

Original Research Article

Protective effect of *Rhus chinensis* Mill. extract against liver cirrhosis in rats

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Sent for review: 27 July 2020

Revised accepted: 15 January 2021

Abstract

Purpose: To study the effect of *Rhus chinensis* Mill. extract (RCME) on diethylnitrosamine (DEN)-induced liver cirrhosis in rats.

Methods: RCME was obtained by extracting the dried *Rhus chinensis* Mill. in water. Liver cirrhosis rat model was prepared by injecting with DEN once a week for 8 weeks. After 8th-week of RCME treatment, biochemical index and oxidative stress were determined in DEN-induced liver cirrhosis in rats.

Results: Compared with model group, plasma concentrations of alanine transaminase (ALT, 125.3 ± 4.1 U/L) and aspartate aminotransferase (AST, 152.4 ± 3.5 U/L) decreased significantly ($p < 0.01$) in the 8th week. *Rhus chinensis* Mill. extract (RCME) significantly decreased malondialdehyde (MDA, 0.18 ± 0.02 μ mol/L) and superoxide dismutase (SOD, 0.76 ± 0.05 U/mg protein) in DEN-induced liver cirrhosis in rats ($p < 0.01$) when compared with model group.

Conclusion: RCME protects against diethylnitrosamine-induced liver cirrhosis in rats. However, further investigations are required to ascertain the plant extract's suitability for the clinical management of liver cirrhosis.

Keywords: *Rhus chinensis* Mill., Liver cirrhosis, Diethylnitrosamine, Alanine transaminase, Aspartate aminotransferase

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INTRODUCTION

Liver fibrosis is a multi-step process resulting from various factors such as viral hepatitis, alcohol abuse, biliary atresia and hepatotoxins. Liver cirrhosis is the end-stage of this reaction. Hepatic fibrosis is reversible, associated with apoptosis of activated hepatic stellate cells [1]. The characteristics of liver cirrhosis include cell viability and redox ratio decrease, reactive

oxygen species formation, lipid peroxidation, DNA fragmentation, and formation of apoptotic bodies, which provides potential therapeutic targets [2]. Inhibition of oxidative stress play important role in the prevention of the progression of liver cirrhosis [3]. It has been found that DEN-induced liver cirrhosis in rats is similar to those of human cirrhosis [4,5].

Pharmacological experiments have demonstrated that *Rhus chinensis Mill.* possess wide-reaching biological functions including dilating coronary artery, improving myocardial ischemia, modulating immune system, anticoagulation and antithrombosis, antioxidation, anti-aging, antihypoxia, antifatigue, anti-inflammation, anti-hepatic fibrosis, antitumor, analgesia, etc [6-11]. Due to the traditional use of *Rhus chinensis Mill.* in the prevention of liver cirrhosis [12], this study was therefore performed to study the effect of *Rhus chinensis Mill.* extract against liver cirrhosis in rats.

EXPERIMENTAL

Plant material

Herbal samples of *Rhus chinensis Mill.* were collected from Liupanshui City, Guizhou Province in China in October 2019. Taxonomic identification of the plant was performed by Professor Lin Zhang of college of pharmacy, Xiamen University in China. A voucher specimen of herbarium (no. RCME 201910023) was deposited in the College of Pharmacy, Xiamen University, China for future reference. *Rhus chinensis Mill.* (RCME) was obtained by steeping the dried *Rhus chinensis Mill.* Three times in water at 60 °C, each for one hour before first drying in an oven and then freeze-drying the last extract thus obtained. One gram powder was equivalent to about 2.7 g crude samples. The yield was 38.0 %.

Preparation of animal model and grouping

Male Wistar rats weighing 190 – 210 g were provided by the Experimental Animal Center of Fujian Province (Certificate no. SYXK 2009 - 0006). The animals had free access to feed and water, and were allowed to acclimatize for at least one week before use. The rat experiment was approved by the Animal Care and Use Committee of Xiamen Haicang Hospital (approval ref no. 20160502) and was carried out in compliance with Directive 2010/63/EU on the handling of animals used for scientific purposes [13].

Liver cirrhosis model were prepared by injecting with DEN (60 mg/kg) in rats once a week for 8 weeks. The rats were randomly divided into 3 groups of ten rats each, normal rats with oral saline solution (normal group), Liver cirrhosis model rats with oral saline solution (model group), Liver cirrhosis model rats with oral RCME (50 mg/kg/rat, once every day) (RCME group). All administration lasted for 8 weeks.

Assessment of serum biochemical parameters

At the 4th and 8th weeks of treatment, blood samples (0.5 mL) of rats were collected by moving the eyeball. Blood was immediately processed for serum by centrifugation at 3000 g for 15 min. Serum levels of ALT and AST were measured using spectrophotometry, using commercially available kits (Nanjing Jiancheng Bioengineering Institute).

Determination of oxidative stress parameters in liver tissue

The rats were sacrificed using cervical dislocation and their livers were excised, rapidly washed and homogenized in ten volumes (v/w) of ice-cold saline solution. The homogenates were centrifuged at 3000 rpm for 10 min.

The levels of malondialdehyde (MDA), a biomarker of lipid peroxidation, were determined spectrophotometrically by measuring thiobarbituric acid reactive substances (TBARS). Then 1 mL of 10 % trichloroacetic acid and 1 mL of 0.67 % thiobarbituric acid were added to 0.2 mL of 10 % homogenate of the tissue samples. The mixture was incubated at 100 °C for 15 min. After cooling and centrifugation, the supernatant was aspirated and the absorbance was determined at 532 and 600 nm. The SOD activity was measured using spectrophotometry, using commercially available SOD kits A001-1 (Nanjing Jiancheng Bioengineering Institute).

Statistical analysis

Values are presented as mean \pm standard deviation (SD) and were statistically analyzed using one-way ANOVA, followed by Tukey's multiple comparison using SPSS 16.0 software for Windows. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Experimental fibrosis and cirrhosis preparation

With the weekly DEN-injection, the body weight of rats dropped gradually. After 4 weeks of weekly DEN injections, there were marked increases in the activities of serum ALT and AST in rats ($p < 0.01$) when compared with the normal group. The increase of ALT and AST was ameliorated by RCME. After the 8th week of DEN injection, liver cirrhosis model rats were prepared. Serum concentrations of ALT and AST in the 8th-week model group significantly

decreased when compared with that in the 4th-week model group ($p < 0.05$), but they were still higher than in the normal group. Compared with model group, serum concentrations of ALT and AST decreased significantly ($p < 0.01$) in the 8th-week (Table 1).

Table 1: Effect of RCME on serum ALT (U/L) and AST (U/L)

Group	1 week	4 weeks	8 weeks
ALT			
Normal	82.5 ± 1.8	85.3 ± 3.0	94.6 ± 2.8
Model	137.2 ± 3.4	458.2 ± 9.8**	242.4 ± 6.4*
RCME	101.4 ± 2.6	139.1 ± 3.8 ^{△△}	123.7 ± 3.8 ^{△△}
AST			
Normal	112.4 ± 3.3	88.2 ± 4.5	94.6 ± 4.6
Model	108.6 ± 4.4	310.5 ± 6.3**	175.3 ± 4.9*
RCME	106.3 ± 3.6	128.1 ± 5.0 ^{△△}	148.2 ± 3.3 ^{△△}

* $P < 0.05$, ** $p < 0.01$ vs. normal group; [△] $p < 0.05$, ^{△△} $p < 0.01$ vs. model group

Effect of RCME on MDA and SOD

This study showed that the MDA level was significantly increased in DEN-treated rats ($p < 0.01$), which was significantly inhibited by RCME treatment ($p < 0.01$). Conversely, the levels of SOD were significantly decreased in the livers of DEN-treated rats, which was reversed by the RCME administration significantly (Table 2).

Table 3: Effect of RCME on MDA and SOD level in rat's liver

Group	MDA (umol/L)	SOD (U/mg protein)
Normal	0.12 ± 0.04	0.98 ± 0.06
Model	0.48 ± 0.03**	0.41 ± 0.03**
RCME	0.15 ± 0.02**	0.82 ± 0.04**

* $p < 0.05$, ** $p < 0.01$ vs. model group

DISCUSSION

Recently, many researchers have focused on the anti-fibrotic properties of herb medicines. In our research, oral administration of RCME showed the protective effect on liver fibrosis and cirrhosis induced by DEN in rats. The mechanisms included anti-necrosis, anti-apoptosis [14], anti-inflammatory effects and the activation of hepatic stellate cells [15].

Malondialdehyde (MDA), as an end product of lipid peroxidation, was significantly increased by DEN treatment in liver cirrhosis rats [16]. RCME inhibited the MDA formation and increased the enzyme activity of SOD in DEN-treated rats. And more, RCME inhibited DEN-induced fibrosis process by regulating the anti-oxidant activity.

And the serum levels of ALT and AST were reduced by RCME at the 4th and the 8th weeks when compared with non-treated DEN-induced rats. The results showed that RCME attenuated the apoptosis, oxidative stress in fibrotic liver. Therefore, RCME may be an effective antifibrotic agent.

CONCLUSION

In this study, RCME was effective in the treatment of chemically-induced liver fibrosis in rats, and it could be developed into a new drug in future.

DECLARATIONS

Acknowledgement

The present study was supported by the Project of Medical Innovations Topic in Fujian Province (grant no. 2019-CXB-34) and the Project of Xiamen Scientific and Technological Plan (grant no. 3502ZZ20174023).

Conflict of interest

No conflict of interest is associated with this work

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

Jianmin Zhuang designed all the experiment and revised the paper. Zirong Pan, Qiang Cheng, Heyan Chen, Longhai Lin and Weijia Liao performed the experiments, and Donglai Guo wrote the manuscript. Zirong Pan and Qiang Cheng contributed equally to this work, and they are co-first authors.

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