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**Original Research Article**

# Protective effect of Radix Bupleuri extract against liver cirrhosis in rats

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## Abstract

***Purpose:*** *To explore the effect of Radix Bupleuri extract (RBE) on diethylnitrosamine (DEN)-induced liver cirrhosis in rats.*
***Methods:*** *Rats were injected with DEN once a week for 8 weeks to induce liver cirrhosis. Some DEN-induced rats were also treated with RBE, which was obtained by extracting dried Radix Bupleuri in water, for 8 weeks. Afterwards, biochemical indices and oxidative stress markers were assessed.*
***Results:*** *RBE significantly decreased serum concentrations of both alanine transaminase (137.3 ± 4.4 U/L, $p < 0.01$) and aspartate aminotransaminase (152.1 ± 3.4 U/L, $p < 0.01$) in DEN-induced rats at week 8. In addition, RBE significantly decreased malondialdehyde (0.13 ± 0.02 umol/L, $p < 0.01$) and superoxide dismutase (0.73 ± 0.04 U/mg protein, $p < 0.01$) levels in DEN-induced rats ($p < 0.01$).*
***Conclusion:*** *RBE exhibits a protective effect against DEN-induced liver cirrhosis in rats. Thus, it may have the potential to be used to treat liver cirrhosis in clinical settings.*

***Keywords:*** *Radix Bupleuri, Liver cirrhosis, Anti-oxidant, Diethylnitrosamine, Alanine transaminase, Aspartate aminotransaminase*

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## INTRODUCTION

Liver fibrosis is a multi-step process that results from various factors, such as viral hepatitis, alcohol abuse, biliary atresia, and hepatotoxins. Liver cirrhosis is the end stage of this reaction. In general, liver fibrosis is reversible and is associated with the apoptosis of activated hepatic stellate cells [1]. The characteristics of liver cirrhosis include cell viability and a decrease in redox ratios, along with reactive oxygen species formation, lipid peroxidation, DNA fragmentation, and apoptotic body formation. All of these factors are potential targets for therapy [2]. The inhibition of oxidative stress has been shown to play an important role in preventing the progression of liver cirrhosis [3]. In addition, the degree of liver cirrhosis observed in humans is similar to that observed in diethylnitrosamine (DEN)-induced rats [4,5].

Pharmacological experiments have demonstrated that *Radix Bupleuri* possesses wide-reaching biological activities, including coronary artery dilation, immune system modulation, anti-inflammatory properties and analgesia [6-11]. Due to the traditional use of *Radix Bupleuri* in the prevention of liver cirrhosis

[12], this study was performed to investigate the protective effect of *Radix Bupleuri* extract (RBE) against liver cirrhosis in rats.

## EXPERIMENTAL

### Plant material

Herbal samples of *Radix Bupleuri* were collected from Bozhou City, Anhui Province (China), in May 2015. Taxonomic identification of the plant was performed by Professor Hui Chu (College of Pharmacy, Wuhan University, China). A voucher specimen (no. RB 201505016) was deposited in the College of Pharmacy (Wuhan University, China) herbarium. RBE was obtained by steeping dried *Radix Bupleuri* in water at 60 °C for 1 h and then drying it in an oven. This process was performed three times. Finally, the extract was freeze-dried. One gram of extract powder was equivalent to about 2.0 g of crude plant material, i.e., a yield of 50.0 %.

### Preparation of animal models and groups

Male Wistar rats weighing 180 – 220 g were provided by the Experimental Animal Center of Hubei Province (Certificate no. SYXK 2007-0006). The rats had free access to feed and water and were allowed to acclimate for at least 1 week before use. Animal experiments were approved by the Animal Care and Use Committee of Wuhan University (approval ref no. 20110908) and were performed in compliance with Directive 2010/63/EU on the handling of animals used for scientific purposes [13].

To generate liver cirrhosis model rats, rats were injected with DEN (70 mg/kg body weight/rat) once a week for 8 weeks. Rats were randomly divided into 3 groups containing 10 rats each. Group I consisted of normal rats that were treated with oral saline solution for 8 weeks (normal group). Group II consisted of liver cirrhosis model rats that were treated with oral saline solution for 8 weeks (model group). Group III consisted of liver cirrhosis model rats that were treated with oral RBE (40 mg/kg body weight/rat, once daily) for 8 weeks (RBE group).

### Determination of biochemical indices

In weeks 4 and 8, blood samples (0.5 mL) were collected by puncturing the retro-orbital sinus. Serum was obtained by immediately centrifuging blood samples at 3,500 g for 15 min. Serum levels of alanine transaminase (ALT) and aspartate aminotransaminase (AST) were measured spectrophotometrically, using commercially available kits (Nanjing Jiancheng Bioengineering Institute).

### Determination of oxidative stress parameters in liver tissues

Rats were sacrificed by cervical dislocation, after which the livers were excised, rapidly washed, and homogenized in ten volumes (v/w) of ice-cold saline solution. The homogenate was centrifuged at 3,000 rpm for 10 min.

Levels of malondialdehyde (MDA), a biomarker of lipid peroxidation, were determined spectrophotometrically by measuring thiobarbituric acid reactive substances (TBARS). Trichloroacetic acid (10 % solution, 1 mL) and 1 mL of 0.67 % thiobarbituric acid were added to 0.2 mL of the 10 % homogenates of the tissue samples. The mixtures were incubated at 100 °C for 15 min. After cooling and centrifuging, the supernatant was aspirated, and its absorbance determined at 532 and 600 nm. The concentration of MDA (µmol/L) was calculated according to Eq 1.

$$MDA = 6.45(A_{532} - A_{600}) \quad \text{..... (1)}$$

Where $A_{532} - A_{600}$ are the absorbance at 532 and 600 nm, respectively.

Superoxide dismutase (SOD) activity was measured spectrophotometrically using commercially available SOD kits (A001-1 (Nanjing Jiancheng Bioengineering Institute).

### Statistical analysis

Data are presented as mean ± standard deviation (SD) and were analyzed by 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

### Induction of fibrosis and cirrhosis

During the weekly DEN injections, the body weights dropped gradually. After 4 weeks of weekly DEN injections, marked increases in the activity of serum ALT and AST ($p < 0.01$) were observed. RBE ameliorated the increase in ALT and AST levels. After the eighth week of DEN injections, liver cirrhosis was induced. Serum concentrations of ALT and AST after 8 weeks of DEN injections were significantly lower than those after 4 weeks of DEN injections ($p < 0.05$)

but were still higher than those in the normal group. Compared with the model group, plasma concentrations of both ALT and AST in RBE group decreased significantly ($p < 0.01$) at 8 weeks (Tables 1 and 2).

**Table 1:** Serum alanine transaminase (U/L) at different time points

| Group | 0 week | 4 weeks | 8 weeks |
|---|---|---|---|
| Normal | 101.3±3.4 | 99.26±3.6 | 100.6±3.5 |
| Model | 102.5±3.3 | 523.1±12.4** | 238.4±9.6* |
| RBE | 102.8±3.4 | 152.6±5.7△△ | 137.3±4.4△△ |

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

**Table 2:** Serum aspartate aminotransaminase (U/L) at different time points

| Group | 0 week | 4 weeks | 8 weeks |
|---|---|---|---|
| Normal | 108.6±4.2 | 104.4±3.8 | 106.1±3.2 |
| Model | 106.4±4.5 | 347.6±10.4** | 198.5±5.3* |
| RBE | 101.5±4.7 | 134.7±6.2△△ | 152.1±3.4△△ |

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

**Table 3:** Malondialdehyde (MDA) and superoxide dismutase (SOD) levels in rat liver

| Group | MDA (μmol/L) | SOD (U/mg protein) |
|---|---|---|
| Normal | 0.08±0.03 | 0.82±0.12 |
| Model | 0.32±0.02** | 0.46±0.06** |
| RBE | 0.13±0.02** | 0.73±0.04** |

*$P < 0.05$, ** $p < 0.01$ vs. control group

### Effect of RBE on MDA and SOD levels

MDA levels were significantly increased in DEN-induced rats ($p < 0.01$), as compared with rats in the normal group. RBE significantly inhibited MDA formation in DEN-induced livers ($p < 0.01$). Conversely, RBE significantly increased levels of SOD in the livers of DEN-induced rats (Table 3).

## DISCUSSION

Recently, many researchers have focused on the anti-fibrotic properties of herbal medicines. We show that the oral administration of RBE exerted a protective effect on DEN-induced liver fibrosis and cirrhosis. Several mechanisms that may participate in the induction of the fibrotic process include the inhibition of necrosis and apoptosis [14], the prevention of inflammation, and the activation of hepatic stellate cells [15].

Levels of MDA, which is an end product of lipid peroxidation [16], were significantly increased in DEN-induced rats. RBE remarkably inhibited MDA formation and increased the activity of SOD in DEN-induced rats. Thereby, RBE acted as an anti-oxidant to inhibit the process of DEN-induced fibrosis. Furthermore, RBE reduced serum levels of ALT and AST at weeks 4 and 8, as compared with saline-treated, DEN-induced rats. These findings demonstrate that RBE played a role in the attenuation of apoptosis and oxidative stress in the fibrotic liver. Therefore, RBE may be used as a potential anti-fibrotic agent in the future.

In the present study, RBE was effective in the treatment of chemically induced liver fibrosis in rats. Its anti-fibrotic mechanisms involve the inhibition of oxidative stress, apoptosis, and metabolic disturbances in DEN-induced liver tissues.

## CONCLUSION

RBE shows protective effect against DEN-induced liver cirrhosis in rats and thus, can potentially be used for the treatment of liver cirrhosis in clinical settings.

## DECLARATIONS

### *Conflict of Interest*

No conflict of interest associated with this work.

### *Contribution of Authors*

The authors 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 them. Wan-he Tang and Quan-wei Yang contributed equally to this work.

### *Open Access*



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