

Original Research Article

Analysis of the effect of Qizhuyigan on liver function in a mouse model of immunological liver injury

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Abstract

Purpose: To assess the protective effect of Qizhuyigan capsules containing an herbal mixture on liver function in a mouse model of immunological liver injury and to explore the mechanism of action.

Methods: One hundred and twenty mice were randomly divided into four groups: control, test, bifendate, and Qizhuyigan. Immunological liver injury was induced in all groups except the control group. Mice in the control group and the test group were gavaged with 2.5 g/kg tap water, mice in the bifendate group were gavaged with 12 mg/kg bifendate in water, and mice in the Qizhuyigan group were gavaged with 1,000 mg/kg of an aqueous solution containing the contents of a Qizhuyigan capsule. The gavage continued for 10 days. Changes in liver function-related indices, such as the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), malonaldehyde (MDA), superoxide dismutase (SOD), tumor necrosis factor α (TNF- α), and interleukin 6 (IL-6), were assessed.

Results: Compared with the test and bifendate groups, the Qizhuyigan group exhibited lower serum ALT ($98.3 \text{ U/L} \pm 8.7 \text{ U/L}$ vs. $224.7 \text{ U/L} \pm 18.4 \text{ U/L}$ vs. $132.8 \text{ U/L} \pm 9.4 \text{ U/L}$); AST ($165.5 \text{ U/L} \pm 12.1 \text{ U/L}$ vs. $362.6 \text{ U/L} \pm 16.6 \text{ U/L}$ vs. $224.6 \text{ U/L} \pm 12.2 \text{ U/L}$); and MDA levels ($12.7 \pm 2 \mu\text{mol/L}$ vs. $31.3 \pm 6.60 \mu\text{mol/L}$ vs. $14.4 \pm 2.3 \mu\text{mol/L}$); a higher SOD level; and reduced TNF- α and IL-6 levels. The differences of the above indices had statistical significance ($p < 0.05$).

Conclusion: Qizhuyigan exerted a protective effect in a mouse model of immunological liver injury.

Keywords: Qizhuyigan capsule, Immunological liver injury, Biochemical parameters

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INTRODUCTION

Viral hepatitis has various routes of infection, and the population suffering from viral hepatitis is widespread. China has the largest population of hepatitis B patients worldwide and the morbidity associated with hepatitis C is increasing annually [1]. Persistent infection and repeated viral attacks are important factors in liver cirrhosis and primary hepatic carcinoma (PHC) [2].

Currently, Western medicine usually treats viral hepatitis by inhibiting virus replication, protecting hepatocytes, reducing transaminases, and

improving immune function. Drugs such as nucleosides and interferon have good short-term curative effects, but their long-term effects are unsatisfactory. Moreover, the high medical expenses, the severe toxic side effects of drugs, and viral drug resistance, are problematic [3,4]. It would be very useful to identify a drug that inhibits acute liver immunoreaction and lipid peroxidation, removes free radicals, reduces the levels of cytotoxic factors, and modulates liver metabolism [5,6].

Schemes based on traditional Chinese medicine have long been used to treat liver disease, and

valuable experience has been accumulated. Recently, the pharmacological mechanisms and the clinical medicinal value of some Chinese herbal medicines have been clarified, and their lower costs, fewer toxic effects, and reliable long-term curative effects make them worthy of study [7].

Qizhuyigan capsule contains seven traditional Chinese medicinal materials, including the root of red-rooted salvia, felwort, white atractylodes rhizome, *Astragalus membranaceus*, and *radix isatidis*. The preparation can eliminate damp-heat, and regulates the liver and spleen. The capsule is used clinically to treat various types of chronic hepatitis, and has good curative effects, but its mechanism of action has not been determined [8].

The study mainly explored the mechanism of action of Qizhuyigan on liver function in a mouse model of immunological liver injury to provide a theoretical foundation for the treatment of immunological liver injury and offer an experimental basis for further development of the Qizhuyigan capsules.

EXPERIMENTAL

Drugs and reagents

Qizhuyigan capsules formulated from 15 g of the root of red-rooted salvia, 15 g rough gentian, 15 g *Rhizoma Atractylodis Macrocephalae*, 15 g *Astragalus membranaceus*, 12 g *Radix isatidis*, 10 g Semen brassicae, and 6 g *Curcuma zedoary* were obtained from the Binzhou People's Hospital. Bifendate pills (batch number: 2014122081) were purchased from Guangzhou Baiyun Mountain Xingqun Pharmacy Co., Ltd. (China), malondialdehyde (MDA), superoxide dismutase (SOD), allophanamide (Shanghai Hongshun Biotech. Co., Ltd., China), tumour necrosis factor- α (TNF- α) enzyme-linked immunosorbent assay (ELISA), and interleukin 6 (IL-6) kits (Beijing Biolab Science and Technology Ltd., China) were also purchased.

Animals and instruments

One hundred and twenty male mice (body weight range 18 – 22 g) were obtained from the Experimental Animal Centre of Binzhou Medical College, Shandong, China, and were acclimated for 7 d in the Binzhou People's Hospital, Shandong, China. Instruments used included an AU5800 automatic biochemical analyser (Beckman Coulter, Inc., USA), a TG18G-II high-speed centrifuge (Shanghai Yanqi Biotechnology Co., Ltd., China), an RX-MBY enzyme-labelling

instrument (Beijing Ruixi Science and Technology Ltd., China), a UV-765 ultraviolet and visible spectrophotometer (Shanghai Junke Biotech. Co., Ltd., China), and an AUW120D analytical balance (Shimadzu Inc., Japan).

Grouping and modelling

The 120 mice were divided randomly into control, test, bifendate, and Qizhuyigan groups. The model used was reported previously [9]. Specifically, except for the control group, the other groups were gavaged with tap water at 2.5 g/kg. Mice in the bifendate group were gavaged with 12 mg/kg of the drug, and mice in the Qizhuyigan group were gavaged with water containing the contents of a Qizhuyigan capsule (1,000 mg/kg), for 10 days. On the last day of the experiment, all mice except those in the blank control group were injected intravenously with lipopolysaccharides (0.4 mg/kg) through the tail vein after the last dosing. Furthermore, each mouse was fixed with a fixator, with its tail outside the fixators. The tail was repeatedly wiped with of 75 % (v/v) ethyl alcohol. The tail vein was punctured with a syringe at an angle of 30° and a white line was seen along the vein. The absence of resistance during drug injection indicated that the tip of the needle had penetrated the vein, and residual drug was then injected continuously. Then, the needle was removed and the injection site was pressed with a cotton swab to stop the bleeding. Mice were provided with water, but no food, after the injection. They were assessed 12 h after the injection.

This study was approved by the Medical Ethics Committee of Binzhou People's Hospital (approval no. ZXQ20160115BZ) and followed the protocols set out in the Declaration of Helsinki [10].

Determination of biochemical parameters

After the mice were anaesthetised with diethyl ether, the eyeballs were removed with tweezers. Next, 1.5 mL of blood was collected using a sterile blood collection tube and centrifuged at (5,000 revolutions/min at a centrifugal radius of 6 cm for 10 min, and serum was recovered. An automatic biochemical analyser was used to measure levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Liver tissues were collected, washed with normal saline at 4°C, and dried with filter paper. Samples (0.2 g) of liver tissues were taken and 10 % (w/v) homogenates were prepared, and centrifuged at 4,500 revolutions/min at a

centrifugal radius of 6 cm for 10 min. The supernatants were removed and the levels of MDA, SOD, TNF- α , and IL-6 measured following standard operating instructions provided with the kits.

Statistical analysis

Analyses were conducted using SPSS version 20.0. Data are expressed as means \pm standard deviation (SD). Enumerated data were assessed using χ^2 tests, whereas categorical data were processed using *t*-tests. $P < 0.05$ was taken to indicate statistical significance.

RESULTS

Serum ALT and AST levels

The serum ALT and AST levels of the test group were significantly higher than those of the control group (both $p < 0.05$), indicating that the animal model had been established successfully. The serum ALT and AST levels of the bifendate and Qizhuyigan groups were much lower than those of the test group (both $p < 0.05$). The ALT and AST levels were lowest in the Qizhuyigan group (Table 1).

Table 1: Serum ALT and AST levels in the various groups (mean \pm SD, U/L, N = 30)

Group	ALT	AST
Control	43.10 \pm 6.30	98.40 \pm 10.40
Test	224.70 \pm 18.40 [*]	362.60 \pm 16.60 [*]
Bifendate	132.80 \pm 9.40 [#]	224.60 \pm 12.20 [#]
Qizhuyigan capsule	98.30 \pm 8.70 [#]	165.50 \pm 12.10 [#]

* $p < 0.05$ compared with that of the blank control group; # $p < 0.05$ compared with that of the test group

MDA and SOD contents of liver homogenates

Compared with the control, SOD levels in the hepatic tissue homogenate of the test group decreased, but MDA levels increased ($p < 0.05$), indicating peroxidation damage. Compared with the bifendate group, the Qizhuyigan group exhibited clearly reduced MDA levels in hepatic tissue homogenate ($p < 0.05$) and increased SOD levels ($p < 0.05$). MDA and SOD contents changed the most in the Qizhuyigan group, indicating that Qizhuyigan enhanced anti-lipid peroxidation ability (Table 2).

TNF- α and IL-6 contents of liver homogenates

Compared with the control group, the TNF- α and IL-6 levels in the liver homogenate of the test group were significantly increased (both $p < 0.05$), indicating more serious damage to liver

tissues in the former group. Compared with the test group, the bifendate group and the Qizhuyigan group exhibited significantly reduced TNF- α and IL-6 levels (both p values < 0.05); this was especially true of the Qizhuyigan group, suggesting that the anti-hepatic injuryability of Qizhuyigan may be related to inhibition of the release of the cytotoxic factors TNF- α and IL-6 (Table 3).

Table 2: MDA and SOD contents of liver homogenates of the groups (mean \pm SD, N = 30)

Group	MDA (μ mol/L)	SOD (U/g)
Control	4.70 \pm 1.40	33.70 \pm 4.70
Test	31.30 \pm 6.60 [*]	10.20 \pm 2.40 [*]
Bifendate	14.40 \pm 2.30 [#]	27.50 \pm 3.70 [#]
Qizhuyigan capsule	12.70 \pm 2 [#]	29.70 \pm 4 [#]

* $p < 0.05$ compared with the blank control group; # $p < 0.05$ compared with the test group

Table 3: Comparison of TNF- α and IL-6 contents in liver homogenates of the various groups (mean \pm SD, ng/L, N = 30)

Group	TNF- α	IL-6
Control	112.90 \pm 21.70	53.20 \pm 12.70
Test	364.90 \pm 40.90 [*]	229.20 \pm 33.90 [*]
Bifendate	156.70 \pm 25.90 [#]	154.70 \pm 18.40 [#]
Qizhuyigan capsule	138.30 \pm 23.70 [#]	139.40 \pm 16 [#]

* $p < 0.05$ compared with the blank control group; # $p < 0.05$ compared with the test group

DISCUSSION

Viral hepatitis is a relatively common infectious disease. The occurrence of immunoreactions in the liver is an important factor causing liver injuries in patients with viral hepatitis. Currently, effective drugs for treating chronic viral hepatitis are lacking, but good curative effect may be achieved if the treatment is focused on regulating immunity. The occurrence of liver injury in patients with chronic viral hepatitis appears to be significantly correlated with immune dysfunction and immunodeficiency [11]. The animal model of immunological liver injury used in this study was associated with extensive cytokine release (e.g., TNF- α and IL-6) and oxygen radicals, similar to the pathogenesis of liver injury induced by chronic viral hepatitis. Thus, the mouse model was suitable for the purpose [12].

Traditional Chinese medicine suggests that liver injury is caused by insufficient anti-pathogenic energy, damp-heat, and pestilent toxin. The basic therapies used to treat Chronic Hepatitis B (CHB) are based on toxin elimination, phlegm reduction, relief of fatigue, correction of deficiencies, and tonification. Traditional Chinese

medicine can help treat various difficult diseases, and scholars are focusing on applying traditional Chinese medicine to develop drugs to treat liver diseases.

The Qizhuyigan capsule used in this study was developed based on clinical experience using traditional Chinese medicine to treat viral hepatitis, along with features of traditional Chinese medicine, such as diagnosis and treatment based on an overall analysis of the illness and the patient's condition, as well as modern pharmacology. The capsule consists of seven traditional Chinese medicine materials, including the root of red-rooted salvia, felwort, *white atractylodes rhizome*, *Astragalus membranaceus*, and *radix isatidis*. *Astragalus membranaceus* is frequently used in traditional Chinese medicine, with effects such as tonifying qi, lifting yang, strengthening the exterior, reducing sweat, inducing diuresis to alleviate oedema, and promoting fluid and blood production. Work using modern pharmacological techniques [13,14] has revealed that *Astragalus membranaceus* contains active ingredients, such as astragaloside, which can improve cardio-pulmonary function, enhance immunity and promote the body's resistance to disease, specifically its ability to resist viruses.

ALT and AST levels can directly reflect the degree of liver injury. Previous research has found that damage to 1 % of hepatocytes can double the levels of ALT and AST in the liver [15]. Levels of ALT and AST in the test group were clearly increased compared with the blank control group, and those in the bifendate and Qizhuyigan groups were significantly decreased, indicating that Qizhuyigan had a protective effect in this mouse model of immunological liver injury.

Lipid peroxidation plays an important role in the occurrence and development of immunological liver injury. MDA is the end product of lipid peroxidation, the content of which is positively proportional to the degree of lipid peroxidation [16].

SOD is important in balancing oxidant and antioxidant content in the body, and can remove superoxide anion free radicals and protect cells from damage [17]. Thus, levels of SOD and MDA can reflect the degree of damage caused by peroxidation. Our results indicate that Qizhuyigan can significantly lower MDA and increase SOD in mice, indicating positive anti-lipid peroxidation effects.

TNF- α not only directly kills hepatocytes, but also promotes liver injury [18]. IL-6 has functions in

regulating immunoresponses and promoting the growth of hepatocytes [19]. The results of this study suggest that Qizhuyigan may effectively protect the liver by significantly lowering TNF- α and IL-6 content and reducing the release of inflammatory factors.

CONCLUSION

Qizhuyigan protected mouse liver function in animals with induced immunological liver injury. The mechanism of action appeared to be related primarily to the inhibition of immune function and lipid peroxidation in the liver, thereby providing some theoretical pharmacological foundation for the clinical treatment of chronic viral hepatitis using Qizhuyigan capsules.

DECLARATIONS

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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.

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