Effect of Shuanghuanglian Oral Solution on Liver Function in a Mouse Model of Non-alcoholic Steatohepatitis

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Abstract [**Objectives**] To observe the effect of Shuanghuanglian oral solution on liver function in BABL/cJ mice in non-alcoholic steatohepatitis (NASH) model. [**Methods**] The BABL/cJ mice were randomly divided into three groups: a control group, a model group, and an experimental group. The experimental group was administered with 10% Shuanghuanglian oral solution at a dose of 0.1 mL/(10 g · d), while the control group and experimental group received an equivalent dosage of normal saline. All three groups were treated for a period of 28 d. The liver function of the mice in each group was examined after the treatment. [**Results**] The body mass, liver index, triacylglycerol (TG), total cholesterol (TC), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels were all significantly reduced compared to the model group ($P < 0.05$). [**Conclusions**] Shuanghuanglian oral solution has a beneficial effect on liver function in BABL/cJ mice.

Key words BABL/cJ mice, Shuanghuanglian oral solution, Non-alcoholic steatohepatitis model, Liver function

1 Introduction

Non-alcoholic steatohepatitis (NASH), also known as metabolic steatohepatitis, is a common cause of liver fibrosis and cirrhosis[1]. NASH is similar to alcoholic hepatitis, but its etiology remains unclear. Potential contributing factors include obesity, insulin resistance, oxidative stress-induced lipid peroxidation damage, intestinal bacterial ecological imbalance, gene or gene receptor polymorphisms, which collectively lead to steatohepatitis[2]. Therefore, it is of great significance to prevent and treat NASH to prevent or delay the evolution of liver fibrosis to acute liver failure caused by cirrhosis and liver cancer, and to improve the survival time and quality of life of patients. The objective of this experiment was to observe the effect of Shuanghuanglian oral solution on liver function in NASH model mice, to explore the possible mechanism of Shuanghuanglian oral solution against NASH, and to provide new ideas for the clinical treatment of NASH.

2 Materials and methods

2.1 Experimental animals Twenty-four newly weaned BABL/cJ mice, male, 3 weeks of age, body mass (13.0 ± 0.5) g, were utilized in this study. Throughout the modeling and experimental phases, the 3R principle was adhered to, and humane care was provided to ensure that the subject mice could fully enjoy animal welfare. During the experiment, the animals were permitted to drink water ad libitum, the feeding room was changed approximately 10 times per hour, and the bedding was changed once a day. The environmental temperature of the animal room was maintained at 22 - 26 ℃, the relative humidity was controlled at 40% - 70%, and the light and shade were alternated between 12 h of lighting and 12 h of darkness.

2.2 Main drugs and instruments The Shuanghuanglian oral solution (batch No.: Z10920053) was manufactured by Harbin Pharmaceutical Group Sanwei Pharmaceutical Co., Ltd. The kits for aspartate ammonia (AST), alanine aminotransferase (ALT), triacylglycerol (TG), and total cholesterol (TC) were purchased from Shanghai Yisheng Biotechnology Co., Ltd. (batch No.: H16133, H161024, H16127, H16409, H167216).

2.3 Mice grouping and NASH model construction The subject mice were numbered in a random number table for the control, model, and experimental groups ($n = 8$). The nutrient solution was prepared according to the formula of Chen Yiping *et al.*[3], which consisted of a high-fat enteral nutrient solution containing 20% lard, 20% sucrose, 1% cholate, and 2% cholesterol. This resulted in an energy supply ratio of carbohydrate of 30.8% and fat of 69.2%. All mice in the three groups were fed with a standard diet, while the model group and the experimental group were administered an enteral high-fat nutrient solution for 12 weeks. In the morning and afternoon of every day, a high-fat enteral nutrient solution was infused at a rate of 0.3 mL/(10 g · d). The control group received an equal volume of saline as a blank control. All three groups were fed for 12 weeks. The NASH standard referred to the pre-experimental results. After 12 weeks, the mice exhibited slight growth in size compared with the control group, accompanied by a sluggish response, lethargy, and a yellowish hue to their hair.. The above pathological changes in the liver were indicative of the successful establishment of the NASH standard[4].

2.4 Treatment methods According to the human and animal conversion method of pharmacological experimental methodology and previous experience in the laboratory, the experimental group received Shuanghuanglian oral solution by gavage at a dose of 0.1 mL/(10 g · d) body mass, administered twice daily, while the control and model groups received 0.1 mL/(10 g · d). All groups were treated with gavage for a period of 28 d.

2.5 Test index

2.5.1 Liver index determination. Following the administration of an anesthetic agent (*e.g.*, ether), the eyeballs of the mice were

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excised and approximately 0.2 mL of blood were collected and frozen at a temperature of 40 ℃. All mice in the 3 experimental groups were euthanized, and the abdomen was opened to find the liver. The size, color and shape of the liver were observed, and then the liver was harvested, dried, and the liver index was calculated [Liver index = Liver weight (g)/Body mass (g)].

2.5.2 Liver function detection in mice. A sample of frozen mouse blood (0.1 mL) was submitted to the laboratory for analysis using the UniCel DxC 600 Synchron automatic biochemical analysis system to detect the levels of AST, ALT, TG and TC.

2.6 Statistical methods The statistical processing was performed using the SPSS 17.0 software, and the quantitative data were expressed as mean ± standard deviation ($\bar{x} \pm s$). The one-way analysis of variance and the pairwise t-test with two independent samples were employed for the pairwise comparisons between the groups. $P < 0.05$ was considered to be statistically significant.

3 Results and analysis

3.1 Effects on body mass and liver index in NASH model mice After the treatment, the body quality and liver index of the mice in the experimental group were observed to be lower than those in the control group. This difference was found to be statistically significant ($P < 0.05$, Table 1).

Table 1 Comparison of body mass and liver index of mice in 3 groups ($n = 8$, $\bar{x} \pm s$)

Group	Body mass//g	Liver index//%
Control	28.67 ± 2.73	4.52 ± 0.94
Model	33.02 ± 3.26[a]	7.25 ± 1.07[a]
Experimental	29.43 ± 2.90[b]	4.71 ± 0.86[b]

NOTE Compared with control group, [a]$P < 0.05$; compared with the model group, [b]$P < 0.05$. The same below.

3.2 Effects on liver function and lipid in NASH model mice The levels of AST, ALT, TG and TC in the experimental group were significantly reduced compared with the model group, and the difference between the groups was statistically significant ($P < 0.05$, Table 2).

Table 2 Comparison of liver function and lipid profile of mice in 3 groups ($n = 8$, $\bar{x} \pm s$)

Group	AST//U/L	ALT//U/L	TG//pg/mL	TC//pg/mL
Control	17.39 ± 2.81	17.23 ± 1.06	0.94 ± 0.12	1.67 ± 0.10
Model	90.27 ± 2.95[a]	30.04 ± 4.57[a]	3.25 ± 0.27[a]	2.89 ± 0.17[a]
Experimental	18.21 ± 2.83[b]	18.84 ± 4.02[b]	1.02 ± 0.14[b]	1.73 ± 0.12[b]

4 Discussion

The latest research in the field of modern medicine indicates NASH is a hepatic fatty lesion caused by a variety of diseases and causes. The onset and long course of the disease, as well as the trend of younger age due to life and dietary habits, have been identified as key factors[5]. NASH is not a DNA virus infection in hepatitis, but its pathological changes exhibit similarities with both. Eighty percent of patients present with no overt symptoms, and they are frequently identified and diagnosed during routine health examinations. As the disease progresses, the patients may develop liver fibrosis and cirrhosis, and in some cases, hepatocellular carcinoma[6]. Among the various causes of liver disease, progressive liver fibrosis and persistent inflammatory reaction are the most significant, eventually leading to liver cirrhosis[7]. NASH not only directly affects the quality of life of patients, but also has a close relationship with atherosclerosis, diabetes, cerebrovascular disease, coronary heart disease and other conditions[8]. NASH exhibits considerable overlap with the syndromes of sputum, threat pain, accumulation, accumulation syndrome, and less fat gas, as defined in traditional Chinese medicine. The majority of Chinese medicine scholars concur that the underlying causes of NASH and appetite fat gain are "qi stagnation, phlegm, blood stasis, qi deficiency and phlegm poison", phlegm turbidity and blood stasis, and other endogenous factors that impede the normal flow of qi and blood. These factors may also result from emotional imbalances, loss of vital energy, damp and humid epidemic poisons, liver loss, and other factors that disrupt the normal functioning of the liver and spleen[9].

The experimental observation found that the body mass, liver index, AST, ALT, TG and TC levels were significantly lower than those observed in the model group. The results of this experiment suggest that Shuanghuanglian oral solution may be beneficial for improving liver function in BABL/cJ mice. Concurrently, it facilitates the recovery of liver function by enhancing the metabolic processes within liver tissue, thereby exerting a therapeutic effect in the treatment of NASH.

The Shuanghuanglian oral solution is composed of Chinese herbal medicine, including honeysuckle, forsythia, and baicalensis. The analysis indicates that forsythia and honeysuckle can evacuate wind heat and detoxification, while the bitter cold baicalensis can clear lung fire and the solid heat of coke can relieve cough. These three drugs play a role in detoxification, relieving toxicity and clearing heat. However, they also have anti-fat effects that relieve liver and spleen congestion, phlegm, and blood stasis[10].

In conclusion, the Shuanghuanglian oral solution has been demonstrated to improve liver function in a NASH model of BABL/cJ ASH mice. The mechanism may be to promote the recovery of liver function by improving the energy metabolism of liver tissue, thereby playing a therapeutic role in NASH. It is evident that further experimental research is required to ascertain the efficacy of this role in the prevention and control of NASH. In order to achieve this, it is necessary to conduct further studies and explore the pharmacological effects of this formula.

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(To page 106)

drugs on serum motilin (MLT) and vasoactive intestinal peptide (VIP) levels in normal mice, only the serum MLT level in high concentration group of *P. fallax* Hemsl had statistical significance, indicating that the drug contained substances that stimulated MTL release, but the concentration needed to be at 0.5 g/mL of the raw material to take effect. However, the serum VIP content was not statistically significant, indicating that the effect of *P. fallax* Hemsl was not related to VIP. In the study on the effects of different drugs on the tissue morphology of the gastric and small intestinal mucosa in normal mice, no significant changes were observed in each group compared to the blank group. Therefore, it can be concluded that the mechanism of enhancing normal gastrointestinal motility by various concentrations of *P. fallax* Hemsl water solution and domperidone solution would not cause changes in tissue morphology.

In the small intestine propulsion rate experiments of liquid and semi-solid paste in gastric motility disorder model mice, the high, medium, and low concentration groups of *P. fallax* Hemsl, the domperidone group, and the blank group had higher small intestine propulsion rates than the atropine group. Among them, the low concentration group was the most significant, and it did not show a linear relationship with concentration. It can be inferred that there may be a substance concentration in the low concentration group that can reactivate the M receptor blocked by atropine, causing the low concentration of the drug to reverse the effect of atropine and result in enhanced small intestine motility. In the residue rate experiment of semi-solid paste in gastric motility disorder model mice, the gastric residue rate showed a similar situation to the small intestine propulsion rate. The gastric residue rates of the high, medium, and low concentration groups of *P. fallax* Hemsl, the domperidone group, and the blank group were lower than that of the atropine group, with the low concentration group showing the strongest performance. It can be inferred that the low concentration of this drug can reverse the effect of atropine and result in enhanced gastric motility.

In summary, *P. fallax* Hemsl water extract had a promoting effect on gastrointestinal motility. One of the specific mechanisms by which *P. fallax* Hemsl promoted gastrointestinal motility in normal mice may be related to the content of MLT in mouse serum. The mechanism of action in atropine induced gastric paresis mice may be related to the reactivation of M receptors, and the action mechanism of *P. fallax* Hemsl did not change the original histological basis. It can be inferred that there are other synergistic effects of *P. fallax* Hemsl water extract on gastrointestinal motility, but it is not fully understood and further in-depth research is needed.

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(From page 100)

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