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Original article

Treatment of Kangxian Pills to Chronic Liver Injury in Mice Induced with Carbon Tetrachloride

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ABSTRACT

Objective Kangxian Pills, containing *Angelicae Sinensis*, *Chuanxiong Rhizoma*, *radix paeoniae rubra*, and 12 other kinds of Chinese materia medica, have the functions of softening and dispersing blood stasis. It has been used for liver injury and liver fibrosis. The current study was designed to evaluate the anti-hepatic injury activity and the mechanism of Kangxian Pills on a CCl_4-induced animal model. **Methods** To induce chronic liver injury, mice were treated with CCl_4 twice a week for four weeks. Kangxian Pills (6 or 12 g/kg) and Compound Biejia Ruangan Tablet (0.901 g/kg) were ig given to mice once daily for four weeks after CCl_4 was withdrawal. The anti-hepatic injury activities and mechanisms of Kangxian Pills were assessed by hepatic histology and by measuring the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin (ALB), and total protein (TP) of serum as well as superoxide dismutase (SOD) and glycogen (Gn) of the liver. **Results** Kangxian Pills significantly decreased the levels of liver index, ALT, and AST in mice liver injury models in treatment group. Moreover, Kangxian Pills and Compound Biejia Ruangan Tablet inhibited the CCl_4-induced reduction of SOD and Gn levels in the liver. The histological study showed that Kangxian Pills could reduce cellular swelling and infiltration of inflammatory cells in liver injury. **Conclusion** Kangxian Pills possess the potent abilities to alleviate chronic liver injury, suggesting that Kangxian Pills exert this effect by enhancing the anti-oxidant ability and metabolism of the liver.

Key words

Angelica sinensis; carbon tetrachloride; Kangxian Pills; *Ligusticum wallichii*; liver injury

1. Introduction

Liver injury can be induced directly from hepatotoxicity or indirectly from immune mediation by biological factors (hepatitis virus, bacteria, parasite, etc.), chemical factors (medicine, industrial poisons, alcohol, etc.) and environmental factors (Holt and Ju, 2006). These factors would induce apoptosis and necrosis of hepatic cells (Wang, 2014; Friedman et al, 1985; Maher and McGuire, 1990). Long term repeated hepatic cells necrosis leads to hepatic fibrosis, liver cirrhosis, and even hepatocellular carcinoma. Therefore, the prevention of liver injury is a critical step for protecting liver

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against the occurrence of cirrhosis and liver function failure.

Chinese materia medica (CMM) has been universally practiced in China for thousands of years and has been widely used in the treatment of chronic disease based on less side-effects and high efficiency. The species in *Astragalus* Linn. have been used in the prevention and treatment of CCl_4-induced liver fibrosis (Wang et al, 2010). *Bupleurum chinense* DC, *Carthamus tinctorius* L., and *Szechwan Lovage Rhizome* could decrease the expression of TGFβ1 and PDGF and the ratio of TIMP-1 to MMP-1 to prevent liver injury (Zhang and Liu, 2006). Decursin could reduce CCl_4-induced liver fibrosis by inhibiting the TGF-β1 induced NOX activation and Smad signal in hepatic stellate cells (Choi et al, 2014). Baoganning could up-regulate the cAMP response element binding (CREB) protein phosphorylation hepatic stellate cells to prevent liver injury (Tan et al, 2006).

Kangxian Pills, produced by Tianjin Second People's Hospital, containing *Angelica sinensis* (Oliv.) Diels, *Ligusticum wallichii* Franch., *Paeoniae Rubra Radix* and 12 kinds of other CMM, have the functions of softening and dispersing blood stasis. Although Kangxian Pills have been wildly used in the hospital for the treatment of liver injury and liver fibrosis, the mechanism of Kangxian Pills preventing liver injury remains unknown. The aim of this study was to investigate the effect of Kangxian Pills on the prevention of chronic liver injury induced by CCl_4 in mice and evaluate the possible mechanism of anti-hepatic injury. Compound Biejia Ruangan Tablet, a commonly used anti-hepatic injury medicine, was used as a positive control.

The anti-hepatic injury activities of Kangxian Pills were assessed by hepatic histology and by measuring levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin (ALB), and total protein (TP) of serum, which were frequently-used parameters of liver function. Moreover, superoxide dismutase (SOD) and glycogen (Gn) of mice were investigated in an effort to elucidate the possible mechanisms by which Kangxian Pills exert their hepatic protective activity.

2. Materials and methods

2.1 Animal and reagent

ICR mice were purchased from Beijing HFK Bioscience Company; Kangxian Pills were purchased from Tianjin Second People's Hospital; Compound Biejia Ruangan Tablet was purchased from Nei Monggol Furuizhong Drug Science Company; Carbon tetrachloride (CCl_4), alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), total protein (TP), superoxide dismutase (SOD), and glycogen (Gn) test kits were purchased from Nanjing Jiancheng Biological Engineering Institute.

2.2 Methods

2.2.1 Animals and treatments

Mice were housed at room temperature of (23 ± 1) ^oC with a 12 h light-12 h dark cycle (lights on from 6:00 am to 6:00 pm). Food and water were available ad libitum. The experiments were carried out according to the institutional regulations and national criteria for animal experimentation. The Institution Animal Ethics Committee reviewed the entire animal protocol prior to conducting the experiments.

Mice were randomly divided into five groups: Group A: control group (n = 6); Group B: CCl_4 model group (n = 10); Group C: positive control group (n = 10); Group D: low-dose Kangxian Pills group (n = 10); Group E: high-dose Kangxian Pills group (n = 10).

After 3 d of acclimatization, chronic liver injury was induced as described previously (Ohta and Sahashi, 2002). In brief, the mice in Groups B−E received ip injection of 20% CCl_4 solution diluted in olive oil (2 mL/kg body weight), twice weekly for a four-week period. The mice in Group A received an ip injection of olive oil (2 mL/kg body weight) instead of CCl_4. After four weeks when CCl_4 was withdrawal, the mice in Groups A and B received normal saline orally for four weeks daily, and the mice in Group C were given Compound Biejia Ruangan Tablets (0.901 g/kg body weight) diluted in normal saline orally for four weeks daily. The mice in Groups D and E received Kangxian Pills (6 and 12 g/kg body weight, respectively) orally for four weeks daily.

At the end of the four weeks Compound Biejia Ruangan Tablets and Kangxian Pills administration, mice were anesthetized with ether and blood was obtained from the retrobulbar plexus for serum biochemistry analysis. Blood samples were centrifuged at 3000 r/min for 15 min and serum was collected and kept at – 80 ^oC for analysis.

Animals were then decapitated; Livers and spleens were removed and weighed. Half of the livers were then fixed in 10% formalin for histological analysis, others were stored at −80 ^oC for anti-oxidant analysis.

2.2.2 Serum and tissue analysis

ALT, AST, ALB, TP in serum, and SOD and Gn in liver were measured on a Plate Reader using diagnostic reagent kit.

2.2.3 Histological analysis

Liver tissue sections were dissected and fixed in 10% formalin, then embedded in paraffin, sectioned to 5 μm thickness, and stained with hematoxylin and eosin (H&E). The extent of CCl_4-induced liver injury was evaluated by assessing morphological changes in liver sections.

2.2.4 Statistics

Quantitative data were expressed as $\bar{x} \pm s$ and compared using ANOVAK independent samples test when variances were heterogeneous. Data were considered significant difference when $P < 0.05$.

3. Results

3.1 General statement

The initial body weight was not significantly different in the five groups (Figure 1a). The body weight of mice treated