Volume 21, No 4 | International Journal of Radiation Research, October 2023

Hepatoprotective effects of the mixed decoction of Inula cappa and Serissa japonica in acute liver injury mouse model

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► ***Original article***

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Received: February 2022
Final revised: January 2023
Accepted: January 2023

Int. J. Radiat. Res., October 2023; 21(4): 765-768

DOI: 10.52547/ijrr.21.4.23

Keywords: *Inula cappa, Serissa japonica, mixed decoction, CCl4, acute liver injury.*

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ABSTRACT

Background*:** To explore the effectiveness of the mixed decoction of Inula cappa and Serissa japonica (IS) on acute liver injury model. ***Materials and Methods*:** Sixty carbon tetrachloride (CCl4)-induced mice models were divided into bifendate group (0.2 g/kg) and IS (2, 1and 0.5 g/kg) groups, model group, and control group randomly. Liver function biomarkers aspartate aminotransferase (AST), alanine amiotransferase (ALT), and oxidative stress biomarkers superoxide dismutase (SOD), malonic dialdehyde (MDA) were measured in each group. Hematoxylin and eosin (HE) staining was used to detect the liver tissues. ***Results: The necrosis degree and scope, and the structural damage of hepatocyte were significantly ameliorated in the bifendate, IS (2 g/kg) and IS (1 g/kg), compare to model group. IS and bifendate could significantly inhibit the liver index, reduce liver function biomarkers levels and the content of MDA in the liver of acute liver injury mice, and improve the activity of SOD. The serum level of AST was 147.162 (Control), 736.023 (Model), 370.285 (Bifendate), 325.589 (High-dose IS), 407.205 (Middle-dose IS), and 438.631 U/L (Low-dose IS) while the level of ALT was 44.804 (Control), 474.825 (Model), 156.812 (Bifendate), 157.02 (High-dose IS), 217.399 (Middle-dose IS), and 255.649 U/L (Low-dose IS). The content of MDA increased in model and treatment groups compared to control, while the activity of SOD was 69.362 (Control), 53.208 (Model), 64.77 (Bifendate), 73.389 (High-dose IS), 65.173 (Middle-dose IS), and 61.755 (Low-dose IS). Compared to the model group, high-dose IS group exerted significant hepatoprotective effect ($p<0.05$). ***Conclusion*:** The mixed decoction of Inula cappa and Serissa japonica was able to protect acute liver injury.

INTRODUCTION

The liver is the largest substantial and digestive organ that has complicated functions [1, 2]. However, the liver is susceptible to pathogenic factors from both *in vivo* and *in-vitro* [3]. Virus infection, alcohol addiction, industrial chemicals, surgical trauma, drug abuse and adverse reactions and other factors can cause a variety of liver diseases [4]. Effective drugs are still being explored to improve the liver function and repair the damaged hepatocytes [5]. Therefore, liver disease has become a global disease with high morbidity and mortality, and has increasingly become an "invisible killer" of human health.

InuIacappa (Buch. - Ham) DC., a Chinese folk medicinal herb, is the fresh or dry whole plant of Compositae in South China, also known as bainiudan, dahurian angelica, etc. [6]. The whole herb or root can be used as medicine [7]. It has been reported that the main components of *InuIacappa* are flavonoids, phenolic acids, coumarins and volatile oil [8-11]. *Serissajaponica* (Thunb.), a traditional Chinese medicine, is a plant of Serissa Serissoides of the family Rubiaceae. It tastes bitter, slightly sweet and has a cool nature, which contains volatile oil, terpenoids, steroids, lignans and other chemical components [12]. It can activate blood circulation, cool blood, soothe the liver and relieve dampness, detumescence and pain [13]. Studies have showed that *Inula cappa* and *Serissa japonica* could protect acute liver injury, and *Serissa japonica* has an anti-inflammatory effect [14, 15]. Carbon tetrachloride (CCl_4) is a common reagent applied in generation of liver-damage model and increase the level of liver damage markers. It is similar to the damage model induced by radiation in which these damage markers are upregulated greatly [16, 17].

To explore the effect of the mixed decoction of *Inula cappa* and *Serissa japonica* (IS) on liver-damage model, we detect the typical liver damage markers and liver morphology. For the first time, this study will provide us a new decoction of *Inula cappa* and *Serissa japonica* to protect liver damage.

MATERIALS AND METHODS

Preparation of drugs

The root of *Inula cappa* (1 kg, Yulin harmony Pharmaceutical Management Co., Ltd., Yulin, China) and the whole plant of *Serissa japonica* (1 kg, Yulin harmony Pharmaceutical Management Co., Ltd., Yulin,

[DOI: 10.61186/ijrr.21.4.765]

China) were crushed and soaked in distilled water. Then, the mixture solution was performed boiling extraction for 2 times, 2 hour each time. After discarding the residue, the supernatant was concentrated to the mixture stock solution with rotary evaporator. The final concentration was 0.2 g/ml. The prepared drug was stored at -20 °C.

Animals and protocols

The animal research protocol of this work was given permission by Guangdong Medical University Ethics Committee. Sixty 6-week old SPF Kunming mice with sex ratio 1:1, weighing (21.5 ± 5) g, were used in this research. The environment is temperature of (20-23) ˚C and the humidity of 50% - 60%. The mice can feed and drink freely. Sixty mice were randomly divided into bifendate group (0.2 g/kg, Zhejiang Wepon Co., Ltd., Hangzhou, China) and IS (2, 1and 0.5 g/kg) groups, model group, and control group with an average of 10 mice per group. Mice in each group were received intragastric administration with different drugs for 7 days (one time per day). Distilled water (10 ml/kg) was given to mice in the control and model groups. At two hours after the last administration, the acute liver injury was induced by intraperitoneal injection of 40% CCl4 peanut oil solution (Beijing Chemical Industry Group Co., Ltd., Beijing, China) except the mice in control group. The CCl4 dosage was set to 10 ml/kg body weight. The blood sample was collected after 24 hour of fasting and stored at -80°C after centrifugation (3500g) at 4°C. Finally, the liver tissue was also harvested for further analysis.

Calculation of liver index

During the experiment, the body weight, mental state, activity, food and water intake of the mice were observed daily. The change curve of body weight was drawn. The liver index was liver weight divided by body weight and multiplied by 100%.

Measurement of serum and liver variables

After thawing the serum, liver function biomarkers aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured accordance with protocol of kits (Beijing Century ward Biotechnology Co., Ltd., Beijing, China). The contents of SOD and MDA were also measured according with the protocol of kits (Nanjing Jiancheng Bioengineering Institute Co., Ltd., Nanjing, China). All experiments were repeated three times.

Histopathological analysis

The staining method was hematoxylin and eosin (HE) staining [18]. The liver tissue was fixed for 24 hours and embedded in paraffin. The thickness of the slices was 5 μm, followed by H&E staining. After covering with neutral balsam, the slice was observed under microscope (100 x, OLYMPUS, Tokyo, Japan).

Statistical analysis

Means with standard deviation (SD) was adopted to describe the measure results. The overall difference among groups was tested by one-way analysis of variance (ANOVA), and post-hoc test was used to adjust the results of pairwise comparisons. $P<0.05$ indicated significantly different. The computer software SPSS (IBM, NY, USA) used for analysis; the version was 24.0.

RESULTS

Effect of the mixed decoction of IS on activity state of mice

With increasing time, the body weight of all mice increased (figure 1). After giving CCl_4, the mice in the model group and 0.5 g/kg IS group were dispirited and their hair were arched. The state of mice in 2 g/kg IS and 1 g/kg IS and bifendate groups had significantly improved compare with model group mice.

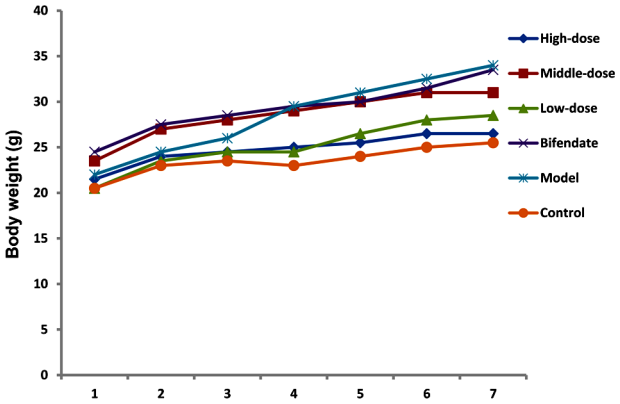


Figure 1. Body weight of mice during 7 days.

Effects of the mixed decoction of IS on liver index of mice

The liver indexes of the all IS, bifendate, and model groups were significantly higher than control ($P<0.05$). The liver index in the all IS groups showed significant downward trends compare with model group ($P<0.05$) (table 1).

Table 1. Body weight and liver weight indexes in each group mice.

Groups	Body weight/g	Liver weight/g	Liver indexes /%
Control group	31.00±1.97	1.60±0.26	5.14±0.54
Model group	27.62±2.16	1.67±0.17	6.05±0.37※
Bifendate group	26.53±1.50	1.51±0.18	5.67±0.38#※
High-dose SI group	26.44±2.21	1.50±0.30	5.67±0.81#※
Middle-dose SI group	24.93±2.84	1.37±0.26	5.46±0.41#※
Low-dose SI group	23.34±2.64	1.21±0.11	5.88±0.24#※

※P<0.05: compared with control group; #P<0.05: compared with model group.

[DOI: 10.61186/ijrr.21.4.765]

Effects of the mixed decoction of IS on liver histopathological features

In control group, the liver showed normal properties that smooth, shiny, soft, and dark red. In the model group, the liver was increase in volume, and there were white dots on the surface, with rough texture and white color. In the bifendate group, the liver surface was smooth with a few white spots. In 2 g/kg IS group, the surface of liver was similar with that in control group. In the 1 g/kg IS group, the surface of liver was smoother and softer than that in the 0.5 g/kg IS group. In the 0.5 g/kg IS group, the liver had white color with white spots on the surface (figure 2).

In the control group, the liver tissue had normal morphology microscopically with intact lobules, clear outline and nucleolus, order arranged cells. There was no inflammatory cell infiltration. In the model group, a lot of necrotic hepatocytes were observed and arranged disorderly. The nuclei were different in size with enlargement and deformation, and hepatocytes were seriously damaged. Bifendate can significantly reduce the degree of liver injury, as showed in bifendate group, compare with model group. IS mixed decoction could obviously improve the damage of liver tissue. Particularly, in the 2 g/kg IS group, the liver lobules were clear and complete, and the cell necrosis was significantly improved (figure 3).

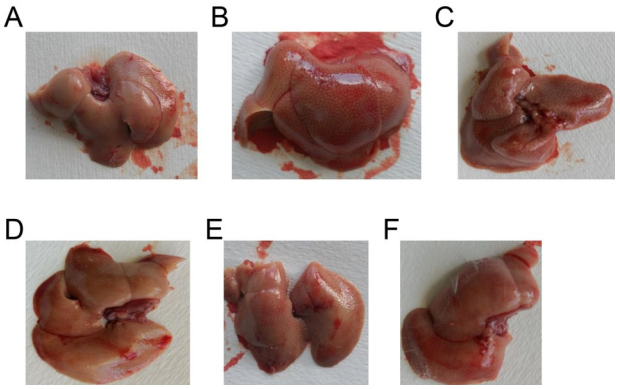
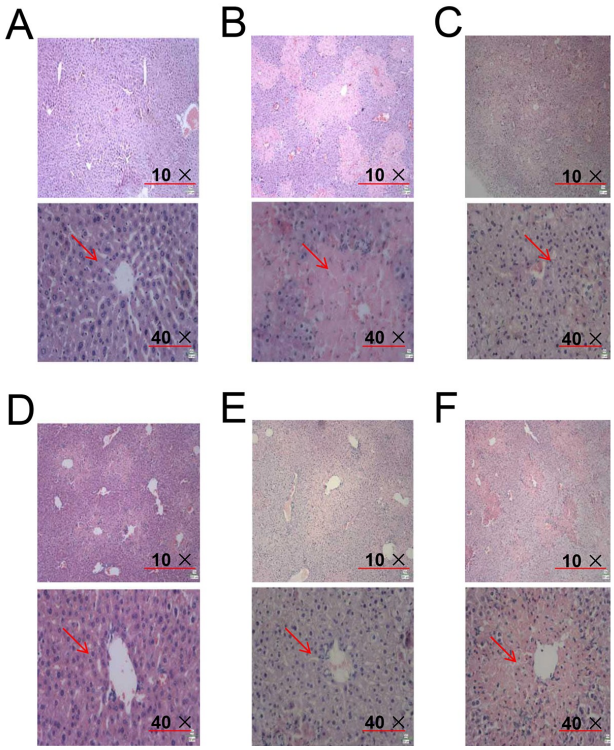


Figure 2. Mice liver morphology in each group mice. **(A)** Control group; **(B)** Model group; **(C)** Bifendate group; **(D)** High-dose SI group; **(E)** Middle-dose SI group; **(F)** Low-dose SI group; Images are representative images of 3 independent samples.

Effects of IS on oxidative stress biomarkers and liver function index

The model group showed more severe liver injury than control that the AST and ALT significantly increased (P<0.01). And the model group also showed more severe liver injury than the 2 g/kg and 1 g/kg IS groups (P<0.05, figure 4A and B). The effect of IS was similar to that of bifendate group.

For oxidative stress, the model group showed more severe oxidative stress than control that the SOD activity was significant lower (P<0.01, figure 4C). IS intervention significantly increased the SOD activity (P<0.01 or P<0.05). Compare bifendate group, 2 g/kg IS group had significant higher SOD activity (P<0.05). The acute liver injury model also significantly increased MDA level (P<0.01, Figure 4D). Meanwhile, the MDA level was obviously reduced after 2 g/kg IS, 1 g/kg IS, and bifendate interventions (P<0.01).



Fiure 3. The pathological observation of mice liver in each group mice. **(A)** Control group; **(B)** Model group; **(C)** Bifendate group; **(D)** High-dose SI group; **(E)** Middle-dose SI group; **(F)** Low-dose SI group; Images are representative images of 3 independent samples. The red arrows indicate the cell arrangement of liver tissues in each group.

DISCUSSION

This work showed that 2 g/kg and 1 g/kg IS administration brought significantly lower levels of AST, ALT, and MDA, and higher SOD activity. HE staining showed that the cell necrosis and structural disorder in the 2 g/kg and the 1 g/kg IS groups were improved significantly than those in the model group, and the degree of liver injury was significantly reduced.

Acute liver injury due to a variety of factors, such as toxic chemicals, drugs, viruses, bacteria, etc., seriously threaten human health. In recent years, people have been committed to finding phytochemicals with prevention or treatment of acute liver injury. Our work found that IS had a protection on Cl_4-induced acute liver injury.

With the change of human life style, the incidence of liver diseases has increased year by year. Liver injury is the basis of other severe liver diseases.

[DOI: 10.61186/ijrr.21.4.765]

Therefore, the prevention, treatment and mechanism of liver injury have become the focus of global health workers. At present, it is generally believed that oxidative stress, inflammatory response and autophagy were important in the progress of liver injury [19]. In addition, ionizing radiation is another critical environmental factor causing liver damage. During our daily life, exposure to radiation is not uncommon due to medical treatments such as radiotherapy and clinical diagnosis. Of the important organs, liver plays various critical functions in metabolism and is very sensitive to radiation [20, 21]. Once injured, a serious of pathological responses will occur and severely reduce the quality of life. In this study, we demonstrated that high-dose SI could protect the liver and reduce the damage induced by CCL4. This will provide a promising way to protect the patients from being damaged when diagnosed or treated in clinics.

The components of traditional Chinese medicine have the characteristics of multiple active components acting together and influencing the disease states. Traditional Chinese medicine compound preparation can protect Cl_4-induced liver injury by reducing oxidative stress injury, inhibiting hepatocyte apoptosis and the release of inflammatory factors and regulating related signaling pathways, with the characteristics of multi-target and multi-channel interaction [22].

As a hepatophilic cytotoxic substance, CCl_4 can be rapidly absorbed by liver and brain tissues. Under the action of cytochrome P450 in liver microparticles, CCl_4 can generate free radicals such as trichloromethyl, destroy the structure of biofilm, increase the permeability of biofilm, and release ALT and AST into the circulation. In addition, MDA, a product of membrane lipid peroxidation, can aggravate cell membrane damage. At the same time, when CCl_4 induced liver injury occurs lipid peroxidation injury, the activities of antioxidant enzymes, such as SOD and glutathione (GSH), will be also reduced [23, 24].

CONCLUSION

In this work, the results suggested that the mixed decoction of *Inula cappa* and *Serissa japonica* protected acute liver injury induced by CCl4. However, the specific mechanism needs further study.

ACKNOWLEDGMENT

Thank you for the all help from our research group.

***Funding*:** Special Topic on Unsponsored Science and Technology Research in Zhanjiang City, 2021B01394，2021B01067 Guangdong Medical University Youth Research and Cultivation Fund Project，GDMUQ2021008.

***Conflicts of interests*:** The authors declare that there is no conflicts of interests.

***Ethical consideration*:** The study was approved by the Committee on Animal Research and Ethics of (GDY2102595, 08/09/2021).

***Author contribution*:** X.L. designed the whole study and reviewed the paper. X.Q. acquired the experimental data and wrote the draft. Y.J., B.C., and P.Z. analyzed the data, reviewed the draft, and made literature review.

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[DOI: 10.61186/ijrr.21.4.765]