

# Hepatitis: A Global Health Concern

## Chapter 3

### Traditional Chinese Medicine Treatment for Chronic Hepatitis B

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#### Abstract

Chronic hepatitis B (CHB) is a chronic infectious disease caused by hepatitis B virus, which represents a significant challenge to public health. A long-term CHB treatment using the antiviral agents such as nucleos(t)ide analogues and interferon-alpha raise up obvious drug resistance and side effects. Traditional Chinese medicine (TCM) as an important part of complementary and alternative medicine, is using in clinical for CHB treatment in China. The effective CHB treatment is based on the accurate TCM syndrome (ZHENG) differentiation. Here, we reviewed the situation of TCM application in CHB treatment, and summarized the potential effects and mechanisms of Chinese herbal formulae (CHF), Chinese herbal medicines (CHMs) and their extracts or bioactive compounds *in vitro* and *in vivo*.

**Keyword:** Chronic hepatitis B; Treatment; Traditional Chinese Medicine; TCM syndrome

#### 1. Introduction

Chronic hepatitis B (CHB) is a chronic infectious disease caused by hepatitis B virus (HBV). More than two billion people have been infected worldwide and about 240 million people are chronically infected persons [1]. It becomes a serious clinical problem because of its subsequent diseases such as hepatic decompensation, cirrhosis and hepatocellular carcinoma (HCC) as well as liver failure and other complications [2]. According to WHO 2015 Global hepatitis report, hepatitis B resulted in 887000 deaths, mostly from cirrhosis and HCC.

As pointed out by the “Guidelines for prevention and treatment of chronic hepatitis B”, the ultimate goal of treatment is to prevent hepatic decompensation, halt progression to cir-

rhosis and/or HCC, and prolong survival. And the short-term goal is to permanently suppress HBV replication [2,3]. Currently, available options for the treatment of chronic HBV infection include four nucleos(t)ide analogue (NUCs) antiviral agents such as lamivudine, adefovir, entecavir and telbivudine and two interferons including interferon alpha (IFN- $\alpha$ ) and pegylated IFN- $\alpha$ -2a [4].

NUCs are able to suppress HBV replication, alleviate the liver histological changes and improve its function. However, these agents failed to eradicate HBV genomes from the liver, and may elicit mutation for drug-resistance with long-term use [5,6]. Only 30-50% cases in CHB who have received treatments with IFN- $\alpha$  or lamivudine achieved viral, biochemical and histological remission [7]. IFN- $\alpha$  alone or in combination with a NUC has serious side effects with a high incidence of recurrence and is also very expensive. Hence, these drugs are not widely used in developing countries.

Traditional Chinese medicine (TCM), which is an important part of the complementary and alternative medicine, has been used in clinical for more than 3000 years and accepted by a large portion of people [8]. In China, over 90 % of CHB patients received the TCM therapy. Compared with the interferon or lamivudine treatment, TCM treatment may have equivalent or even better effect in CHB treatment in terms of HBeAg and HBsAg seroconversion as well as HBV DNA clearance, suggested by long-term TCM clinical trials [8,9].

TCM treatment is based on syndrome (ZHENG) differentiation, called “Bian Zheng Shi Zhi” in Chinese, which is in essence a characteristic profile of all clinical manifestations, identified by a TCM practitioner. The accurate treatments rely on the successful TCM syndromes differentiation [9]. A series of studies has revealed that the TCM-assessed syndrome differs significantly in patients in different phases of CHB [10]. In addition, patients with different syndromes respond differently to the same TCM therapy, with varying clinical outcomes [10].

Patients with CHB are classified into five types according to their clinical manifestations, including Liver Qi stagnation and spleen deficiency syndrome (LSSDS), Damp heat stasis syndrome (DHS), Liver and kidney Yin deficiency syndrome (LKYDS) and Blood stasis syndrome (BSS) [11]. TCM syndrome lay the foundation for getting optimum efficacy in CHB treatment and the prevention of HBV-related complications.

Here we summarized the advances in research of TCM treatment for CHB. Firstly, the clinical effects and their mechanisms of common Chinese herbal formulae (CHF) according to TCM syndrome differentiation, as well as the effects of the CHF combined with anti-HBV drugs were listed (**Table 1**). Then potential effects and mechanisms of single Chinese herbal medicines (CHM) and their active ingredients or bioactive compounds *in vitro* and *in vivo* were summarized. We hope this review will contribute to an understanding of TCM treatment, and provide useful information for the development of more effective drugs for CHB.

## 2. TCM Syndrome-Based Treatment for CHB

### 2.1. LSSDS

According to the TCM theory, taking the analogy with natural phenomena like, Liver corresponds to Wood (*Mu*) and Wood tends to spread out freely. The Liver regulates the flow of Qi and Blood. Appropriate Liver function is the prerequisite for normal Qi movement, Blood circulation as well as meridian smoothness. The dysfunction of Liver would result to Qi stagnation and affect the Spleen that activates digestion system. That is, Liver stagnation may lead to Spleen deficiency, become the LSSDS. The major symptoms of LSSDS are (1) distending pain of lateral thorax; (2) abdominal distension and loose stools. The minor symptoms include (1) chest distress and depression; (2) lassitude and fatigue; and (3) a pink and tooth-marked tongue.

The inclusion criteria for LSSDS diagnosis [12] is as following:

- (1) Cases that have all the major symptoms;
- (2) Cases that have the major symptom (1) and the minor symptoms (2) and (3);
- (3) Cases that have the major symptom (2) and the minor symptom (1).

Xiao-Chai-Hu-Tang (XCHT) is clinically used to treat CHB patients with LSSDS. XCHT is a classical CHF derived from *Shang-Han Lun* (Treatise on Febrile Diseases), which was prescribed by Zhong-Jing Zhang (150-219 A.D.) to treat liver diseases nearly two thousand years ago. Currently, it is still commonly used to treat chronic hepatitis in China and Japan. XCHT consists of seven herbs: Chaihu (*Radix bupleuri*), Banxia (*pinellia tuberifera tenore*), Renshen (*Ginseng*), Gancao (*Glycyrrhiza uralensis Fisc*) Huangqin (*Scutellaria baicalensis*), Shengjiang (*Zingiber officinale Roscoe*) and Daza o (*Fructus Ziziphi Jujubae*) [12]. They work synergistically to tonify Qi and fortify the Spleen, thus strengthening the body to eliminate pathogens; Banxia and Shengjiang work together to regulate the stomach Qi and stop vomit; and the “guide” Gancao is used to harmonize the other CHMs [13,14].

Previous clinical studies have shown the beneficial effects and safety of XCHT for CHB treatment. In a clinical study, Hirayama C *et al.* found that XCHT could significantly declined serum AST and ALT levels and tend to decrease the HBeAg and increase of the levels of Anti-HBe antibodies [15]. Qin *et al.* have summarized the studies on combination of XCHT and IFN in a meta-analysis included 16 randomized trials (1,601 cases), and found that in the combination group, both the HBV DNA and HBeAg levels reduced significantly and the serum ALT also decreased significantly [16]. These results suggested that XCHT combined with antiviral drugs (e.g., lamivudine and IFN- $\alpha$ ) was more effective in serum loss of hepatitis B viral markers and in improving liver function compared to antiviral drugs alone.

To clarify the effective mechanisms of XCHT, Chiang et al. have tested the anti-HBV activity of XCHT using HepG2.2.15 cells, and found it could inhibit the production of HBV and decrease the expression of HBeAg [17]. Yamashiki *et al.* have found the XCHT promoted granulocyte colony-stimulating factor production in peripheral blood mononuclear cells [18]. Tseng et al. have found XCHT could suppresses HBsAg production in HepA2 cells, and suppressed HBV core promoter activity [19].

In addition, Xiao-Yao-San was also used for the treatment of CHB patients with LSSDS [20].

## 2.2. DHS

Under the guidance of TCM theory, HBV belongs to the pathological toxin. It is close to Dampness and Heat in the analogy with natural phenomena like the characteristics of lingering and hotness. Dampness would transform to Heat may generate the both Dampness and Heat, then all together they delay the recovery. The major symptoms of DHS are (1) yellow skin and eyes; (2) a yellow and greasy tongue coating. The minor symptoms are (1) nausea and anorexia ; (2) lateral thorax distension and epigastria depression; and [3] yellow urine. The inclusion criteria for DHS [12] is as following:

- (1) cases that have all the major symptoms;
- (2) cases that have the major symptom (1) and two minor symptoms;
- (3) cases that have the major symptom (2) and the minor symptoms (1) and (2).

Yin-Chen-Hao decoction (YCHD) is used in clinical to treat the CHB patients with DHS. YCHD, firstly described in the Shang-han-Lun, is comprised of Yinchen (*Artemisia capillaris* Thunb.), Zhizi (*Gardenia jasminoides* Ellis) and Dahuang (*Rheum palmatum* L.) [21].

Previous clinical studies have reported that YCHD can reduce serum transaminase activity, elevate serum albumin and reduce the ratio of albumin and globulin (A/G) to improve liver function and provide satisfactory long-term effects [20]. These effects could benefit CHB treatment. HBV infection activated the immune system to produce and release cytokines, which promoted liver inflammation and caused liver damage. Cai et al. have found the aqueous extract of YCHD inhibited the elevation in transaminase and lactic dehydrogenase activity, and reduced liver DNA fragmentation and caspase-3 levels. Moreover, YCHD alleviated histological changes including inflammatory infiltration, hepatocyte necrosis and degeneration and Kupffer cell hyperplasia, and inhibited NF- $\kappa$ B activation [22].

Zhanget al. has identified three major active compounds of YCHT, 6,7-Dimethylesculetin (D), geniposide (G) and rhein (R) can synergistically regulate molecular networks through

activating both intrinsic and extrinsic pathways to obtain intensified therapeutic effects [23]. Geng et al. have found 90% ethanol extract and 3 constituents from Yinchen revealed anti-HBV activity. They all inhibited the HBsAg and HBeAg secretions and HBV DNA replication [24].

In addition, Long-Dan-Xie-Gan-Tang were also used for the treatment of CHB patients with DHS [20,25].

### 2.3. LKYDS

LKYDS refers to the fluid deficiency of Liver and Kidney, which lead to deficient heat stirring up inside. LKYDS is frequently caused by insufficient innate constitution and long-term disease would also exhaust the Yin of Liver and Kidney. It's a common TCM syndrome in the later stage of many chronic diseases. The major symptoms of LKYDS are (1) dizziness and dry eyes; (2) weakness of loins and knees; and (3) a red and dry tongue or with fissure. The minor symptoms are (1) vexing heat of the chest, palms and soles; (2) insomnia; (3) dull pain of lateral thorax, aggravated by labor; and (4) thready and rapid pulse.

The inclusion criteria for LKYDS is as following(12):

- (1) cases that have all the three major symptoms;
- (2) cases that have two major symptoms and two minor symptoms;
- (3) cases that have one major symptom and three minor symptoms;
- (4) cases that have all the four minor symptoms.

Yi-Guan-Jian decoction (YGJD), first described in "Liu Zhou medical treatment", includes 6 herbs, Beishashen (*Glehnia littoralis*), Maidong (*Ophiopogon japonicus*), Danggui (*Angelica sinensis*), Fresh Dihuang (*Rehmannia glutinosa*), Gouqiji (*Fructus Lycii*) and Chuanlianzi (*MeLia toosendan Sieb. et Zucc.*).

YGJD is clinically used to treat CHB and other liver diseases with LKYDS. Furthermore, it has reported that YGJD improves liver fibrosis by inhibiting the migration of bone marrow cells into the liver as well as inhibiting their differentiation and suppressing the proliferation of both progenitors and hepatocytes in the injured liver [26,27].

### 2.4. BSS

BSS indicates the blood blockage in organs or collaterals that failed to dissipate immediately. Trauma, Qi stagnation and coldness may block blood circulation and elicit BSS. Blood concentration caused by excessive heat and insufficient Qi movement also could lead

to BSS. The major symptoms of BSS are (1) a long period of jaundice with a dark color; and (2) itchy skin. The minor symptoms are: (1) a dark red tongue; (2) distending pain at the right hypochondrial region; and (3) stool with a light color or gray color. The inclusion criteria for BSS is as following (1) major symptom (1) and (2) [28];

(2) major symptom (1) and two minor symptoms.

Fuzheng Huayu recipe/capsule (FZHY) was used to CHB patients with BSS. It can strengthen body to remove blood stasis. FZHY is composed of Danshen(*Salvia miltiorrhiza* Bge.), Chongcao (*Cordyceps mycelium*), Taoren (*Prunus persica* (L.) Batsch), Songhua powder (*Pinus massoniana* Lamb.), Jiaogulan (*Gynostemma pentaphyllum* (Thunb.) Makino) and Wuweizi (*Schisandra chinensis* (Turcz.) Baill ). FZHY has been shown to have good effect in removing blood stasis and protected the Liver and Kidney in TCM [29]. Specifically FZHY has been effectively treated the CHB and other liver diseases with BSS. It has been well reported that, after treated with FZHY, ALT level was brought back to the normal state, and HBV-DNA replication was inhibited, which finally alleviated liver tissue inflammation, necrosis and liver fibrosis [30]. Moreover, FZHY combined with IFN or tenofovir was effective in the treatment of liver fibrosis caused by CHB. A clinical study showed that FZHY markedly decreased ALT and total bilirubin levels, and significantly improved serum albumin and A/G ratio, lowered serum monamine oxidase activities, tissue inhibitor of metalloproteinase-1(TIMP-1), type III procollagen (P-III-P) and LM and also increased urine Hyp content [30].

### 3. Effects of CHMs and Their Compounds on CHB

At present, more and more researches on TCM have been conducted and the effective mechanisms of CHMs on CHB treatment is being gradually clarified. Treatment based on TCM syndrome differentiation can make CHMs achieve the best curative effect. Different combinations of CHMs and/or their compounds showed the multiple activities such as anti-viral activity, liver protection, anti-inflammation, immune regulation and prevention of hepatic fibrosis (**Table 2**).

#### 3.1. Anti-HBV

A number of CHMs show the anti-HBV effect, such as Chaihu (*Bupleuri Radix*), Hangqin (*Scutellaria radix*), Dahuang (*Rheum palmatum* L.), Zhexie (*Alisma orientalis*), Huzhang (*Polygonum cuspidatum*), Yexianzhu (*Phyllanthus niruri*, *amarus* and *nanus*), Kushen (*Sophora flavescens*) and so on.

Some saikosaponins derived from Chaihu, have been found to suppress HBV infection [15]. The aqueous extract of Hangqin was also able to suppress the replication of lamivudine-resistant HBV mutant in human hepatoma cells by suppressing HBV core promoter activity

[19]. Wogonin, an active compound isolated from Hangqin, was found to inhibit HBV DNA by inactivating DNA polymerase [31]. Chrysophanol 8-O- $\beta$ -D-glucoside, an active compound isolated from Dahuang, significantly inhibited HBV DNA replication and viral antigens expressions by suppressing DNA polymerase activity [32]. Jiang, *et al.* have reported that protostane triterpenes derived from Zhexie inhibited the expressions of HBsAg and HBeAg without cytotoxicity *in vitro* [33]. The ethanol or aqueous extract of Huzhang could dose-dependently inhibit the production of HBsAg and HBeAg or HBeAg only [34].

Yexianzhu, including *Phyllanthus niruri*, *amarus* and *nanus*, has been used to treat chronic liver disease for thousands of years. Recently several basic or clinical studies have been assessed the beneficial effects and safety of Yexianzhu for CHB treatment. *Phyllanthus amarus* was reported to significantly increase the negative conversion rate of serum HBeAg compared with control [35]. Liu *et al.* have conducted a systematic review to showed that *Phyllanthus niruri* plus interferon was better than interferon alone, and *Phyllanthus niruri* was better than nonspecific treatment or other CHMs for the negative conversion of HBsAg, HBeAg and HBV DNA [36]. The extract of *Phyllanthus nanus* decreased HBsAg secretion, HBsAg mRNA expression, and HBV replication *in vitro* and in HBV transgenic mice by affecting HBV polymerase and decreasing HBV mRNA accumulation [37,38].

Kushen is traditional to treat several diseases including inflammations such as enteritis, hepatitis and atopic dermatitis. Ye *et al.* have found that the aqueous extract of Kushen possessed anti-HBV activity, the effective substances were oxymatrine, sophoranol and matrine [39]. Other studies also demonstrated the oxymatrine anti-HBV effects *in vitro* or *in vivo* [40, 41]. Ma *et al.* have found that combination of lamivudine with oxymatrine or matrine showed significant inhibitory effects on the secretion of HBsAg, HBeAg, and HBV-DNA than the use of lamivudine alone [42].

In addition, Bufalin derived from the *Cinobufacini* also can inhibit the levels of HBsAg, HBeAg and HBcAg in HepG2.2.15 cells [43].

### 3.2. Liver protection

During the anti-HBV treatment for CHB therapy, the hepato-protective effect is very important. Chaihu showed liver protective effects against  $\text{CCl}_4$  induced liver injury through decreasing the levels of AST, ALT, and the effects of vinegar-baked Chaihu were better than that of raw Chaihu [46]. Saikosaponins from Chaihu, showed remarkable inhibition of D-galactosamine through decreasing the activity of glucose-6-phosphatase and NADPH-cytochrome C reductase and increasing 5-nucleotidase activity [44].

Huangqin decreased the levels of AST, ALT, hyaluronic acid, laminin, and procollagen type III (PCIII), hydroxyproline and MMPs in  $\text{CCl}_4$ -induced liver fibrosis model [45]. Glycyrr-

rhizin derived from Gancao (LicoriceRoot) also can reduce ALT level in chronic HBV carrier and CCL<sub>4</sub> induced acute hepatic injury mice [46-48]. Water extracts of Huzhang improved microcirculation of injured liver tissue and inhibited the adhesion of white blood cells, blood plaque, and liver endothelial cells [49]. Osthole extracted from Shechuangzi (Cnidium monnieri) exerted protective effects against hepatitis, which inhibited anti-Fas antibody-induced elevation of plasma ALT through affecting caspase-3 activity and preventing the development of apoptosis in mice [50]. In addition, Cucurbitacin E also showed a hepato-protective effect through reduced the levels of serum ALT/AST levels,  $\alpha$ -SMA, TIMP-1, and collagen I protein in TAA-treated mice [51].

Periplocoside A (PSA), isolated from Haibiaoxiao (*P. sepium* Bge.) can dramatically decrease the levels of IL-4, IFN- $\gamma$  and serum ALT secretion, which further inhibited the hepatocyte necrosis and protected the liver function *in vivo* [52,53]. Active ingredients of Danshen (*Salvia miltiorrhiza* Bunge) can also exert hepato-protective effect from CCl<sub>4</sub>-induced liver injury in rats. The protective effect may be due to inhibition of NF-kB and p38 [54]. It can accelerate liver cell regeneration by modulating IL-6 and TNF- $\alpha$  mediated signaling pathways, and suppressing the phosphorylation of NF-kB, JNK and ERK [55].

### 3.3. Anti-inflammation

Chronic inflammation is one of the main characteristics of CHB. Therefore, the treatment of CHB must eliminate inflammation. Lin *et al.* have found that Zhizhi extracts reduced LPS-induced nitric oxide (NO), interleukin (IL)-1, IL-6, reactive oxygen species (ROS), and prostaglandin (PGE<sub>2</sub>) production and decreased serum AST and ALT levels in LPS-treated rats, and through suppression of JNK1/2 signaling pathways in BV-2 cell [56]. Saikosaponins from Chaihu also showed the anti-inflammation effects *in vitro* or *in vivo*. SSa have found to inhibit pro-inflammatory cytokines expression in LPS-stimulated macrophages, which may be regulated by MAPK and NF-kB signals pathways or nucleotide-binding oligomerization domain 2 (NOD2)/NF-kB signaling pathway [57,58]. And it also inhibited the production of ROS, TNF- $\alpha$ , IL-8, COX-2, and iNOS in LPS-stimulated human umbilical endothelial cells (HUVECs) by up-regulating of the LXR  $\alpha$ -ABCA1 signaling pathway [59]. Moreover, SSa was also shown to inhibited LPS-induced apoptosis in HUVECs via inhibition of caspase-3 activation and caspase-3-mediated-FAK degradation [60]. Additionally SSd has been reported to inhibit PGE<sub>2</sub> production and intracellular free Ca<sup>2+</sup> concentration ([Ca<sup>2+</sup>]<sub>i</sub>) in C6 rat glioma cells [61].

Other studies show similar anti-inflammatory effect of Huzhang, which can inhibit mRNA expressions of cyclooxygenase 2 (COX-2), TNF-alpha, IL-6, and C-reactive protein [62,63].

### 3.4. Immunomodulation

The main mechanism of chronic HBV infection is that the host has a different degree of specific immune response to the various antigens of HBV. CHB patients had abnormalities in both natural and specific immunity. TCM treatment of CHB is mainly to strengthening body resistance and eliminating evil, which is to inhibit HBV replication by regulating the function of the immune system.

Eugenin and saikochrome, A isolated from the Chaihu MeOH extracts possessed immunosuppressive effect on human peripheral blood T cells via inhibiting CD28-costimulate-dactivation [64]. SSd significantly activated peritoneal macrophages in terms of enhancing phagocytic activity, increased level of cellular lysosomal enzyme, and suppressed the response of plaque-forming cells to heterologous erythrocytes by stimulating T and B cells [65]. And it also modulated lymphocyte activity through suppressing the T cell response and increasing the B cell response to different mitogens and the IL-2/IL-4 production through a receptor-bypassed pathway [66]. When macrophages were treated with ginsan, the production of reactive oxygen/nitrogen components such as NO and hydrogen peroxide ( $H_2O_2$ ) were enhanced. The expression of CD14 and 1-Ab on murine peritoneal macrophages was increased by the treatment with ginsan, while the expression of CD11b was decreased [67]. Huangqi (*Astragalus radix*) has also been traditionally used to strengthen the immune system. The extracts improved the B cells and macrophage activity while decreased the IFN- $\gamma$  levels [68]. Moreover, water-soluble extracts of Huangqi were able to stimulate the proliferation of splenic lymphocytes, as well as increase the mRNA expression of the cytokines (IL-1, IL-6 and TNF) [69]. And it can also increase macrophage count, promote opsonization, via the C3 complement component [70].

### 3.5. Anti-liver fibrosis

There are varying degrees liver fibrosis induced by inflammatory factors in CHB process. The liver fibrosis is the basis of liver cirrhosis, and the control of liver fibrosis is an important link in preventing disease development.

Sun *et al.* have showed that baicalein dose dependently decreased levels of AST, ALT, hyaluronic acid, laminin, and procollagen type III (PCIII) in serum as well as hydroxyproline and MMPs in liver in  $CCl_4$ -induced liver fibrosis model [71]. Moreover, baicalein also alleviated inflammation, destruction of liver architecture, collagen accumulation and expression of PDGF- $\beta$  receptor, indicated baicalein can prevent the activation of stellate cells and liver fibrosis [71]. Chen *et al.* have found Zhizhi attenuates hepatocellular injury and fibrosis in bile duct ligation (BDL) rats and TGF- $\beta$ 1-stimulated human hepatic stellate cells, and decreased serum ALT and AST as well as hydroxyproline in the BDL rats. Additionally a study showed that Zhizhi significantly suppressed the up-regulation of TGF- $\beta$ 1, Smad2 phosphorylation, Col I and  $\alpha$ -SMA in TGF- $\beta$ 1 stimulated LX-2 cells [72].

Moreover, it has been reported that SSd from Chaihu significantly reduced collagen I deposition and ALT level on liver fibrosis rats, and decreased the concentration of TGF- $\beta$ 1 [73]. Similarly, bupleurosides III, VI, IX and XIII and saikosaponin b3 isolated from Chaihu have also the protective effect on the D-galactosamine induced cytotoxicity in primary cultured rat hepatocytes [74]. Salvianonic acid B, a major water soluble component in Danshen, relieves the CCl<sub>4</sub>-induced fibrosis and reverses DMN-induced liver fibrosis in rats, it inhibited the plasmic and nuclear protein expression of Small Mothers Against decapentaplegic deleted 2/3 (Smad 2/3) and significantly inhibited intracellular phosphorylation of Smad2, decreased type I receptor expression and T $\beta$ R binding [29]. Shuifeiji (*Silybum marianum*) has been shown some additional mechanism, which increased the cellular glutathione, regulated cell membrane permeability, and inhibited the transformation of stellate hepatocytes into myofibroblasts in liver [75]. In additional, cucurbitacin E could ameliorate hepatic fibrosis through reduced levels of p-Erk/MAPK,  $\alpha$ -SMA, TIMP-1 and collagen I in activated HSC-T6 cells [51].

#### 4. Conclusions

TCM is a holistic approach to treat liver diseases. It is developing rapidly in clinical practice and theoretical and basic research. However, the mechanisms of function and safety remain incomprehensive and even controversial. Herein, we summarize some CHMs and CHFs and related active compounds, and tried to find out multiple therapeutic effects and their mechanisms. TCM treatment is based on syndrome (ZHENG) differentiation, different TCM syndrome therapy will get different effects although using the same formulas. TCM treatment is not as effective as western medicine in the treatment of anti-HBV drugs, it might be more effective in the treatment of comprehensive regulation thought multi-effects including inhibition of HBV replication, liver protection, anti-inflammation, immune regulation and prevention of hepatic fibrosis. Although most people consider herbal products as natural and safe agents, there have been some clinical case-reports about side-effects or toxicity of herbal products in recent years [76]. More research is not only preparation, standardization, identification of active compounds, effects and toxicological evaluation and mechanisms clarification of CHMs and CHFs, and also investigate how to increase curative effect, reduce side effect and drug resistance by TCM treatment combination with anti-HBV drugs. Moreover, further investigation in well-designed trials with a better understanding of mechanisms, therapeutic effects, and the safety profile, will be helpful for developing effective drugs for CHB treatment.

**Table 1:** Effects and mechanisms of TCM syndrome-based treatment on CHB

TCM syndromes (ZHENGs)	Formulae	Constituents	Effects	Mechanisms	Combination effects	References
Liver Qi stagnation and spleen deficiency syndrome (LSSDS)	Xiao-Chai-Hu-tang	Bupleurum falcatum, Scutellaria baicalensis, Panax ginseng, Zizyphus jujube, Pinellia ternate, Zingiber officinale, and Glycyrrhiza glabra.	Anti-Hepatitis B virus, anti-inflammation, anti-oxidation, immunomodulation, hepatoprotective, anti-hepatic fibrosis properties	Inhibiting HBV DNA and decrease the expression of HBeAg in HepG2.2.15. promoting the clearance of HBeAg; decreasing AST, ALT, hyaluronic acid, laminin, and procollagen type III (PCIII), modulating lymphocyte activity, suppressing the T cell response and increasing the B cell response to different mitogens and the IL-2/IL-4 production	Combated with lamivudine and IFN- $\alpha$ exhibits more effectiveness in serum loss of HBV markers and in improving liver function	(15-18 77-79)
Damp heat stasis syndrome (DHS)	Yin-Chen-Hao-Tang	Artemisia capillaris, Gardenia jasminoides and Rheum rhabarbarum	Anti-HBV activity, hepatoprotective, anti-inflammation,	Exhibiting activity against the secretions of HBsAg and HBeAg, and HBV DNA replication; suppressing the upregulation of TGF- $\beta$ 1, Smad2 phosphorylation, Col I and $\alpha$ -SMA in TGF- $\beta$ 1 stimulated LX-2 cells; reducing nitric oxide (NO), interleukin (IL)-1, IL-6, reactive oxygen species (ROS), and prostaglandin (PGE2) production; decreasing AST and ALT	Combined with entecavir was significantly better than entecavir treatment alone in reducing serum transaminase activity, elevating serum albumin and decreasing the proinflammation cytokines such as TNF- $\alpha$ and IL-6	(21-24, 80)
Liver and kidney Yin deficiency syndrome (LKYDS)	Yi-guan-Jian	Radices glehniae, Radices ophiopogonis, Radix angelicae sinensis, Dried rehmannia root, Lycium barbarum L and Fructus meliae toosendan	Hepatoprotective and immunomodulation,	Inhibiting the migration of bone marrow cells, suppressing the proliferation of both progenitors and hepatocytes; increasing the ratio of OKT4 / OKT8,	Combination of lamivudine to improve immune function, decrease the HBsAg level in patient serum	(26, 27)
Blood stasis syndrome (BSS)	Fu-Zheng-Huan-Yu capsule	Bupleurum falcatum, Cordyceps mycelium, Peach kernel, Gynostemma, Schisandra, Pine pollen	Anti-HBV, anti-inflammatory and	Inhibiting HBV-DNA replication, HBsAg and decreasing the level of serum ALT and AST,	Combinated with adefovir or interferon or tenofovir can significantly improve the liver function	(29, 30)

**Table 2:** Effective mechanisms of CHMs extracts or compounds on CHB treatment

Effects	CHMs	Extracts or compounds	Mechanisms	Cell line/animal model	References
Anti-HBV	Bupleuri radix	Saikosaponin c,	Reducing HBV DNA replication and HBeAg secretion	HepG2.2.15 cells	(17)
		Crude Saikosaponins	Inhibiting the replication of HBV; decreasing the expression of HBsAg	HepG2.2.15 cells	(64)
		Baicalensis	Suppressing HBV core promoter activity	HepG2(32).2.15 cells	(19)
	Scutellaria radix	Wogonin	Inhibiting HBV DNA by inactivating DNA polymerase	HepG2.2.15 cells and DHBV-infected ducks	(31)
	R. palmatum L.	Chrysophanol 8-O-D-glucoside	Inhibiting HBV–DNA polymerase activity; inhibiting HBV DNA replication and expression of viral antigens	HepG2.2.15 cells	(32)
	Artemisia scoparia	90% ethanol extract and 4-pyridone glucoside and two polyacetylene glucosides	Inhibiting HBsAg and HBeAg secretions and HBV DNA replication	HepG2.2.15 cells	(24)
	Alisma orientalis	Protostane triterpenes	Inhibiting HBsAg and HBeAg	HepG2.2.15 cells	(33)
	Polygonum cuspidatum	Extract	Inhibiting HBV DNA production, secretion of HBsAg and HBeAg	HepG2.2.15 cells	(34)
	Phyllanthus niruri	Extract	Inhibiting HBV DNA replication; reducing the HBsAg and HBeAg	CHB patients	(35, 36)
	Phyllanthus nanus	Ethanol extract	Inhibiting HBsAg secretion, HBsAg mRNA expression and HBV replication	Alexander cells, HepG2 2.2.15 cells and duck primary hepatocytes	(37)
	Phyllanthus amarus	Extract	Inhibiting hepatitis B virus polymerase activity; suppressing virus release	HepG2.2.15 cell line and HBV transgenic mice	(38)
	Sophora flavescens	Aqueous extracts and oxymatrine, sophoranol and matrine	Anti-hepatitis B virus activity	HBV infected duck and HepG2.2.15 cells	(39)
		Oxymatrine	Inhibiting HBsAg, HBeAg, and HBcAg expressions	HBV transgenic mice	(41)
		Oxymatrine or matrine	Supressing the HBsAg, HBeAg secretion and HBV-DNA into culture medium	HepG2.2.15 cells	(42)
	Cinobufalini	Extract and Bufalin	Inhibiting the secretion of HBsAg, HBeAg, and HBcAg. also inhibit HBV DNA	HepG2.2.15 cell line	(43)
	Radix bupleuri	Extract	Reducing the hepatic enzyme levels (GOT,GPT, and ALP) and the lipid peroxidation	CCL4 induced acute hepatic injury rat	(81)
		Saikosaponins, especially SSa or SSd	Inhibiting D-galactosamine	CCL4 induced acute hepatic injury rat	(44)

Liver protection	Scutellaria radix	Extract and baicalein	Decreasing AST, ALT, hyaluronic acid, laminin, and procollagen type III (PCIII), hydroxyproline and MMPs	CCL4 induced acute hepatic injury rat	(45)
	Licorice	Glycyrrhizin	Reducing ALT level	carriers, CCL4 induced acute hepatic injury rat	(46-48)
	Polygonum cuspidatum	Water extracts	Improving microcirculation of injured liver tissue ; inhibiting the adhesion of cells	CCL4 induced acute hepatic injury rat; white blood cells, blood plaque and liver endothelial cells	(49)
	Cnidium monnieri	Osthole	Reducing ALT; preventing the development of apoptosis may affect caspase-3 activity.	Anti-Fas antibody induced hepatitis mice	(50)
Anti-inflammation	Cucurbitaceae	Cucurbitacin E	Reducing serum ALT/AST levels, $\alpha$ -SMA, TIMP-1 and collagen I protein expressions	TAA-treated mice	(51)
	Bupleuri radix	Saikosaponin a	Inhibiting pro-inflammatory cytokines such as TNF $\alpha$ , IL-6, IL-8, COX-2, ROS and iNOS through MAPK and NF- $\kappa$ B signals pathways or (NOD2)/NF- $\kappa$ B signaling pathway	Macrophages, HU-VECs or the intestines of septic rats	(57-59)
		Saikosaponin c	Inhibiting LPS-induced apoptosis	HUVECs	(60)
		Saikosaponin d	Inhibiting PGE2 production, activating peritoneal macrophages in terms of enhancement of phagocytic activity,	Rat glioma cells	(61)
	Scutellariae radix	Baicalein	Suppressing the phosphorylation of NF- $\kappa$ B, JNK and ERK; modulating IL-6 and TNF- $\alpha$ mediated signaling pathways	Acute liver failure mouse model	(55)
	Gardenia jasminoides	Extract	Inhibiting LPS-induced iNOS mRNA expression and NO production and cyclooxygenase 2 (COX-2)mRNA expression.	BV-2 microglial cells and LPS treated SD rats	(56)
	Polygonum cuspidatum	Extract	Reducing the expression of IFN- $\beta$ , COX-2, and IL-6	RAW 264.7 macrophages	(62)
		Containing 20% resveratrol	Decreasing mRNA expressions of TNF- $\alpha$ , IL-6, and C-reactive protein and decreasing NF- $\kappa$ B pathways	Mononuclear cell from healthy human	(63, 66)
	Periploca sepium bge	Periplocoside A (PSA)	Reducing the cytokines level including TNF- $\alpha$ , IL-1 and IL-6	CCL4-induced acute liver injury rat	(52, 53)
Salvia miltiorrhiza bunge	Extract	Inhibiting p38 and NF- $\kappa$ B signaling in Kupffer cells.	Primary Kupffer cells	(54)	
Immuno-modulation	Radix bupleuri	Eugenin and saikochrome A	Possessing immunosuppressive effect on human peripheral blood T cells via inhibiting CD28-costimulated activation	Human peripheral blood T cells	64
		Saikosaponin d	Modulating lymphocyte activity through suppressing the T cell response and increasing the B cell response to different mitogens, inhibit OKT3/CD28-costimulated human T cell proliferation and PMA, PMA/ionomycin	Human T cell, and Con A-induced mouse	(65, 66)
	Panax ginseng	Polysaccharide fraction	Inducing phagocytic activity; increasing the expression of CD14 and 1-Ab; decreasing the expression of CD11b	Murine peritoneal macrophages	(67)

Anti- liver fibrosis	Scutellaria radix	Baicalein	preventing the activation of stellate cells and liver fibrosis	CCl4-induced liver fibrosis model	(71)
		Saikosaponins SSd	Reducing collagen I deposition and alanine aminotransferase level; decreasing the concentration of transforming growth factor $\beta$ 1 (TGF- $\beta$ 1)	Liver fibrosis rat	(74)
		Bupleurosides III, VI, IX, and XIII and saikosaponin b3	Exhibiting protective effect on the D-galactosamine	Primary rat hepatocytes	(74)
	Gardenia jasminoides	Extract	Reducing serum alanine aminotransferase, aspartate aminotransferase and hydroxyproline;decreasingTGF-b1, Smad2 phosphorylation, Col I and $\alpha$ -SMA	Bile duct-ligated rats and TGF-b1 stimulated LX-2 cells	(72)
	Salvia miltiorrhiza	Salvianonic acid B	Inhibiting intracellular phosphorylation of Smad2;decreasingtype I receptor expression and T $\beta$ R binding	Hepatic stellate cells	(29, 75)
	Silybum marianum	Flavonolignans	Inhibiting the deposition in collagen fibers	Hepatic stellate cells	(75)
	Cucurbitaceae	Cucurbitacin E	Regulating the expression of $\alpha$ -SMA, TIMP-1 and collagen I	Hepatic stellate cells	(51)

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