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Effect of *Rheum palmatum* L. on NF-κB signaling pathway of mice with acute liver failure

Rong-Zhen Zhang, Hua Qiu, Na Wang, Fu-Li Long, De-Wen Mao*

First Affiliated Hospital of Guangxi University of TCM, Nanning 530021, China

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ABSTRACT

Objective: To explore the regulation effect of *Rheum palmatum* (*R. palmatum*) L. on NF-κB signaling pathway of ALF mice.

Methods: The intraperitoneal injection of D-GalN/LPS was employed for the model building. Mice in the treatment group and positive control group were given the *R. palmatum* L. and bifendate before the model building. Mice in the normal group were given the intraperitoneal injection of equivalent normal saline for continuously 3 d. After 16 h of model building, the blood was collected from eyeballs of mice and then mice were executed. The measurement was performed on the content of ALT, AST, NO and Il-1β in the serum of mice in each group, as well as the activity of Caspase 3 and Caspase 8 in the liver tissue. HE staining was employed to detect the pathological morphology of liver; and the western blot was used to detect the expression of iNOS, COX-2, Bax, Bcl-2, PCNA, NF-κB p65 and IκBα.

Results: The content of ALT, AST, NO and Il-1β in the serum and the activity of Caspase 3 and Caspase 8 in the liver tissue were increased in the mice of ALF model group. Besides, the expression of iNOS, COX-2 and Bax was increased, the expression of Bcl-2 and PCNA was decreased, the phosphorylation of NF-κB p65 and IκBα was significant and the treatment group of *R. palmatum* L. could inhibit such change.

Conclusions: Through NF-κB signaling pathway, the *R. palmatum* L. could reduce the content of enzyme of liver function and inflammation factor in the serum of ALF mice, regulate the expression of cell apoptosis-related protein and improve the symptoms of ALF mice.

1. Introduction

The acute liver failure (ALF) refers to the clinical syndrome of abundant necrosis of liver cells, infiltration of inflammatory cells and severe failure of cells and organs that are caused by various factors. It can result in the serious damage to the human health and its clinical mortality is over 50%. ALF that is induced by different factors will eventually cause the activation of inflammatory cells and thus aggravate the necrosis of liver cells, lead to the serious disorders of synthesis, metabolism, transport and excretion of liver and different kinds of clinical symptoms [1,2].

The liver failure belongs to yellow fever in traditional Chinese medicine. Its basic pathology is the combination of 'toxin', 'blood stasis' and 'phlegm' and the key to the treatment is the detoxification and stasis-removing. The *Rheum palmatum* (*R. palmatum*) L. is the root or stem of *R. palmatum* of Polygonaceae family. According to the traditional Chinese medicine, the *R. palmatum* L. is bitter in taste and cold in nature, with the effect of heat-clearing, fire-purging, blood-cooling, detoxification, blood circulation-promoting and blood stasis-removing. Results of modern pharmacological studies showed that *R. palmatum* L. could relieve the liver injury [3–6] and it is also widely applied in the treatment of hepatitis. In the treatment of post-operative ALF rats, the *R. palmatum* L. could reduce the expression of Caspase 3, Fas and FasL to inhibit the apoptosis of liver cells [3]. Besides, it could also increase the expression of PCNA [4] to promote the regeneration of liver cells and relieve the liver injury. In the treatment of mice with the acute liver injury that is induced by concanavalin, the *R. palmatum* L. and licorice decoction can reduce the content of alanine

*Corresponding author: De-Wen Mao, PhD., Professor, Department of Hepatology, First Affiliated Hospital of Guangxi University of TCM, No. 89-9 Dongge Road, Nanning, Guangxi, China.
Tel: +86 13132905888
E-mail: Mdwboshi2005@163.com

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aminotransferase (ALT) and aspartate aminotransferase (AST) to improve the liver function [5]. In the treatment of rats with the acute intrahepatic cholestasis, compared with the model group, the *R. palmatum* L. infusion could inhibit the activity of NF-κB and reduce the content of ALT and AST to play its role of protecting the liver [6].

NF-κB can be activated by the lipopolysaccharide, bacteria and virus and it can be transported from the cytoplasm to the nucleus to regulate the expression of related genes [7,8]. There is a close relationship between the apoptosis and inflammation of liver cells that is caused by ALF and the NF-κB signaling pathway. Through such pathway, it can regulate the expression of inflammation factors of iNOS and COX-2 and the apoptosis proteins of Bax, Bcl-2 and PCNA [9–13].

Under such theoretical background, it's assumed that the *R. palmatum* L. can reduce the enzymes, decrease the secretion of inflammatory factors and inhibit the apoptosis of liver cells through NF-κB signaling pathway, in order to play its role of protecting the liver. Accordingly, this study aims to explore the effect of *R. palmatum* L. on the expression of ALF inflammatory factors, ALF apoptosis-related proteins and ALF NF-κB signaling pathway related proteins.

2. Materials and methods

2.1. Animals

ICR mice were purchased from Shanghai SLAC laboratory Animal Co., Ltd., SPF, with the qualified certificate of 2012-0002 and the weight of (20 ± 2) g. The room temperature was controlled at (23 ± 2) °C and mice were given the diet and water freely.

2.2. Drugs and reagents

The *R. palmatum* L. was purchased from Guoda Drugstore and bifendate dropping pills from Zhejiang Medicine Co., Ltd.

D-galactosamine (D-GalN) was purchased from Sigma (Item No.: G0500); lipopolysaccharide (LPS) from Sigma (Item No.: L2630); BCA protein assay kit from Beyotime Biotechnology (Item No.: P0009); ECL Western blotting kit from Beyotime Biotechnology (Item No.: P0018); β-actin from Beyotime Biotechnology (Item No.: AA128); NO test kit from Applygen Technologies Inc. (Item No.: E1030); Caspase 3 from Nanjing KeyGen Biotech. Co. Ltd. (Item No.: KGA204); Caspase 8 test kit from Nanjing KeyGen Biotech. Co. Ltd. (Item No.: KGA304); ALT test kit from Nanjing Jiancheng Bioengineering Institute (Item No.: C010-2); AST test kit from Nanjing Jiancheng Bioengineering Institute (Item No.: C009-2); interleukin-1β (IL-1β) test kit from Nanjing Jiancheng Bioengineering Institute (Item No.: H002); rabbit anti COX-2 and iNOS from Wuhan Boster Biological Technology Co., Ltd.; and rabbit anti Bcl-2, Bax, PCNA, NF-κB p65, pp65, IκBα, p-IκBα antibodies from Epitmics.

2.3. Instruments

The paraffin slicing machine was purchased from Optical; automatic biochemical analyzer from Abbott; electrophoresis instrument, Trans-blot electrophoresis tank and ChemiDoc™ XRS gel imaging system Bio-Rad; ELISA kit from TECAN; and −80 °C refrigerator from Thermo.

2.4. Modeling

A total of 40 mice were randomly divided into the normal group, model group, *R. palmatum* L. treatment group and bifendate positive control group, with 10 mice in each group. According to the previous researches [7,8], mice were given the intraperitoneal injection of D-GalN (400 mg/kg)/LPS(10 μg/kg) for the modeling. Before the modeling, mice in the treatment group and positive control group were given the intraperitoneal injection of *R. palmatum* L. (1.5 $g \cdot kg^{-1} \cdot d^{-1}$) and bifendate (150 $mg \cdot kg^{-1} \cdot d^{-1}$) respectively, while mice in the normal group were given the intraperitoneal injection of equivalent normal saline for continuously 3 d. After 16 h of model building, the blood was collected from eyeballs of mice and then mice were executed. Samples were taken from the liver tissue and then stored at −80 °C. Afterwards, the measurement was performed on different indicators.

2.5. HE staining

After being immersed in 10% neutral formalin, liver samples were embedded with paraffin and then cut into slices and dried. They were deparaffinaged using dimethylbenzene and dehydrated with water-free 95% and 80% ethanol. They were washed with running water and immersed in the hematoxylin and hydrochloride alcohol. When being turned to be antiblue, they were stained with eosin. Afterwards, they were washed with running water, dehydrated with ethanol at gradient degree. They were transparented with dimethylbenzene. After being dried, they were mounted with the neutral resin and then observed under the microscope.

2.6. Detection of serum ALT, AST, NO, Il-1β, Caspase 3 and Caspase 8 in the liver tissue

The detection was performed according to the instruction manual of kit respectively.

2.7. Western blot

Liver samples were collected from each group. Then the RIPA lysis buffer was added and centrifuged to obtain the protein. BCA kit was employed to detect the protein concentration. SDS gel electrophoresis was performed on protein samples and then it was transferred with the wet method. Then the film was immersed into the primary antibody solution for the incubation at 4 °C over night. After being washed, it was immersed into the secondary antibody solution (1:100) for the incubation at the room temperature for 1–2 h. Afterwards, the film was taken out and washed, while ECL reagent was added on the film for the exposure in the gel imaging system. Statistics was performed on the gray value of each antibody band using 'Quantity one' software.

2.8. Data analysis

The results were expressed as mean ± SD, with three repeats for each set of data at least. The t test was employed for the significant difference between two groups and $P < 0.05$ was regarded to be significant difference. All data were treated using SPSS 17.0.

3. Results

3.1. Effect of R. palmatum L. on the liver tissue of ALF mice

The liver tissue of mice in the normal group had the even staining and clear and complete structure (Figure 1A); for mice in the model group, the number of liver cells was decreased and the normal tissue structure was disappeared, being accompanied with the infiltration of abundant inflammatory cells (Figure 1B); compared with the model group, for mice in the *R. palmatum* L. treatment group and bifendate group, the number of liver cells was increased and the cell necrosis was decreased, with limited infiltration of inflammatory cells (Figure 1C, D).

3.2. Effect of R. palmatum L. on the level of ALT and AST in serum of mice with ALF

As shown in Table 1, compared with the normal group, the level of ALT and AST in the serum of mice in the model group was significantly increased ($P < 0.01$), with the statistical significance; the level of ALT and AST was significantly inhibited for mice in the *R. palmatum* L. treatment group ($P < 0.01$).

3.3. Effect of R. palmatum L. on the level of NO and Il-1β in mice serum with ALF

As shown in Table 2, compared with the normal group, the level of NO and Il-1β in the serum of mice in the model group was significantly increased ($P < 0.01$), with the statistical significance; the level of NO and Il-1β was significantly inhibited for mice in the *R. palmatum* L. treatment group ($P < 0.01$).

3.4. Effect of R. palmatum L. on level of Caspase 3 and Caspase 8 in mice serum with ALF

As shown in Table 3, compared with the model group, the level of Caspase 3 and Caspase 8 was significantly inhibited for mice in the *R. palmatum* L. treatment group ($P < 0.01$).

3.5. Effect of R. palmatum L. on expression of iNOS and COX-2 in mice with ALF

As shown in Figure 2, compared with the normal group, the expression of iNOS and COX-2 for mice in the model group was significantly increased, while the expression of iNOS and COX-2 for mice in the *R. palmatum* L. treatment group was significantly inhibited.

Table 1
Effect of *R. palmatum* L. on level of ALT and AST in serum of mice with ALF (mean ± SD).

Group	Dosage ($mg \cdot kg^{-1} \cdot d^{-1}$)	ALT (IU/L)	AST (U/L)
Normal group	–	45.12 ± 4.59	160.11 ± 34.11
Model group	–	265.84 ± 556.32*	645.34 ± 67.84*
R. palmatum L.	1000	124.35 ± 43.28△	389.56 ± 56.45△
Bifendate	150	122.00 ± 35.89△	367.33 ± 45.86△

*$P < 0.01$ *vs.* normal control; △$P < 0.05$ *vs.* model control.

Table 2
Effect of *R. palmatum* L. on level of NO and Il-1β in mice serum with ALF (mean ± SD).

Group	Dosage ($mg \cdot kg^{-1} \cdot d^{-1}$)	NO (μM)	Il-1β (ng/L)
Normal group	–	0.54 ± 0.22	7.27 ± 0.37
Model group	–	12.08 ± 1.21*	18.11 ± 0.97*
R. palmatum L.	1000	2.83 ± 0.28△	8.30 ± 1.04△
bifendate	150	1.14 ± 0.13△	8.36 ± 0.57△

*$P < 0.01$ *vs.* normal control; △$P < 0.05$, *vs.* model control.

Table 3
Effect of *R. palmatum* L. on level of Caspase 3 and Caspase 8 in mice serum with ALF (mean ± SD).

Group	Dosage ($mg \cdot kg^{-1} \cdot d^{-1}$)	Caspase 3 (μg)	Caspase 8 (μg)
Normal group	–	1.54 ± 0.37	0.97 ± 0.06
Model group	–	12.08 ± 7.21*	9.11 ± 1.35*
R. palmatum L.	1000	4.83 ± 6.45△	3.30 ± 1.11△
Bifendate	150	4.14 ± 5.73△	3.36 ± 0.57△

*$P < 0.01$ *vs.* normal control; △$P < 0.05$, *vs.* model control.

3.6. Effect of R. palmatum L. on expression of apoptotis related protein in mice with ALF

As shown in Figure 3, compared with the normal group, the expression of Bax was significantly increased and the expression of Bcl-2 and PCNA was significantly decreased for mice in the model group; while the expression of Bax, Bcl-2 and PCNA was significantly inhibited for mice in the *R. palmatum* L. treatment group.

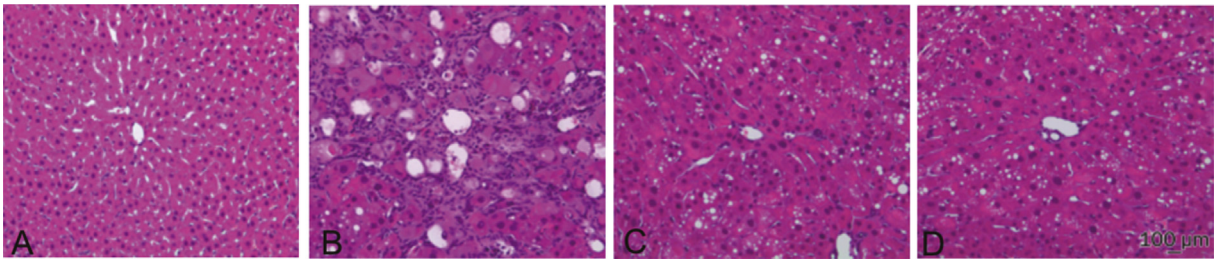

Figure 1. Protective effect of *R. palmatum* L. on liver in mice with ALF. A: normal control, B: model control, C: *R. palmatum* L., D: bifendate.

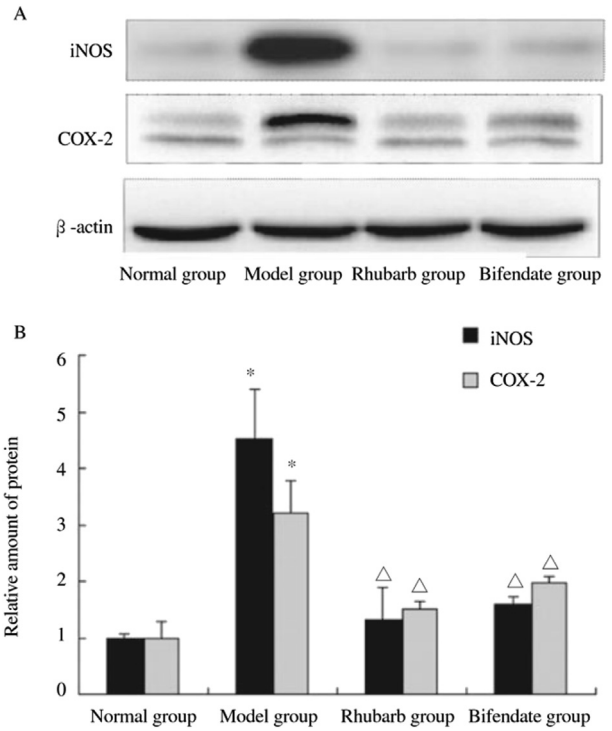

Figure 2. Effect of *R. palmatum* L. on expression of iNOS and COX-2 in mice with ALF.
A: Protein grayscale, B: Statistical graph. $^{*}P < 0.01$ *vs.* normal control; $^{\triangle}P < 0.01$, *vs.* model control.

3.7. Effect of R. palmatum L. on expression of NF-κB signaling pathway related protein in mice with ALF

As shown in Figure 4, compared with the model group, *R. palmatum* L. treatment group could significantly inhibit the phosphorylation of NF-κB p65 and IκBα in mice with ALF and not affect the expression of NF-κB p65 and IκBα no matter the dosage and modeling were performed or not.

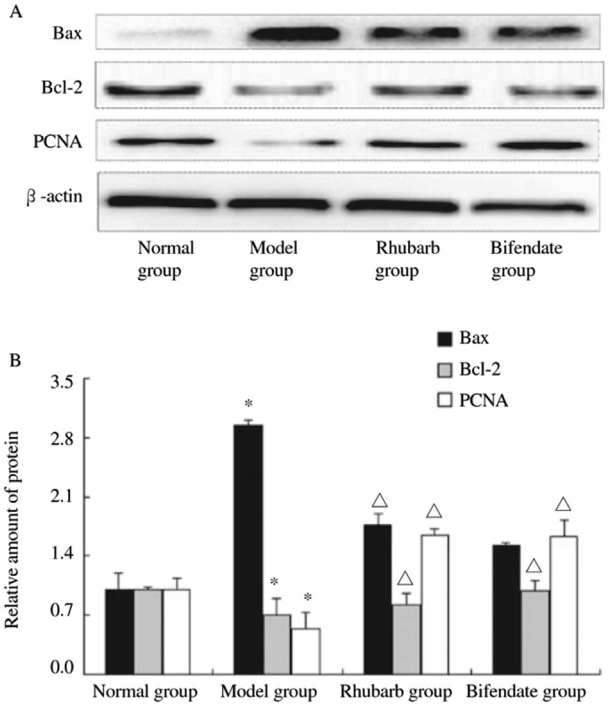

Figure 3. Effect of *R. palmatum* L. on expression of apoptotis related protein in mice with ALF.
A: Protein grayscale, B: Statistical graph. $^{*}P < 0.01$ *vs.* normal control; $^{\triangle}P < 0.01$, *vs.* model control.

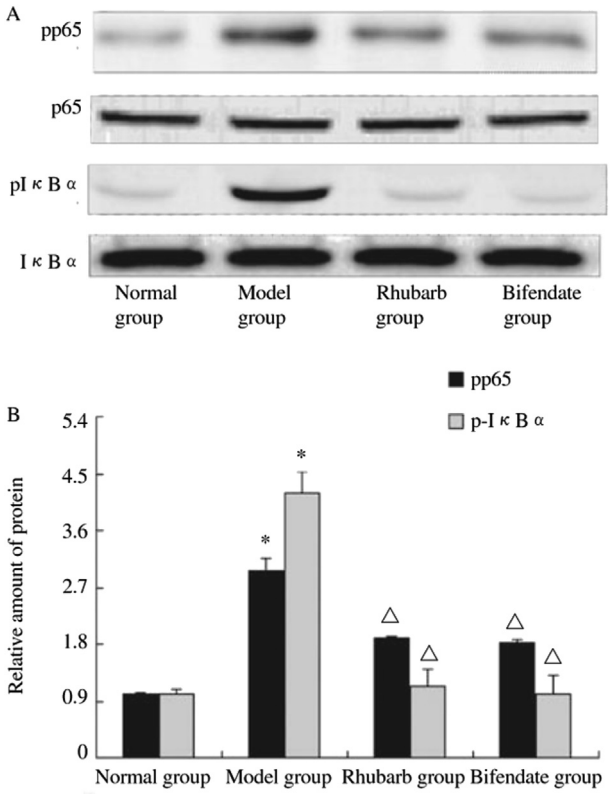

Figure 4. Effect of *R. palmatum* L. on expression of NF-κB signaling pathway related protein in mice with ALF.
A: Protein grayscale, B: Statistical graph. $^{*}P < 0.01$ *vs.* normal control; $^{\triangle}P < 0.01$, *vs.* model control.

4. Discussion

In China, the main cause of ALF is the virus. Thus it is applicable to choose the chemical drugs to induce ALF, where the mostly reported on at home and abroad is D-GalN/LPS [9,10], with the modeling characteristics of reproducibility, reversibility and little harm to experimenters. GalN can greatly digest the pyridinoline nucleosides, inhibit the synthesis of proteins related to the proliferation of liver cells, increase the sensitivity of liver cells to the inflammation factor, and limit the regeneration of cell organelles and supplement and synthesis of enzymes to seriously damage the structure and function of liver cells and thus cause ALF in mice. LPS can lead to the symptoms of liver injury, endotoxic shock and multiple organ failure. Furthermore, the synergism between LPS and GalN can also make the immune cells produce more inflammatory factors and toxin to apply to liver cells and thus result in the liver injury. Therefore, in this study, GalN and LPS were jointly employed to induce ALF for the modeling. In the model group, the level of AST, ALT and inflammatory factors was significantly increased and the expression of proteins related to the apoptosis was also significantly increased. Results of pathological detection indicated the fuzzy structure of liver cells, decreased number of normal liver cells, necrosis, gas shape and infiltration of abundant inflammatory factors. All results showed that the modeling of D-GalN/LPS-induced ALF was successful.

The liver function test on the change in the serum transaminase is a sensitive indicator to reflect the hepatocyte injury. Most common ones in the clinical practice are ALT and AST. The level of ALT is richest in the liver, which is only distributed in the cytoplasm and has the higher activity inside the liver cells than the one outsides. When 1% liver cells are damaged, the level of ALT in the serum will be significantly increased. AST is a functional enzyme in the liver cells. When cells are damaged, it will be transferred through the cell membrane into the serum and thus the level of AST in the serum will be significantly increased. According to a great number of researches, the reduction in the level of ALT and AST in the serum of ALF can relieve the liver injury [14,15]. In this study, compared with the model group, *R. palmatum* L. treatment group could significantly reduce the level of ALT and AST and its inhibition degree was similar with the positive control group, which indicated that *R. palmatum* L. can reduce the enzymes to relieve the liver injury.

The apoptosis and inflammation are regulated by multiple signaling pathways, where NF-κB signaling pathway is one of them. NF-κB is some kind of protein that can be specifically bound to multiple gene promoters or enhancer κB and can promote the transcription. The action form in cells is the dimer consisting of p65 and p50. When it's not stimulated, it will be bound to IκB; when it's stimulated, IκB will be degraded and phosphorylated to relieve NF-κB. Then its transcription activity will be recovered and it will be transferred from the cytoplasm to nucleus to regulate the expression of inflammation and apoptosis-related genes. In ALF, the activation of NF-κB can promote the abundant expression of apoptosis and inflammation genes, accelerate the release of inflammatory mediator and apoptosis of liver cells, which has a close relationship with the prognosis [14,16–19]. When stimulating the mice with NF-κB knockout and wild mice using thioacetamide, it's found that wild mice had the obvious cerebral edema, swelling of astrocytes, significant expression of iNOS and non-significant symptoms because of gene knockout, which indicated that there was the negative correlation between the content of NF-κB and the improvement on ALF symptoms [20]. According to Wu *et al.* [11], in GalN/LPS model of rats, the expression of NF-κB p65 and ALT was significantly increased. After the administration, it can inhibit the phosphorylation and degradation of IκB-α, as well as the transport of NF-κB/p65 from the cytoplasm to nucleus. Results of this study also indicated that, in the model group, the phosphorylation of NF-κB p65 and IκBα was significant; while being given the *R. palmatum* L., the degree of phosphorylation was reduced, which pointed out that *R. palmatum* L. might relieve ALT symptoms through NF-κB signaling pathway.

ALF will induce the immune cells of macrophages and neutrophils to release a great number of inflammatory factors and thus further cause the injury and necrosis of liver cells. NO is some kind of unstable free radical. In the catalysis of iNOS, it is produced by the nitrogen oxidation of guanidyl at the end of L-arginine. NO is common in all kinds of cells. As the intercellular signal molecule, it can mediate many physiological and pathological processes. In GalN/LPS-induced ALF animal model, the expression of iNOS was increased and the level of NO was increased to cause the cytotoxicity and inhibit the synthesis of NO and iNOS to protect the ALF [12,21]. In this study, it's found that the content of NO and iNOS was significantly increased in the model group, while in the treatment group, *R. palmatum* L. could inhibit the increase of its content. Il-1β plays a key role in the transmission of information, activation of immune cells, medication in activation, proliferation and differentiation of T, B cells and inflammatory response [22]. In the model group, the level of Il-1β was increased [23,24], while in *R. palmatum* L. treatment group, the secretion of Il-1β was decreased. COX-2 is the key enzyme in the stages of synthesis and initiation of prostate. The expression of COX-2 is limited in normal cells, but it will be rapidly expressed in case of the stimulation by the inflammatory factors to cause the inflammation [25]. The liver injury was less in LPS-induced COX-2 transgenic mice than the one in LPS-induced wild mice [26]. In mice with liver injury, the expression of COX-2 was too high [27]. In this study, compared with the control group, the expression of COX-2 was down-regulated in both *R. palmatum* L. treatment group and positive control group, which indicated that *R. palmatum* L. could inhibit the secretion of inflammation factor to improve the injury of ALF mice.

In the thioacetamide-induced ALF rat model, the inhibition of NF-κB signaling pathway could significantly reduce the level of inflammatory factors of IL-1 and COX-2 and thus relieve the liver injury [28]. In the hepatectomy-induced ALF rat model, the inhibition of NF-κB activity could significantly reduce the level of IL-1 [19,29]. In mice with the multiple organ dysfunction syndrome caused by the zymosan, the inhibition of NF-κB signaling pathway could down-regulate the expression of proteins such as iNOS [13]. In GalN/LPS-induced mouse model, the inhibition of NF-κB signaling pathway could reduce the level of NO and iNOS [9,30]. All these findings full indicate that *R. palmatum* L. can reduce the level of NO and Il-1β in the serum of ALF mice, while the inhibition on the expression of iNOS and COX-2 is realized through NF-κB signaling pathway.

According to previous researches [31,32], in case of acute liver failure, the remained liver cells still had the strong ability of proliferation, which indicated that the inhibition of apoptosis and promotion of proliferation of liver cells could promote the generation of liver cells to the certain extent and then recover the liver function. Caspase family is the important performer of cell apoptosis and it usually exists in the cytoplasm in the form of procaspase. It would be activated under the stimulation of multiple apoptosis signals through the proteolysis, which could thus degrade the many protein substrates and play a key role in the process of cell apoptosis [33]. Where, Caspase 3 and 8 had the higher activity in ALF model than the one in the normal group [10,12,34,35]. After being treated with *R. palmatum* L., its activity could be significantly reduced. The cell apoptosis is strictly under the control of apoptosis-related proteins. For instance, members of Bcl-2 family can be interacted with ones of Bax subfamily to induce the apoptosis signal in cells and cause the apoptosis. In GalN/LPS mouse model, the expression of Bcl-2 was significantly decreased, while the expression of Bax was increased [11,32]. After being treated with *R. palmatum* L., the expression of Bcl-2 was increased, while the expression of Bax was inhibited. The proliferating cell nuclear antigen (PCNA) was found in the serum of patients with the systemic lupus erythematosus. It is closely related to the synthesis of DNA and regarded as one of markers for the cell proliferation. In the acute liver injury, the expression of PCNA was significantly decreased [32,36]. Results of this study showed that, after being treated with *R. palmatum* L., the expression of PCNA was increased, which indicated that *R. palmatum* L. could inhibit the expression of

proapoptosis proteins and promote the expression of anti-apoptosis proteins to improve the apoptosis of liver cells that was caused by ALF.

A great number of findings have shown that, in GalN/LPS mouse model, the reduction in the activity of NF-κB could down-regulate the expression of Bax [11,13], promote the expression of Bcl-2 [11,13] and PCNA [37], and reduce the activity of Caspase 3 and 8 [10,12]. In consequence, *R. palmatum* L. could up-regulate the expression of Bcl-2 and PCNA, down-regulate the expression of Bax, reduce the activity of Caspase 3 and 8, and thus inhibit the apoptosis of liver cells in mice that was caused by ALF, which was realized through NF-κB signaling pathway.

R. palmatum L. can reduce the level of ALT and AST in serum of ALF mice through NF-κB signaling pathway, inhibit the secretion of NO and Il-1β, reduce the activity of Caspase 3 and 8, down-regulate the expression of iNOS, COX-2 and Bax and up-regulate the expression of Bcl-2 and PCNA to improve the injury in mice that is caused by ALF.

Though this study drew the conclusion that *R. palmatum* L. could rely on NF-κB signaling pathway to inhibit the secretion of inflammation factors and regulate the expression of apoptosis-related proteins to relieve the liver failure in mice, there were deficiencies in the experimental design. First of all, this study was to discuss the effect of *R. palmatum* L. on NF-κB signaling pathway at the protein level, but not the gene level. The synthesis of mRNA and protein may not be at the same level and the resulting error may affect the results of this study. Besides, there was no NF-κB signaling pathway inhibitor administration group in this study to further prove the conclusion that how *R. palmatum* L. relieve the liver failure in mice through NF-κB signaling pathway.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Siddiqui MS, Stravitz RT. Intensive care unit management of patients with liver failure. *Clin Liver Dis* 2014; **18**(4): 957-978.

[2] Zhang Z, Zhao YC, Cheng Y, Jian GD, Pan MX, Gao Y. Hybrid bioartificial liver support in cynomolgus monkeys with D-galactosamine-induced acute liver failure. *World J Gastroenterol* 2014; **20**(46): 17399-17406.

[3] Chen X. Effect of *Rheum palmatum* L. on apoptosis of liver cells in rats with acute liver failure. *Chin J Gerontol* 2014; **33**(8): 1841-1842.

[4] Zhang R, Mao D, Li D. Effect of *Rheum palmatum* L. and radix paeoniae rubra on PCNA in liver tissue of rats after operation of liver failure. *Liaoning J Trad Chin Med* 2013; **40**(2): 355-356.

[5] Cui J, Shan L, Zhang P. Protection of *Rheum palmatum* L. and licorice decoction on mice with concanavalin-induced liver injury and rats with adenine-induced renal injury. *Chin Trad Patent Med* 2013; **35**(7): 1539-1542.

[6] Zheng T, Yu F, Yao J. Effect of *Rheum palmatum* L. infusion on expression of NF-κB in the liver of rats with acute intrahepatic cholestasis. *Zhejiang J Trad Chin Med* 2013; **48**(5): 380-381.

[7] Yu JH, Kim H. Oxidative stress and inflammatory signaling in cerulein pancreatitis. *World J Gastroenterol* 2014; **20**(46): 17324-17329.

[8] Eguchi K. Apoptosis in autoimmune diseases. *Intern Med* 2001; **40**(4): 275-284.

[9] Wu YL, Lian LH, Wan Y. Baicalein inhibits nuclear factor-κB and apoptosis via c-FLIP and MAPK in D-GalN/LPS induced acute liver failure in murine models. *Chem Biol Interact* 2010; **188**(3): 526-534.

[10] Jin Q, Jiang S, Wu YL. Hepatoprotective effect of cryptotanshinone from Salvia miltiorrhiza in D-galactosamine/lipopolysaccharide-induced fulminant hepatic failure. *Phytomedicine* 2014; **21**(2): 141-147.

[11] Wu Z, Kong X, Zhang T. Pseudoephedrine/ephedrine shows potent anti-inflammatory activity against TNF-α-mediated acute liver failure induced by lipopolysaccharide/D-galactosamine. *Eur J Pharmacol* 2014; **724**: 112-121.

[12] Lin X, Zhang S, Huang R. Protective effect of tormentic acid from Potentilla chinensis against lipopolysaccharide/D-galactosamine induced fulminant hepatic failure in mice. *Int Immunopharmacol* 2014; **19**(2): 365-372.

[13] Di Paola R, Galuppo M, Mazzon E. PD98059, a specific MAP kinase inhibitor, attenuates multiple organ dysfunction syndrome/failure (MODS) induced by zymosan in mice. *Pharmacol Res* 2010; **61**(2): 175-187.

[14] Wu MJ, Gong X, Jiang R. Polydatin protects against lipopolysaccharide-induced fulminant hepatic failure in D-galactosamine-sensitized mice. *Int J Immunopathol Pharmacol* 2012; **25**(4): 923-934.

[15] Brodsky M, Hirsh S, Albeck M. Resolution of inflammation-related apoptotic processes by the synthetic tellurium compound, AS101 following liver injury. *J Hepatol* 2009; **51**(3): 491-503.

[16] Taniguchi M, Takeuchi T, Nakatsuka R. Molecular process in acute liver injury and regeneration induced by carbon tetrachloride. *Life Sci* 2004; **75**(13): 1539-1549.

[17] Chen LY, Yan BZ, Bi MR. Expression and significance of NF-κB p65 in rats with acute liver failure. *Zhonghua Gan Zang Bing Za Zhi* 2011; **19**(12): 942-944.

[18] Park JH, Kim KH, Lee WR. Protective effect of melittin on inflammation and apoptosis in acute liver failure. *Apoptosis* 2012; **17**(1): 61-69.

[19] Tan YC, Xie F, Zhang HL. Hydrogen-rich saline attenuates post-operative liver failure after major hepatectomy in rats. *Clin Res Hepatol Gastroenterol* 2014; **38**(3): 337-345.

[20] Jayakumar AR, Bethea JR. NF-κB in the mechanism of brain edema in acute liver failure: studies in transgenic mice. factor-κB (NF-κB). *Neurobiol Dis* 2011; **41**(2): 498-507.

[21] Jiang W, Desjardins P, Butterworth RF. Minocycline attenuates oxidative/nitrosative stress and cerebral complications of acute liver failure in rats. *Neurochem Int* 2009; **55**(7): 601-605.

[22] Singhal G, Jaehne EJ, Corrigan F. Inflammasomes in neuroinflammation and changes in brain function: a focused review. *Front Neurosci* 2014; **8**: 315.

[23] Steinebrunner N, Mogler C, Vittas S. Pharmacologic cholinesterase inhibition improves survival in acetaminophen-induced acute liver failure in the mouse. *BMC Gastroenterol* 2014; **14**: 148.

[24] Li Y, Han MF, Li WN, Shi AC, Zhang YY, Wang HY, et al. SOCS3 expression correlates with severity of inflammation in mouse hepatitis virus strain 3-induced acute liver failure and HBV-ACLF. *J Huazhong Univ Sci Technolog Med Sci* 2014; **34**(3): 348-353.

[25] Ashour TH. Therapy with interleukin-22 alleviates hepatic injury and hemostasis dysregulation in rat model of acute liver failure. *Adv Hematol* 2014; **2014**: 705290.

[26] Han C, Li G, Lim K. Transgenic expression of cyclooxygenase-2 in hepatocytes accelerates endotoxin-induced acute liver failure. *J Immunol* 2008; **181**(11): 8027-8035.

[27] Domitrović R, Skoda M, Vasiljev Marchesi V. Rosmarinic acid ameliorates acute liver damage and fibrogenesis in carbon tetrachloride-intoxicated mice. *Food Chem Toxicol* 2013; **51**: 370-378.

[28] Demirel U, Yalniz M, Aygün C. Allopurinol ameliorates thioacetamide-induced acute liver failure by regulating cellular redox-sensitive transcription factors in rats. *Inflammation* 2012; **35**(4): 1549-1557.

[29] Antoniades CG, Khamri W, Abeles RD. Secretory leukocyte protease inhibitor: a pivotal mediator of anti-inflammatory responses

in acetaminophen-induced acute liver failure. *Hepatology* 2014; **59**(4): 1564-1576.

[30] Tanaka H, Uchida Y, Kaibori M. Na+/H+ exchanger inhibitor, FR183998, has protective effect in lethal acute liver failure and prevents iNOS induction in rats. *J Hepatol* 2008; **48**(2): 289-299.

[31] Sun L, Fan X, Zhang L. Bone mesenchymal stem cell transplantation via four routes for the treatment of acute liver failure in rats. *Int J Mol Med* 2014; **34**(4): 987-996.

[32] Xu Y, Wang H, Bao S. Amelioration of liver injury by continuously targeted intervention against TNFRp55 in rats with acute-on-chronic liver failure. *PLoS One* 2013; **8**(7): e68757.

[33] Porter AG, Janicke RU. Emerging roles of caspase-3 in apoptosis. *Cell Death Differ* 1999; **6**(2): 99-104.

[34] Lu J, Chen YP, Wan R. Protective effects of ulinastatin on acute liver failure induced by lipopolysaccharide/D-galactosamine. *Dig Dis Sci* 2012; **57**(2): 399-404.

[35] Shirozu K, Hirai S, Tanaka T. Farnesyltransferase inhibitor, tipifarnib, prevents galactosamine/lipopolysaccharide-inducedacute liver failure. *Shock* 2014; **42**(6): 570-577.

[36] Nie XH, Han T, Ha FU. Comparison of the effects of the pretreatment and treatment with RhIL-11 on acute liver failureinduced by D-galactosamine. *Eur Rev Med Pharmacol Sci* 2014; **18**(8): 1142-1150.

[37] Zhu H, Zhang Y, Hu X. The effects of high-dose qinggan huoxue recipe on acute liver failure induced by D-galactosamine in rats. *Evid Based Complement Alternat Med* 2013; **2013**: 905715.