*Research Paper*

***Afr. J. Traditional*, Complementary and Alternative Medicines**
www.africanethnomedicines.net

**ISSN 0189-6016©2009**

# CHINESE MEDICINAL HERBS IN TREATING MODEL RATS WITH HEPATIC FIBROSIS

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## Abstract

The objective of this study was to examine the effects of Chinese medicine formula-Yu Zhang Dan (YZD, composed of *Herba Lysimachiae*, *Rhizoma Polygoni Cuspidati, Radix Curcumae*) on the model rats with hepatic fibrosis. Forty male Sprague-Dawley (SD) rats were used in the present study, and they were separated randomly into 4 groups: a normal control group (Group A, n=5), a model control (Group B, n=15), a high dose of YZD (Group C, n=10), and a low dose of YZD (Group D, n=10). Hepatic fibrosis in rats was induced by carbon tetrachloride ($CCl_4$). The variation of the serum alanine transaminase (ALT), aspartate aminotransferase (AST), hyaluronate acid (HA), laminin (LN), type Ⅲ procollagen peptide (PⅢ NP), L-Glutathione (GSH) was respectively measured with radioimmunoassay (RIA) and detection of transforming growth factor-beta 1 (TGF-β1) and smooth muscle alpha actin (α-SMA) was conducted with immunohistochemistry. The ALT, AST HA, LN and PIII NP levels in the serum of the model control group were significantly higher than those of the normal control group ($P<0.05$), and both of the high dose of YZD and low dose of YZD significantly decreased the ALT, AST HA, LN and PIII NP levels of the model rats ($P<0.05$). The TGF-β1 and α-SMA levels of the model control group were significantly higher than those of the normal control group ($P<0.05$), and both of the high dose of YZD and low dose of YZD significantly decreased the TGF-β1 levels of the model rats ($P<0.05$) , and only the high dose of YZD significantly decreased the α-SMA levels of the model rats ($P<0.05$). The expression of TGF-β1 and α-SMA in the liver tissues of the rats were in the cytoplasm of the cells. It may be through decreasing the ALT, AST, HA, LN and PIII NP levels in the serum of the model rats and decreasing the expression of TGF-β1 and α-SMA in the liver tissues of the model rats that YZD significantly relieved the hepatic fibrosis.

**Key words:** Chinese medicinal herbs, Yu Zhang Dan (YZD), Hepatic fibrosis.

**Abbreviations:** TCM, Traditional Chinese Medicine; ALT: alanine aminotransferase; AST: aspartate aminotransferase; α-SMA, smooth muscle alpha actin; TGF-β: transforming growth factor-beta.

## Introduction

Hepatic fibrosis, as the consequence of any types of liver injury, is often caused by alcohol abuse, viral hepatitis, non-alcoholic steatohepatitis, autoimmunity and drug intoxication (Bataller and Brenner, 2005). In recent years, Chinese medicinal herbs have been found to lead to satisfactory curative effects in treating the disease (Hu, 2006; Liu, 2006).

A recent study found that TCM 319 recipe, a Chinese medicine, attenuated hepatic fibrosis induced by carbon tetrachloride ($CCl_4$) in rats and the anti-fibrotic effect of TCM 319 recipe was found to be associated with the down-regulation of mRNA expression of tissue inhibitor of metalloproteinase-1(TIMP-1), platelet derived growth factor (PDGF-B) and PDGF-Rβ, and with suppression of protein expression of PDGF-Rβ and transforming growth factor-β1.(TGF-β1) (Cheung et al., 2009). JinSanE decoction, another chinese herbal medicine, was also found to inhibit expression of TGF-β1/Smads in experimental hepatic fibrosis in rats (Song et al., 2006). Bushen Rougan Recipe, a Chinese medicinal formula was found to inhibit the advancement of hepatic fibrosis (Zhang et al., 2004).

The present study was designated to explore the effects of Chinese medicine formula-Yu Zhang Dan (YZD) on the model rats with hepatic fibrosis. The formula of YZD originated from the authors' group.

## Material and methods

Forty(40) male Sprague–Dawley (SD) rats (weighing 160 ± 20 g) were provided by The Animal Laboratory Center, Zhejiang Medical Institute (Hangzhou, China). Serum alanine transaminase (ALT), aspartate aminotransferase (AST) reagents was provided by Shanghai Chemical Reagents Co.Ltd., (Shanghai, China). Hyaluronate acid (HA), laminin (LN), and type Ⅲ procollagen peptide (PⅢ NP) were provided by Beifang biological technology institute, (Beijing, China). Yu Zhang Dan (YZD, composed of 20g of *Herba Lysimachiae*, 20g of *Rhizoma Polygoni Cuspidati,* and 20g of *Radix Curcumae*) was provided by the 1st Affiliated Hospital, Zhejiang University (Hangzhou, China). The guidelines for animal care and use were approved by the committee on animal research. Hepatic fibrosis was induced by oral administration of $CCl_4$ at a dose of 1ml/kg body weight twice-weekly as described previously (Proctor and Chatamra, 1983).

The rats were then separated randomly into 4 groups: a normal control group (Group A, n=5), a model control (Group B, n=15), a high dose of YZD(Group C, n=10), and a low dose of YZD (Group D, n=10). Hepatic fibrosis in rats was induced by carbon tetrachloride ($CCl_4$). The variation of the serum alanine transaminase (ALT), aspartate aminotransferase (AST), hyaluronate acid (HA), laminin (LN), type Ⅲ procollagen peptide (PⅢ NP), L-Glutathione (GSH) was respectively measured with radioimmunoassay (RIA) and detection of transforming growth factor-β 1 (TGF-β1) and smooth muscle alpha actin (α-SMA) was conducted with immunohistochemistry. Hematoxylin and eosin staining and pico-sirius red staining were conducted for collagen visualization. For hematoxylin and eosin staining, liver tissues were fixed in 10% natural buffered formalin for 24 hrs. Then the tissues were embedded in paraffin, and the blocks were sectioned at 4-m thickness using microtome. Seven fields were taken into account for each liver section. Before collection of the samples, three rats respectively from a model control (Group B), a high dose of YZD(Group C), and a low dose of YZD (Group D) were sacrificed and the samples were not included in the statistical analysis of the results.

The statistical analysis in the research was respectively conducted by three university-based postgraduates in our university. The data is expressed in mean±SD and tested by analysis of variance. For all hypothesis tests, a 5% significance level ($p < 0.05$) and two-tailed tests were used. Ninety-five percent (95%) Mann-Whitney confidence intervals (CI) for the median difference between groups were determined.

## Results

### Group serum comparison: ALT and AST

As shown in Table 1, the ALT and AST levels in the serum of the model control group were significantly higher than those of the normal control group (P<0.05), and both of the high dose of YZD and low dose of YZD significantly decreased the ALT and AST levels of the model rats (P<0.05).

**Table1:** Group serum comparison: ALT and AST(U/L, Mean±SD)

| Group | N | ALT | AST |
|---|---|---|---|
| The normal control group (A) | 5 | 63±11* | 205±127* |
| The model control group (B) | 14 | 435±136 | 752±370 |
| High dose of YZD(C) | 9 | 277±192* | 555±230* |
| Low dose of YZD (D) | 9 | 261±142* | 473±177* |

Note: Compared with the model control group: *P < 0.05

### Group serum comparison: HA, LN and PIII NP

As shown in Table 2, the HA, LN and PIII NP levels in the serum of the model control group were significantly higher than those of the normal control group (P<0.05), and both of the high dose of YZD and low dose of YZD significantly decreased the HA, LN and PIII NP levels of the model rats (P<0.05).

**Table2:** Group serum comparison: HA, LN and PIII NP(ug/L, Mean±SD)

| Group | N | HA | LN | PIII NP |
|---|---|---|---|---|
| The normal control group (A) | 5 | 183±50* | 46.3±3.2* | 90±13* |
| The model control group (B) | 14 | 553±208 | 54.6±4.4 | 267±73 |
| High dose of YZD(C) | 9 | 367±101* | 48.1±5.2* | 114±29* |
| Low dose of YZD (D) | 9 | 373±96* | 43.5±4.9* | 133±39* |

Note: Compared with the model control group: *P < 0.05

**Group serum comparison: TGF-β1 and α-SMA**

As shown in Fig 1, TGF-β1 and α-SMA in the liver tissues of the rats were expressed in the cytoplasm of the cells.

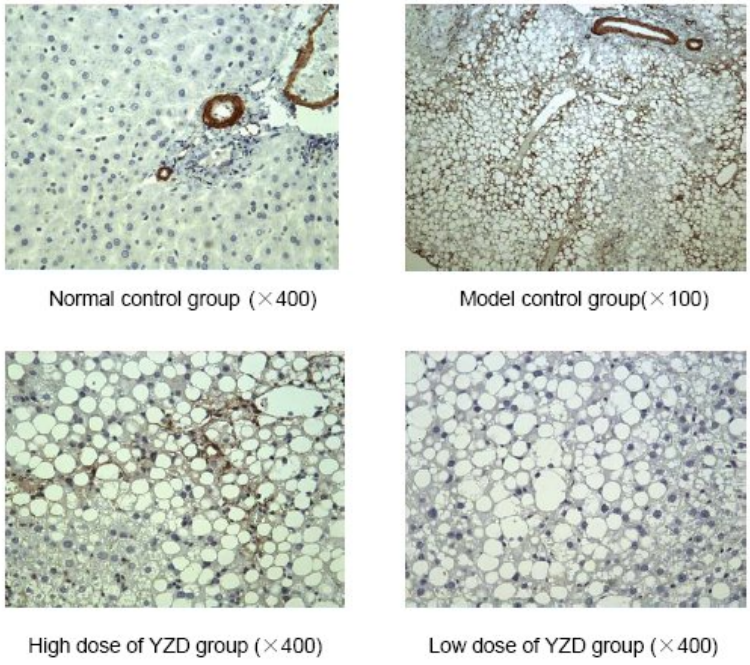


**Fig 1 (A) The expression of α-SMA in the Liver tissue**

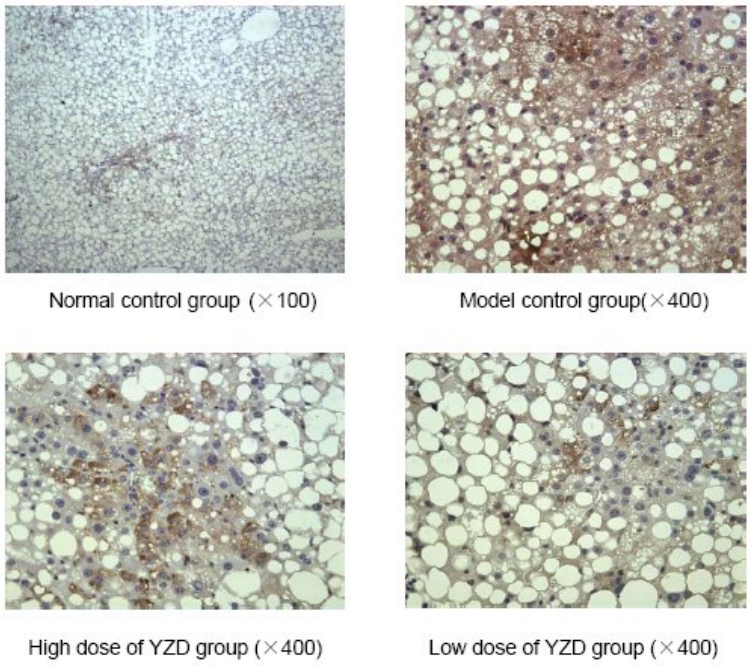


**Fig 1 (B) The expression of TGF-β 1 in the Liver tissue**

As shown in Table 3, the TGF-β1 and α-SMA levels in the serum of the model control group were significantly higher than those of the normal control group ($P<0.05$), and both of the high dose of YZD and low dose of YZD significantly decreased the TGF-β1 levels of the model rats ($P<0.05$) , and only the high dose of YZD significantly decreased the α-SMA levels of the model rats ($P<0.05$).

**Table 3:** Group serum comparison: TGF-β1 and α-SMA (Mean±SD)

| Group | N | TGF-β1 | α-SMA |
|---|---|---|---|
| The normal control group (A) | 5 | 0.0±0.0* | 0.0±0.0* |
| The model control group (B) | 14 | 6.4±2.6 | 8.4±3.5 |
| High dose of YZD(C) | 9 | 3.4±1.9* | 4.2±2.4* |
| Low dose of YZD (D) | 9 | 2.6±2.1* | 6.4±4.2 |

Note: Compared with the model control group: *P < 0.05

## Discussion

The present study has shown that ALT, AST HA, LN and PIII NP levels in the serum of the model control group were significantly higher than those of the normal control group, and both of the high dose of YZD and low dose of YZD significantly decreased the ALT, AST HA, LN and PIII NP levels of the model rats. Moreover, the TGF-β1 and α-SMA levels of the model control group were significantly higher than those of the normal control group, and both of the high dose of YZD and low dose of YZD significantly decreased the TGF-β1 levels of the model rats and only the high dose of YZD significantly decreased the α-SMA levels of the model rats. The expression of TGF-β1 and α-SMA in the liver tissues of the rats were in the cytoplasm of the cells. It may be through decreasing the ALT, AST, HA, LN and PIII NP levels in the serum of the model rats and decreasing the expression of TGF-β1 and α-SMA in the liver tissues of the model rats that YZD significantly relieved the hepatic fibrosis.

In a study to investigate the value of α-SMA, an indicator of stellate cell activation, in predicting fibrosis in chronic hepatitis B (CHB) patients, the liver biopsy specimens of 30 patients with a clinical diagnosis of CHB were obtained before treatment and scored by Knodell's histological activity index. The specimens were then immunohistochemically stained with α-SMA and semiquantitatively evaluated. Fibrosis and the immunoreactivity of α-SMA in the periportal, perisinusoidal and pericentral areas were compared. It was then concluded that in liver biopsy samples, α-SMA may prove to be a valuable marker in the evaluation of stellate cell activation and fibrosis progression and an early indicator of the development of fibrosis (Akpolat et al., 2005). Another study indirectly confirmed that, *in vivo*, α-SMA expression is a reliable marker of hepatic stellate cells activation which precedes fibrous tissue deposition even in the setting of recurrent HCV chronic hepatitis after liver transplantation, and it could be useful to identify the earliest stages of hepatic fibrosis and monitoring the efficacy of the therapy. In the presence of advanced cirrhosis other factors, rather thanα-SMA -positive hepatic stellate cells, may sustain fibrosis deposition (Carpino et al., 2005). In a Chinese study to measure the plasma levels of TGFβ1, the protein expression of α-SMA in hepatic stellate cells and urokinase plasminogen activator (uPA) and plasminogen activator inhibitor-1 (PAI-1), and study on the relationships between the plasma levels of TGFβ1, the protein expression and the serum HA in patients with different grades of hepatic fibrosis, they finally found that with the the progression of hepatic fibrosis, the plasma levels of TGFβ1 and the protein expression of a-SMA, uPA and PAI-1 in fibrotic liver tissue were increased. In grade 3 and 4, the plasma levels of TGFβ and the protein expression of a-SMA and PAI-1 in fibrotic liver tissue were significantly increased, but the protein expression of uPA in cirrhosis liver tissue did not increase. They concluded that TGFβ1, a-SMA, uPA and PAI-1 play an important role in the progression of hepatic fibrosis. Inhibiting the early activation of latent TGFβ1 or increasing uPA and inhibiting PAI-1 over express may contribute to matrix degradation and retard the progression of hepatic fibrosis (Wu et al., 2004). The present study found that TGF-β1 and α-SMA levels of the model control group were significantly higher than those of the normal control group ($P<0.05$), and both of the high dose of YZD and low dose of YZD significantly decreased the TGF-β1 levels of the model rats ($P<0.05$) , and only the high dose of YZD significantly decreased the α-SMA levels of the model rats ($P<0.05$). The expression of TGF-β1 and α-SMA in the liver tissues of the rats were in the cytoplasm of the cells.

In the formula of ZYD, *Herba Lysimachiae* is sweet and salty in flavour, and slightly cold in nature (Lei, 1994). It acts on the liver, gallbladder, kidney and urinary bladder channels (Lei, 1994). Being sweet and tasteless for inducing diuresis and excreting dampness, salty for softening hard masses, and slightly cold for clearing heat, the herb can eliminate damp-heat, treat stranguria and jaundice (Lei, 1994). It is particularly good at softening hard masses and removing urinary calculus. Therefore, the herb is often used to treat stranguria due to heat, stranguria caused by urinary stone, jaundice due to damp-heat, and calculus of the liver and gallbladder (Lei, 1994; Zheng, 2005). Radix Curcumae has a bright yellow color and mustard like taste, and it is often used in the preparation of various curries. Lately, *Radix Curcumae* has received a great deal of scientific attention because of the many medicinal properties it yields. Clinical studies demonstrate that Turmeric and the curcuminoids it contains act as an antioxidant, anticarcinogenic and antimutagenic, (inhibiting carcinogenesis and mutagenesis in laboratory animals), anti-inflammatory (comparable in strength to steroidal drugs and nonsteroidal drugs such as idomethacin and phenylbutazone), antimicrobial (inhibiting the growth of numerous gram positive and gram negative bacteria, fungi and the intestinal parasite *Entamoeba histolytica*) (Xiao, 2007).

*Radix Curcumae* also shows potential for reducing the risk of heart disease and slowing the degeneration often accompanying AIDS. Curcumin, a curcuminoid, also exhibited antibacterial characteristics by inhibiting production of aflatoxins, a toxin produced by a mold of poorly preserved foods, which causes damage to the liver and could result in liver cancer(Xiao, 2007; Zheng, 2005). Turmeric is thought to be especially important in aiding the body's natural regenerative and restorative processes (especially in the liver) having been linked to protein production and metabolism recently by Asian science. It is also thought useful in clearing the circulatory system. (Xiao, 2007; Zheng, 2005). The active agents, the curcuminoids, appear to preserve the life of critical biomolecules within the body either by shielding its activity, preventing their oxidation, or by the elimination of wastes which could destroy them. The same chemical process in which meat is preserved by Turmeric, preventing it from going rancid, appears to work on the body's tissues as well, preventing degradation of cellular tissues and systems. *Rhizoma Polygoni Cuspidati* acts on the lung, liver, gallbladder and large intestine channels. Being bitter for dispersion and purgation, and cold for reducing fever and sending down abnormally ascending Qi (Lei, 1994; Zheng, 2005), it effects on the liver channel and the blood system promoting blood circulation, relieves blood stasis and pain. Acting on the Qi division of lung and intestines, it clears away heat from lung and large intestine, resolves phlegm and cough, expels the pathogenic heat to loose the bowles. Acting on the liver and gallbladder channels. It can clear away damp-heat to cure jaundice (Lei, 1994; Zheng, 2005). Therefore, it is used to treat pathogenic heat accumulated in the blood system, cough due to the lung-heat, constipation caused by accumulation of heat jaundice due to damp-heat and other syndromes (Lei, 1994; Zheng, 2005). All the herbs together in the formula of YZD functioned to clear heat and dampness, eliminate potential danger and soften hard masses.

The study demonstrated that the Chinese medicinal formula-YZD showed promise in relieving hepatic fibrosis and merits further study. It may be through decreasing the ALT,AST, HA, LN and PIII NP levels in the serum of the model rats and decreasing the expression of TGF-β1 and α-SMA in the liver tissues of the model rats that YZD significantly relieved the hepatic fibrosis. Further researches with larger samples and with medication control groups need to be conducted next on the mRNA and protein levels.

**Conclusion**

It may be through decreasing the ALT,AST, HA, LN and PIII NP levels in the serum of the model rats and decreasing the expression of TGF-β1 and α-SMA in the liver tissues of the model rats that YZD significantly relieved the hepatic fibrosis.

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