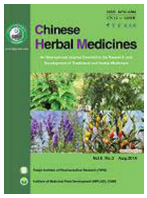

Available online at SciVarse ScienceDirect

Chinese Herbal Medicines (CHM)

ISSN 1674-6384

Journal homepage: www.tiprpress.com E-mail: chm@tiprpress.com

Original article

Hepatoprotective Effects of Yintian Granule on Experimental Liver Injury in Mice

Yan-jun Li [1, 2, 3], Sheng Yang[1, 2, 3], Yan-jing Zhou[1, 2, 3], Fang-hong Shang[1, 2, 3], Xiao-yu Xu[1, 2, 3*]

1. College of Pharmaceutical Sciences & College of Traditional Chinese Medicine and Pharmacology, Southwest University, Chongqing 400715, China

2. Institute of Chinese Medicine, Southwest University, Chongqing 400715, China

3. Pharmacology of Chinese Materia Medica, Key Constructing Discipline by the State Administrative Bureau of Traditional Chinese Medicine, Chongqing 400715, China

ARTICLE INFO

Article history

Received: February 19, 2014

Revised: May 8, 2014

Accepted: July 1, 2014

Available online:

August 20, 2014

DOI:

10.1016/S1674-6384(14)60036-8

ABSTRACT

Objective Yintian Granule (YTG), as a type of local preparation and applied for Chinese patent, is mainly composed of several traditional Chinese herbs used as both drug and food such as *Lonicera macranthoides*, *Gardenia jasminoides*, and *Asparagus cochinchinenis*, and has been reported to demonstrate the beneficial effects on human health in other researches. In this paper, the protective effects of YTG against experimental acute liver injury of mice were investigated to assess the value of this innovative Chinese herbal compound. **Methods** Carbon tetrachloride (CCl_4) and 50% ethanol were used respectively to induce the acute liver injury model in mice pre-administered with YTG. Lai's method was used to detect the level of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum, Coomassie brilliant blue method was used for the determination of superoxide dismutase (SOD) activity and malondialdehyde (MDA) content, and hematoxylin-eosin (HE) staining was used for the observation of liver histomorphometry. **Results** YTG significantly lowered the elevated ALT and AST levels, increased the SOD activity, decreased the MDA content, and inhibited the deterioration of liver. **Conclusion** YTG exerts protective effected against hepatocyte damage in mice induced by CCl_4 and 50% ethanol, respectively.

Key words

Hepatoprotection; liver injury; Yintian Granule

1. Introduction

Liver is a vital organ in human body and easy to be attacked by various factors, such as poisoning, metabolic disturbance, and circulatory disturbance. Liver injury is a process that involves variation in multiple kinases, free radical injury, oxidative stress, and lipid peroxidation (Sanjoy et al, 2013; Carmen et al, 2013). Recently, the usage of Chinese materia medica (CMM) on the treatment of liver disease is getting more and more attention worldwide. The idea of

* **Corresponding author: Xu XY** Tel: +86-23-6825 0761 Fax: +86-23-6825 1225 E-mail: xuxiaoyu@swu.edu.cn

Fund: Ministry of Education Doctoral Program Special Fund (20110182110012); Key Projects of Chinese Medicine Research of Chongqing Municipal Health Bureau (2010[60] 2010-1-4); Fundamental Research Funds for Central Universities (XDJK2014C058)

"preventive care" focusing on health has been recently brought in this field. Yintian Granule (YTG) is a prescription of Chinese potent medicine firstly produced by our team (Zhang hui et al, 2012). Application for national technology invention patent has been filed. YTG was made from *Lonicera macranthoides* Hand.-Mazz*, Gardenia jasminoides* Ellis, and *Asparagus cochinchinensis* (Lour.) Merr., all of which were Chinese herbal medicine, fitting for medicinal and edible use for thousands of years. YTG contains many major active ingredients, such as chlorogenic acid (CGA), geniposide, *A. cochinchinensis* glycoside, polysaccharides, etc. CGA exhibits potent anti-oxidant and anti-inflammatory activities, reduces blood pressure and liver injury, and improves the oxidation of low density lipoprotein (LDL) (Jennifer et al, 2007; Yun et al, 2012; Joao, 1994). Geniposide is from the fruit of *G. jasminoides* and is useful against hyperlipidemia and fatty liver. It can significantly inhibit liver fibrosis and possess anti- inflammatory and anti-angiogenic activities as well (Kojima et al, 2011; Ma et al, 2011; Koo et al, 2006). *A. cochinchinensis* glycoside was extracted from the roots of *A. cochinchinensis*. Recent studies have suggested that *A. cochinchinensis* glycoside could show anti-oxidant activity and protect mice liver against oxidative damage by inhibiting the hepatic microsomal lipid peroxidation (Chun et al, 2011; Jian et al, 2013; Xiong et al, 2011). The main objective of this study was to investigate the protective effects of YTG against liver injury. Carbon tetrachloride (CCl_4) and 50% ethanol were used to induce acute liver injury model (Handa and Sharma 1990; Tsukamoto et al, 1995) in mice pre- administered with YTG. The results suggested that YTG could be used in designing new anti-hepatic disease agents.

2. Materials and methods

2.1 Preparation of YTG

Dried *Lonicera macranthoides* Hand.-Mazz*, Gardenia jasminoides* Ellis, and *Asparagus cochinchinensis* (Lour.) Merr. were obtained from Southwest Hosptal (Chongqing, China) and the voucher specimens were deposited in Natural Medicinal Chemistry Research Center of this Institute. Water extract was obtained at 70 ^{o}C (1 h × 3 times). The extracts were filtered through Whatman No. 1 filter paper and the filtrate was concentrated under vacuum using a rotary evaporator (RE−2000A, Shanghai, China). The concentrated extracts were dried at 80 ^{o}C. TLC chromatogram was used for identification of the extracts, and the active constituents were tested by HPLC (Agilent−1200 series, USA).

The CGA standard (0.0108 g) was taken into 100 mL volumetric flask, added with 50% methanol, and shaked. The CGA standard solution (108 μg/mL) was obtained. Geniposide (0.0110 g) was accurately weighed and added with 50% methanol into 100 mL volumetric flask, mixed and then the geniposide standard solution (110 μg/mL) was obtained.

Dried YTG (0.3 g) was taken into 25 mL volumetric flask, added with 20 mL of 50% methanol, and the sample was treated by ultrasonic for 30 min and filtered through a 0.45 μm membrane filter, the filtrate was detected by HPLC.

A Platisil-ODS column (250 mm × 4.6 mm, 5 μm) was installed and the column temperature was kept at 25 ^{o}C. The mobile phase composed of water containing acetonitrile and 0.2% phosphoric acid, and the split ratio to HPLC was 2:8. The flow rate was 1.0 mL/min, injection volume was 20 μL, and the detection wavelength was 326 nm. The retention time of CGA which was identified in *Lonicera macranthoides* Hand.-Mazz by HPLC was 5.5 min (Figure 1A).

An Agilent Zorbax SB-C18column (250 mm × 4.6 mm, 5 μm). The mobile phase composed of water containing acetonitrile and deionized water, the split ratio to the HPLC was 1:9 and the column temperature was kept at 25 ^{o}C. A flow rate was 1.0 mL/min, injection volume was 20 μL and detection wavelength was 238 nm. The retention time of geniposide was 19.2 min (Figure 1B).

2.2 Animals and experiment protocol

Male ICR mice with body weight of 20.0 ± 2.0g were obtained from the Experimental Animal Center, Chongqing Medical University (China) and housed under controlled environment [(22 ± 2) ℃, 12 h light/dark cycle, diet and water *ad libitum*] in the Experimental Animal Center, College of Pharmaceutical Sciences & College of Chinese Medicine, Southwest University (Chongqing, China). All animal protocols were performed in accordance with international guidelines for care and use of laboratory animals.

2.3 Protective effects of YTG on experimental acute liver injury induced by CCl_4

Sixty ICR mice were randomly divided into normal control, model, positive control [0.01 g/kg bifendatatum (DDB)], low-, mid-, and high-dose (6.6, 13.2, and 26.4 g/kg, ig administration) YTG groups (n = 10). The mice in nomal control and model groups were ig administered equal volume of normal saline. All animals were ig administered for 14 d. After 2 h of the last treatment, all animals except those in the normal control group got acute experimental hepatic injury induced by 0.10% CCl_4 solution (20 mL/kg), fasting but water *ad libitum* for 16 h, and then all mice were sacrificed and the whole blood was collected from the suborbital vein under chloral hydrate anesthesia. The liver, kidney, and spleen were removed and weighed.

2.4 Protective effects of YTG on experimental acute liver injury induced by 50% ethanol

Sixty ICR mice were grouped using the method consistent with liver injury induced by CCl_4. All animals except tose in the normal control group were ig administered for 14 d. The mice in normal control group were ig administered with 50% ethanol solution in doses of 14 mL/kg body weight at intervals of 2 h. At the last treatment, the mice in all groups stopped feeding for 12 h, water *ad libitum*. All animals were sacrificed and the whole blood was collected