Acute Toxicity of Jinchuan Formula Plum Wine Extract and Its Protective Effect on Mice with Liver Injury

Hairong ZHONG[1], Yun TENG[2], Limin LI[3*], Yuan LIU[4*]

1. College of Pharmacy, Sichuan College of Traditional Chinese Medicine, Mianyang 621000, China; 2. Chengdu Brilliant Pharmaceuticals Co., Ltd., Chengdu 610095, China; 3. Sichuan Academy of Chinese Medicine Sciences, Chengdu 610041, China; 4. Ethnic Medicine Institute, Southwest Minzu University, Chengdu 610041, China

Abstract [**Objectives**] To investigate the acute toxicity and hepatoprotective effect of Jinchuan formula plum wine extract on mice, determine its safety range, and evaluate its hepatoprotective effect. [**Methods**] The median lethal dose (LD_{50}) was determined by acute toxicity test with the toxic reaction and mortality of mice as indexes. Sixty Kunming mice were randomly divided into 6 groups: normal control group, model group (ConA-induced liver injury model), Jinchuan formula plum wine high, medium and low dose groups (1.0, 0.5, 0.25 g/kg) and silybin group (0.1 g/kg). The levels of ALT, AST, LDH in serum and TG, VLDL in liver were measured. After HE staining, the pathological changes of liver tissue in mice were observed, and the liver protective effect of Jinchuan formula plum wine extract was analyzed and evaluated. [**Results**] LD_{50} was 11.18 g/kg, and the 95% confidence limit of LD_{50} was 10.31 - 12.05 g/kg. The high-dose group of Jinchuan formula plum wine extract could significantly reduce the serum ALT and AST activities of ConA-induced liver injury mice ($P < 0.05$). [**Conclusions**] Jinchuan formula plum wine extract is relatively safe, and also has a protective effect on liver injury.

Key words Jinchuan formula plum wine extract, Acute toxicity test, ConA-induced liver injury, Liver protective effect

1 Introduction

Green plum is an immature fruit of *Prunus mume* (Sieb.) Sieb. et Zucc., a plant of *Armeniaca*, Rosaceae. It is native to China and is a dual-purpose medicinal and edible resource with multiple health benefits. Its medicinal value has a long history, first seen in the *Sheng Nong's Herbal Classic*. In the Ming Dynasty's *Compendium of Materia Medica*, it was recorded that smoked plum has the effects of relieving cough, reducing phlegm, expelling roundworms, and detoxifying. Green plums have a sour and astringent taste, rich in nutrients, mainly containing amino acids, vitamin B_2, organic acids, and various mineral elements. Among them, organic acids have a high content, which can maintain weak alkalinity in body fluids, decompose acidic and toxic substances in the blood to improve blood circulation. It is rich in minerals such as calcium, phosphorus, iron, and zinc, with a reasonable calcium phosphorus ratio that can promote calcium absorption[1]. Modern research has shown that green plums have various pharmacological effects, including improving blood circulation, relieving fatigue, inhibiting bacteria, anti-tumor, and antiviral effects[2-4]. Therefore, green plums and their processed products are known as the "king of cold fruits" and "natural health food".

Green plum wine has a long history in China, and its production process has been continuously improved over the long term, mainly including fermentation and soaking methods. At present, the widely used method for making green plum wine is represented by the soaking method in some areas of southwestern Sichuan. During the soaking process, active ingredients such as polyphenols, flavonoids, and esters gradually dissolve out of the green plum fruit, which can effectively preserve its activity and greatly improve the nutritional value of the fruit wine[5-8].

During a visit and investigation in Jinchuan County, Aba Tibetan and Qiang Autonomous Prefecture, Sichuan, it was found that the wild plum resources in Jinchuan County are abundant, but the effective utilization rate of green plum fruits in Jinchuan County is low. Due to the limited scale and poor sales of local plum products, there are no production and processing enterprises involved, resulting in a large amount of wild plum resources being wasted. The homemade plum wine brewed by local methods has a long history. In this study, the famous corn wine from Jinchuan County was used as the base wine, and a new type of plum wine product, Jinchuan formula plum wine, was developed in a ratio of 100 : 20 : 1 : 1 (solid/liquid = kg/L) with fresh green plum fruit, jujube (dried), and goji berries (dried). Based on various versions of the *Catalogue of Medicines and Foodstuffs with Same Origin* and traditional medical literature research, goji berries and jujube were scientifically and reasonably mixed to increase the nutritional efficacy of green plum wine.

This paper aimed to explore the toxicity of Jinchuan formula plum wine extract and its protective effect on the liver through acute toxicity tests in mice and the establishment of a ConA induced mice liver injury model. This paper could provide a pharmacological basis for its safe use, promote the utilization of plum resources by local farmers, and drive the development of the plum industry.

2 Materials and methods

2.1 Experimental animals and reagents SPF Kunming mice, weighing 19 - 22 g, purchased from the Laboratory of Experimental Animals, Sichuan University of Traditional Chinese Medicine, with production license number of SCXK (Sichuan) 2013-19. They were raised at the Sichuan Center for New Drug Screening and Pharmacology of Traditional Chinese Medicine

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* Corresponding author. E-mail: 392009773@qq.com

(a key research institution of the Sichuan Administration of Traditional Chinese Medicine). The environment temperature was between 19 and 21 ℃, the relative humidity was between 40% and 70%, and no more than 5 mice were kept in each cage. The indoor air was adjusted through an automatic ventilation device, and the fluorescence lighting was manually controlled to be bright at 08:00—20:00 and dark at 20:00—08:00, with free drinking water. Regular full-price feed was fed in a fixed and quantitative manner, and adaptive feeding was carried out for one week. 0.9% sodium chloride injection (lot No.: C151110 B); silybin capsule; concanavalin A (ConA); alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), triglycerides (TG), and very low density lipoprotein (VLDL) test kits; Yihong dyeing solution and hematoxylin dyeing solution (Chengdu Kelong Chemical Reagent Factory).

2.2 Instruments JA1003 electronic balance; EB-3200D electronic balance; BP211D precision electronic balance (Sedolis, Germany); VARIOSKAN FLASH microplate reader (Thermo Fisher Scientific, USA); overspeed freezing centrifuge (Beckman, USA); TSJ-Q fully automatic closed tissue dehydrator, BMJ-III embedding machine (Changzhou Zhongwei Electronic Instrument Factory); rotary slicer (LEICA, Germany); CX31 optical microscope (OLYMPUS, Japan).

2.3 Preparation of extracts The extract of Jinchuan formula plum wine is a brownish extract. The extraction process was as follows: fresh plum fruit : goji berries (dried) : jujube (dried) : corn wine = 100 : 20 : 1 (solid/liquid = kg/L), extracted for one year, filtered and clarified. The supernatant was taken and steamed under reduced pressure until the extract was in the form of a paste. The brownish semi dry extract of Jinchuan formula plum wine was obtained by freeze-drying. 4 L of Jinchuan formula plum wine sample was taken, with a freeze-dried yield of 70.1% and an extract content of 6.75 g/L.

2.4 Acute toxicity test of extract

2.4.1 Drug formulation. A certain amount of extract was weighed, and an appropriate amount of distilled water was added to let it stand for 2 – 4 h. After the extract was completely dissolved, an appropriate amount of distilled water was added until the gavage needle can inhale. A measuring cylinder was used to measure the final volume of the obtained solution, and 77.88% of the solution was obtained for experimental use.

2.4.2 Acute toxicity pre-test. Mice (half male and half female) were randomly divided into 5 groups, with 4 mice in each group. After a single gavage of 0.4, 0.5, 0.6, 0.7, and 0.8 mL/mice, the toxic reactions and deaths of each group of mice were observed for one week continuously. The results were shown in Table 1.

2.4.3 LD_{50} determination. Mice (half male and half female) were randomly divided into 5 groups, with 10 mice in each group. Each group was administered by gavage in the same volume at different concentrations of 8.21, 9.66, 11.37, 13.37, and 15.73 g/kg. The ratio of gavage volume to mouse body weight was 0.03 mL/g. The toxic reactions and deaths of the mice were monitored for 2 consecutive weeks, and the acute toxicity LD_{50} value of the mice was calculated.

Table 1 Pre-experimental animal death

Group	Volume of drug solution//mL	Number of animals	Death toll	Mortality rate//%
1	0.8	4	4	100
2	0.7	4	4	100
3	0.6	4	3	75
4	0.5	4	2	50
5	0.4	4	1	25

2.5 Hepatoprotective effect of extracts on ConA-induced liver injury mice

2.5.1 Animal modeling and processing methods. Mice (half male and half female) were randomly divided into a normal control group, a model group, and high-, medium-, and low-dose groups of Jinchuan formula plum wine extract (1.0, 0.5, 0.25 g/kg) and silybin group (0.1 g/kg). The mice in normal control group and model group were given an equal amount of distilled water by gavage, while the other groups were given daily gavage for 2 consecutive weeks. After 30 min of the last administration, except for the normal control group, all treatment groups received tail vein injection of ConA at a dose of 16 mg/kg. After the experiment finished, mouse eyeball was extracted for blood collection, and serum was separated and stored at 4 ℃. Liver tissue was taken, and the largest lobe of the liver was fixed in 10% formaldehyde[9].

2.5.2 Indicator measurement. An appropriate amount of mouse serum was taken, and the levels of ALT, AST, and LDH were measured according to the recommended usage method of the reagent kit. Liver tissue was taken from formalin immersion solution for pathological morphology examination.

2.6 Statistical methods of data SPSS 21.0 software and *t*-test method were used for inter group comparison, and the results were represented by $\bar{x} + s$ ($P < 0.05$).

3 Results and analysis

3.1 Acute toxicity test results After a large dose of Jinchuan formula plum wine extract was administered orally to mice, there were reactions such as delayed movement, closed eyes, prone position, and open mouth breathing in a short period of time. Most of the dead animals died within 5 – 30 min of administration. The dead mice were immediately dissected and observed with the naked eye. Except for the presence of medicinal liquid in the stomach, all other organs were found to be normal under naked eye observation. The death situation of each group of animals was shown in Table 2.

Table 2 Death of mice in acute toxicity test

Group	Dosage g/kg	Number of animals	Death toll	Mortality rate//%
1	15.73	10	10	100
2	13.37	10	9	90
3	11.37	10	4	40
4	9.66	10	3	30
5	8.21	10	0	0

According to Table 2, when the dosage of Jinchuan formula plum wine extract was 8.21 g/kg, no animal death occurred, indicating that the acute toxicity LD_{50} of Jinchuan formula plum wine extract was greater than 8.21 g/kg. After calculation, LD_{50} of Jinchuan formula plum wine extract was 11.18 g/kg, which was equivalent to a volume of 1.656 L/kg of Jinchuan formula plum wine. The 95% confidence limit range of LD_{50} was between 10.31 and 12.05 g/kg.

3.2 Liver protective effect of ConA-induced liver injury mice

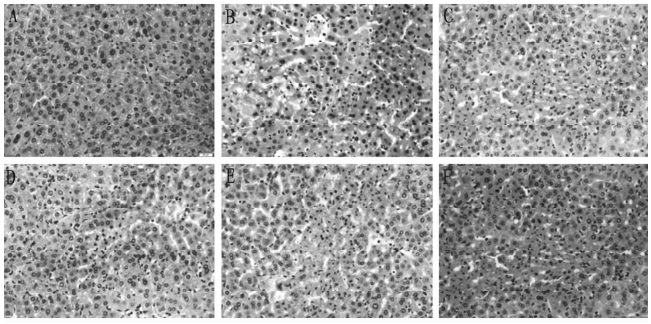
3.2.1 Effects on serum biochemical indicators in mice. According to Table 3, compared with the normal group, the activity of ALT, AST, and LDH in the model group was significantly increased ($P < 0.01$), indicating that the model replication was successful. Compared with the model group, ALT and AST activities of mice serum showed a significant decrease in the high-dose group of Jinchuan formula plum wine extract and the silybin group ($P < 0.05$), indicating that Jinchuan formula plum wine extract had a liver protective effect similar to the positive control drug silybin[10-11].

Table 3 Effects of Jinchuan formula plum wine extract on ALT, AST and LDH levels in mice with ConA induced liver injury ($\bar{x} \pm s$)

Group	Dosage g/kg	Number of animals	ALT//U/L	AST//U/L	LDH//U/L
Normal control	–	10	26 ± 10**	23 ± 11**	6 271 ± 556**
Model	–	10	304 ± 79	145 ± 39	7 012 ± 247
Low dose	0.25	10	279 ± 103	119 ± 42	7 010 ± 449
Medium dose	0.50	10	253 ± 99	112 ± 47	7 280 ± 664
High dose	1.00	10	211 ± 82*	89 ± 47*	7 319 ± 465
Silybin	0.10	10	207 ± 89*	97 ± 35*	7 071 ± 431

NOTE * shows $P < 0.05$; ** shows $P < 0.01$.

3.2.2 Effects on mouse tissue pathology. Normal mouse liver is composed of liver capsule, liver parenchyma, and stroma[12]. Through microscopic observation, the liver capsule of mice in the normal control group was smooth and intact, without infiltration of inflammatory cells. The structure of liver lobules was neat. Except for a few mice with a small amount of inflammatory cell infiltration in the liver interstitium, the morphology of other liver cells was normal. Compared with the normal control group, the model group showed significant swelling of liver cells, infiltration of inflammatory cells (mainly lymphocytes and neutrophils) in the stroma, and more necrotic lesions in the field of vision, mainly manifested as liver cell nucleus pyknosis, cytoplasmic staining, and hepatic sinus dilation and congestion, but no accumulation of inflammatory cells was observed in the lesions. Compared with the model group, each treatment group showed varying degrees of improvement in liver cell lesions (Fig. 1).



NOTE A. Normal control group; B. Model group; C. Low dose group (0.25 g/kg); D. Medium dose group (0.5 g/kg); E. High dose group (1.0 g/kg); F. Silybin (0.1 g/kg).

Fig. 1 Effect of Jinchuan formula plum wine extract on histopathology of mice with acute liver injury

4 Conclusions and discussion

Acute toxicity test is a basic test for detecting and evaluating the toxic effects of test substances. It can provide a preliminary understanding of the toxicity level of the test substance, classify its acute toxicity, and provide a basis for other toxicological experiments[13-15]. At present, no relevant reports have been made on the toxicological safety evaluation of Jinchuan formula plum wine extract. The results of this experiment showed that the acute toxicity LD_{50} of Jinchuan formula plum wine extract was 11.18 g/kg (equivalent to Jinchuan formula plum wine volume of 1.656 L/kg), and the 95% confidence limit range of LD_{50} was 10.31 – 12.05 g/kg. There were no significant pathological changes in all organs, indicating that the wine is relatively safe and can be consumed appropriately. This acute toxicity test was based on the short-term toxicity reactions of animals after a single dose, and the toxicity level of Jinchuan formula plum wine extract can be determined preliminarily. However, due to the short trial schedule, there was no significant pathological changes in tissues observed only through naked eye. Therefore, the toxic effects of Jinchuan formula plum wine extract were not clear, and further exploration was needed.

The main characteristics of ConA induced acute liver injury model are rapid onset, obvious liver injury, simple modeling process, and no need for pre sensitization. Therefore, it is an ideal animal experimental model for studying the liver protective effect of drugs[16-17]. Research[18-19] has shown that after ConA is injected into mice, there are significant pathological changes in the liver and a sharp increase in transaminase levels in the blood within a short period of time. Therefore, indicators such as ALT and AST have high sensitivity in identifying early liver injury. The results of this experiment indicated that high-dose Jinchuan formula plum wine extract can alleviate liver cell damage and significantly reduce serum ALT and AST activities in liver injury mice. It showed that Jinchuan formula plum wine extract has a certain liver protective effect.

In summary, Jinchuan formula plum wine has a certain liver protective effect, and reasonable consumption within a certain range is beneficial to physical health. Jinchuan formula plum wine is beneficial for the development and utilization of plum resources in Jinchuan County, promotes local economic development, and has certain nutritional, social, and economic value. It should vigorously develop the fruit wine industry, which can not only reduce the high loss rate of fruits, save resources, but also drive the up-

(To page 37)

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(From page 32)

grading of China's planting industry, create new employment opportunities, especially increase the income of the agricultural population.

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