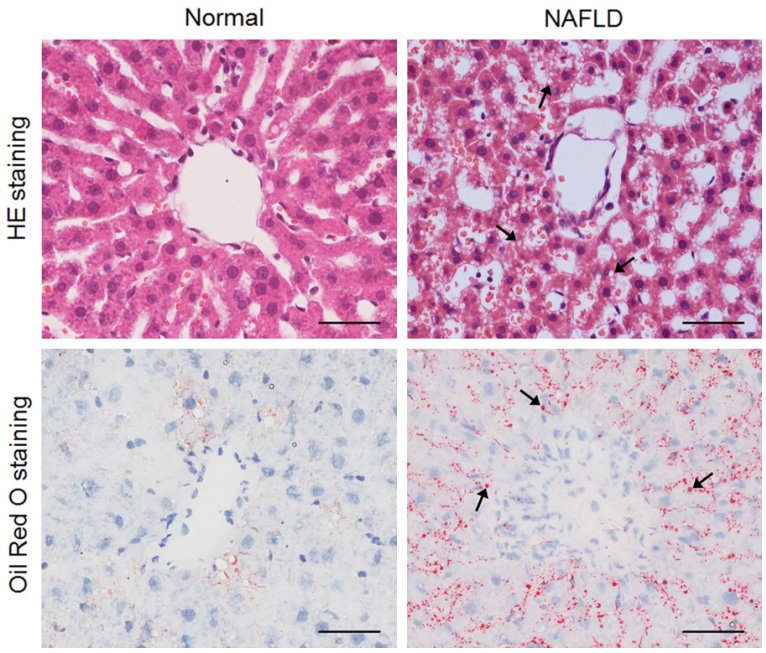

Figure 2. High-fat diet for 4 weeks leads to lipid accumulation in hepatocytes (HE staining, Oil Red O staining, ×200). White bubbles show lipids in HE staining, and red areas show lipids in Oil Red O staining (as shown by arrows).

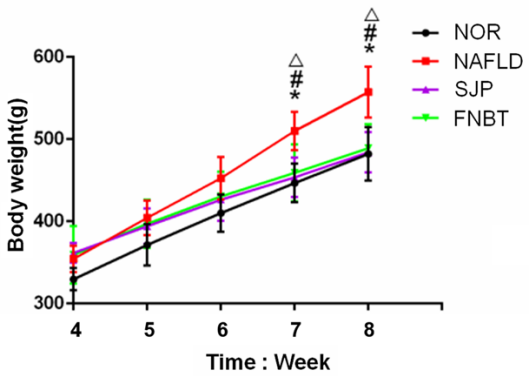

Figure 3. SJP reduces the body weight of NAFLD rats significantly. *P<0.05 compared with NOR group at the same time point; #P<0.05 compared with SJP group; ΔP<0.05 compared with FNBT group.

significant differences in TC, ALT and AST levels between groups (**Figure 4B-D**).

SJP decreases the content of TG and TC in liver tissue of NAFLD rats

In order to study the effect of SJP on lipid content in rat liver tissue, we examined the content of TG and TC in liver tissue (**Figure 5**). Compared with NOR group, the contents of TG and TC in liver tissue of NAFLD group were significantly increased. Compared with the NAFLD group, the liver TG and TC levels in the SJP group and the FNBT group were significantly lower, and the SJP group was the lowest.

SJP improves hepatic steatosis in NAFLD rats

In order to show the effect of SJP on hepatic steatosis in NAFLD rats, we performed liver HE staining and Oil Red O staining (**Figure 6**). From the HE staining and Oil Red O staining, it can be seen that there is a large amount of lipid accumulation in the cytoplasm of rat hepatocytes in the NAFLD group. Hepatic cytoplasmic lipids were rare in SJP rats and liver steatosis was significantly improved compared with NALFD group. Hepatic steatosis in FNBT group also improved compared with NALFD group, but the degree was less than SJP group.

SJP increases the expression of p-AMPKα protein and decreases the expression of p-mTOR protein

Immunohistochemistry (**Figure 7**) and Western blot (**Figure 8A**, **8B**) showed that the expression of p-AMPKα protein in the liver of the rats of NAFLD group was significantly reduced, and the expression of p-AMPKα protein was significantly increased after intervention of SJP and fenofibrate. The expression of p-mTOR protein (**Figure 8C**, **8D**) in the liver of NAFLD rats was significantly increased, and the expression of p-mTOR protein was significantly decreased after intervention of SJP. No significant recovery was observed after the intervention with finofibrate.

Discussion

In this study, we found that after 8 weeks of high-fat diet, the body weight of the model group increased significantly. Serum TG and liver tissue TG, TC levels were significantly

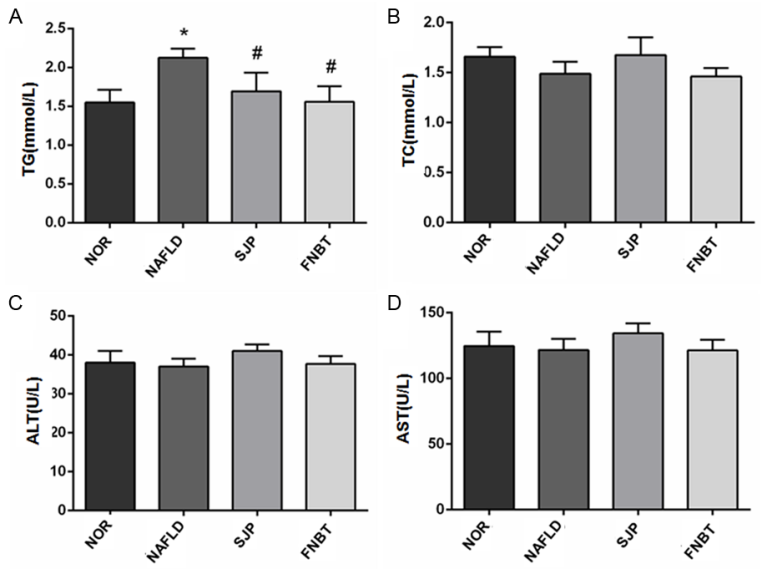

Figure 4. SJP reduces serum TG levels in NAFLD rats significantly. A. The content of serum TG; B. The content of serum TC; C. The content of serum ALT; D. The content of serum AST. **P*<0.05 versus NOR group, #*P*<0.05 versus NAFLD group.

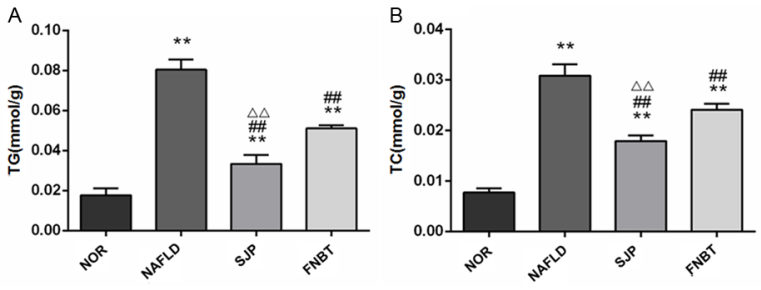

Figure 5. SJP reduces liver TG and TC levels in NAFLD rats significantly. A. The content of liver TG; B. The content of liver TC. ***P*<0.01 versus NOR group; ##*P*<0.01 versus NAFLD group; ΔΔ*P*<0.01 versus FNBT group.

increased, and a large number of lipids accumulated in the liver cells. After SJP intervention, rats' body weight and serum TG levels were down-regulated, liver tissue TG and TC contents were significantly decreased, and liver tissue pathological changes were significantly restored. The above results show that SJP can regulate blood lipids and improve hepatic steatosis in rats, which has a therapeutic effect on rat NAFLD.

According to traditional Chinese medicine theory, the basic pathogenesis of high-fat diet-induced NAFLD is a dysfunction of "Qi". Spleen dysfunction leads to abnormal motion of "Qi", further causing "Qi" stagnation, phlegm retention, and blood stasis, which finally induces NAFLD. SJP improves NAFLD by regulating functional activity of "Qi", ascend lucidity and descend turbidity. Evidence showed that curcuma longa and rhubarb have a role in lowering blood lipids. Some researchers have found that SJP plus bupleurum root and citrus aurantium can reduce serum TG, TC, and LDL levels in hyperlipidemic rats, and regulate lipid metabolism disorders. NAFLD is closely linked with hyperlipidemia, suggesting that SJP may have a treatment effect on NAFLD by regulating lipid metabolism. The results of this study also showed that SJP can reduce serum TG levels, which again confirms that therapeutic effect of SJP on NAFLD is related to its regulation of lipid metabolism.

In this study, the established NAFLD model was in the early stage. At present, diet control and proper exercise are mainly suggested in the early stage of NAFLD. However, the compliance is not satisfactory, and the patients with severe hyperlipidemia are treated with lipid-lowering therapy. Fenofibrate is a common lipid-lowering drug, which mainly reduces serum triglyceride levels. Considering the main characteristics of NAFLD is the dysfunction of triglycerides metabolism, this study set the fenofibrate group as positive control group. The results of this study showed that after intervention with fenofibrate, rats' body weight, serum TG, liver tissue TG and TC, and liver pathological changes all recovered to varying degrees, but the decrease degree of TG and TC in liver tissue were not as significant as SJP group. Similarly, the pathological recovery of liver tissue is also not as obvious as SJP group. The above results suggest that fenofibrate has a significant effect in reducing blood lipids, but it is not ideal for the reduction of intrahepatic

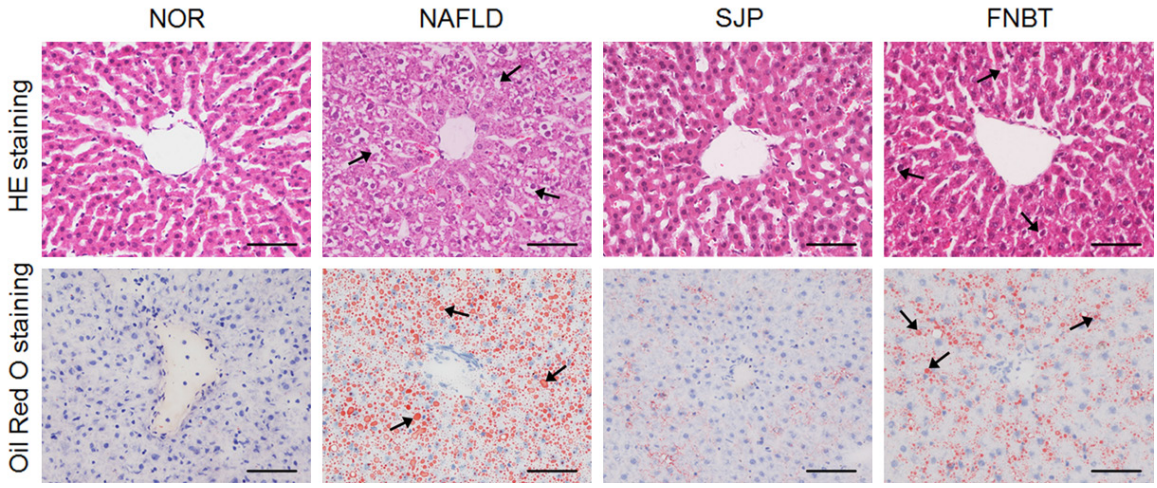

Figure 6. SJP improves hepatic steatosis in NAFLD rats (HE staining, Oil Red O staining, ×200). White vacuoles show lipids in HE staining, and red areas show lipids in Oil Red O staining (as shown by arrows).

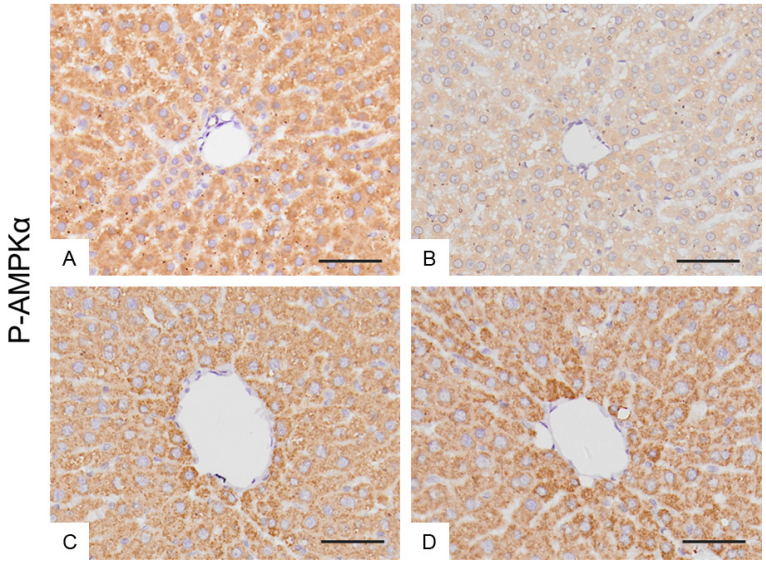

Figure 7. SJP increases p-AMPKα protein expression in NAFLD rats (immunohistochemistry, ×200). A. NOR group; B. NAFLD group; C. SJP group; D. FNBT group. Brown-yellow shows positive expression.

lipid levels. As a traditional Chinese medicine compound, SJP may be better than fenofibrate in regulating the overall lipid metabolism in NAFLD.

The pathogenesis of NAFLD is not yet fully understood, but lipids such as triglycerides caused by various causes are deposited in large numbers of hepatocytes, resulting in steatosis of hepatocytes. This is one of the most widely recognized mechanisms [28-30]. AMP-activated protein kinase (AMPK) is the key to energy metabolism in the body [31-33]. It is activated by the low-energy state of the cell (phosphorylation). After activation, it triggers the downstream catalytic process to promote ATP production while inhibits the anabolic metabolism of ATP, facilitating the restoration of cellular energy metabolism homeostasis. Energy metabolism and lipid metabolism are closely linked. Hong-Ai Zhang et al. [34] found in *in vitro* experiments that overexpression of activated AMPKα in hepatocytes caused a decrease of triglyceride levels in hepatocytes. Studies by Eunhui Seo et al. [35] showed that overexpression of activated AMPKα in the liver downregulated the expression of lipogenic genes in hyperlipidemia type 2 diabetic rat models, further reducing liver triglyceride levels, resulting in reduced hepatic steatosis in rats. This suggests that AMPKα may become a therapeutic target for NAFLD. In recent years, studies have shown that mTOR plays a key role in the promotion of lipid biosynthesis [36]. After mTOR is activated (phosphorylated), it regulates downstream molecules or helps regulate other related pathways to promote lipid biosynthesis [37, 38]. In addition, studies have shown that activation of

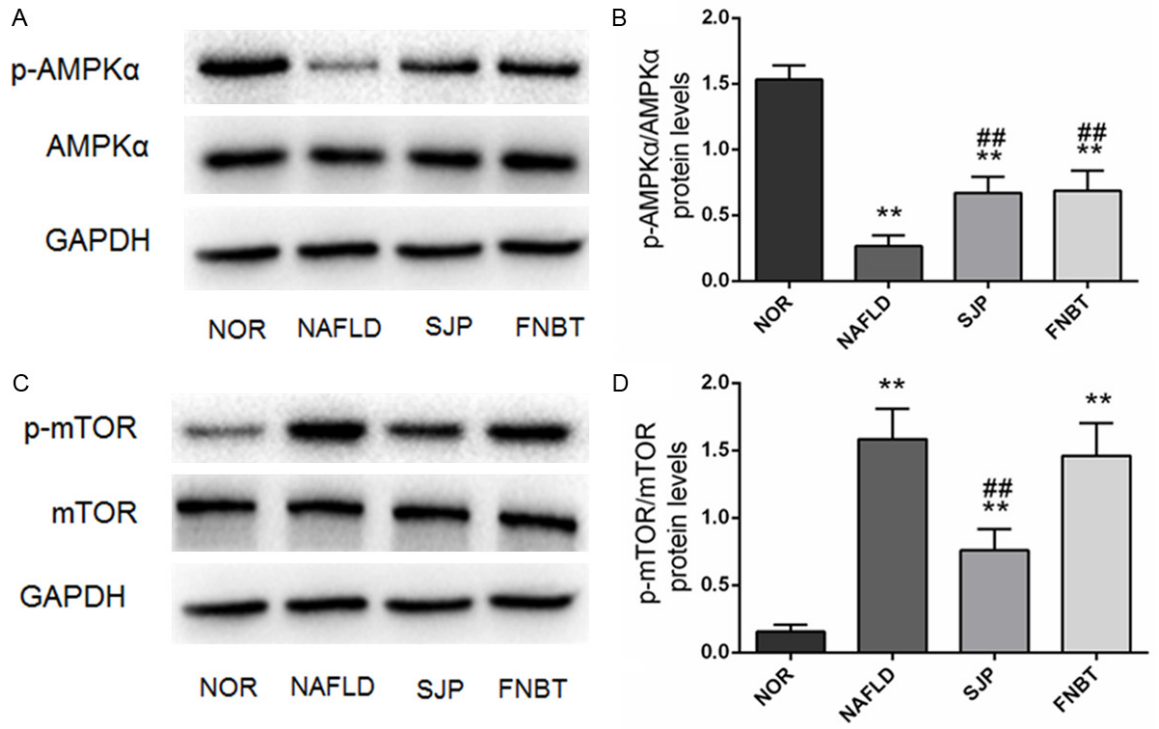

Figure 8. SJP increases the p-AMPKα protein level in the liver of NAFLD rats and decreases the p-mTOR protein level. A. The protein band of p-AMPKα and AMPKα in rat liver; B. The quantification of p-AMPKα/AMPKαin rat liver; C. The protein band of p-mTOR and mTOR in rat liver; D. The quantification of p-mTOR/mTOR in rat liver. $**P<0.01$ versus NOR group, $##P<0.01$ versus NAFLD group.

AMPK has an inhibitory effect on p-mTOR [39]. Therefore, this study focuses on the above pathogenesis and possible therapeutic targets, exploring the mechanisms of SJP's treatment effect on NAFLD. The results of this study showed that the expression of p-AMPKα protein in the liver of NAFLD rats was significantly lower than that in normal rats, and the expression of p-mTOR protein was significantly increased. After SJP intervention, the expression of p-AMPKα protein was significantly increased, and the expression of p-mTOR protein was significantly decreased. This suggests that SJP may treat rat NAFLD by regulating the AMPK/mTOR signaling pathway, inhibiting lipid synthesis, and reducing hepatic lipid deposition.

In this study, we also found that after the intervention of fenofibrate, the expression of p-AMPKα in liver recovered, but p-mTOR did not fall back. This suggests that fenofibrate treatment of NAFLD may be related to the up-regulation of p-AMPKα, but p-mTOR is not much affected.

In conclusion, this study shows that SJP can improve the lipid and hepatic lipid accumulation of rat NAFLD induced by high-fat diet, and its therapeutic effect may be achieved by regulating the AMPK/mTOR signaling pathway. In this study, the mechanisms of SJP in the treatment of NAFLD is preliminarily explored, and the mechanisms of SJP will be further confirmed and thoroughly studied at the cellular level through the AMPK/mTOR pathway inhibitors and other methods in the following studies. In addition, we will look for effective ingredients to further enhance and optimize the prescription so as to provide new methods and new targets for clinical treatment of NAFLD.

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Disclosure of conflict of interest

None.

Address correspondence to: Li Wang, Laboratory of Organ Fibrosis Prophylaxis and Treatment, Research Center of Integrated Chinese and Western Medicine, Affiliated Traditional Medicine Hospital of Southwest Medical University, Luzhou, Sichuan, China. Tel: +86 0830 3161222; E-mail: wangli120@swmu.edu.cn

References

[1] Adams LA, Lymp JF, St Sauver J, Sanderson SO, Lindor KD, Feldstein A, Angulo P. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. Gastroenterology 2005; 129: 113-121.

[2] Angulo P, Kleiner DE, Dam-Larsen S, Adams LA, Bjornsson ES, Charatcharoenwitthaya P, Mills PR, Keach JC, Lafferty HD, Stahler A, Haflidadottir S, Bendtsen F. Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. Gastroenterology 2015; 149: 389-397, e310.

[3] Bugianesi E, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, Musso A, De Paolis P, Capussotti L, Salizzoni M, Rizzetto M. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. Gastroenterology 2002; 123: 134-140.

[4] McPherson S, Hardy T, Henderson E, Burt AD, Day CP, Anstee QM. Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: implications for prognosis and clinical management. J Hepatol 2015; 62: 1148-1155.

[5] Mollard RC, Sénéchal M, MacIntosh AC, Hay J, Wicklow BA, Wittmeier KD, Sellers EA, Dean HJ, Ryner L, Berard L, McGavock JM. Dietary determinants of hepatic steatosis and visceral adiposity in overweight and obese youth at risk of type 2 diabetes. Am J Clin Nutr 2014; 99: 804-812.

[6] Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 2016; 64: 73-84.

[7] Adams LA, Sanderson S, Lindor KD, Angulo P. The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. J Hepatol 2005; 42: 132-138.

[8] Fassio E, Alvarez E, Domínguez N, Landeira G, Longo C. Natural history of nonalcoholic steatohepatitis: a longitudinal study of repeat liver biopsies. Hepatology 2004; 40: 820-826.

[9] Li Z, Xue J, Chen P, Chen L, Yan S, Liu L. Prevalence of nonalcoholic fatty liver disease in mainland of China: a meta-analysis of published studies. J Gastroenterol Hepatol 2014; 29: 42-51.

[10] Wong VW, Wong GL, Choi PC, Chan AW, Li MK, Chan HY, Chim AM, Yu J, Sung JJ, Chan HL. Disease progression of non-alcoholic fatty liver disease: a prospective study with paired liver biopsies at 3 years. Gut 2010; 59: 969-974.

[11] Mantovani A, Byrne CD, Bonora E, Targher G. Nonalcoholic fatty liver disease and risk of incident type 2 diabetes: a meta-analysis. Diabetes Care 2018; 41: 372-382.

[12] Lallukka S, Yki-Järvinen H. Non-alcoholic fatty liver disease and risk of type 2 diabetes. Best Pract Res Clin Endocrinol Metab 2016; 30: 385-395.

[13] Ballestri S, Zona S, Targher G, Romagnoli D, Baldelli E, Nascimbeni F, Roverato A, Guaraldi G, Lonardo A. Nonalcoholic fatty liver disease is associated with an almost twofold increased risk of incident type 2 diabetes and metabolic syndrome. Evidence from a systematic review and meta-analysis. J Gastroenterol Hepatol 2016; 31: 936-944.

[14] Kim JS, Lê KA, Mahurkar S, Davis JN, Goran MI. Influence of elevated liver fat on circulating adipocytokines and insulin resistance in obese Hispanic adolescents. Pediatr Obes 2012; 7: 158-164.

[15] Wei C, Ford A, Hunt L, Crowne EC, Shield JP. Abnormal liver function in children with metabolic syndrome from a UK-based obesity clinic. Arch Dis Child 2011; 96: 1003-1007.

[16] Radmard AR, Rahmanian MS, Abrishami A, Yoonessi A, Kooraki S, Dadgostar M, Hashemi Taheri AP, Gerami Seresht M, Poustchi H, Jafari E, Malekzadeh R, Merat S. Assessment of abdominal fat distribution in non-alcoholic fatty liver disease by magnetic resonance imaging: a population-based study. Arch Iran Med 2016; 19: 693-699.

[17] Ebrahimi-Mameghani M, Jamali H, Mahdavi R, Kakaei F, Abedi R, Kabir-Mamdooh B. Conjugated linoleic acid improves glycemic response, lipid profile, and oxidative stress in obese patients with non-alcoholic fatty liver disease: a randomized controlled clinical trial. Croat Med J 2016; 57: 331-342.

[18] Lonardo A, Nascimbeni F, Mantovani A, Targher G. Hypertension, diabetes, atherosclerosis and NASH: cause or consequence? J Hepatol 2018; 68: 335-352.

[19] Misciagna G, Del Pilar Díaz M, Caramia DV, Bonfiglio C, Franco I, Noviello MR, Chiloiro M, Abbrescia DI, Mirizzi A, Tanzi M, Caruso MG, Correale M, Reddavide R, Inguaggiato R, Cisternino AM, Osella AR. Effect of a low glycemic index mediterranean diet on non-alcoholic fatty liver disease. A randomized controlled clinici trial. J Nutr Health Aging 2017; 21: 404-412.

[20] Babu PS, Srinivasan K. Hypolipidemic action of curcumin, the active principle of turmeric (Curcuma longa) in streptozotocin induced diabetic rats. Mol Cell Biochem 1997; 166: 169-175.
[21] Inzaugarat ME, De Matteo E, Baz P, Lucero D, García CC, Gonzalez Ballerga E, Daruich J, Sorda JA, Wald MR, Cherñavsky AC. New evidence for the therapeutic potential of curcumin to treat nonalcoholic fatty liver disease in humans. PLoS One 2017; 12: e0172900.
[22] Arun N, Nalini N. Efficacy of turmeric on blood sugar and polyol pathway in diabetic albino rats. Plant Foods Hum Nutr 2002; 57: 41-52.
[23] Zhao YL, Zhou GD, Yang HB, Wang JB, Shan LM, Li RS, Xiao XH. Rhein protects against acetaminophen-induced hepatic and renal toxicity. Food Chem Toxicol 2011; 49: 1705-1710.
[24] Li J, Zhang YM, Li JY, Zhu L, Kang HX, Ren HY, Chen H, Yuan L, Miao YF, Wan MH, Tang WF. Effect of Sheng-jiang powder on obesity-induced multiple organ injuries in rats. Evid Based Complement Alternat Med 2017; 2017: 6575276.
[25] Zhang T, Xiao M, Wong CK, Mok KC, Zhao X, Ti H, Shaw PC. Sheng Jiang San, a traditional multi-herb formulation, exerts anti-influenza effects in vitro and in vivo via neuraminidase inhibition and immune regulation. BMC Complement Altern Med 2018; 18: 150.
[26] Hong XZ, Li LD, Wu LM. Effects of fenofibrate and xuezhikang on high-fat diet-induced nonalcoholic fatty liver disease. Clin Exp Pharmacol Physiol 2007; 34: 27-35.
[27] Frias FT, Rocha KCE, de Mendonça M, Murata GM, Araujo HN, de Sousa LGO, de Sousa É, Hirabara SM, Leite NC, Carneiro EM, Curi R, Silveira LR, Rodrigues AC. Fenofibrate reverses changes induced by high-fat diet on metabolism in mice muscle and visceral adipocytes. J Cell Physiol 2018; 233: 3515-3528.
[28] Malaguarnera M, Di Rosa M, Nicoletti F, Malaguarnera L. Molecular mechanisms involved in NAFLD progression. J Mol Med (Berl) 2009; 87: 679-695.
[29] Della Pepa G, Vetrani C, Lombardi G, Bozzetto L, Annuzzi G, Rivellese AA. Isocaloric dietary changes and non-alcoholic fatty liver disease in high cardiometabolic risk individuals. Nutrients 2017; 9.
[30] Puri P, Baillie RA, Wiest MM, Mirshahi F, Choudhury J, Cheung O, Sargeant C, Contos MJ, Sanyal AJ. A lipidomic analysis of nonalcoholic fatty liver disease. Hepatology 2007; 46: 1081-1090.
[31] Ke R, Xu Q, Li C, Luo L, Huang D. Mechanisms of AMPK in the maintenance of ATP balance during energy metabolism. Cell Biol Int 2018; 42: 384-392.
[32] Auciello FR, Ross FA, Ikematsu N, Hardie DG. Oxidative stress activates AMPK in cultured cells primarily by increasing cellular AMP and/or ADP. FEBS Lett 2014; 588: 3361-3366.
[33] Bronisz A, Chiocca EA, Godlewski J. Response to energy depletion: miR-451/AMPK loop. Oncotarget 2015; 6: 17851-17852.
[34] Zhang HA, Yang XY, Xiao YF. AMPKalpha1 overexpression alleviates the hepatocyte model of nonalcoholic fatty liver disease via inactivating p38MAPK pathway. Biochem Biophys Res Commun 2016; 474: 364-370.
[35] Seo E, Park EJ, Joe Y, Kang S, Kim MS, Hong SH, Park MK, Kim DK, Koh H, Lee HJ. Overexpression of AMPKalpha1 ameliorates fatty liver in hyperlipidemic diabetic rats. Korean J Physiol Pharmacol 2009; 13: 449-454.
[36] Han J, Wang Y. mTORC1 signaling in hepatic lipid metabolism. Protein Cell 2018; 9: 145-151.
[37] Tremblay F, Gagnon A, Veilleux A, Sorisky A, Marette A. Activation of the mammalian target of rapamycin pathway acutely inhibits insulin signaling to Akt and glucose transport in 3T3-L1 and human adipocytes. Endocrinology 2005; 146: 1328-1337.
[38] Huffman TA, Mothe-Satney I, Lawrence JC Jr. Insulin-stimulated phosphorylation of lipin mediated by the mammalian target of rapamycin. Proc Natl Acad Sci U S A 2002; 99: 1047-1052.
[39] Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, Vasquez DS, Turk BE, Shaw RJ. AMPK phosphorylation of raptor mediates a metabolic checkpoint. Mol Cell 2008; 30: 214-226.