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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.

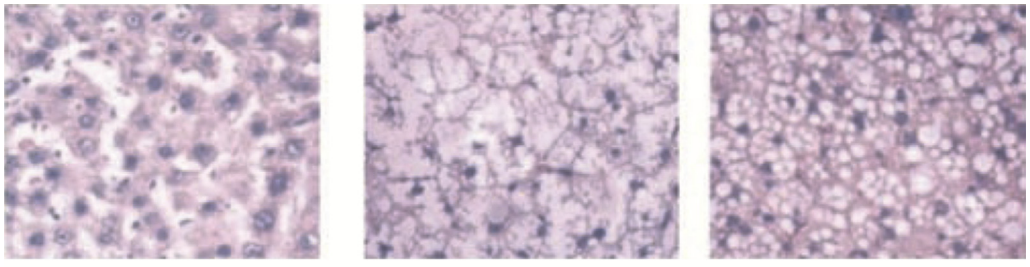

Figure 1. Results of histopathological sections of liver tissues of rats in each group (×400).

Table 1
Comparison of AST, ALT, TC, TG, HDL-C and LDL-C in the serum of rats (n = 7).

Group	AST (μkat/L)	ALT (μkat/L)	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
Control group	2.185 ± 0.173	0.359 ± 0.124	1.25 ± 0.21	0.92 ± 0.22	1.12 ± 0.15	0.18 ± 0.05
Model group	4.238 ± 0.702*	1.037 ± 0.112*	3.51 ± 0.72*	1.15 ± 0.14	0.61 ± 0.07*	0.71 ± 0.19*
Intervention group	3.301 ± 0.215$^{\#}$	0.796 ± 0.083$^{\#}$	1.72 ± 0.57$^{\#}$	1.07 ± 0.20	0.78 ± 0.16	0.41 ± 0.11$^{\#}$

Note: Compared with the control group, *$P < 0.05$; compared with the model group, $^{\#}P < 0.05$.

Table 2
Effect of trigonelline on levels of TG, TC, SOD and MDA in liver issues of NAFLD Rats (n = 7).

Group	TG (mmol/L)	TC (mmol/L)	SOD (U/mgprot)	MDA (nmol/mgprot)
Control group	1.65 ± 0.33	1.12 ± 0.33	376 ± 42	1.53 ± 0.23
Model group	3.85 ± 0.25*	1.83 ± 0.76*	201 ± 32*	3.81 ± 1.42*
Intervention group	2.55 ± 0.36$^{\#}$	1.31 ± 0.64$^{\#}$	282 ± 36$^{\#}$	1.79 ± 0.61$^{\#}$

Note: Compared with the control group, *$P < 0.05$; compared with the model group, $^{\#}P < 0.05$.

After the treatment of trigonelline, rats in the intervention group showed the significant decreased levels of AST, ALT, TC and LDL-C in the serum, but no significant change in the levels of HDL-C and TG. Thus the trigonelline could significantly reduce the levels of AST, ALT, TC and LDL-C in the serum of modeled rats, but have limited effect on HDL-C and TG, which indicated that the trigonelline could protect the liver.

3.3. Change in TG, TC, SOD, MDA

As shown in Table 2, levels of TG, TC and MDA in the liver tissues of rats in the model group were significantly increased than the one in the control group, while the level of SOD decreased. After the treatment of trigonelline, levels of TG, TC and MDA in the liver tissues of rats in the intervention group were significantly reduced than the one in the model group, while the level of SOD showed the significant increase.

3.4. Expression of Bcl-2 and Bax

The expression of Bcl-2 in rats of model group was lower than the one in the control group, while the expression of Bax was higher. After the treatment of trigonelline, the expression of Bcl-2 in rats of intervention group was significantly higher than the one in the model group, while the expression of Bax was lower (Figure 2, Table 3).

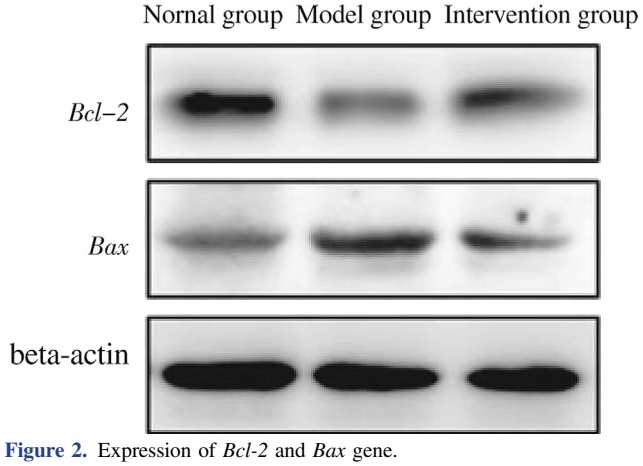

Figure 2. Expression of *Bcl-2* and *Bax* gene.

Table 3
Expression of proteins of Bcl-2 and Bax.

Group	Bcl-2/β-actin	Bax/β-actin	Bcl-2/Bax
Control group	0.56 ± 0.13	0.58 ± 0.17	0.95 ± 0.21
Model group	0.25 ± 0.08*	0.93 ± 0.13*	0.37 ± 0.15*
Intervention group	0.39 ± 0.15$^{\#}$	0.67 ± 0.16$^{\#}$	0.55 ± 0.22$^{\#}$

Note: Compared with the control group, *$P < 0.05$; compared with the model group, $^{\#}P < 0.05$.

4. Discussion

The trigonelline is a sort of alkaloid. Recent researches showed that the trigonelline possesses the functions of anti-oxidation and can reduce the blood sugar and cholesterol [11–13], but there is no research on the therapeutic effect of trigonelline against NAFLD. In this study, results of pathological detection showed that the damage degree of liver of rats in the trigonelline intervention group was reduced and steatosis rate of liver cells was also significantly decreased than the model group, with the visible hepatic lobule. Besides, the levels of ALT, AST, TC and LDL-C in the serum were significantly decreased, as well as the levels of TG, TC and MDA in the liver tissues, but the level of SOD was increased. These results all indicate that the trigonelline can effectively improve the symptoms of NAFLD. So far as we know, it is the first study to adopt the trigonelline in the treatment of NAFLD, evaluate a series of indicators of liver and also preliminarily confirm the protection mechanism of trigonelline for the liver with NAFLD.

The hepatocyte apoptosis is closely related to the diseases such as the inflammation and fibrosis, as well as the occurrence and development of NAFLD. In the signal transduction of apoptosis, the component ratio of Bcl-2 family members is the critical factor to regulate the apoptosis, where Bcl-2 and Bax are the typical members of Bcl-2 family and the ratio between them Bcl-2/Bax is regarded as the “molecular switch” to start the apoptosis [14].

Bcl-2 gene is also named as the apoptosis inhibition gene. According to the immunohistochemical staining and electron microscopic observation, *Bcl-2* is mainly distributed in the endoplasmic reticulum membrane, outer mitochondrial membrane and perinuclear membrane, which can resist the stimulation of apoptosis and thus play the role of protection for cells.

The low expression of such gene was always accompanied with the occurrence of apoptosis [15,16]. *Bax* (Bcl-2 associated protein X) is distributed in the cytoplasm, intracellular membrane and nucleus. The high expression of such gene is the indication of apoptosis. The normal liver tissue shows the certain expression of *Bcl-2* and *Bax* and its ratio between *Bcl-2*/*Bax* keeps balanced. In case of the apoptosis, such ration would be disordered [17]. This study adopts the experimental techniques of molecular biology to detect the function of trigonelline. Results showed that, compared with the model group, the expression of Bcl-2 in the liver tissue of rats in the trigonelline intervention group was significantly increased, with the significant decrease in the expression of Bax and the significant increase in the ratio Bcl-2/Bax, but still lower than the one in the control group. It indicates that the trigonelline may affect the relative expression of Bcl-2 and Bax in the liver tissues and thus reduce the hepatocyte apoptosis and realize the treatment of NAFLD. This research will provide the reference for our further exploration in the treatment of NAFLD using trigonelline. In the succeeding works, we will continue to evaluate the effect of trigonelline in the treatment of NAFLD.

Conflict of interest statement

We declare that we have no conflict of interest.

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