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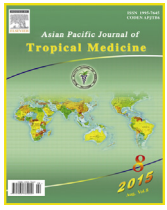

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Protection effect of trigonelline on liver of rats with non-alcoholic fatty liver diseases

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ABSTRACT

Objective: To study the effect of trigonelline on the change of indicators of serum transaminase, lipoprotein and liver lipid of model rats with non-alcoholic fatty liver diseases and on the expression level of Bcl-2 and Bax proteins.

Methods: A total of 45 SD rats were randomly divided into the control group, model group and trigonelline intervention group. Rats in the control group were fed with the common diet, while rats in the model group and intervention group were fed with the high fat diet. 8 weeks later, the intervention group received the intragastric administration of trigonellin e (with the dosage of 40 mg/kg/d) for 8 weeks; while control group and model group received the intragastric administration of saline with the equal dosage. Blood was taken from the abdominal aorta of rats 8 weeks later, detecting the level of a series of indicators of ALT, AST, TG, TC, HDL-C and LDL-C in the serum. After the rats were sacrificed, detect the indicators of TG, TC, SOD and MDA in the liver tissue of rats, as well as the expression of Bcl-2 and Bax in the liver tissue.

Results: Results of histopathologic examination showed that the damage degree of liver for rats in the trigonelline intervention group was smaller than the one in the model group, with significantly reduced hepatic steatosis and the partially visible hepatic lobule. The levels of ALT, AST, TC and LDL-C in the serum of rats in the trigonelline group were significantly reduced, while the change in the levels of TG and HDL-C was not significantly different. The levels of TG, TC and MDA in the liver tissues were significantly decreased, while the level of SOD significantly increased; the expression of Bcl-2 protein in the liver tissues of rats in the trigonelline intervention group was significantly increased, while the expression of Bax protein significantly decreased.

Conclusions: The trigonelline contributes to the therapeutic effect of non-alcoholic fatty liver diseases. It can also increase the expression of Bcl-2 protein and decrease the expression of Bax protein in the liver tissues, which can protect the liver.

1. Introduction

In recent years, the incidence of non-alcoholic fatty liver diseases (NAFLD) and non-alcoholic steatohepatitis is increasing year by year [1,2] and there is no medicine with the proved effect at the moment. In China, with the increased living standard, dietary structure and lifestyle of Chinese people show great change, while the incidence of NAFLD shows the tendency of increase year by year [3,4]. NAFLD is a sort of metabolic syndrome and its pathological features include the degeneration of liver cells and lipid particles in the liver cells [5].

Trigonelline is one of major alkaloids that exists in the seeds of fenugreek, is also the main effective component of such seeds [6,7]. According to the modern medical researchers, the trigonelline can reduce the blood sugar and cholesterol, has

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antioxidation function and can promote the regeneration of neural tissues [8–10], but there is no research on the effect of trigonelline on NAFLD. This study took the lipid oxidation and cell apoptosis as the starting point, fed SD rats with high-fat diet for the preparation of NAFLD model, then discussed the specific mechanism of trigonelline and the effect on liver of NAFLD rats.

2. Materials and methods

2.1. Preparation of animal model and grouping

After one week of adaptive feeding, 45 SPF-level SD male rats were randomly divided into 3 groups, with 15 subjects in each group, including the control group, model group and intervention group. Rats in the control group were fed with the normal diet, while rats in the model group and intervention group were fed with the high fat diet for 8 weeks. Eight weeks later, the intervention group received the intragastric administration of trigonelline (with the dosage of 40 mg/kg/d) for 8 weeks; while control group and model group received the intragastric administration of saline with the equal dosage.

2.2. Detection of biochemical indicators in blood

After the final feeding, rats in each group were fasted for 12 h. A total of 15 experimental animals were taken from the control group, model group and intervention group respectively for the blood collection from the abdominal aorta. The levels of a series of indicators of ALT, AST, TC, TG, HDL-C and LDL-C in the serum were detected.

2.3. Detection of Bcl-2 and Bax

Parts of liver tissues were cut off for the detection of liver lipid, while other part of liver tissues for the detection of *Bcl-2* and *Bax*. They were stored in the refrigerator at −80 °C.

2.4. Western blot test

Samples of liver tissues were unfrozen. RIPA lysis buffer was chosen for the cell lysis. The loading buffer was added. After being boiled, the polyacrylamide gel electrophoresis was performed to separate the protein. After the electrophoresis, the protein was transferred on the gel to PVDF film. When it was finished, the PVDF film was blocked using the solution with 5% skimmed milk powder for 1 h. The concentration of 1:200 was set to dilute the primary antibody of Bcl-2 and Bax (the primary antibody of Bcl-2 was purchased from Santa Cruz, with the item number of sc-492; the primary antibody of Bax was purchased from Santa Cruz, with the item number of sc-493; the primary antibody of β-actin was purchased from Santa Cruz, with the item number of sc-1616). The overnight incubation was performed at 4 °C. On the next day, PBST was used to wash PVDF film for 3 times and then the secondary antibody was added for the incubation. Finally, the chemiluminescence solution purchased from Millipore was employed for the coloration and the chemiluminescence apparatus (purchased from Biorad) for the detection.

2.5. Statistical analysis

The experimental data was expressed by mean ± SD. SPSS17.0 was employed for the analysis of variance between groups. The t test was used for the comparison of means between two groups, while $P < 0.05$ was considered as the significant difference.

3. Results

3.1. Pathological slices of liver tissues of NAFLD rats

According to the visual inspection, the liver morphology of rats in the control group, showed the normal size, soft texture and red color; while the liver of rats in the model group showed the increased volume, hard texture, relatively oily section and creamy yellow color. The liver of rats in the trigonelline intervention group was better than the one in the model group, showing the light red surface. As shown in Figure 1, the liver cells of rats in the control group showed the normal structure and clearly visible hepatic lobule, without any obvious inflammation, steatosis or necrosis; a great number of liver cells in the liver tissue of rats in the model group showed the steatosis and invisible hepatic lobule, with a great number of lipid droplets in the cytoplasm. The damage of liver cells of rats in the trigonelline intervention group was significantly relieved and only part of liver cells showed the steatosis, with the smaller lipid droplet and the certain amount of hepatic lobule.

3.2. Changes in AST, ALT, TC, TG, HDL-C and LDL-C in the serum

As shown in Table 1, compared with the control group, rats in the model group showed the significantly increased level of AST, ALT, TC and LDL-C in the serum, significantly decreased level of HDL-C, but no significant difference in the level of TG.

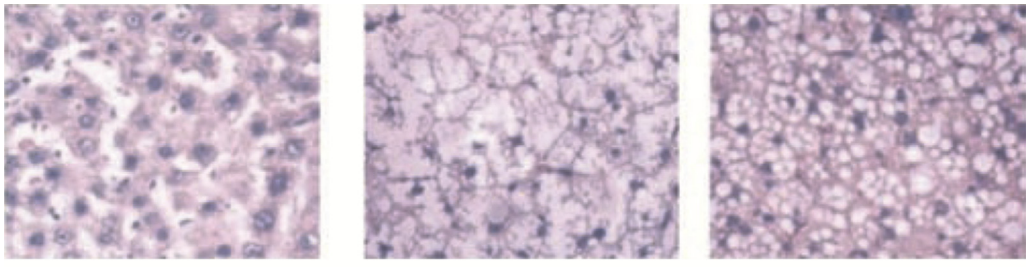

Figure 1. Results of histopathological sections of liver tissues of rats in each group (×400).