Protective Effects of Siwu Xuanhu Decoction on the Liver of Mice with Acute Liver Injury

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Abstract [**Objectives**] To investigate the protective effects of Siwu Xuanhu decoction on the liver of mice with acute liver injury induced by CCl_4. [**Methods**] A total of 48 ICR mice were randomly assigned to six groups: a blank control group, a model group, a low dose group of Siwu Xuanhu decoction (100 mg/kg), a medium dose group of Siwu Xuanhu decoction (200 mg/kg), a high dose group of Siwu Xuanhu decoction (300 mg/kg), and a positive control group (silymarin 2 mg/kg). Each group comprised 8 mice. Each dosing group received the designated dose of the drug (10 mL/kg) via continuous gavage, while the blank group and the model group were administered an equivalent volume of normal saline for four weeks, three times per week. An acute liver injury model was established through the intraperitoneal administration of a 20% CCl_4 olive oil solution at a dosage of 2 mL/kg in all experimental groups, with the exception of the control group, which received an equivalent volume of the olive oil solution. After 24 h fasting with water intake, blood samples were collected from the ocular region. The blood samples were allowed to stand and subsequently subjected to centrifugation to isolate the upper layer of serum, and a diagnostic kit was employed to measure the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and interleukin-6 (IL-6), and the activity of superoxide dismutase (SOD) in the serum. [**Results**] In comparison to the control group, serum levels of AST and ALT were significantly elevated in the model group. Furthermore, when compared to the model group, treatment with Siwu Xuanhu decoction resulted in a reduction of serum levels of ALT, AST, and IL-6, while simultaneously increasing SOD activities. [**Conclusions**] Siwu Xuanhu decoction exhibits a protective effect against acute liver injury induced by CCl_4 in mice.

Key words Siwu Xuanhu decoction, Angelicae Sinensis Radix, Rhizoma Corydalis, Liver injury, Protective effect

1 Introduction

The liver is one of the largest and most complex organs in the human body, performing a multitude of essential functions, including metabolism, detoxification, synthesis, and storage. The proper regulation of numerous physiological activities within the human body is intricately linked to the support provided by the liver. Acute liver injury (ALI) is characterized by the sudden damage or necrosis of hepatocytes, resulting in a significant deviation of liver function from its normal state. In severe instances, this condition may result in liver failure and is classified within a category of critical liver function disorders[1]. Common factors contributing to ALI encompass recent drug use, short-term heavy alcohol consumption, long-term alcohol abuse, viral infections, and autoimmune reactions. In recent years, influenced by economic development, advancements in science and technology, and shifts in lifestyle, the incidence of liver diseases has steadily increased, rendering it a significant public health concern that warrants attention.

Prolonged damage to the liver significantly increases the likelihood of progression to liver fibrosis, cirrhosis, or even hepatocellular carcinoma, along with other severe hepatic conditions. Nevertheless, the liver possesses a remarkable capacity for self-repair, and many diseases resulting from liver damage can be reversed prior to the onset of cirrhosis. Consequently, to effectively impede the advancement of liver disease, it is essential to prioritize early intervention alongside systematic prevention and control strategies. These approaches aim to mitigate the risk of disease progression through the implementation of scientifically grounded and standardized preventive measures[2].

Siwu Xuanhu decoction is a composite formulation derived from several classical recipes, primarily incorporating Angelicae Sinensis Radix, Rhizoma Chuanxiong, Radix Paeoniae Alba, Radix Rehmanniae, and Rhizoma Corydalis[3]. This traditional formula is recognized for its efficacy in tonifying blood, promoting blood circulation, and regulating menstrual cycles, and it is esteemed as the "first formula in gynecology". Angelicae Sinensis Radix is utilized for the regulation of liver function and the nourishment of blood. Radix Paeoniae Alba serves to astringe the liver and alleviate pain[4]. Rhizoma Chuanxiong is known to enter the liver and gallbladder meridians, exhibiting properties that activate blood circulation, remove blood stasis, dispel wind, and relieve pain[5]. Additionally, Radix Rehmanniae is characterized by its thick and nourishing qualities, contributing to the nourishment of both nutrients and blood. Collectively, the combined application of these four substances demonstrate the efficacy of activating blood circulation, promoting menstruation, and eliminating carbuncles through the facilitation of bowel movements, thereby aiding in the restoration of the liver's excretory function. The present study was conducted to establish an ALI model in mice through the intraperitoneal injection of carbon tetrachloride (CCl_4). Addition-

Received: April 25, 2025 Accepted: July 26, 2025
Supported by Undergraduate Innovation and Entrepreneurship Training Program of Guangxi Zhuang Autonomous Region (S202310599089); Open Fund Project of Key Laboratory of Guangxi Universities on National Medicine in Youjiang River Basin (yykf2024-04).

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ally, the study aimed to investigate the protective effects of Siwu Xuanhu decoction on the liver of mice experiencing ALI, following the administration of the decoction. This research seeks to provide a reference for the clinical application of Siwu Xuanhu decoction.

2 Materials and methods

2.1 Experimental animals A total of 48 male ICR mice, aged 8 weeks and weighing (40 ± 5) g, were utilized in this study. The mice, along with their bedding and feed, were procured from Guangdong Charles River Laboratory Animal Technology Co., Ltd., under certificate No.: SCXK (Y) 2022-0063. The mice were acclimatized for one week prior to the formal experiments, during which they had ad libitum access to food and water.

2.2 Instruments, reagents and consumables The instruments utilized in the study comprised the following: FA12048 Electronic Balance (Shanghai Tianmei Balance Instrument Co., Ltd.); JSB-15 Electronic Weighing Scale (Shanghai Puchun Measuring Instrument Co., Ltd.); L-530 Low-Speed Centrifuge (Hunan Xiangyi Laboratory Instrument Development Co., Ltd.); HH-ZK600 Intelligent Constant Temperature Water Bath (Gongyi Yingyu High-tech Instrument Factory); HHS-11-4 Digital Display Electrothermal Constant Temperature Water Bath (Shanghai Baidian Instrument Equipment Co., Ltd.); RE-3000A Rotary Evaporator (Shanghai Yarong Biochemical Instrument Factory); DLSB-5/20 Low Temperature Coolant Circulation Pump and AHB-III Circulating Water Multi-Purpose Vacuum Pump (Zhengzhou Greatwall Scientific, Industrial & Trade Co., Ltd.); and TriStar LB941 Multifunctional Microplate Reader (Berthold, Germany). The reagents utilized in this study comprised carbon tetrachloride (analytically pure, Tianjin Fengchuan Chemical Reagent Technology Co., Ltd., batch No.: 090516); Oleo Bella extra virgin olive oil (Jiangsu Goldland Oils & Grains Food Co., Ltd., lot No.: F20220906); normal saline (Sichuan Kelun Pharmaceutical Co., Ltd., product batch No.: N22102901); and silymarin (Legalon capsule, lot No.: H20160336). The alanine aminotransferase (ALT) kit, aspartate aminotransferase (AST) kit, and total superoxide dismutase (SOD) assay kit were procured from Nanjing Jiancheng Bioengineering Research Institute (batch No.: C009-2-1, C010-2-1, and A001-3-2, respectively). Additionally, the mouse interleukin 6 (IL-6) enzyme immunoassay kit was obtained from Shanghai Yuanju Biotechnology Center (batch No.: YJ009936).

2.3 Preparation of Siwu Xuanhu decoction A total of 25 g of Angelicae Sinensis Radix powder and Rhizoma Corydalis powder, along with 12.5 g each of Rhizoma Chuanxiong powder, Radix Paeoniae Alba powder, and Radix Rehmanniae powder, were combined with 2 000 mL of ultrapure water. The mixture was then sealed in an airtight container and allowed to soak for 30 min. Subsequently, the mixture was heated and boiled for 2 h, after which it was allowed to cool naturally. Finally, the resulting filtrate was collected and set aside. The filtered dregs were combined with 1 500 mL of ultrapure water and heated for 1.5 h. The mixture was then filtered, and the filtrate was collected. This process was repeated three times. The filtrates obtained were combined, and the excess water was removed through evaporation using a rotary evaporator. The resulting filtrate was concentrated to a volume of 50 – 100 mL, which was subsequently transferred into a beaker. The concentrated filtrate was then evaporated to a paste at a constant temperature of 50 ℃ using an electrically heated thermostatic water bath. Following natural cooling, the filtrate was stored in a refrigerator at 4 ℃ for freezing and subsequent use. Prior to the experiment, the required concentration was prepared.

2.4 Animal grouping and administration The experimental mice were labeled with a 5% solution of picric acid utilizing the random number table method. The mice were categorized into six distinct groups: a blank group, a model group, a positive control group (silymarin group), and three treatment groups receiving low (100 mg/kg), medium (200 mg/kg), and high (300 mg/kg) doses of Siwu Xuanhu decoction. Each group comprised eight mice, which were housed in separate cages. The experimental groups received their respective doses, while the model control group was administered 0.9% normal saline. The positive control group (silymarin group) was treated with silymarin at a concentration of 2 mg/kg. To ensure the accuracy of the gavage dosing and to uphold the scientificity and precision of the experiment, the researchers measured the body weight of each experimental mouse on a daily basis. Each group received the appropriate drug solution based on a dosage of 10 mL/kg of body mass. The administration was conducted at a precisely scheduled time interval, specifically at 17:00 on the day of gavage, with the drug being administered continuously over four weeks, three times per week (on Monday, Wednesday, and Friday) to ensure the continuity and stability of the drug's effects. All experimental procedures were carried out in strict adherence to ethical standards for animal research, fully respecting and safeguarding the rights and welfare of the experimental animals.

2.5 Establishment of ALI model To develop a mouse model of ALI, all groups received continuous administration of the respective drugs for four weeks, followed by a gavage of the corresponding drugs for 2 h. Subsequently, all groups, except the control group, were injected intraperitoneally with 20% CCl_4 at a dosage of 2 mL/kg, while the control group received an equivalent volume of normal saline.

2.6 Observation indicators and methods

2.6.1 Detection of liver index in mice. Twenty-four hours following the administration of CCl_4 via injection, the mice were weighed using an electronic balance, and their body weights were recorded. Subsequently, the mice were anesthetized with urapidil hydrochloride, and blood samples were obtained through the retro-orbital venous plexus collection method. Following this, cervical dislocation was performed to euthanize the animals. The abdominal cavities of the mice were then opened, and the livers were excised

and rinsed with pre-cooled normal saline. The wet weight of the liver was measured immediately after being blotted with filter paper, and the liver index was subsequently calculated.

Liver index (%) = Liver weight/ Body weight of mouse × 100%

2.6.2 Determination of biochemical parameter levels in mouse serum. Following the final gavage, the experimental animals were subjected to a 24 h fasting period with access to water. Blood samples were collected from the ocular region and allowed to stand at room temperature for 30 min. Subsequently, the samples were centrifuged at 3 000 r/min for 10 min. The supernatant serum was then stored at −20 ℃ for future analysis. The levels of serum AST, ALT, and IL-6 were measured in strict accordance with the provided protocols.

2.7 Statistical methods An analysis of variance (ANOVA) was conducted utilizing SPSS 27.0 software.

3 Results and analysis

3.1 General situation At the conclusion of the modeling process, the mice in the control group exhibited a stable physiological state, demonstrated active behavior, maintained a neat and shiny coat, and displayed no observable abnormalities. In contrast, the mice in the model group presented with a poorer overall condition, characterized by fluffy and dull fur, a curled posture, reduced activity, and slow response. The general health of the mice in the low, medium, and high dose groups of Siwu Xuanhu decoction, as well as the positive control group, was found to be superior to that of the model group.

3.2 Effects of Siwu Xuanhu decoction on liver index and serum ALT, AST, IL-6 and SOD in mice with liver injury The findings indicated that both low and high doses of Siwu Xuanhu decoction significantly decreased the liver index, reduced serum levels of ALT and AST, increased the activity of SOD, and lowered serum levels of IL-6, as presented in Table 1.

Table 1 Effects of Siwu Xuanhu decoction on liver index and serum ALT, AST, IL-6 and SOD in mice with liver injury

Group	Liver index//%	ALT//U/L	AST//U/L	SOD//U/mL	IL-6//pg/mL
Blank	4.58 ± 0.63	49.69 ± 38.06	83.59 ± 3.23	23.24 ± 1.31	34.12 ± 3.77
Model	5.55 ± 0.25$^{\#\#}$	302.90 ± 23.10$^{\#\#}$	365.47 ± 9.29$^{\#\#}$	13.94 ± 4.30$^{\#\#}$	49.35 ± 2.85$^{\#\#}$
Positive control	5.50 ± 0.53	263.25 ± 5.84*	152.71 ± 21.01**	21.00 ± 2.52*	44.82 ± 8.51
Low dose	4.92 ± 0.21*	218.57 ± 14.25**	95.52 ± 28.23**	19.18 ± 1.35*	41.42 ± 3.52*
Medium dose	5.09 ± 0.24	211.73 ± 49.88**	146.19 ± 21.51**	21.32 ± 4.29*	37.55 ± 1.27*
High dose	4.60 ± 0.34**	220.81 ± 16.33**	169.89 ± 33.76**	18.74 ± 4.90*	39.63 ± 5.92*

NOTE Compared to the normal group, $^{\#}P<0.05$, $^{\#\#}P<0.01$; compared to the model group, $^{*}P<0.05$, $^{**}P<0.01$.

3.2.1 Liver function in mice. In comparison to the control group (Table 1), the serum activities of AST and ALT, as well as the liver index of mice in the CCl_4 model group, exhibited significant increases ($P<0.01$). Conversely, the serum activities of AST and ALT and the liver index in the positive control group, as well as the low and high dose groups, demonstrated significant reductions when compared to the CCl_4 model group ($P<0.05$). Furthermore, the serum activities of AST and ALT in the medium dose group also showed a substantial decrease ($P<0.01$). These findings suggest that Siwu Xuanhu decoction may mitigate the liver function abnormalities and hepatomegaly induced by CCl_4 in mice.

3.2.2 Oxidative stress in mouse liver. In comparison to the blank group (Table 1), the activity of SOD in the liver of mice in the CCl_4 model group exhibited a highly significant decrease ($P<0.01$), indicating a reduction in the body's antioxidant capacity in the model group. Conversely, when compared to the model group, SOD activity in the positive control group, as well as in the low, medium, and high dose groups of Siwu Xuanhu decoction, was significantly increased ($P<0.05$). This finding suggests that Siwu Xuanhu decoction may mitigate oxidative stress in mice with induced liver injury, enhance the liver's antioxidant capacity, and provide a protective effect on the liver.

3.2.3 Inflammatory response in mouse liver. In comparison to the blank group (Table 1), mice in the CCl_4 model group exhibited a significantly elevated expression of IL-6 in the liver ($P<0.01$). Furthermore, mice administered low, medium, and high doses of Siwu Xuanhu decoction demonstrated a significant reduction in IL-6 expression relative to the model group ($P<0.05$). These findings suggest that Siwu Xuanhu decoction may mitigate inflammatory responses in mice with CCl_4-induced ALI. The underlying anti-inflammatory mechanisms may involve the modulation of inflammatory signaling pathways, the inhibition of inflammatory cell activation, and the suppression of inflammatory factor secretion.

4 Discussion

CCl_4, as a hepatotoxic agent, serves as a standard model for studying ALI due to its ability to induce lipid peroxidation via its metabolites, specifically trichloromethyl radicals and trichloromethyl peroxyl radicals. These free radicals interact with unsaturated fatty acids present in cellular membranes, mitochondria, and endoplasmic reticulum, thereby damaging the structural integrity of the membranes[6-7]. ALT and AST are two enzymes synthesized by the liver, which typically maintain stable serum levels under normal physiological conditions. However, when hepatocyte rupture occurs, ALT, which is located in the cytoplasm, and AST, found in the mitochondria, are released into the bloodstream through damaged cell membranes. This process leads to a marked elevation in the serum activity of these two transaminases. Consequently, alterations in serum ALT and AST activities can serve as effective and indirect indicators of the extent and severity of liver injury, thereby facilitating an objective assessment of the degree of liver dam-

age[8]. The experimental results indicated that, in comparison to the model group, the various dosage groups (low, medium, and high) of Siwu Xuanhu decoction significantly reduced serum levels of ALT and AST ($P<0.01$). This finding suggests that Siwu Xuanhu decoction may exert hepatoprotective effects by stabilizing the structural integrity of liver cell membranes and reducing enzyme leakage. The hepatoprotective effect observed may be attributed to the cytoprotective properties of Angelicae Sinensis Radix and Rhizoma Chuanxiong, as utilized in Siwu Xuanhu decoction[9]. Experimental findings have demonstrated that Siwu Xuanhu decoction can significantly mitigate liver injury induced by CCl_4 and reduce the severity of liver damage through mechanisms that involve hepatoprotection and the reduction of enzyme levels.

Contemporary research has established that inflammatory factors play a significant role in the process of cellular damage. This damage is characterized by the rupture of cellular membranes, which results in the release of cellular contents into the extracellular environment, thereby initiating an inflammatory response[10]. IL-6 is a prevalent inflammatory cytokine that is maintained at low concentrations under physiological conditions. However, during pathophysiological states, the body is prompted to release various inflammatory mediators, including IL-6, which play a role in pro-inflammatory responses, apoptosis, oxidative stress, lipid peroxidation, and other related processes. Consequently, serum levels of IL-6 can become significantly elevated, leading to hepatocellular damage. The findings of this experiment indicated a significant increase in the serum levels of IL-6 in the model mice. In contrast, the IL-6 levels in all dosage groups of Siwu Xuanhu decoction exhibited a marked decrease when compared to the model group. These results suggest that the decoction may confer a protective effect on the liver by inhibiting inflammatory responses and mitigating the destruction of inflammatory factors on the organism.

SOD is a crucial endogenous molecule within the antioxidant system and serves as a significant antioxidant enzyme in the human body. It possesses the capability to scavenge oxygen free radicals, thereby contributing to the maintenance and repair of liver cells, which is indicative of the antioxidant capacity of these cells. The excessive generation of free radicals induced by CCl_4 results in an imbalance within the antioxidant system, characterized by the accumulation of lipids, a reduction in the activity of antioxidant enzymes, and an increase in lipid peroxidation products[11]. This phenomenon is primarily manifested through a decrease in SOD activity, which is synthesized by the organism to exert antioxidant effects and provide protection against cellular damage. The experimental results indicated that the SOD levels in all dosage groups of Siwu Xuanhu decoction were significantly elevated compared to those in the model group. This finding suggests that Siwu Xuanhu decoction possesses a notable capacity to inhibit lipid peroxidation.

In conclusion, Siwu Xuanhu decoction exhibits a protective effect against CCl_4-induced liver injury in mice. The underlying mechanisms may involve the inhibition of oxidative stress, a reduction in the secretion of pro-inflammatory cytokines such as IL-6, which subsequently alleviates the inflammatory response. Additionally, the decoction appears to stabilize the structural integrity of liver cell membranes, thereby minimizing the leakage of intracellular enzymes, including AST and ALT. Currently, our understanding of the mechanisms of action remains at a macro level. Therefore, further in-depth investigations into the mechanisms of liver injury are warranted to inform clinical treatment strategies for liver diseases and to aid in drug development.

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