Protective Effect of Gegen Hawthorn Ginseng Granules on Alcoholic Liver Injury in Mice

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Keywords: Gegen Hawthorn Ginseng Granules, Alcoholic Liver Injury, Oxidative Stress, Inflammation, Mechanism of Action.

Abstract: Gegen hawthorn ginseng granules (GHGG) is composed of 6 Chinese medicinal materials including pueraria lobata, hawthorn and ginseng. This study aims to explore the protective effect of GHGG on alcoholic liver injury, and the correlation between oxidative stress, lipid metabolism, inflammation and alcoholic liver disease. The mice were randomly divided into control group and model group, diammonium glycyrrhizinate positive drug group, and GHGG low and high dose groups (GHGG-L and GHGG-H). Oral alcohol was used to establish an acute alcoholic liver injury model. Determination of liver function index ALT and AST levels after administration; Determination of superoxide dismutase (SOD), malondialdehyde (MDA) and reduced glutathione in liver tissue (GSH), TNF-α, IL-1β, IL-6 and other biochemical indicators; HE staining to analyze the morphological changes of tissue sections. Experimental results show that GHGG can significantly reduce the levels of ALT, AST, and MDA in mice; increase the level of liver tissue The activity of SOD and GSH, and GHGG can significantly inhibit the levels of biochemical indicators such as pro-inflammatory factors TNF-α, IL-1β, IL-6. The degree of pathological changes in the liver tissue of mice in the GHGG group was significantly reduced. In summary, GHGG can improve ALD by anti-oxidation, inhibiting inflammation, and promoting liver cell regeneration.

1 INTRODUCTION

In recent years, the global incidence of alcoholic liver disease (ALD) has been increasing year by year, and it has become a major disease that seriously endangers human health. The main clinical symptoms are hepatitis and liver fibrosis, and severe cases can lead to liver cirrhosis and liver cancer (Kong 2019). Treatment is mainly through drug intervention and liver transplantation (Bloom Patricia 2021). So far, there have been many researches and developments of alcoholic liver disease drugs at home and abroad, but they have not yet been able to meet the clinical needs. Its pathogenesis is complex and diverse, among which oxidative stress, abnormal lipid metabolism, disturbance of intestinal flora, and inflammation are currently considered to be the main causes of ALD (Kong 2019, Natalia 2017, Woodhouse 2018). In this experiment, by studying the effects of GHGG on the antioxidant enzyme activity, liver function index levels in mice with acute alcoholic liver injury and detecting the level of related inflammation in mice with acute alcoholic liver injury, the GHGG can relieve alcohol The liver-protecting effect and its mechanism of action are preliminarily discussed to provide experimental evidence for its clinical application.

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2 MATERIALS AND METHODS

2.1 Materials

2.1.1 Medicines and Reagents

GHGG (Homemade in the laboratory); diammonium glycyrrhizinate (China Zhengda Tianqing Pharmaceutical Group Co., Ltd.); alanine aminotransferase (ALT), aspartate aminotransferase (AST), malondialdehyde (MDA), Superoxide dismutase (SOD), reduced glutathione (GSH) detection kit (Nanjing Jiancheng Institute of Biological Engineering). Tumor Necrosis Factor (TNF-α), Interleukin-6 (IL-6), IL-1β (Jiangsu Enzyme Industry Co., Ltd.); BCA protein concentration determination kit (Shanghai Biyuntian Biotechnology Limited company).

2.1.2 Animals

50 SPF KM mice, provided by (Liaoning Changsheng Biotechnology Co., Ltd., production license number: SCXK (Liao) 2020-0001; certificate number: 210726200100461748). The animals are maintained under a 12h/12h light/dark cycle at 25±3℃ and a relative humidity of 50±20%. This study was conducted in accordance with the Declaration of Helsinki, and the protocol has been approved by the Animal Health and Welfare Committee of Changchun University of Chinese Medicine (20190123). The procedures involving animals and their care comply with the institutional guidelines of national and international laws and policies.

2.2 Methods

2.2.1 Design of Animal Experiment

Fifty mice were randomly divided into 5 groups, namely control group, model group, diammonium glycyrrhizinate positive drug group (150 mg/kg bw) (Zhang 2020) and GHGG low and high dose groups (800, 2000mg/kg bw), each group has 10 animals. Gavage is given once a day for 10 consecutive days. Four hours after the last administration, except the control group, the mice in each group were given 56% ethanol (12mL/kg bw) to establish an acute alcoholic liver injury model. The blank control group mice were given an equal volume of distilled water.

2.2.2 Measurement of Liver Index

The liver of the mice was aseptically removed, the final body mass and organ mass of the mice were weighed, and the organ coefficient was calculated according to the ratio of the organ mass to the body mass.And weighed to measured organ index according to thefollowing formula:

$$\text{Organ index (\%)} = \text{organ mass/final body mass} \times 100\% \quad (1)$$

2.2.3 Determination of Biochemical Indicators of Liver Function

Blood was collected from the eyeballs of mice. After clotting at 4℃ for 1 hour, the blood was centrifuged at 4000 r/min for 15 minutes. The supernatant was collected as serum. The ALT and AST levels were determined according to the kit instructions.

2.2.4 Determination of Oxidative Stress Indicators

Take the liver, place it in a glass tissue homogenizer, add appropriate amount of physiological saline, prepare a 10% (m:V) tissue homogenate, centrifuge at 4000r/min for 15 minutes, collect the supernatant, and determine SOD, MDA and GSH according to the kit instructions level.

2.2.5 Determination of Hepatic Proinflammatory Cytokines

Take the liver, place it in a glass tissue homogenizer, add an appropriate amount of physiological saline, prepare a 10% (m:V) tissue homogenate, centrifuge at 4000r/min for 15 minutes, collect the supernatant, and according to the kit instructions to determine the levels of IL-6, IL-1β and TNF-α.

2.2.6 Histological Investigation of Liver

Samples of liver were separated from each mouse and fixed in formalin solution (10%) for 24 h, and after that dehydrated using graded alcohol and xylene, and implanted in paraffin. Paraffin-embedded sections, stained with hematoxylin and eosin (H&E) for histological investigation.

2.2.7 Statistical Analysis

The data of the animal experiment was evaluated by using Graph Pad Prism version 5 (La Jolla, CA, USA). One-way analysis of variance (ANOVA) was used to compare variations between groups,

followed by Duncan's multiple range test. Differences between groups were found statistically significant at $p < 0.01$ or $p < 0.05$ and the data was expressed as mean ± SD.

3 RESULTS

3.1 Effect of GHGG on Body Weight and Organ Index in ALD Mice

The food intake of each group of mice was recorded, and the calculation formula was intake g/day. The results in Table 1 show that compared with the normal group, the food utilization rate of the model group was significantly reduced ($p<0.05$), and the feed intake of GHGG-H was significantly improved ($P<0.05$). The liver index results are shown in Table 1. The liver index of the model group was significantly higher than that of the control group ($p<0.05$). However, compared with the model group, giving different doses of GHGG (GHGG-L and GHGG-H) can reduce liver swelling. There was no significant difference between GHGG-L and GHGG-H groups ($p>0.05$).

Table 1: Body weight and organ index in ALD mice.

	Control	Model	Positive drug	GHGG-L	GHGG-H
Food intake g/day	6.30±0.25	4.94±0.25$^{\#}$	5.67±044*	5.24±0.18	5.47±0.40*
Liver index %	4.01±0.27	6.07±0.33$^{\#}$	4.60±0.17*	4.31±0.43*	4.46±0.53*

$p^{\#}<0.05$ represents compared with the control group, and $p^{*}<0.05$represents compared with the model group.

3.2 Effects of GHGG on Liver Function Indexes of ALD Mice

AST and ALT are biochemical markers for clinical evaluation of liver function. When liver function is impaired, the levels of AST and ALT increase sharply compared with normal physiological conditions (Katrine 2021, Sun 2021). Figure 1-2 shows the effect of GHGG on serum AST and ALT in ALD mice. The levels of AST and ALT in the model group were significantly higher than those in the blank group ($P<0.05$), indicating that the liver was damaged after a large amount of alcohol intake, which led to a significant increase in serum ALT and AST. GHGG in different dose groups can significantly reduce the serum content level and achieve the effect of protecting the liver.

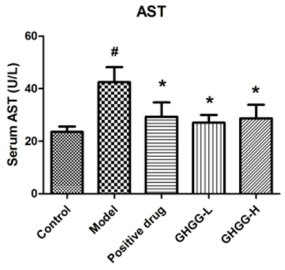

Figure 1: AST levels in serum of ALD mice.

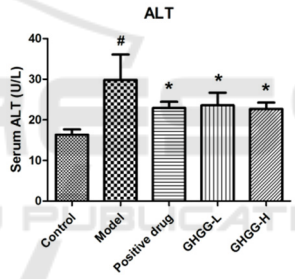

Figure 2: ALT levels in serum of ALD mice.

3.3 Effects of GHGG on Antioxidant Capacity of Liver Tissue in ALD Mice

Oxidative stress is one of the main mechanisms of the pathogenesis of alcoholic liver disease, and it has an important impact on the initial liver fibrosis and hepatocellular carcinoma (Zhang 2018, NWFLALD 2018, Xia 2017). MDA, SOD and GSH play an important role in protecting alcohol-induced liver damage and oxidative stress. Figure 3-5 shows the effect of GHGG on liver antioxidant enzymes MDA, SOD and GSH. Compared with the control group, the liver MDA level of the model group was significantly increased ($p<0.05$). Compared with the model group, the MDA content of the GHGG treatment group (GHGG-L and GHGG-H) was significantly reduced ($p<0.05$). In addition, compared with the control group, the liver SOD and

GSH levels in the model group were significantly lower ($p<0.05$), and the GHGG administration group (GHGG-L and GHGG-H) could increase their levels. The experimental results show that GHGG can reduce the content of MDA and increase the activity of antioxidant enzymes SOD and GSH to protect the liver in mice with alcoholic liver injury.

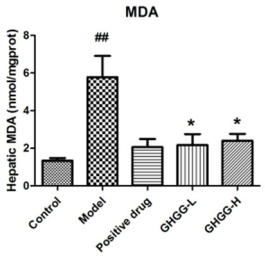

Figure 3: MDA levels in tissues of ALD mice.

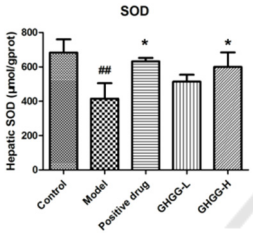

Figure 4: SOD levels in tissues of ALD mice.

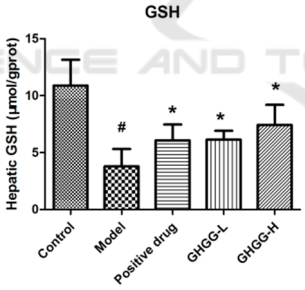

Figure 5: GSH levels in tissues of ALD mice.

3.4 Effects of GHGG on the Level of Pro-inflammatory Cytokines in the Liver of ALD Mice

The results of pro-inflammatory cytokines such as IL-6, IL-1β and TNF-α in mouse liver tissue are shown in Figure 6-8. Compared with the blank group, the liver TNF-α, IL-1β and IL-6 of the mice in the model group all increased significantly ($p<0.05$). Compared with the model group, the GHGG administration group (GHGG-L and GHGG-H) can significantly reduce the levels of TNF-α, IL-1β and IL-6 ($p<0.05$). The above results indicate that GHGG can protect liver cells by reducing the occurrence of alcohol-induced liver inflammation.

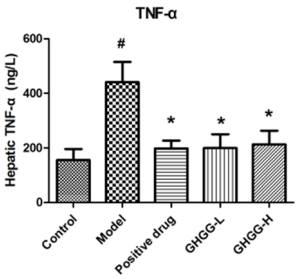

Figure 6: TNF-α levels in tissues of ALD mice.

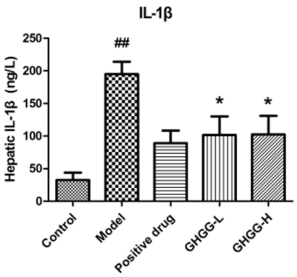

Figure 7: IL-1β levels in tissues of ALD mice.

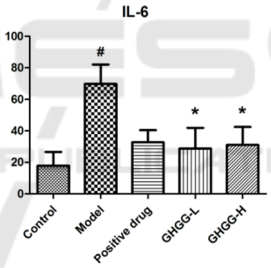

Figure 8: IL-6 levels in tissues of ALD mice.

3.5 Effects of GHGG on Histopathological Characteristics of Liver in ALD Mice

The H&E staining method was used to study the pathological changes of liver tissue in ALD mice. As shown in Figure 9, the structure of the liver lobules in the control group was complete and clear, the liver cells were arranged in an orderly manner, and the nuclei were complete and clear. The liver tissue sections of mice in the model group showed obvious inflammatory cell infiltration, fatty vacuoles and hepatocyte enlargement. Compared with the model group, the GHGG administration group (GHGG-L and GHGG-H) had less edema, fatty vacuoles and inflammatory cell infiltration.

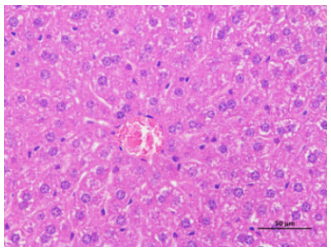

Figure 9: Liver tissue slices of mice in the control group

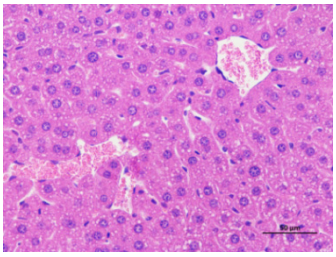

Figure 10: Liver tissue slices of mice in the model group

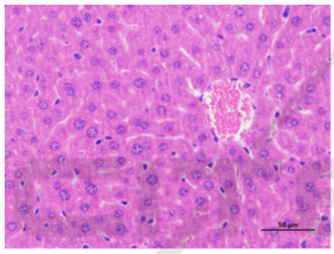

Figure 11: Liver tissue slices of mice in the positive drug group

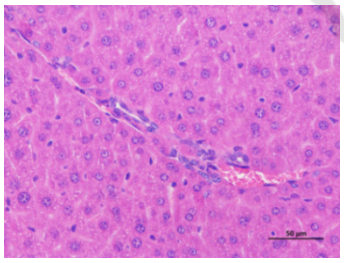

Figure 12: Liver tissue slices of mice in the GHGG-L group

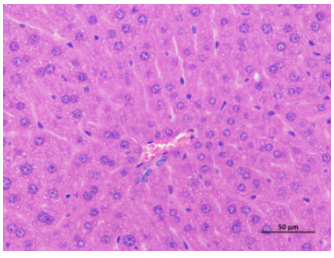

Figure 13: Liver tissue slices of mice in the GHGG-H group

4 DISCUSSION

The liver is one of the main organs for alcohol metabolism and the largest gland in the human body. It has very important physiological functions (Li 2019). When the human body consumes excessive alcohol, a large amount of metabolic waste cannot be excreted in time, which will cause degeneration and necrosis of liver cells, which will cause disorders of related metabolic pathways in the cells, cause liver cells to produce inflammation and oxidative stress, fibrosis and lead to liver lipids. and its can induce liver cell damage (Xie 2021, Tu 2019, TESCHKE 2018). For oxidative stress, excessive alcohol intake leads to the weakening of SOD's antioxidant capacity, and the body's oxidation and antioxidant balance is disrupted, causing oxidative stress in the body, leading to a chain reaction of lipid peroxidation, and damage to mitochondrial function. Liver damage caused by endoplasmic reticulum stress and immune inflammatory response (Zhang 2021). MDA is an important product of lipid peroxidation, and its content can reflect the degree of lipid peroxidation in the body (Rani 2016), GSH is an important antioxidant substance in the body. It is a substrate of two enzymes, GSH-Px and GSH-ST. It is a low-molecular scavenger that can remove O2, H2O2, so the level of GSH content is a measure of the body's antioxidant capacity (Wu 2021). The balance of pro-inflammatory cytokines and anti-inflammatory cell levels is essential for maintaining human health (Sun 2021). In this experiment, we tested the levels of three inflammatory cytokines, TNF-α, IL-1β and IL-6. TNF-α is significantly increased during alcoholic liver injury compared to normal physiological levels (Shen 2018). It can be activated by binding to caspase3 to induce apoptosis of hepatocytes (Dalia 2019). At the same time, it also activates the NF-κB signaling pathway, triggers the secretion of inflammatory factors such as IL-1β or IL-6, further aggravates liver inflammation and induces the occurrence of alcoholic liver disease (Liu 2017).

5 CONCLUSIONS

In summary, GHGG can effectively reduce liver lipid peroxidation levels by inhibiting the increase of liver function indicators in mice, regulate liver metabolic disorders, and improve the antioxidant capacity of mice, thus playing a protective role on alcohol-induced acute liver injury in mice. This

research lays an experimental foundation for the in-depth development of drugs for the treatment of alcoholic liver injury, provides a basis for clinical medication, and also broadens the use of medicinal materials for both food and medicine.

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