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ScienceDirect
Journal of Ethnopharmacology 109 (2007) 318–324
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

# Herb medicine Yin-Chen-Hao-Tang ameliorates hepatic fibrosis in bile duct ligation rats

Tzung-Yan Lee [a,*], Hen-Hong Chang [a,b], Jenn-Han Chen [c], Ming-Lung Hsueh [a], Jong-Jen Kuo [b]

[a] *Graduate Institute of Traditional Chinese Medicine, Chang Gung University, Tao-Yuan, Taiwan, ROC*
[b] *Center for Traditional Chinese Medicine, Chang Gung Memorial Hospital, Tao-Yuan, Taiwan, ROC*
[c] *School of Dentistry, National Defense Medical Center, Taipei, Taiwan, ROC*

Received 25 February 2006; received in revised form 12 July 2006; accepted 31 July 2006
Available online 5 August 2006

**Abstract**

The accumulation of hydrophilic bile acids in the liver is considered to play a pivotal role in the induction of hepatic injury. Yin-Chen-Hao-Tang (YCHT) decoction is an aqueous extract from three different herbs: *Artemisia capillaries* Thunb (Compositae), *Gardenia jasminoides* Ellis (Rubiaceae), *Rheum officinale* Baill (Polygonaceae), which has been recognized as a hepatoprotective agent for various types of liver diseases. Therefore, we used an experimental of biliary atresia model to test that YCHT plays a regulatory role in the pathogenesis of hepatic fibrosis.

Hepatic damage with fibrosis was produced by common bile duct ligation (BDL) for 27 days in experimental cholestasis animal model. After surgery, YCHT (250 and 500 mg/kg BW) oral administration once a day continued for 27 days. BDL caused a prominent liver collagen deposition that was supported by the increased α-SMA protein and mRNA expression of procollagen I. YCHT significantly decreased hepatic α-SMA protein levels and decreased in hydroxyproline and thiobarbituric acid reactive substances (TBARS) levels of BDL rats. On the other hand, the normalizing effect of YCHT (250 mg/kg) on the TGF-β1 mRNA expression was independent on the dose of YCHT, 500 mg/kg was not effectively changed the quantitative composition of mRNA levels. The study shows that hepatic hydroxyproline accumulation caused by hydrophilic bile acids accompanied by elevated hepatic lipid peroxidation, and hepatic collagen levels can be decreased in the presence of YCHT. In conclusion, long-term administration of YCHT in rats ameliorated the hydropholic bile acids induced hepatic injury that probably related to a reduced oxidant stress and degree of hepatic fibrosis.


*Keywords:* Yin-Chen-Hao-Tang; Bile duct ligation; Hepatic fibrosis

## 1. Introduction

Liver fibrosis is major features of a wide range of chronic liver injuries including metabolic, viral, cholestatic and genetic disease. The failure of bile salt excretion in cholestasis leads to retention of hydrophobic bile salts within the hepatocytes (Greim et al., 1972a,b) and causes apoptosis and/or necrosis (Myoshi et al., 1999). Although the mechanism of bile salt mediated fibrosis is not completely understood, the involvement of the transforming growth factor-β1 (TGF-β1) expression has been suggested by *in vitro* studies, as well as by *in vivo* studies using an animal model with bile duct ligation (BDL).

The substances react with membrane lipids to propagate a chain reaction, leading to lipid peroxidation in cellular membranes that induces cell injury (Recknagel et al., 1989). Following hepatic injury, activation of hepatic stellate cells (the key fibrogenic cells) is the dominant event in liver fibrogenesis, causing an excessive accumulation of extracellular matrix (Friedman, 2000; Friedman et al., 2000). Additionally, oxidative stress is a direct fibrogenic stimulus (Pratico et al., 1998; Yamamoto et al., 1998). During the fibrogenic process, matrix production by stellate cells is markedly increased through the activation of TGF-β1 (Friedman, 2000; Friedman et al., 2000).

Additionally, a number of studies have shown that administration of anti-oxidants or Chinese herb lead to a decrease in hepatic TGF-β1 expression and severity of fibrosis in rats (Wasser et al., 1998; Vendemiale et al., 2001; Lee et al., 2003). Yin-Chen-Hao-Tang (YCHT/ Japanese herbal medicine TJ-135) decoctions has long been used in China and Japan as anti-

* Corresponding author at: Graduate Institute of Traditional Chinese Medicine, Chang Gung University, No. 259 Wen-Hwa 1st Road, Kwei-Shan, Tao-Yuan 333, Taiwan, ROC. Fax: +886 3 211 8421.
*E-mail address:* joyamen@mail.cgu.edu.tw (T.-Y. Lee).

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doi:10.1016/j.jep.2006.07.042

inflammatory, antipyretic, choleretic and diuretic agent for liver disorders and jaundice and several studies provide clinical evidence of its effectiveness in treatment of various liver diseases. YCHT is an aqueous extract from 3-herbs; *Artemisia capillaries* Thunb (Herba *Artemisiae Capillaris*, Yin-Cen-Hao), *Gardenia jasminoides* Ellis (*Fructus Gardeniae*, Zhi-zi) and *Rheum officinale* Baill (Radix Rhei Officinalis, Da-huang) with a ratio of 4:3:1 in weight. *Artemisia capillaries* and *Gardenia jasminoides* are effective for liver diseases, and *Rheum officinale* is a laxative (Komiyama et al., 1976; Okuno et al., 1988; Yamamoto et al., 1996, 2000). Among components of *Artemisia capillaries*, capillarisin can act as choleretic (Komiyama et al., 1976; Okuno et al., 1988) and capillene and capillin inhibit apoptosis of hepatocytes induced by TGF-β (Yamamoto et al., 1996). Genipin, an aglycone converted in the gut from geniposide is proven to suppress Fas-induced liver injury in mice (Yamamoto et al., 2000). Recently, YCHT was reported to suppress liver fibrosis in rats induced by choline-deficient diet (Sakaida et al., 2003), but the mechanisms in such suppression are to be elucidated. Considering that YCHT seems to affect the signaling derived from TGF-β in hepatocytes (Yamamoto et al., 1996), YCHT can regulate HSC activity. However the question may arise from this anti-fibrotic effect of whether YCHT will ameliorate hepatic lipid peroxidation after extrahepatic biliary obstruction.

These studies have resulted in considerable interest in YCHT as a therapeutic agent in chronic liver disease. The aim of the current study was to investigate the beneficial effects of long-term YCHT administration on oxidative stress and liver fibrosis in rats with biliary obstruction mediated hepatic injury. The hepatic peroxidation products estimated mainly as thiobarbituratic acid reactive substances (TBARS) were measured in BDL rats receiving different doses of YCHT. In addition, molecular markers of liver fibrogenesis, including TGF-β-1 and procollagen I, were also measured.

## 2. Materials and methods

### 2.1. Preparation of YCHT

Yin-Chen-Hao-Tang extract power consists of crude ingredients extracted from the following three medicinal herbs mixed in the ratio in parenthesis: *Artemisia capillaries* Thunb (Herba Artemisiae Capillaris, Yin-Cen-Hao), *Gardenia jasminoides* Ellis (Fructus Gardeniae, Zhi-zi) and *Rheum officinale* Baill (Radix Rhei Officinalis, Da-huang) with a ratio of 4:3:1 in weight. YCHT was prepared by boiling the dried power with distilled water for 5 h. The extract was filtered, freeze-dried, and kept at 4 °C. The extraction yield was approximately 10.1% (w/w). The dried extract was dissolved in distilled water before use.

### 2.2. Biliary obstruction and animal treatments

Adult male Sprague-Dawley rats weighing between 220 and 250 g were used in all experiments. Hepatic damage with fibrosis was produced by common bile duct ligation (BDL), as ours previously described (Yang et al., 2001). In brief, under ether anesthesia, the common bile duct was ligated with 3–0 silk and sectioned between the ligatures. The midline abdominal incision was closed with catgut. Sham-operated rats had their bile duct exposed but not ligated or sectioned. All rats were caged at 24 °C with a 12:12 h light–dark cycle and were allowed free access to food and water. Animal studies were approved by the Animal Experiment Committee of the Chang-Gung University and were conducted humanely. After surgery, YCHT was dissolved in distilled water and administered daily (250 and 500 mg/kg, po) over the experimental period. Control rats received equal volumes of the solvent and sham-operated animals were used as day 0 controls.

### 2.3. Histopathology assay and serum biomarkers measurement

The liver tissue was fixed in 10% formalin and then embedded in paraffin, cut into 5-μm thick sections, and stained with Masson's trichrome, and examined under light microscopy by an experienced pathologist. Blood was obtained for serum biochemical analysis. The activities of alanine aminotransferase (ALT) were measured using an Auto Dry Chemistry Analyzer (Hitachi 736-60, Tokyo, Japan).

### 2.4. Hepatic TBARS measurements

The liver homogenate for lipid peroxidation was prepared with 2 mL of 50 mM potassium phosphate buffer, pH 7.4, and TBARS were determined (Fraga et al., 1988). The fluorescence of the samples was detected at an excitation wavelength of 515 nm and an emission wavelength of 555 nm in a F4500 fluorescence spectrophotometer (Hitachi, Japan). 1,1,3,3-Tetramethoxypropane was used as the TBARS standard. Results were expressed in nmol/mg protein, and protein concentrations were determined by the method of Lowry et al. (1951).

### 2.5. Hepatic hydroxyproline content

Hepatic hydroxyproline content was measured using a modified version of the method of Jamall et al. (1981). Briefly, liver samples were homogenized and hydrolyzed in 6N HCl at 110 °C for 18 h. After filtration of the hydrolysate through a 0.45-mm Millipore filter (Millipore, Bedford, MA), chloramine T was added to a final concentration of 2.5 mM. The mixture was then treated with 410 mM paradimethyl-amino-benzaldehyde and incubated at 60 °C for 30 min. After cooling to room temperature, the samples were read at 560 nm against a reagent blank which contained the complete system without added tissue. The concentration of hydroxyproline in each sample was determined from a standard curve generated from known quantities of hydroxyproline.

### 2.6. Western blot analysis for α-SMA

Freshly isolated liver tissue was homogenized in a buffer containing 10 mmol/L Tris–Hcl (pH 7.4) and 1% sodium dodecyl sulfate (SDS), 1% sodium deoxycholate, 1 mmol/L PMSF,