

## Effects of *Chai Hu* (Radix Burpleuri) Containing Formulation on Plasma $\beta$ -endorphin, Epinephrine and Dopamine in Patients

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**Abstract:** *Chai Hu* (Radix Burpleuri), a major ingredient in many traditional Chinese medicine formulas, such as *Xiao Yan Wan*, is used in the treatment of liver stagnation and spleen deficiency syndrome (LSSDS). The objectives of this study were to examine the effects of *Xiao Yao Wan* containing *Chai Hu* on the changes of plasma indices in patients with LSSDS. Fifty-eight cases of LSSDS were randomly divided into two groups: 41 cases in the experimental group were treated with *Xiao Yao Wan* containing *Chai Hu* and 17 cases in the control group were treated with *Zhi Bai Di Huang Wan* for one consecutive month in a single blind design. Before and after treatment, high performance liquid chromatography (HPLC) was applied to determine the changes of plasma norepinephrine (NE), epinephrine (E) and dopamine (DA). Radioimmunoassay was performed to measure the amount of plasma  $\beta$ -endorphin ( $\beta$ -EP), adrenocorticotropin hormone (ACTH), estradiol ( $E_2$ ) and testosterone (T), and laser nephelometry was also conducted to measure plasma immunoglobulin A (Ig A) and G (Ig G). Compared to baseline levels, plasma  $\beta$ -EP was significantly increased ( $p < 0.01$ ), while E and DA were markedly decreased ( $p < 0.01$ ) after the administration of *Xiao Yao Wan* in the experimental group. The other indices did not change. This is the first evidence showing that the effect of *Xiao Yao Wan* containing *Chai Hu* on the treatment of patients with LSSDS may be through enhancing plasma  $\beta$ -EP and decreasing E and DA release. We conclude that *Xiao Yao Wan* containing *Chai Hu* regulates nervous and endocrine systems and contributes to the improvement of the clinical status of patients with LSSDS.

**Keywords:** *Chai Hu*; *Dang Gui*; *Bai Shao*; *Bai Zhu*; *Bo He*; *Xiao Yao Wan*; Liver Stagnation and Spleen Deficiency Syndrome.

## Introduction

Liver stagnation and spleen deficiency syndrome (LSSDS) is a common syndrome, which may be seen in many diseases in clinical traditional Chinese medicine. LSSDS can be an indication of general pathological changes in multiple systems, including nerve, endocrine, circulation, digestive, immune, sensory and locomotor. These conditions may result from psychological stress, the negative emotion of depression and/or anxiety (Chen, 1995). *Xiao Yao San*, also called *Xiao Yan Wan*, the patent medicine containing mainly *Chai Hu*, is likely to play a regulating role in nervous, endocrine and immune systems. It has been used in the treatment of LSSDS for thousands of years (Zhang, 1991; Wu, 1993; Zhang, 1998). There are about 308 symptoms and/or signs related to about 165 different kinds of diseases in TCM associated with LSSDS which are treated by *Xiao Yao Wan* (Ji *et al.*, 2001). However, the mechanisms involved in *Xiao Yao Wan* for the treatment of LSSDS are not clear. There are no common indices for the treatment of these symptoms and/or signs. The purpose of the present study was to assess plasma norepinephrine (NE), epinephrine (E), dopamine (DA),  $\beta$ -endorphin ( $\beta$ -EP), adrenocorticotropin hormone (ACTH), estradiol ( $E_2$ ), testosterone (T), immunoglobulin A (Ig A) and G (Ig G) as relative indices for the treatment of LSSDS. These indices were specifically used to determine the mechanisms of *Xiao Yao Wan* for the treatment of LSSDS in nervous endocrine immune systems.

## Patients and Methods

### *Criteria of Inclusion*

Fifty-eight patients were recruited from the department of TCM at the Beijing Railway Chief Hospital and the *Shandong Zhucheng* County People's Hospital from June 2000 to March 2001. The patients were not receiving any herbal medicine for soothing liver and regulating qi and/or invigorating spleen and blood tonic within two weeks before the study. The clinical diagnosis standard was as follows: depression, distending/pain in flanks, listlessness and lassitude, flatulence and loose stool. In the meantime, all subjects were independently diagnosed with LSSDS by three traditional Chinese medicine practitioners.

### *Criteria of Exclusion*

Patients less than 18 and over 69 years old; women in pregnancy or lactation; patients with the allergic constitution or allergic to *Xiao Yao Wan*; patients with cardiovascular diseases, cerebrovascular diseases, severe complications in liver, kidney and hematopoietic system; psychiatric patients; patients out of accord with the diagnostic standard; and patients who were not in compliance with the treatment protocol.

### *General Data*

Among 58 cases, ten were male and 48 were female, age range is from 25 to 69 years and the mean age is 47.18 years. The following diseases are observed: 23 cases of menopause syndrome; 11 cases of hypertension; nine cases of irregular menstruation; five cases of chronic gastritis; five cases of diabetes; five cases of neurasthenia; four cases of mammary hyperplasia; three cases of cerebral angiopathy sequela; three cases of knee joint osteoarthropathy; three cases of uterus myoma; three cases of chronic pharyngitis; three cases of neurosis; three cases of depression; two cases of arrhythmia; two cases of insufficient blood circulation in vertebral arteria basilaris; two cases of cervical vertebra disease; two cases of chronic enteritis; two cases of intercostal neuropathy; one case of cholecystitis; one case of hepatoangioma; one case of coronary heart disease; one case of chronic hepatitis B; one case of cholecyst polypus; one case of thyroid cyst; and one case of stomach prolapse.

### *Grouping and Treatment Protocols*

All patients signed human subjects consent form. Patients were randomized into two groups with a ratio of 3 in the experimental group to one in the control group. Equation and comparability test before treatment showed that the subjects matched well in sex and age in the two groups.

Forty-one subjects in the experimental group received *Xiao Yao Wan* orally (*Wuhu Zhanghengchun* Pharmacy Limited Corporation, licensed certification number: 201027) and 17 subjects in the control group received *Zhi Bai Di Huang Wan* orally (*Wuhu Zhanghengchun* Pharmacy Limited Corporation, licensed certification number: 201012). Patients received eight pills once three times daily for one consecutive month. No other herbs or patent medicines for soothing liver, regulating qi and invigorating spleen, blood tonic were permitted during the study. Blood samples were taken before and after the administration to perform the laboratory tests of the relative indices.

### *Laboratory Tests*

Fifteen-milliliter venous blood samples were taken from each subject in the morning before and after administration and placed in the relative tubes added with inhibitory peptidase (500 units for 1 ml blood) and 0.3 M disodium edetate ( $\text{EDTA}_2\text{Na}$ ), and centrifuged within 2 hours (at 4°C, 3500 rpm, for 10 minutes). Plasma was then carefully removed and restored in the freezer at -80°C for further measurement.

Measurements of NE, E and DA: The following instruments and reagents were applied to measure the plasma NE, E and DA. They are: Beckman high performance chromatography with 114 M high pressure pump; Beckman high liquid performance chromatography

integrator; electrochemical testing apparatus LC4 BAS (USA), with glass-carbon electrode (BASP/N MF100 USA) and Ag/AgCl control electrode; and LKB2220 recorder (Sweden), C<sub>18</sub> reverse phase chromatography column (Ultrasphere 4.6 × 250 mm). Biphenobenzidine was used as the interval standard.

Radioimmunoassay was performed to measure plasma β-EP, ACTH, E<sub>2</sub> and T, and laser nephelometry was used to test plasma Ig A and Ig G.

### Statistical Analysis

All statistics were performed with the software SAS 6.12. Data were presented as mean ± standard error mean (SEM). Wilcoxon test was performed to compare the difference between the two groups,  $p < 0.05$  was accepted as the level of significant difference.

## Results

### Comparison of the Curative Effect for Patient's Clinical Symptoms and Signs Between the Two Groups

Patients' clinical symptoms and signs ranged from none, mild, moderate to severity, which were quantified as 0, 2, 4 and 6, respectively as previously described (Chen and Qu, 1999; Chen, 2001). The following clinical symptoms and signs markedly improved after administration of *Xiao Yao Wan* containing *Chai Hu* in the experimental group: hot temper,

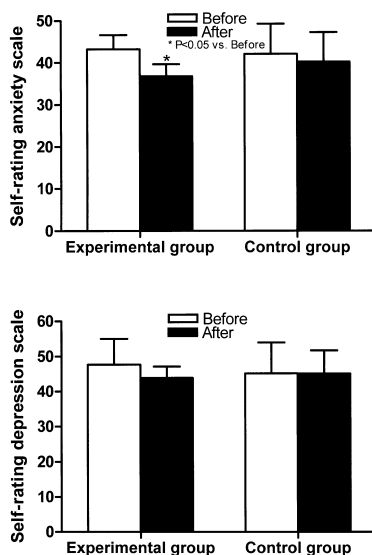


Figure 1. Changes of self-rating anxiety scale and self-rating depression scale in patients with LSSDS before and after treatment in the experimental and control groups. Each bar represents the mean values and vertical bars represent SEM. \* $p < 0.05$ , compared with before treatment.

sadness, dizziness, distending feeling in head, dryness and discomfort in eyes, foreign body feeling in throat, distending feeling and compression in the chest and flanks, hidden pain in flanks and ribs, distending pain in the breast, distending and compression in the upper abdomen after food intake, fatigue, often sighing, irritability before menstruation, distension and abdominal pain during menstruation, sexual dysfunction, insomnia, alternatively loose stools or constipation, no gloss in complexion. Figure 1 shows the total marks in the self-rating anxiety scale in experimental group significantly decreased after treatment.

#### *Comparison of the Curative Effect for All the Indices Between the Two Groups*

The changes before the first and after the last administration taken to evaluate the curative effect of treatment are shown in Table 1. Among all involved indices, there is a significant difference ( $p < 0.01$ ) in  $X_2$  ( $\beta$ -EP),  $X_4$  (E) and  $X_5$  (DA), but no significant difference in  $X_1$  (ACTH),  $X_3$  (NE),  $X_6$  ( $E_2$ ),  $X_7$  (T),  $X_8$  (Ig A) and  $X_9$  (Ig G) in the curative effect of subjects in the experimental group compared to that of the control group.

#### *Effects of Xiao Yao Wan Containing Chai Hu on Plasma $\beta$ -EP, E and DA in Patients with LSSDS*

Compared to the baseline (before treatment), Fig. 2 shows that the amount of plasma  $\beta$ -EP was significantly increased ( $p < 0.05$ ), while E and DA were markedly decreased ( $p < 0.05$ ,  $p < 0.01$ , respectively) after the administration of *Xiao Yao Wan* containing *Chai Hu* in the experimental group. The results demonstrate that *Xiao Yao Wan* containing *Chai Hu* can enhance plasma  $\beta$ -EP and decrease E and DA. These findings suggest that the effects of *Xiao Yao Wan* containing *Chai Hu* on the treatment of patients with LSSDS may be through regulating plasma  $\beta$ -EP, E and DA.

#### *Effects of Xiao Yao Wan Containing Chai Hu on Plasma ACTH, NE, $E_2$ , T, Ig A and Ig G in Patients with LSSDS*

Compared to the baseline (before treatment), there is no statistical difference in the content of plasma ACTH, NE,  $E_2$ , T, Ig A and Ig G in patients with LSSDS after the treatment both in the experimental group and control groups (Fig. 3).

## **Discussion**

In TCM, the liver is one part of the five-zang system and its function is to restore the essence and qi. The liver has a close relationship with the spleen and stomach as it can play a direct regulatory role in the ascending and descending of the spleen and stomach. The manifestations of LSSDS include depression, distending/pain in flanks, listlessness and lassitude, flatulence and loose stool, etc.

**Table 1. Comparison of Curative Effect in Two Groups**

Variables	Experimental			Control			S	Z	P
	Mean	SD	n	Mean	SD	n			
X <sub>1</sub>	30.48	58.45	41	27.15	58.45	17	461.5	-0.68	0.4992
X <sub>2</sub>	24.38	58.54	41	41.85	58.54	17	711.5	3.6	0.0003**
X <sub>3</sub>	31.71	58.54	41	24.18	58.54	17	411.0	-1.5	0.1242
X <sub>4</sub>	34.90	58.53	41	16.47	58.53	17	280.0	-3.8	0.0002**
X <sub>5</sub>	34.35	58.53	41	17.79	58.53	17	302.5	-3.4	0.0007**
X <sub>6</sub>	29.83	58.54	41	28.65	58.54	17	487.0	-0.2	0.8110
X <sub>7</sub>	31.09	58.54	41	25.68	58.54	17	436.5	-1.1	0.2705
X <sub>8</sub>	31.80	58.53	41	23.94	58.53	17	407.0	-1.6	0.1083
X <sub>9</sub>	32.16	58.54	41	23.09	58.54	17	392.5	-1.9	0.0638

\*\*  $p < 0.01$ , Comparison of total curative effect in two groups.

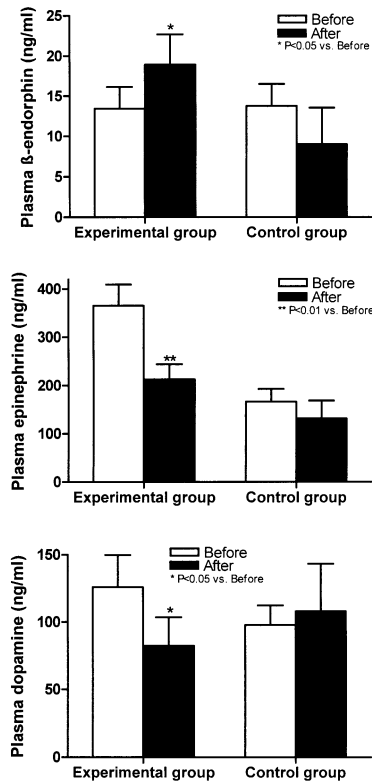


Figure 2. Changes of plasma  $\beta$ -EP, E and DA in patients with LSSDS before and after treatment in the experimental and control groups. Each bar represents the mean values and vertical bars represent SEM. \*  $p < 0.05$ , \*\*  $p < 0.01$ , compared with before treatment.

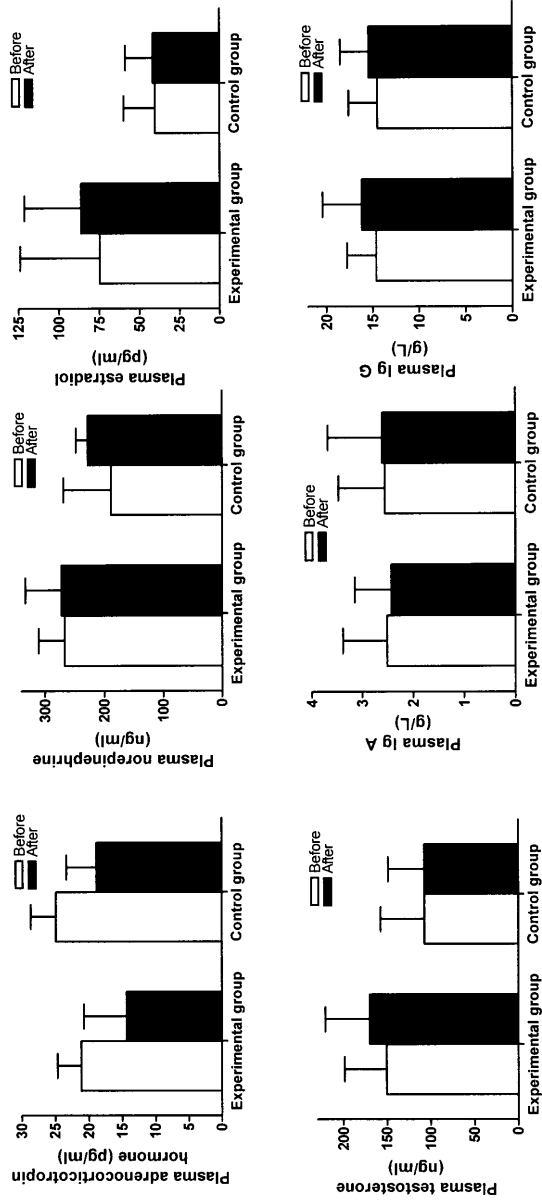


Figure 3. Changes of plasma ACTH, NE, E2, T, Ig A and Ig G in patients with LSSDS before and after treatment in the experimental and control groups. Each bar represents the mean values and vertical bars represent SEM.

*Xiao Yao Wan* which is composed of *Chai Hu* (Radix Bupleuri), *Bai Zhu* (Rhizoma Atractylodis Albae), *Bai Shao Yao* (Radix Paeoniae Albae), *Dang Gui* (Radix Angelicae Sinensis), *Fu Ling* (Sclerotium Poriae), *Bo He* (Herba Menthae), *Sheng Jiang* (Rhizoma Zingiberis Recens) and *Zhi Gan Cao* (Radix Glycyrrhizae Praeparata), originated from the book of *Tai Ping Hui Min He Ji Ju Fang* in the Song Dynasty (960–1127 AD), is mainly used for the treatment of LSSDS. *Xiao Yao Wan*, whose main ingredient is *Chai Hu*, acts as a nervous system sedative and regulates menstrual disorders through its action as a pituitary adrenocortical stimulant. Some studies suggested that LSSDS has a close relationship with the dysfunction of the nervous, endocrine and immune as well as other systems (Chen, 1985; Cheng, 1997; Jin, 1997). Catecholamines including E, nearly all of which is from the adrenal medulla, and NE as well as dopamine are mainly from the sympathetic nerve terminal in the vascular wall. Measurements of catecholamine can be used to partly reflect the function of the peripheral sympathetic adrenal medulla system. Some studies suggested that plasma NE and E are significantly increased in patients with the syndrome of liver stagnation or LSSDS (Jin, 1997). *Xiao Yao Wan* could play a selective role in the central catecholaminergic nervous system (Wu, 1993).

In the present study, the changes of plasma NE, E, DA,  $\beta$ -EP, ACTH, E<sub>2</sub>, T, Ig A and Ig G were observed in 58 cases of patients with LSSDS, which were separately administered with *Xiao Yao Wan* (41 cases) and *Zhi Bai Di Huang Wan* (17 cases), to explore the effects of *Xiao Yao Wan* in regulating the nervous endocrine immune systems. The results showed a significant difference in the effect on  $\beta$ -EP, E and DA, but no statistical difference effect in the rest of the indices between the experimental and the control groups. Plasma E and DA markedly decreased after the treatment, suggesting that they may be implicated in LSSDS.

$\beta$ -EP, which has a close relationship with conditions of emotional disorder, is one of nervous endocrine hormones secreted by the hypothalamus and the pituitary gland. It is one of the five opioid peptides which have been identified. One study (Hu, 2000) investigated the relationship between psychological features and plasma  $\beta$ -EP in patients with the syndrome of flaring-up of liver *Yang*, found that the level of plasma  $\beta$ -EP in psychological stress increased and main manifestations were depression-predominated emotional disorder which was accompanied with increased plasma  $\beta$ -EP release. In the present study, there was a significant difference in the changes of plasma  $\beta$ -EP in the experimental group compared to that in the control group. On the other hand,  $\beta$ -EP was significantly increased after the treatment, which suggests that  $\beta$ -EP also has a close association with the condition of LSSDS. This is the first evidence showing that *Xiao Yao Wan* containing *Chai Hu* used in the treatment of patients with LSSDS may act by enhancing plasma  $\beta$ -EP and decreasing E and DA. We conclude that *Xiao Yao Wan* containing *Chai Hu* regulates the nervous, endocrine systems and contributes to the treatment of patients with LSSDS.

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