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# Aqueous extract of Yin-Chen-Hao decoction, a traditional Chinese prescription, exerts protective effects on concanavalin A-induced hepatitis in mice through inhibition of NF-$\kappa$B

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## Abstract

In traditional oriental medicine, Yin-Chen-Hao decoction is used for the remedy of liver diseases such as hepatitis, fatty liver, hepatocirrhosis and jaundice. However, despite extensive pharmacological study, the molecular mechanism of the anti-inflammatory effect of Yin-Chen-Hao decoction is poorly understood. In this study, we have investigated the pharmacological action on the mechanism of concanavalin A-induced T cell-dependent hepatitis in mice. Concanavalin A administration resulted in a severe liver injury. This was shown through increased levels of serum transaminase and lactic dehydrogenase, and increased liver DNA fragmentation and caspase-3 activity. Pretreatment with the aqueous extract from Yin-Chen-Hao decoction dose-dependently inhibited the elevation in transaminase and lactic dehydrogenase activity, and reduced liver DNA fragmentation and caspase-3 levels. There was an improvement in histological changes including inflammatory infiltration, hepatocyte necrosis and degeneration, and Kupffer cell hyperplasia. In addition, Yin-Chen-Hao decoction significantly inhibited tumour necrosis factor-$\alpha$ (TNF-$\alpha$) production in-vitro and in-vivo. Moreover, the activation of nuclear factor kappa B (NF-$\kappa$B), which regulates TNF-$\alpha$ production, was blocked by Yin-Chen-Hao decoction in-vitro and in-vivo. In conclusion, Yin-Chen-Hao decoction was capable of regulating T-cell-mediated liver injury in-vivo. This event may have depended on the decrease of TNF-$\alpha$ production through the inhibition of NF-$\kappa$B activation.

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## Introduction

T cell-mediated liver damage is a key event involved in the pathogenesis of acute and chronic hepatitis, and the development of cirrhosis. It has been reported that the cellular immune response against viral antigens is responsible for the progression of hepatitis B virus- and hepatitis C virus-related chronic hepatitis (Rehermann 2000; Heneghan & McFarlane 2002; Ichiki et al 2005). It should be considered that elimination and dysfunction of activated T cells, the main effector cells involved in various cellular immune responses, might block the progression of damage in these diseases. In the development of this liver injury, concanavalin A-induced liver injury, as a T cell-dependent model of liver damage, is regarded as an appropriate model of human immunomediated liver disease (Tiegs et al 1992). Concanavalin A administration induced T cell activation and resulted in liver injury, which is thought to be mediated primarily by tumour necrosis factor-$\alpha$ (TNF-$\alpha$) and could be prevented by the use of polyclonal TNF-$\alpha$ antiserum (Mizuhara et al 1994; Gantner et al 1995). In addition, TNF-$\alpha$-induced nuclear transcription factor nuclear factor kappa B (NF-$\kappa$B) activation exerts a critical role in the progress of inflammation and increasing evidence implies that inhibition of NF-$\kappa$B activity may alleviate the degree of inflammatory diseases (Wolf et al 2001; Imose et al 2004). These findings suggest that inhibition of NF-$\kappa$B activation and TNF-$\alpha$ production may be a useful approach to the treatment of T cell-mediated immune diseases.

Yin-Chen-Hao decoction, a traditional Chinese prescription, is widely used for treating various inflammatory diseases, especially hepatic diseases (Chen et al 1998; Yan et al 2001; Wan et al 2003). Yin-Chen-Hao decoction is one representative of the prescriptions derived from "Treatise on febrile diseases", a medical classic written by Zhong-jing Zhang during the East Han Dynasty. The prescription comprises three drugs: Yin-Chen-Hao (Herba Artemisiae Capillaris), Zhi-zi (Fructus Gardeniae) and Da-huang (Radix Rhei Palmati). The ethanol extract of Yin-Chen-Hao decoction has been shown to have a protective role in experimental liver injury induced by $CCl_4$, D-galactosamine, BCG and lipopolysaccharide (Cao et al 2002). Huang (1992) reported that the extract of Yin-Chen-Hao decoction decreased high levels of blood lipids. Besides, the major active substances of *Artemisia capillaris* Thunb. include scoparone, capillarisin, cirsimaritin, $\beta$-pinene, $\beta$-caryophyllene and capillene, most of which possess anti-inflammatory and anti-microbial activity (Cha et al 2005; Jang et al 2005). However, little is known about the molecular mechanism of the anti-inflammatory effect of Yin-Chen-Hao decoction. In this study, we have investigated the effect of the aqueous extract of Yin-Chen-Hao decoction on T cell-mediated liver injury induced by concanavalin A in mice. The mechanism of action was elucidated in terms of TNF-$\alpha$ production and NF-$\kappa$B activation.

## Materials and Methods

### Animals

Female ICR mice (5–6 weeks old; Experimental Animal Center of Nanjing Medical University, Nanjing, China) were maintained in plastic cages at $21 \pm 2$°C with free access to pellet food and water, and kept on a 12-h light/dark cycle. This study complied with current ethical regulations on animal research of the university, and all mice used in the experiments received humane care.

### Drugs and reagents

The crude drugs were purchased from Nanjing Medical Material Co. (Nanjing, China) and were identified as *Artemisia Capillaris* Thunb. (Herba Artemisiae Capillaris, Yin-Cen-Hao), *Gardenia jasminoides* Ellis. (Fructus Gardeniae, Zhi-zi) and *Rheum palmatum* L. (Radix Rhei Palmati, Da-huang) by Dr Chun-Gen Wang (Nanjing University of Traditional Chinese Medicine, Nanjing). They were mixed in a ratio of 10:5:3, respectively, to make up Yin-Chen-Hao decoction. A common method was used to make the prescription into the aqueous extract. Briefly, the material (100 g) was extracted twice with fivefold volumes of water (500 mL) at 100°C for 1 h each time. The supernatants from each extraction were mixed and centrifuged at 1870 *g*, the precipitation was discarded and the supernatant was lyophilized to obtain a powder (yield 21.2%, w/w). The extract was used from the same stock solution in each experiment, thus minimizing the possible variations in the concentration of the active components that occurs between extract preparations. The main components in Yin-Chen-Hao decoction were determined by HPLC as 0.32% (w/w) scoparone, 0.96% geniposide and 0.25% rhein. For the in-vivo assay the powder was dissolved in water for oral administration by gavage to mice. For the in-vitro assay the powder was dissolved in RPMI 1640 medium. Ciclosporin was purchased from Sandoz Ltd (Basel, Switzerland). Concanavalin A was from Sigma (USA), whilst the kits for determining serum alanine transaminase (ALT), aspartate transaminase (AST) and lactic dehydrogenase (LDH) were from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The 96-well culture plates were from Nunclon, diphenylamine was from Shanghai Medical and Pharmaceutical Co. Ltd (Shanghai, China), and Ac-DEVD-pNA was from Alexis Biochemicals (Montreal, Canada). The ELISA kit for tumour necrosis factor-$\alpha$ (TNF-$\alpha$) was from Jingmei Biotech Co. Ltd (Shengzhen, China), RPMI 1640 was from GIBCO BRL, and new bovine serum was from Hangzhou Sijiqing Co. Ltd (Hangzhou, China).

### Induction of concanavalin A hepatitis

Acute liver injury was induced by injecting mice with concanavalin A in phosphate-buffered saline at $22\,mg\,kg^{-1}$ via the tail vein. Eight hours after the injection, the mice were bled. The serum was collected for assaying the activity of ALT and AST by using the commercial kits as the protocols indicated. Portions of liver were fixed in 10% formalin and embedded in paraffin. The tissue sections were stained with haematoxylin–eosin and read on a 0 to 3 scale (0, no change; 1, mild; 2, moderate; and 3, severe) by a pathologist who had no prior knowledge of the induction of liver injury or other experimental data.

### Analysis of DNA fragmentation (Squier & Cohen 1997)

DNA was isolated from frozen liver samples. DNA (10 $\mu$g) was loaded in a 2% agarose gel and electrophoresed. The gel was then stained with ethidium bromide and documented under UV light. The frozen liver tissues were homogenized in 0.5 mL lysis buffer (containing (mM): 10 Tris-HCl (pH 7.5), 1 EDTA (pH 8.0), 0.5% Triton X-100) for 30 min on ice and centrifuged for 10 min at 13 000 *g* (Eppendorf) at 4°C. The supernatants were transferred to other tubes and 0.5 mL 12.5% trichloroacetic acid (TCA) was added to the pellets (P) and the supernatants (S), respectively. The tubes were then incubated at 4°C overnight. The samples were centrifuged for 10 min at 20 000 *g*

(Eppendorf) at 4°C and the pellets were suspended in 80 $\mu$L 5% TCA, followed by incubation at 90°C for 10 min. Subsequently, to each sample 160 $\mu$L diphenylamine solution (150 mg diphenylamine in 10 mL glacial acetic acid, 0.15 mL sulfuric acid and 2.5 $\mu$L acetaldehyde) were added and incubated at room temperature overnight. The proportion of fragmented DNA was calculated from absorbance reading at 595 nm using the formula:

$$\%\ \text{Fragmented DNA} = \text{OD(S)}/[\text{OD(S)}+\text{OD(P)}] \times 100$$

### Caspase-3 activity assay

Mice were killed at 2, 4 or 8 h after concanavalin A injection, and caspase 3-like activity was measured in liver homogenates using Ac-DEVD-pNA as a substrate (Alexis Biochemicals) (Ding et al 2004). Briefly, the frozen liver samples were homogenized in lysis buffer containing 10 mmol $L^{-1}$ HEPES, 5 mmol $L^{-1}$ dithiothreitol, 2 mmol $L^{-1}$ EDTA, 0.1% CHAPS, pH 7.4. The homogenates (100 $\mu$g) were incubated with caspase-3 substrate (Ac-DEVD) that was linked to p-nitroaniline (pNA) at 37°C. Cleavage of the substrate by caspase released p-NA that was then determined by a spectrophotometer (Sunrise, Austria) at 405 nm.

### Preparation of splenocyte suspension

Spleen was aseptically taken from mice, crushed gently and separated into single cells by squeezing in Hank's solution. The cells obtained were passed through eight layers of gauze and centrifuged at 1000 rev $min^{-1}$ for 5 min at 4°C. The pellet was added into 10 mL sterile Tris-$NH_4Cl$ (pH 7.5) followed by centrifugation to remove erythrocytes. After washing twice with RPMI 1640 medium supplied with 100 U $mL^{-1}$ penicillin, 100 $\mu$g $mL^{-1}$ streptomycin and 10% new bovine serum, they were re-suspended in the medium and used for cell culture.

### TNF-$\alpha$ production and assay

TNF-$\alpha$ plasma levels were measured (0, 2, 5, 8 and 24 h) after administration of concanavalin A. After centrifugation, plasma was recovered and samples stored at −80°C until assayed. Spleen cells ($5 \times 10^5$/well) were incubated for 24 h with 5 $\mu$g $mL^{-1}$ concanavalin A, with or without Yin-Chen-Hao decoction added at the same time as concanavalin A. At the end of the incubation period, samples of the supernatant were collected. Plasma and supernatant TNF-$\alpha$ concentrations were measured with a specific ELISA kit.

### Electrophoretic mobility shift assay

Nuclear extracts of the livers were prepared according to Wolf et al (2001). The double-stranded oligonucleotide probe containing a consensus binding sequence for NF-$\kappa$B (5′-GAGGGGACTTTCCCAGGA-3′; Genebase, Shanghai, China) was 3′-end-labelled with digoxigenin-11-ddUTP using a DIG gel shift kit (Roche). Five micrograms of nuclear protein was incubated in a 20-$\mu$L reaction volume on ice for 1 h and then loaded onto a 5.0% nondenaturing polyacrylamide gel in 0.5 × Tris-borate-ethylenediaminetetraacetic acid buffer at 4°C. The bands were detected by anti-digoxigenin-AP and CSPD solution. Specificity of the DNA–protein complex was confirmed by competition with a 100-fold excess of unlabelled NF-$\kappa$B.

### Statistical analysis

One-way analysis of variance for multiple comparisons was used to detect whether there was any significant difference among the different treatments. Once a significant difference was detected ($P < 0.05$), the Student's two-tailed $t$-test was used to evaluate the difference between two groups, and the Dunnett's $t$-test was used between the control group and multiple dose groups. All experimental results were shown as the mean ± s.e.m.

## Results

### Yin-Chen-Hao decoction improved concanavalin A-induced liver injury in mice

Yin-Chen-Hao decoction dose-dependently reduced ALT, AST and LDH levels compared with the control in concanavalin A-induced liver injury (Figure 1). The Yin-Chen-Hao decoction itself had no toxicity to the liver of mice, even at 1200 mg $kg^{-1}$ (Table 1). Histological examination of liver sections from control mice without medication showed marked inflammatory infiltration, severe hepatocyte degeneration, hepatocyte necrosis and Kupffer cell hyperplasia (Figure 2B, Table 1). Pretreatment with Yin-Chen-Hao decoction dose-dependently reduced the extent of liver damage (Figure 2C–E, Table 1). The improvement in ciclosporin-treated mice was also significant (Figure 2F, Table 1). The protective effect was observed only when Yin-Chen-Hao decoction was orally administered by gavage before concanavalin A. Yin-Chen-Hao decoction was ineffective if administered 1 h after concanavalin A injection (data not shown).

### Yin-Chen-Hao decoction suppressed DNA fragmentation and caspase-3 activity in concanavalin A-induced liver injury in mice

Pretreatment with Yin-Chen-Hao decoction almost completely prevented the concanavalin A-induced liver DNA fragmentation in a time-dependent manner (Figure 3A). Moreover, concanavalin A injection caused a DNA ladder pattern in agarose gel electrophoresis that was typical for apoptotic death (Figure 3B, lane 2). Pretreatment with Yin-Chen-Hao decoction prevented DNA laddering induced by concanavalin A in a concentration-dependent manner (Figure 3B, lanes 3–5). Additionally, concanavalin A administration caused a time-dependent increase in

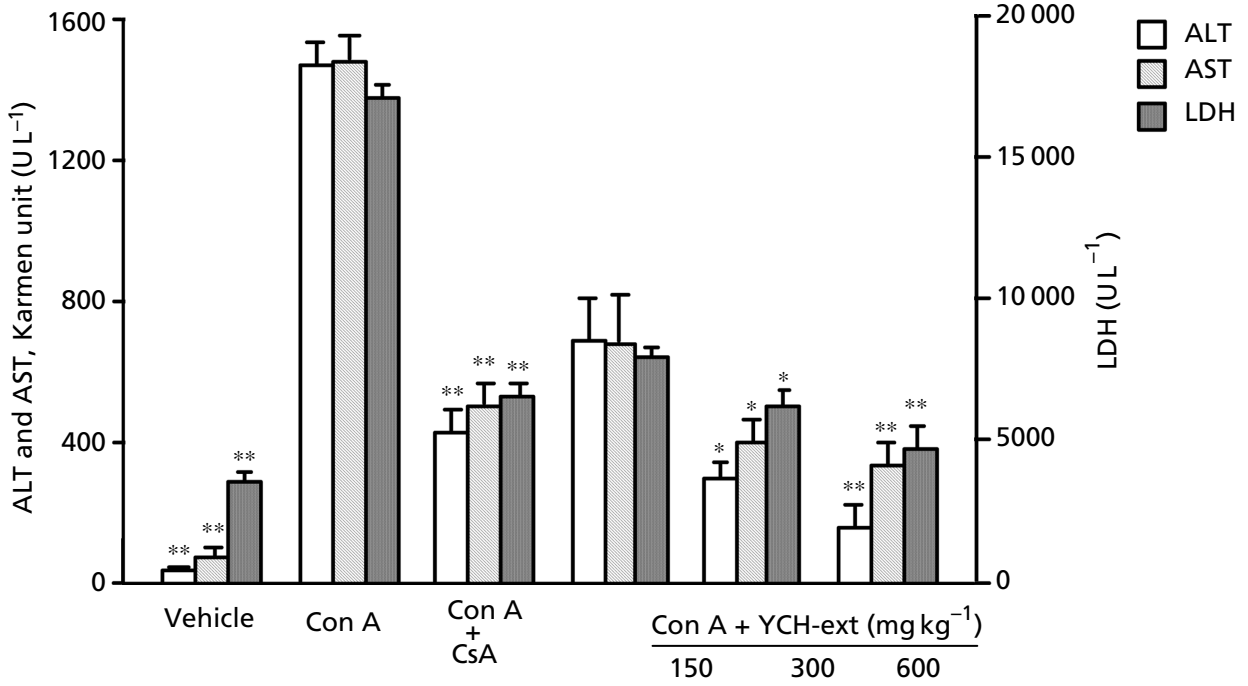


**Figure 1** Effect of Yin-Chen-Hao decoction on changes in serum transaminase activity and LDH level in mice with concanavalin A-induced liver injury. Mice were administered with Yin-Chen-Hao decoction (YCH-ext; 150, 300 and 600 mg kg$^{-1}$) by oral gavage. Ciclosporin (CsA; 45 mg kg$^{-1}$) was given intraperitoneally. One hour after administration, mice were injected intravenously with 22 mg kg$^{-1}$ concanavalin A (Con A), followed 8 h later by bleeding. The serum was used for the measurement of alanine transaminase (ALT), aspartate transaminase (AST) and lactic dehydrogenase (LDH) activity. Each figure indicates the mean ± s.e.m. of eight animals. Concanavalin A: concanavalin A-treated mice without medication. *$P < 0.05$, ** $P < 0.01$ vs concanavalin A group (Dunnett's *t*-test).

**Table 1** Effect of Yin-Chen-Hao decoction on the liver histopathological changes in mice with concanavalin A-induced liver injury

| Group | Dose (mg kg$^{-1}$) | n | Hepatocyte necrosis | Hepatocyte degeneration | Inflammatory infiltration | Kupffer cell hyperplasia |
|---|---|---|---|---|---|---|
| Naive | – | 8 | 0 | 0 | 0 | 0 |
| Yin-Chen-Hao decoction | 600 | 8 | 0 | 0 | 0 | 0 |
| | 1200 | 8 | 0 | 0 | 0 | 0 |
| Concanavalin A | 22 | 8 | $1.87 \pm 0.12^{\#\#}$ | $2.34 \pm 0.19^{\#\#}$ | $1.92 \pm 0.24^{\#\#}$ | $1.76 \pm 0.13^{\#\#}$ |
| Concanavalin A + Yin-Chen-Hao decoction | 150 | 8 | $1.48 \pm 0.22$ | $1.94 \pm 0.23$* | $1.31 \pm 0.16$* | $1.25 \pm 0.17$** |
| | 300 | 8 | $0.52 \pm 0.17$** | $1.18 \pm 0.16$** | $1.03 \pm 0.17$** | $1.01 \pm 0.17$** |
| | 600 | 8 | $0.22 \pm 0.10$** | $0.88 \pm 0.12$** | $0.70 \pm 0.13$** | $0.76 \pm 0.14$** |
| Concanavalin A + ciclosporin | 45 | 8 | $0.25 \pm 0.17$** | $1.03 \pm 0.34$** | $1.04 \pm 0.11$** | $0.89 \pm 0.15$** |

Mice were treated as described in Figure 1. Livers from individual mice were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with haematoxylin–eosin for morphological evaluation. The histological changes were read on a scale of 0–3 (0, no change; 1, mild; 2, moderate; and 3, severe) and expressed as an average score. Each figure indicates the mean ± s.e.m. of eight animals. $^{\#\#}P < 0.01$ vs naive (Student's *t*-test); *$P < 0.05$, **$P < 0.01$ vs concanavalin A (Dunnett's *t*-test).

the liver caspase 3-like activity. Pretreatment with Yin-Chen-Hao decoction significantly reduced liver caspase 3-like activity (Figure 3C).

### Yin-Chen-Hao decoction inhibited TNF-$\alpha$ release in-vitro and in-vivo

Yin-Chen-Hao decoction dose-dependently reduced concanavalin A-induced TNF-$\alpha$ production in naive spleen cells (Figure 4A). The viability of the lymphocytes was more than 96% in the group treated with the high concentration of Yin-Chen-Hao decoction, as measured by Trypan blue exclusion and annexin V–propidium iodide staining (data not shown). On the other hand, concanavalin A injection significantly increased plasma TNF-$\alpha$ level at 2 h. Against this, Yin-Chen-Hao decoction time-dependently inhibited TNF-$\alpha$ production in concanavalin A-injected mice (Figure 4B).

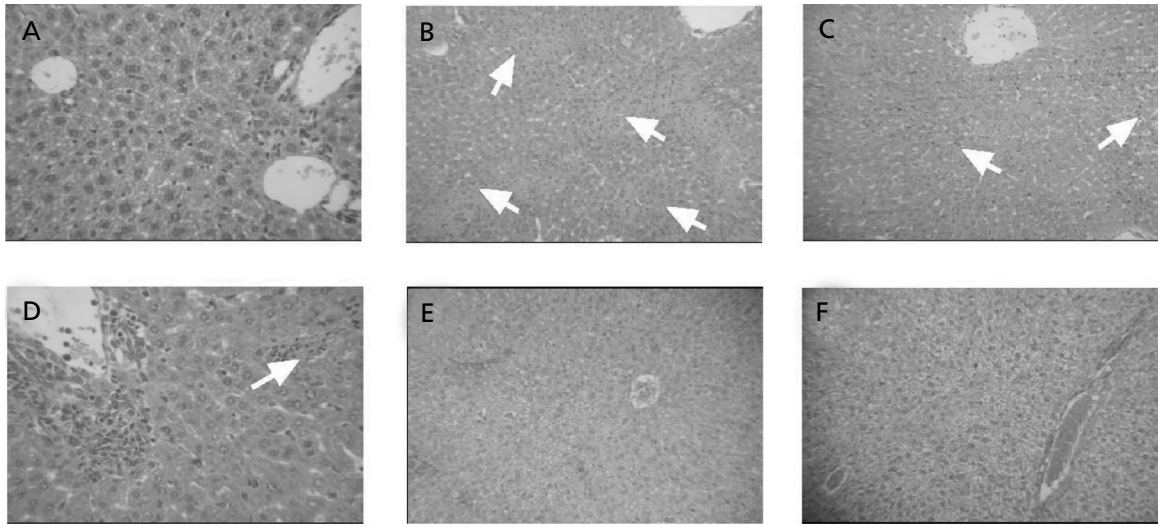


**Figure 2** Effect of Yin-Chen-Hao decoction on the histopathological changes of the injured livers caused by concanavalin A in mice. A. Naive mouse. B. Mouse treated with 22 mg kg$^{-1}$ concanavalin A. C–E. Mouse that was respectively treated with 150, 300 and 600 mg kg$^{-1}$ Yin-Chen-Hao decoction before concanavalin A injection. F. Mouse treated with 45 mg kg$^{-1}$ ciclosporin before concanavalin A injection. White arrows indicate necrosis observed in the liver. Original magnification ×100.

### Yin-Chen-Hao decoction inhibited NF-κB activation in-vitro and in-vivo

Spleen cells isolated from naive mice were activated by concanavalin A for 6 h, and this significantly increased NF-κB activity. Against this, Yin-Chen-Hao decoction dose-dependently suppressed its activation (Figure 5A). Additionally, concanavalin A caused significant activation of NF-κB in the liver and pretreatment with Yin-Chen-Hao decoction blocked its activation markedly (Figure 5B).

## Discussion

Yin-Chen-Hao decoction is currently being tried for the treatment of various liver diseases (Chen et al 1998; Yan et al 2001; Wan et al 2003). However, little is known about the mechanism by which it exerts a protective role in liver disease. In this study, we have provided experimental evidence of the protective role of the aqueous extract of Yin-Chen-Hao decoction in a murine model of T cell-mediated liver injury. T cell-dependent hepatitis can be induced in mice by injection of the T cell mitogenic plant lectin concanavalin A, which rapidly induces clinical and histological evidence of hepatitis, including elevation of transaminase activity, massive granulocyte accumulation, hepatocellular necrosis, and apoptosis in the liver (Tiegs et al 1992). In this study, concanavalin A administration induced acute liver injury, which resulted in increased levels of serum transaminase and lactic dehydrogenase, and liver cell death. Pretreatment with Yin-Chen-Hao decoction was sufficient to protect against concanavalin A-induced liver failure and improve the histological changes including inflammatory infiltration, hepatocyte necrosis and degeneration, and Kupffer cell hyperplasia (Figures 1, 2 and Table 1). However, Yin-Chen-Hao decoction had no protective effect when administered 1 h after concanavalin A injection. This suggested that the major target of the extract was present in the early phase of the activation of T cells rather than in the elicitation phase of the activated T cell. The positive control immunosuppressant ciclosporin significantly reduced ALT, AST and LDH levels, indicating T cell-mediated immune reaction played an important role in concanavalin A-induced liver failure.

To further characterize the protective role of Yin-Chen-Hao decoction on liver damage, we analysed the DNA fragmentation and caspase-3 activity in the liver. Concanavalin A injection induced liver DNA fragmentation in a time-dependent manner and resulted in a marked DNA ladder pattern after agarose gel electrophoresis, typical for cell apoptotic death. Pretreatment with Yin-Chen-Hao decoction prevented DNA laddering induced by concanavalin A in a time- and concentration-dependent manner (Figure 3A and B). In addition, concanavalin A administration caused a time-dependent increase in liver caspase 3-like activity, suggesting that hepatocyte death depended on caspase cascade activation. Yin-Chen-Hao decoction almost completely inhibited the caspase 3-like activity induced by concanavalin A in the liver tissue (Figure 3C). These findings suggested that the inhibition of apoptosis in the liver contributed to the protective effect of Yin-Chen-Hao decoction against concanavalin A-induced acute liver injury.

Concanavalin A-induced hepatitis is thought to be a model of immunologically-induced hepatocyte injury. T cell activation plays a crucial role in the process of

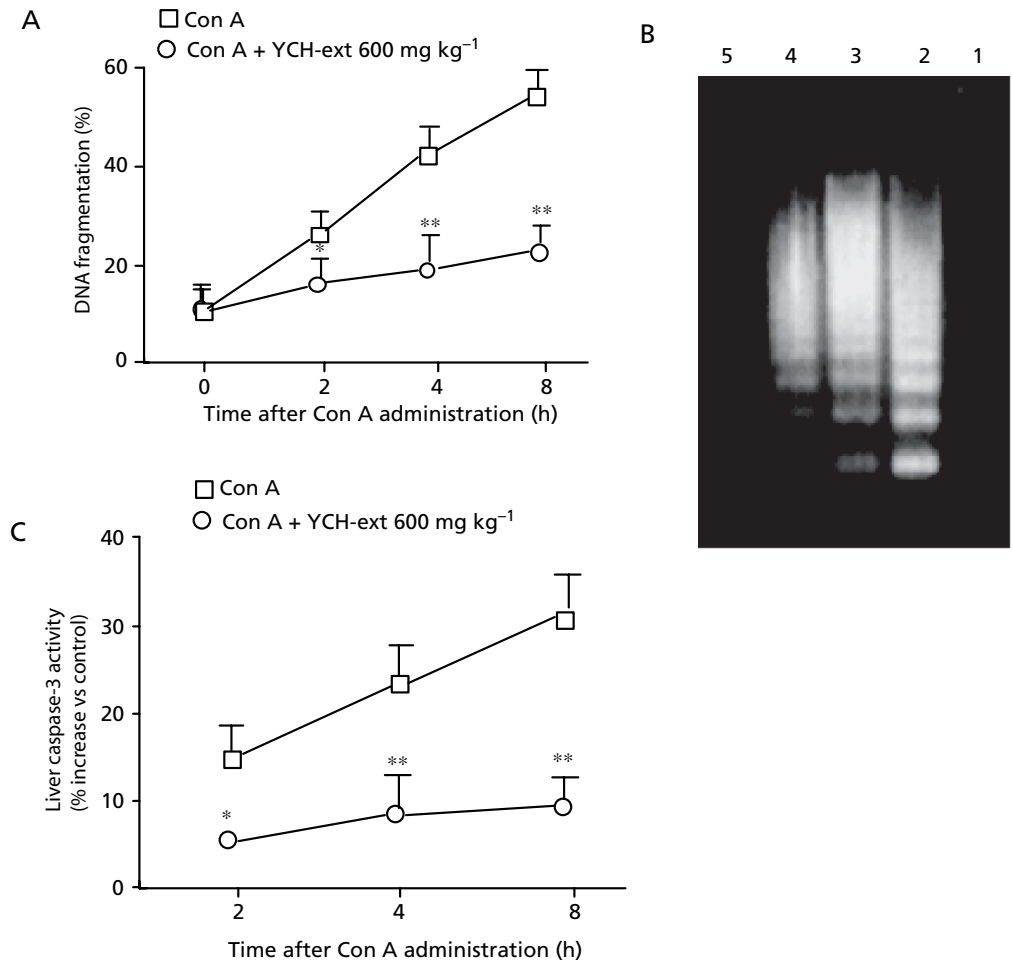


**Figure 3** Effect of Yin-Chen-Hao decoction (YCH-ext) on DNA fragmentation and caspase-3 activity in injured livers induced by concanavalin A in mice. Mice were administered 600 mg kg$^{-1}$ Yin-Chen-Hao decoction orally by gavage. One hour after administration, mice were injected intravenously with 22 mg kg$^{-1}$ concanavalin A. Mice were killed at the indicated times and the livers were assayed for (A) DNA fragmentation and (C) caspase-3 activity. Data were expressed as mean ± s.e.m. of six animals. B. Lane 1: normal mice; lane 2: concanavalin A-treated mice; lanes 3–5: mice treated with Yin-Chen-Hao decoction (150, 300 or 600 mg kg$^{-1}$, respectively) before concanavalin A administration. The figure is representative of three different experiments. *$P < 0.05$, **$P < 0.01$ vs concanavalin A group at each time point (Dunnett's $t$-test).

concanavalin A-induced hepatitis. T cell activation elicited by concanavalin A resulted in the elevation of plasma cytokines, including TNF-$\alpha$, interferon-$\gamma$, and interleukin-2 (Sass et al 2002). Among those cytokines, TNF-$\alpha$ released from activated T cells appears to be a prime suspect in causing liver injury induced by concanavalin A. Mice pretreated with anti-mouse TNF-$\alpha$ antiserum or TNF-$\alpha$ inhibitor or mice deficient for TNFR1 and TNFR2 (Mizuhara et al 1994; Gantner et al 1995; Koerber et al 2002) were resistant to concanavalin A-induced hepatitis. In an attempt to elucidate how Yin-Chen-Hao decoction exerted its protective effect, we found that pretreatment of mice with this extract almost completely prevented concanavalin A-induced TNF-$\alpha$ production in the circulation (Figure 4B). Consistent with the in-vivo result, the in-vitro experiment revealed Yin-Chen-Hao decoction could suppress TNF-$\alpha$ release from spleen cells activated by concanavalin A (Figure 4A). Moreover, the possibility that the cytotoxicity effect of the used extracts caused inhibition of TNF-$\alpha$ could be ruled out based on the fact that cell viability was unaffected when a high concentration of Yin-Chen-Hao decoction was used (data not shown). Given that one important signalling pathway in the induction of TNF-$\alpha$ expression is the activation of nuclear transcription factor NF-$\kappa$B (Van Antwerp et al 1996), we examined the action of Yin-Chen-Hao decoction on NF-$\kappa$B. Consistent with the inhibition on TNF-$\alpha$ production in-vitro (Figure 4A) and in-vivo (Figure 4B), Yin-Chen-Hao decoction remarkably blocked NF-$\kappa$B activation in spleen cells activated by concanavalin A (Figure 5A) and in liver

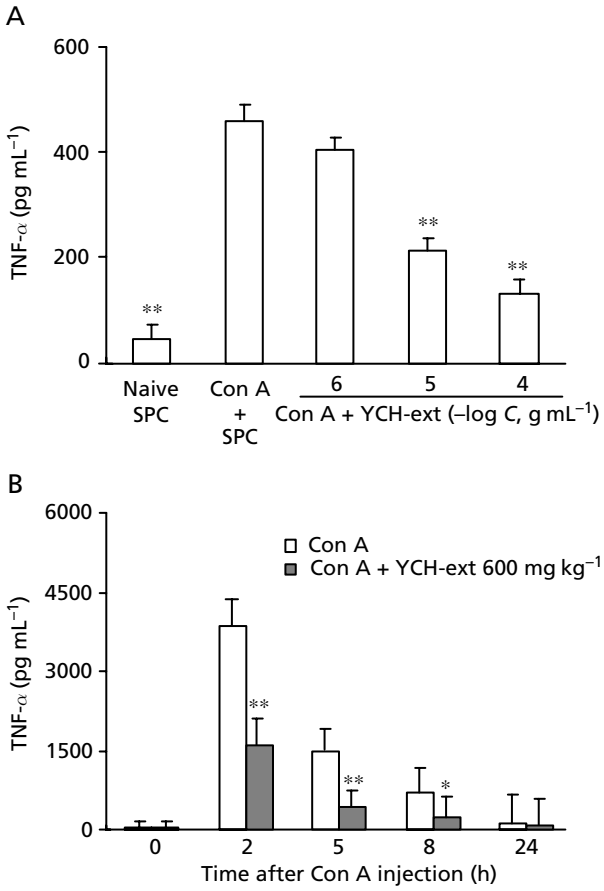


**Figure 4** Effect of Yin-Chen-Hao decoction (YCH-ext) on the TNF-$\alpha$ levels in-vitro (A) and in-vivo (B). Spleen cells (SPC) isolated from naive mice were cultured with 5 $\mu$g mL$^{-1}$ concanavalin A (Con A) for 24 h in the presence or absence of Yin-Chen-Hao decoction. TNF-$\alpha$ level in the culture supernatant was measured by ELISA. Each column represents the mean ± s.e.m. of three experiments and each experiment consists of triplicate sets. $^{**}P < 0.01$ vs concanavalin A group (Dunnett's $t$-test). For the in-vivo experiment, mice were injected intravenously with concanavalin A and bled at the indicated time. TNF-$\alpha$ level in serum was measured by ELISA. Data were expressed as mean ± s.e.m. of six animals. $^{*}P < 0.05$, $^{**}P < 0.01$ vs concanavalin A group at each time point (Dunnett's $t$-test).

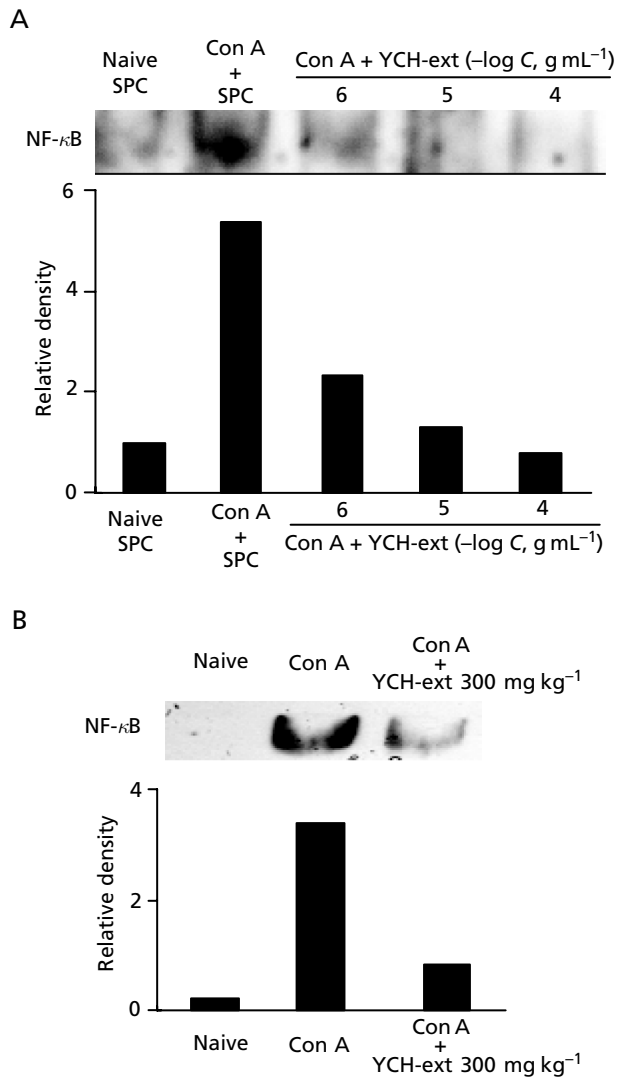


**Figure 5** Effects of Yin-Chen-Hao decoction (YCH-ext) on NF-$\kappa$B activation in-vitro (A) and in-vivo (B). A. Spleen cells (SPC) isolated from naive mice were cultured with 5 $\mu$g mL$^{-1}$ concanavalin A (Con A) for 6 h in the presence or absence of Yin-Chen-Hao decoction. Nuclear protein was extracted and assayed by EMSA. B. Mice were administered 300 mg kg$^{-1}$ Yin-Chen-Hao decoction by oral gavage. One hour after administration, mice were injected intravenously with 22 mg kg$^{-1}$ concanavalin A. Mice were killed 2 h later and the livers were extracted for nuclear proteins. NF-$\kappa$B activity was assayed by EMSA. The figure is representative of three different experiments.

injury induced by concanavalin A (Figure 5B). We speculated that Yin-Chen-Hao decoction blocked NF-$\kappa$B activation in the liver, subsequently inhibited the transcription of TNF-$\alpha$, which was the target gene of NF-$\kappa$B, and finally protected mice from concanavalin A-induced hepatic injury.

In conclusion, our results indicated that Yin-Chen-Hao decoction protected mice against concanavalin A-induced liver injury through downregulation of the production of pro-inflammatory cytokine TNF-$\alpha$ by inhibition of NF-$\kappa$B activation. This effect contributed to the amelioration of T cell-mediated liver disease in clinical conditions.

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