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Comparative Study on the Effects of Several Hepatoprotective Drugs on Acute Liver Injury in Mice

Yanjie Chen*

College of Health Management, Shanghai Jian Qiao University, Shanghai 201306, China

**Corresponding author: Yanjie Chen, 934887427@qq.com*

Abstract: *Objective:* To investigate the protective effects of Gantaile capsule, ammonium glycyrrhizinate injection, and Ginseng Jianpi pill on CCl_4-induced acute liver injury in mice. *Methods:* Forty-eight mice were randomly divided into blank group, model group (15% CCl_4, 10 ml/kg), Gantaile group (150 mg/kg), ammonium glycyrrhizinate group (10 ml/kg), Ginseng Jianpi pill group (150 mg/kg), and positive control group (biphenyl diester, 150 mg/kg), with 8 mice in each group. Each administration group was given the corresponding dose of drugs by gavage, and the blank and model groups were given the corresponding volume of normal saline by gavage, once a day for 14 hours. Two hours after the last administration, the normal group was intraperitoneally injected with normal saline, and the other groups were intraperitoneally injected with CCl_4 peanut oil solution. 24 hours later, blood was collected from the eyeballs to measure the serum levels of alanine aminotransferase, aspartate aminotransferase, tumor necrosis factor-alpha, and interleukin-6. The liver tissue was taken out to calculate the liver index. The morphological changes of the liver tissue and the degree of liver injury were observed by HE staining, and the activities of superoxide dismutase and malondialdehyde in the liver tissue were detected. *Results:* Several hepatoprotective drugs can significantly reduce CCl_4-induced acute liver injury in mice. Gantaile capsule has the most obvious effect, followed by ammonium glycyrrhizinate injection, and Ginseng Jianpi pill is the last. *Conclusion:* This experiment found that Gantaile capsules acted quickly, significantly alleviating liver injury. Ammonium glycyrrhizinate injection demonstrated the best anti-inflammatory effect. Compared to the first two drugs, Ginseng Jianpi pills showed less ideal effects, serving only as an auxiliary treatment.

Keywords: Hepatoprotective drugs; Carbon tetrachloride; Acute liver injury; Protective effect

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1. Introduction

Liver injury [1,2] is one of the serious health threats to human health. Liver injury can be divided into broad and narrow categories. Broadly, liver injury generally includes various inflammatory liver diseases within internal

medicine. In a narrow sense, liver injury typically refers to liver rupture caused by external forces. The main causes of liver injury include factors such as excessive alcohol consumption and drug abuse.

Gantaile capsules, ammonium glycyrrhizinate injections, and Ginseng Jianpi pills are all used in the treatment of patients with liver injury and have demonstrated significant clinical efficacy [3,4]. This study will use a CCl_4-induced acute liver injury model in mice [5–7] to observe how the three drugs protect against acute liver injury in mice and to explore their mechanisms, to provide pharmacological evidence for using liver-protecting drugs in clinical settings and developing new ones [8–10].

2. Materials and methods

2.1. Instruments

HH-6 constant-temperature water bath (Changzhou Tianrui Instrument Co., Ltd.); BX51 optical microscope (Olympus Corporation, Japan); TDL-40B (low-temperature centrifuge).

2.2. Reagents

Gantaile capsules (Hua Zhong Pharmaceutical Co., Ltd., specification: 150mg/capsule); Ammonium glycyrrhizinate injection (Zhengda Tianqing Pharmaceutical Group Co., Ltd., specification: 10 ml/vial); Biphenyl diester drops (Beijing Xiehe Pharmaceutical Factory, specification: 1.5 mg/drop); Ginseng Jianpi pills (Beijing Tongrentang Pharmaceutical Factory, specification: 6 g/pill); Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) test kits (China National Pharmaceutical Group Corporation); Tumor necrosis factor-alpha (TNF-α), Interleukin-6 (IL-6), Superoxide dismutase (SOD), Malondialdehyde (MDA) test kits (Nanjing Jiancheng Bioengineering Institute); other reagents are of analytical grade.

2.3. Experimental animals

48 male Kunming mice, weighing 18–22 g each, clean grade, purchased from the Animal Experiment Center of Zhengzhou University.

2.4. Mouse grouping, modeling, and administration

After feeding the 48 mice for one week, they were randomly divided into: blank group, model group, positive control group (biphenyl diester 150 mg/kg), Gantaile capsule group (150 mg/kg), ammonium glycyrrhizinate group (10 ml/kg), Ginseng Jianpi pill group (150 mg/kg), with 8 mice per group. The positive control group and each treatment group were administered the corresponding reagents by gavage at the aforementioned doses once daily for 7 consecutive days. The blank group and model group were given a certain volume of physiological saline by gavage, at a volume of 10 ml/kg. Two hours after the last dose, the model group and each treatment group were intraperitoneally injected with a 15% CCl_4 peanut oil solution to induce liver damage, with an injection dose of 10 ml/kg, while the blank group received 10 ml/kg of physiological saline.

2.5. Biochemical index detection

2.5.1. Mouse liver index and serum ALT, AST, TNF-α, and IL-6 measurement

After the last dose, the mice were fasted for 12 hours without water. After 12 hours, the body weight of the mice was measured, blood was collected via cardiac puncture, centrifuged at 4°C for 5 minutes to separate the serum, and the serum levels of ALT, AST, TNF-α, and IL-6 were measured [11]. After euthanizing the mice, the liver tissue

was removed, rinsed, and dried before weighing to calculate the liver index. Liver index (%) = liver weight (g) / body weight (g) × 100% [12].

2.5.2. Preparation of liver tissue homogenate and histopathological observation

After assessing the liver's size, color, and smoothness, 0.3 g of the left lobe liver tissue from the mice was fixed in 10% formaldehyde solution, embedded in paraffin after 24 hours, and sliced into 5–8 micrometer thick sections. The paraffin was removed from the sections using xylene, followed by washing with alcohol, and then placed in distilled water for staining with Hematoxylin-Eosin (HE) [13–15]. Finally, the liver tissue histopathological sections were observed and photographed under an optical microscope.

2.5.3. Liver tissue MDA and SOD content

0.3 g of the right lobe of the liver was collected, rinsed with physiological saline, and prepared into a tissue homogenate, centrifuged for 5 minutes, and the supernatant was collected for later use. The contents of SOD and MDA in the liver tissue were measured using an enzyme-linked immunosorbent assay (ELISA) [16].

2.6. Statistical analysis

Statistical analysis was conducted using SPSS 23.0. Serum ALT, AST, liver index, TNF-α, IL-6, and liver tissue MDA and SOD contents were expressed as mean ± standard deviation (SD). Independent sample *t*-tests were used for comparisons between two groups, and one-way ANOVA was used for comparisons among multiple groups [17]. A *P*-value of less than 0.05 was considered statistically significant, and a *P*-value of less than 0.01 was considered highly statistically significant.

3. Experimental results and analysis

3.1. Effects on liver index and serum ALT, AST activity in mice

Data analysis shows that compared to the control group, the model group exhibited a significant increase in serum AST and ALT levels ($P < 0.01$), indicating that the CCl_4 model was successfully established. The liver index of mice in the model group significantly increased ($P < 0.01$), suggesting liver enlargement in these mice. All treatment groups were able to reduce the liver index, ALT, and AST levels in mice ($P < 0.05$), with Gantaile capsules having the most notable effects. Ammonium glycyrrhizinate injection followed, while the Ginseng Jianpi pills group ranked last.

3.2. Effects on serum TNF-α and IL-6 levels in mice

Data analysis showed that serum TNF-α and IL-6 levels were significantly higher in the model group compared to the control group, with statistically significant differences ($P < 0.01$), further confirming successful modeling. Compared to the model group, all treatment groups significantly inhibited the activity of TNF-α and IL-6, with statistically significant differences ($P < 0.01$). Ammonium glycyrrhizinate exhibited strong anti-inflammatory effects, followed by Gantaile capsules, with Ginseng Jianpi pills ranking last.

3.3. Effects on MDA levels and SOD activity in mouse liver tissue

Data comparison analysis showed that compared to the control group, the SOD activity in the liver tissue of mice in the model group gradually decreased ($P < 0.01$), while MDA levels significantly increased ($P < 0.01$), indicating

a marked decline in the antioxidant capacity of the model group mice. Compared to the model group, all treatment groups showed significant increases in SOD activity and decreases in MDA levels, with statistically significant differences ($P < 0.01$). Except for the positive control group, Gantaile capsules had the most pronounced effect in increasing SOD and decreasing MDA, suggesting the strongest antioxidant capacity. Ammonium glycyrrhizinate injection followed, with Ginseng Jianpi pills ranking last.

3.4. Effects on the pathological structure of the liver in mice with acute liver injury

Under microscopic observation, the liver cell cord structure in the control group was clear, and the liver lobule structure was intact. The model group exhibited a large number of degenerative necrotic cells, with vacuolated liver cells of varying sizes in the liver lobules, showing that the liver injury model was successfully created with CCl_4. The liver cell cord structure in all treatment groups and the positive control group was relatively clear, with varying degrees of improvement in cell vacuolation and necrosis, showing cell regeneration and reduced inflammation. The results suggest that all treatment groups had a noticeable improvement effect on CCl4-induced acute liver injury, with the Gantaile capsules showing the most significant effect.

4. Conclusion

CCl_4-induced liver injury mainly involves CCl_4 and its toxic metabolite, trichloromethyl free radicals. This metabolite can cause the release of AST and ALT into the blood. Therefore, the levels of AST and ALT in serum can directly reflect the degree of liver injury. This experiment found that Gantaile capsules, ammonium glycyrrhizinate injection, and Ginseng Jianpi pills all helped lower AST and ALT levels in mice, but to different extents, with Gantaile capsules showing the best effect.

After acute liver injury, an inflammatory response occurs, with ammonium glycyrrhizinate injection demonstrating the best anti-inflammatory effect. The experimental results indicate that these hepatoprotective drugs can all enhance liver SOD activity, reduce MDA levels, and alleviate liver injury, with Gantaile capsules showing the best effect.

This experiment found that Gantaile capsules acted quickly, significantly alleviating liver injury. Ammonium glycyrrhizinate injection demonstrated the best anti-inflammatory effect. Compared to the first two drugs, Ginseng Jianpi pills showed less ideal effects, serving only as an auxiliary treatment. This experiment lasted only seven days, suggesting that the traditional Chinese medicine formulations may not have reached an effective treatment duration, and their effects were not fully manifested. However, we cannot overlook the strong effects of traditional Chinese medicine; both traditional Chinese medicine and Western medicine have their advantages, and appropriate combinations in clinical practice can achieve synergistic effects.

Disclosure statement

The author declares no conflict of interest.

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