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**ORIGINAL ARTICLE**

# Protective activity of Jiang-Zhi-Li-Gan against carbon tetrachloride-induced hepatic injury in mice

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**Abstract**

The administration of carbon tetrachloride ($CCl_4$) to mice produced hepatotoxicity, showing a significant increase in the serum levels of alanine transaminase (ALT) and aspartate aminotransferase (AST). Mice pretreated with Jiang-Zhi-Li-Gan (JZLG, 100–900 mg/kg, p.o.), a domestic remedy for liver disease in Rui-Jin Hospital, showed a significant decrease in serum ALT and AST levels when compared to the group treated with $CCl_4$ alone. The standard drug, bifendate (200 mg/kg, p.o.), also exhibited similar results. In the acute toxicity study, JZLG did not show any mortality up to a dose of 32 g/kg body weight. Based on the results obtained, it can be concluded that JZLG seems to possess hepatoprotective activity in mice. These results support the use of this prescription against chemical hepatic injury.

**Keywords:** *Carbon tetrachloride; $CCl_4$; chemical hepatic injury; hepatoprotective; Jiang-Zhi-Li-Gan*



## Introduction

Jiang-Zhi-Li-Gan (JZLG) comprises: (1) *Radix Bupleuri* (Umbelliferae), the dried roots of *Bupleurum chinense* DC. or *Bulpeurum scorzoneri folium* Willd., used for treatment of liver disease in oriental medicine and possessing anti-tumor properties (Kang et al., 2008); (2) *Radix Astragali* (Leguminosae), the dried roots of *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao or *Astragalus membranaceus* (Fisch.) Bge., widely used for treatment of cardiovascular diseases such as heart failure, angina pectoris, myocardial infarction, and stroke in Asian countries (Zhao et al., 2008); (3) *Fructus Crataegi* (Rosaceae), the dried matured fruits of *Crataegus pinnatifida* Bge. var. *major* N.E. Br. or *Crataegus pinnatifida* Bge., possessing potential against oxidative stress (Chu et al., 2003); (4) *Radix et Rhizoma Salviae Miltiorrhizae* (Labiatae), the dried roots and rhizome of *Salvia miltiorrhiza* Bge. or *Salviae miltiorrhizae Radix* (SMR), an eminent herb in the treatment of cardiovascular disorder (called blood stasis in traditional Chinese medicine) widely used in China, Japan, Taiwan, and Korea (Koo et al., 2004); (5) *Radix et Rhizoma Rhei* (Polyginaceae), the dried roots and rhizome of *Rheum palmatum* L., *Rheum tanguticum* Maxim. ex Balf., or *Rheum officinale* Baill., having a protective capacity both *in vitro* on primary hepatocyte cultures and in *in vivo* in a rat model of $CCl_4$ mediated liver injury (Ibrahim et al., 2008); and (6) *Radix et Rhizoma Glycyrrhizae* (Leguminosae), the dried roots and rhizome of *Glycyrrhiza uralensis* Fisch., *Glycyrrhiza inflata* Bat., or *Glycyrrhiza glabra* L., having long been used worldwide as a herbal medicine and natural sweetener and as a traditional medicine mainly for the treatment of peptic ulcer, hepatitis C, and pulmonary and skin diseases (Asl & Hosseinzadeh, 2008). This prescription is a remedy for liver disease that has been used in Rui-Jin Hospital for years. However, its effect on carbon tetrachloride ($CCl_4$) induced liver injury has never been investigated.

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The present study was designed to investigate the effect of JZLG on acute chemical liver injury by $CCl_4$ in mice.

## Materials and methods

### *Plants*

Air-dried plants were supplied by Shanghai Herbal Medicine Co. Ltd. (Shanghai, China). A voucher specimen of each plant is deposited in the Department of Pharmacy, Rui-Jin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China (reference number 080413:1–6).

### *Reagents*

Alanine transaminase (ALT) and aspartate aminotransferase (AST) assay kits were purchased from Kang-Jian (Shanghai, China). Other chemicals and reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated.

### *Extraction*

Deionized water (1 L) was added to 100.0 g of JZLG prescription, comprising 12.8 g of *Radix Bupleuri*, 19.2 g of *Radix Astragali*, 25.6 g of *Fructus Crataegi*, 19.2 g of *Radix et Rhizoma Salviae Miltiorrhizae*, 3.8 g of *Radix et Rhizoma Rhei*, and 19.4 g of *Radix et Rhizoma Glycyrrhizae*, previously ground and sieved through a mesh No. 50, and was heated until the preparation boiled. Boiling was continued for 2 h. The decoction was then percolated to obtain the filtrate, and the dregs were re-boiled with fresh deionized water (1 L) for 1 h. Collected filtrates were then poured together and evaporated in a vacuum to give a dark brown semi-solid extract (yield 32.2%), which was stored at −20°C until use.

### *Animals*

Kun-Ming mice of either sex (weighing 19–21 g) were supplied by Shanghai Slac Laboratory Animal Co. Ltd. (Shanghai, China). The mice were housed in cages under controlled conditions at 20 ± 3°C and 45–65% humidity with a 12 h light–dark cycle (lights on 6:00 h). Drinking water and food were provided *ad libitum* throughout the study. Animals were acclimatized to their environment for 1 week prior to experimentation. All procedures were performed in accordance with guidelines on the care and use of experimental animals set by Fudan University Cancer Hospital.

### *Hepatoprotective activity*

Hepatic injury was induced in mice by intraperitoneal administration of a single dose of 10 mL/kg 0.1% $CCl_4$. Bifendate (biphenyldicarboxylate), a known hepatoprotective agent, was used as the reference standard (Pan et al., 2006).
The 60 mice were grouped randomly as follows:

- Group I: Control group, treated with vehicle (0.2 mL, p.o.) daily for 7 days.
- Group II: Treated with vehicle (0.2 mL, p.o.) daily for 7 days followed by $CCl_4$ on day 7.
- Group III: Treated with low dose of JZLG (100 mg/kg, p.o.) daily for 7 days followed by $CCl_4$ on day 7.
- Group IV: Treated with medium dose of JZLG (300 mg/kg, p.o.) daily for 7 days followed by $CCl_4$ on day 7.
- Group V: Treated with high dose of JZLG (900 mg/kg, p.o.) daily for 7 days followed by $CCl_4$ on day 7.
- Group VI: Treated with bifendate (200 mg/kg, p.o.) daily for 7 days followed by $CCl_4$ on day 7.

At the end of the treatment, body weight and relative liver weight were measured; blood samples of each animal were collected in sterile centrifuge tubes and allowed to clot. The serum was separated and used for the assay of marker enzymes (ALT and AST).

### *Acute toxicity study*

Mice were divided into groups of 10 animals each. The control group received saline and the other groups received increasing doses of JZLG extract, respectively. Immediately after dosing, the animals were observed continuously for 7 days to record the mortality.

### *Statistical analysis*

Group mean values and standard deviations were calculated. Data are expressed as mean ± SD. Statistically significant differences were determined by Student's *t*-test. Differences were considered statistically significant if $p < 0.05$.

## Results and discussion

The effects of JZLG on the serum enzymes were investigated and the results are given in Table 1. It is well known that $CCl_4$ is used as a hepatotoxic agent in various animals (Gopal & Sengottuvelu, 2008). The administration of $CCl_4$ to mice produced hepatotoxicity, showed by a significant increase in the serum levels of ALT and AST in comparison to the control group. JZLG

**Table 1.** Effect of JZLG on $CCl_4$ induced liver damage in mice.

| Group | ALT (IU/L) | AST (IU/L) |
|---|---|---|
| Group I | 55.9 ± 7.8 | 62.3 ± 10.0 |
| Group II | 458.7 ± 85.8$^{\#\#}$ | 435.9 ± 96.3$^{\#\#}$ |
| Group III | 201.3 ± 60.4$^{**,\#}$ | 209.9 ± 30.1$^{**,\#}$ |
| Group IV | 95.3 ± 60.2$^{**,\#\#}$ | 185.7 ± 33.7$^{**,\#\#}$ |
| Group V | 52.3 ± 12.4$^{\#\#}$ | 160.7 ± 30.0$^{**,\#\#}$ |
| Group VI | 72.5 ± 32.2$^{*,\#\#}$ | 210.2 ± 102.3$^{*,\#}$ |

*Note*. Values are expressed as mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$ compared to Group I; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to Group II.

**Table 2.** Body weight and relative liver weight of mice.

| Group | Body weight (g) | Relative liver weight (%) |
|---|---|---|
| Group I | 19.0 ± 1.8 | 9.9 ± 1.7 |
| Group II | 20.9 ± 2.2 | 9.8 ± 0.9 |
| Group III | 20.1 ± 1.1 | 10.3 ± 1.4 |
| Group IV | 20.0 ± 0.9 | 10.0 ± 1.0 |
| Group V | 19.9 ± 0.8 | 9.9 ± 1.9 |
| Group VI | 20.1 ± 0.5 | 10.9 ± 0.8 |

*Note*. Values are expressed as mean ± SD.

caused a significant decrease in the serum enzymes ALT and AST when compared to the $CCl_4$ group. The standard drug, bifendate (biphenyldicarboxylate, 200 mg/kg, p.o.), also exhibited similar significant results. There were no significant differences in body weight and relative liver weight between all groups (Table 2). In the preliminary acute toxicity study, the JZLG test did not show any mortality up to 32 g/kg (equivalent to 250 times the clinical dose, the maximum administration volume).

On the basis of the results obtained, it can be concluded that JZLG seems to possess hepatoprotective activity in mice. No toxic symptom or mortality was observed in the 48 h study in mice. These results seem to support the traditional use of this prescription against hepatotoxicity.

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