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# A randomized, double-blind, double-dummy control study on Callicarpa capsules in treating chronic hepatitis B

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**Abstract Objective:** To assess the antiviral efficacy and safety of Callicarpa capsules on treating chronic hepatitis B (CHB). **Methods:** Multi-center, randomized, double-blind, double-dummy control study. Four hundred and thirteen cases of CHB were randomly divided into treatment group (Callicarpa capsules) 310 cases and control group (Oxymatrine capsules) 103 cases, medication for 24 weeks. Their serum HBV DNA and HBV markers were detected by the central laboratory at 12th and 24th week, patients were followed up every 4 weeks recording symptom scores to evaluate efficacy of TCM, also recording adverse events and laboratory abnormalities data to monitor the safety of study drug. **Results:** The basic demography, clinical and virological characteristics were similar in two groups. After 24 weeks of treatment, integrated anti-virus response rate was 42. 58% in treatment group, including HBV DNA negative conversion rate was 33.23%, HBeAg negative conversion rate was 31.93%, E antigen seroconversion rate was 13.08%, compared with the control group, no significant difference ($P > 0.05$); improvement rate of TCM syndrome was 88.42% in Callicarpa capsules group, superior to control group ($P < 0.01$); the incidence of adverse events was similar in two groups, 0.63% for Callicarpa capsules group, 2.89% for oxymatrine capsules group. **Conclusion:** Callicarpa capsules have high safety and better inhibitory effect of HBV, and significantly improve the clinical symptoms of traditional Chinese medicine.

**Key Words** hepatitis B; chronic; dampness-heat accumulation; Callicarpa capsules/therapeutic application; Oxymatrine capsules /therapeutic application; case-control studies

Callicarpa Capsule is a new type of Traditional Chinese Medicine (Class III) for the treatment of chronic hepatitis B (CHB). The medicine is made up of 4 traditional Chinese medicines such as phyllanthus urinaria L., and featured by clearing heat, promoting diuresis, detoxification, benefiting qi and activating blood circulation. In order to further evaluate the effectiveness and safety of this medicine, according to No. 2002ZL0151 Approval issued by the National Medical Products Administration (NMPA), this research group performed Phase III clinical trial of Callicarpa Capsule in treatment of CHB. The results are reported as follows.

Table 1 Comparison of general data between two groups of patients before treatment (FAS)

| Group | n | Gender (Male/Female) | Age (years) | Total syndrome scores | Classification of severity (Mild/moderate/severe) | ALT (U/L) | HBV DNA Log value | HBeAg (Positive/negative)) |
|---|---|---|---|---|---|---|---|---|
| Treatment group | 310 | 232/78 | 29.39 ±10.00 | 12.51 ±5.24 | 185/120/5 | 147.03 ±93.58 | 6.62 ±1.27 | 238 /72 |
| Control group | 103 | 78 /25 | 29.50 ±9.65 | 11.47 ±4.72 | 55 /45 /3 | 143.83 ±82.19 | 6.64 ±1.25 | 83 /20 |
| P | | 0.8962 | 0.917 | 0.094 | 0.2124 | 0.759 | 0.881 | 0.5887 |

## 1. Materials and Methods

1.1 Diagnosis criteria The traditional Chinese medicine (TCM) syndrome differentiation standard formulated according to the *Guidelines for the Clinical Research of Chinese Medicine New Drugs*[1], and the western medicine diagnostic standard formulated based on the *Viral Hepatitis Prevention and Treatment Scheme* jointly revised by the Chinese Society of Infectious Diseases and Parasitic Diseases, CMA and the Chinese Society of Hepatology, CMA in 2000[2].

1.1.1 Inclusion criteria Diagnosed with chronic hepatitis B by western medicine; dampness-heat accumulation and blood stasis due to qi deficiency by TCM syndrome differentiation; ALT elevated twice or more within six months (≥1.5 times ther normal value), serum ALT still ≥1.5 times the normal value when included; HBV DNA and/or HBeAg positive; aged 16 - 68 years; antiviral or/and immunomodulators not used within 6 months; volunteering to be the subject of observation and signing an informed consent form.

1.1.2 Exclusion criteria Combined with hepatitis infected with HCV, HDV, HAV, HEV, HGV and other hepatotropic virus, alcoholic hepatitis, autoimmune hepatitis and fatty liver; combined with HIV infection; TBil > 51.1 umol/L, or ALT> 10 times the normal value; chronic severe hepatitis and cirrhosis, or chronic hepatitis that may develop into severe hepatitis; patients combined with cardiovascular, kidney, lung, endocrine, blood system, hereditary, metabolic, neurological diseases and mental illness; pregnant or lactating women; those with allergic constitution or allergic to multiple drugs; those who have participated in clinical studies of similar new drugs within 3 months.

1.2 Trial design A randomized, double-blind, double-dummy, controlled, multi-centered study on Callicarpa Capsules and Oxymatrine Capsules (3:1) was adopted. Patients enrolled were randomly divided into treatment group and control group, entering a 6-month (24-week) double-blind trial period, and were orally administered. At the end of medication, the patients treated effectively were followed up for 6 months (24 weeks). The entire trial process lasted 12 months (48 weeks). At the end of the trial, effectiveness and safety assessments were conducted.

1.3 Randomized scheme The randomization block size is 7, and during randomization, patients were stratified by the negative and positive HBeAg and by center.

1.4 Observation index

1.4.1 Primary index Serum HBV DNA, serum HBV markers (HBV-M). The serums of patients were collected before treatment and at Week 12 and Week 24 of treatment respectively, frozen at -40℃, and sent to the Center Laboratory of Beijing 302 Hospital for uniform testing.

1.4.2 Secondary index Serum ALT and TCM syndrome scores were measured and evaluated before treatment and every 4 weeks after treatment. According to the references[1], the TCM syndrome efficacy is divided into 4 levels, namely cured, marked, effective and ineffective.

1.5 Comprehensive efficacy evaluation According to the references[3], the efficacy is categorized as complete response, partial response and no response, of which the complete response means both HBV DNA and HBeAg turn negative, and ALT recovers; partial response means either HBV DNA or HBeAg turns negative, or ALT of liver function recovers; no response means the above criteria are not met.

1.6 Safety evaluation Record the patient's spontaneous report or directly observations by the physician, or ask the patient about the adverse events through non-induced methods, perform blood routine, urine routine, stool routine and renal function index measurement and electrocardiogram examination before and after treatment, and evaluate its clinical safety.

1.7 Regulations and ethics matters This clinical trial was approved by the NMPA of the People's Republic of China with the approval number of 2002ZL0151, and was approved by the Medical Ethics Committee of the First Hospital of Hunan University of Chinese Medicine. Each patient signed the written informed consent form.

1.8 Statistical methods The t-test was used for the measurement data, the $x^2$ test or nonparametric test was used for the enumeration data, and the covariance analysis considering the central effect, the Wilcoxon rank sum test and the CMH test considering the central effect were used for the efficacy index.

Selection of statistical analysis data set: Full analysis set (FAS), including all followed-up patients who were randomized into groups, and medicated the drug at least once, and had at least one efficacy index record. The missing primary efficacy index was estimated by LOCF (last observation carry forward) method.

Per protocol set (PPS) is a subset of the FAS, including all cases that have completed the CRF (Case Report Form). The missing primary efficacy index is estimated by LOCF method. This trial uses PPS analysis and FAS analysis. Safety set (SS) includes all cases who had taken the drug at least once, and had at least one efficacy index follow-up record.

## 2. Results

2.1 Comparison of general data between two groups of patients is shown in Table 1.

2.2 Comparison of antiviral efficacy between two groups of patients

2.2.1 Comparison of comprehensive efficacy between two groups of patients The response rate of the treatment group is 42.58%, in which the complete response rate is 16.13%; the response rate for the control group is 30.1%, in which the complete response rate is 17.48%. There was no significant difference between the two groups. The results are shown in Table 2.

**Table 2 Comparison of comprehensive antiviral efficacy between two groups of patients [n (%), FAS]**

| Group | n | Complete response | Partial response | No response | P |
|---|---|---|---|---|---|
| Treatment group | 310 | 50 (16.13) | 82 (26.45) | 178 (57.42) | >0.05 |
| Control group | 103 | 18 (17.48) | 13(12.62) | 72 (69.90) | >0.05 |

The CMH test considering the central effect was used to compare the efficacy of the two groups, and the statistics was $x^2_{CMH}$

2.2.2 Comparison of HBV DNA negative conversion rate between two groups of patients is shown in Table 3.

**Table 3 Comparison of HBV DNA negative conversion between two groups of patients**

| Group | Statistical analysis data set | n | HBV DNA drop by 2 logarithmic degrees [n (%)] | *P* | HBV DNA negative conversion [n (%)] | *P* |
|---|---|---|---|---|---|---|
| Treatment group | FAS | 310 | 170 (54.84) | 0.0090 | 103(33.23) | 0.1421 |
| | PPS | 300 | 169 (56.33) | 0.0191 | 102(34.00) | 0.2123 |
| Control group | FAS | 103 | 41 (39.81) | | 26(25.24) | |
| | PPS | 97 | 41 (42.27) | | 26(26.80) | |

The Fisher's exact test was used for the comparison of the two groups

2.2.3 Comparison of HBeAg negative conversion rate between two groups of patients is shown in Table 4.

**Table 4 Comparison of HBeAg negative conversion between two groups of patients**

| Group | Statistical analysis data set | n | HBeAg negative conversion [n (%)] | *p* |
|---|---|---|---|---|
| Treatment group | FAS | 238 | 76(31.93) | 0.1271 |
| | PPS | 229 | 75(32.75) | 0.2012 |
| Control group | FAS | 83 | 19(22.89) | |
| | PPS | 77 | 19(24.68) | |

The Fisher's exact test was used for the comparison of the two groups

2.2.4 Comparison of HBeAg seroconversion rate between two groups of patients is shown in Table 5.

**Table 5 Comparison of HBeAg seroconversion rate between two groups of patients**

| Group | Statistical analysis data set | n | HBeAg serum conversion [n (%)] | *p* |
|---|---|---|---|---|
| Treatment group | FAS | 237 | 31(13.08) | 0.1052 |
| | PPS | 228 | 31(13.60) | 0.1055 |
| Control group | FAS | 82 | 5(6.10) | |
| | PPS | 77 | 5(6.49) | |

The Fisher's exact test was used for the comparison of the two groups

2.2.5 Comparison of ALT recovery status between two groups after treatment is shown in Table 6.

**Table 6 Comparison of ALT recovery status between two groups after treatment**

| Group | Statistical analysis data set | n | ALT [n (%)] | 95% confidence internal of ALT recovery rate difference of two groups | *p* |
|---|---|---|---|---|---|
| Treatment group | FAS | 310 | 116 (37.42) | (-19.60,1.93) | 0.8152 |
| | PPS | 300 | 116 (38.67) | (-21.63,0.67) | 0.7199 |
| Control group | FAS | 103 | 40 (38.83) | | |
| | PPS | 97 | 40 (41.24) | | |

The Fisher's exact test was used for the comparison of the two groups

2.3 Comparison of TCM syndrome efficacy and scores between two groups of patients is shown in Table 7 and Table 8.

**Table 7 Comparison of TCM syndrome efficacy and scores between two groups of patients [n-(%), FAS]**

| Group | n | Marked | Effective | Ineffective | Effective rate (%) | $X^2_{CMH}$ | *p* |
|---|---|---|---|---|---|---|---|
| Treatment group | 310 | 198 (63.87) | 73 (23.55) | 39 (12.58) | 87.42 | 12.43 | <0.0001 |
| Control group | 103 | 43 (41.75) | 32 (31.07) | 28 (27.18) | 72.82 | | |

CMH test considering the central effect was used for the comparison of efficacy and efficiency between the two groups, and the statistics is $X^2_{CMH}$

**Table 8 Comparison of TCM syndrome scores before and after treatment between two groups [n (%), FAS]**

| Group | Syndrome score | | Difference between before and after | Intra-group comparison | | Inter-group comparison | |
|---|---|---|---|---|---|---|---|
| | Before treatment | After treatment | | *S* | *P* | *Z* | *P* |
| Treatment group (n=310) | 12.51 ± 5.24 | 3.30 ± 3.82 | 9.21 ±5.56 | 21129.0 | <0.0001 | 3.69 | <0.001 |
| Control group (n=103) | 11.47 ± 4.72 | 4.63 ± 4.32 | 6.81 ±5.57 | 1876.5 | <0.0001 | | |

The sign rank test was used for intra-group comparison, and the statistics is S; Wilcoxon rank sum test was used for inter-group comparison after treatment, and the statistics is Z.

2.4 Safety evaluation The incidence of adverse events in the two groups of patients during

study is comparable, 0.63% for the Callicarpa Capsule group and 2.89% for the Oxymatrine Capsule group. The main manifestations were abdominal pain, diarrhea, dry stool and other digestive tract reactions. There were no serious adverse events in both groups. The incidence of serious adverse events was 0.73% for the Callicarpa Capsule group, and 1.92% for the Oxymatrine Capsule group. In the Oxymatrine Capsule group, thrombocytopenia occurred in 1 case and increased bilirubin in 2 cases, which, according to the investigators, may be related to drugs. The patient with thrombocytopenia dropped out of the trial on his own; 1 patient in the Callicarpa Capsule group experienced elevated ALT. There were no deaths during the trial period.

## 3. Discussion

Domestic and foreign research results on the natural history of CHB have fully proved that the viral load in patients with CHB is the main reason for determining disease progression and prognosis, and the removal of HBV is the key to the treatment of CHB. At present, there are two main types of antiviral drugs with definite therapeutic effects on CHB: interferon -α and nucleoside (acid) analogues. Nucleoside (acid) analogues are currently the main drugs for the treatment of CHB due to their mild side effects and wide application range. Clinical medications for 10 years have confirmed their definite efficacy, but there are still problems that are difficult to solve. The ideal goal of CHB treatment is to completely remove HBV and achieve a complete immunological response. However, the proportion of nucleoside (acid) analogues to achieve a durable complete immunological response is extremely low, patients who have undergone seroconversion after drug withdrawal have a high recurrence rate and the problem with CHB treatment cannot be solved fundamentally.

In vitro tests confirmed that some traditional Chinese medicines, such as lightyellow sophora root, phyllanthus amarus L., and vietnamese sophora root, have anti-HBV effect, and clinical studies have shown that oxymatrine and interferon have similar antiviral efficacy [4]. Callicarpa Capsule is made up of 4 traditional Chinese medicines such as phyllanthus urinaria L. Pharmacodynamic studies have indicated that the alcohol-extracted lignans and water-extracted tannins of Phyllanthus urinaria L. in Callicarpa Capsule have a good inhibitory effect on the expression of HBV in HepG2.2.15 cells cultured in vitro; previous clinical studies have demonstrated that the drug has a certain clinical effect of inhibiting HBV.

This trial used a randomized, double-blind, double-dummy, parallel-controlled multicenter clinical research method. The results show that Callicarpa Capsule can significantly relieve the symptoms in the CHB patients such as asthenia, bloating, and hypochondriac pain, effectively inhibiting HBV replication. The negative conversion rate of HBV DNA is 35.10%, comparable to the efficacy of interferon-α reported at home and abroad; the negative conversion rate of HBeAg is 35.34%, and the seroconversion rate of e antigen is 10.43%, close to the HBeAg negative conversion rate of nucleoside drugs and seroconversion rate of e antigen [5].

Callicarpa Capsules are safe and well tolerated. No serious adverse events occurred in both groups. In both groups, there were no patients who discontinued trial treatment due to adverse events. Due to limited funding for research, the course of treatment in the study design is 24 weeks, and the analysis of 12-week and 24 -week trial data finds that HBV DNA negative conversion rate, HBeAg negative conversion rate and ALT recovery rate of this drug at Week 24

are significantly higher than those at Week 12. Therefore, more ideal effect may be achieved if the course of treatment is prolonged to 48 weeks or longer.

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