Review

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Traditional Chinese medicine and related active compounds: A review of their role on hepatitis B virus infection

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ABSTRACT: Since the significant public health hazard of Hepatitis B virus (HBV) infection and obvious drug resistance and dose-dependent side effects for common antiviral agents (*e.g.*, interferon-alpha, lamivudine, and adefovir), continuous development of agents to treat HBV infection is urgently needed. Traditional Chinese medicine (TCM) is an established segment of the health care system in China. Currently, it is widely used for chronic hepatitis B (CHB) in China and many parts of the world. Over a long period of time in clinical practice and in basic research progress, the effectiveness and beneficial contribution of TCM on CHB have been gradually known and confirmed. Based upon our review of related papers and because of our prior knowledge and experience, we have selected some Chinese medicines, including Chinese herbal formulas (*e.g.*, Xiao-Chai-Hu-Tang, Xiao-Yao-San, and Long-Dan-Xie-Gan-Tang), single herbs (*e.g.*, *Phyllanthus niruri*, *Radix astragali*, *Polygonum cuspidatum*, *Rheum palmatum*, and *Salvia miltiorrhiza*) and related active compounds (*e.g.*, wogonin, artesunate, saikosaponin, astragaloside IV, and chrysophanol 8-*O*-beta-D-glucoside) and Chinese medicine preparations (*e.g.*, silymarin, silibinin, kushenin, and cinobufacini), which seem effective and worthy of additional and in-depth study in treating CHB, and we have given them a brief review. We conclude that these Chinese herbal medicines exhibit significant anti-HBV activities with improved liver function, and enhanced HBeAg and HBsAg sero-conversion rates as well as HBV DNA clearance rates in HepG2 2.2.15 cells, DHBV models, or patients with CHB. We hope this review will contribute to an understanding of TCM and related active compounds as an effective treatment for CHB and provide useful information for the development of more effective antiviral drugs.

Keywords: Hepatitis B virus (HBV), chronic hepatitis B (CHB), traditional Chinese medicine (TCM), active compounds

1. Introduction

Hepatitis B virus (HBV) infection is a serious global public health problem, which can lead to liver failure, acute and chronic hepatitis, liver cirrhosis, and liver cancer. Approximately 2 billion people worldwide are reportedly infected with HBV, and more than 350 million of them are chronic carriers (*1*). It is estimated that worldwide more than 600,000 individuals die from HBV-related liver disease each year (*2*). Vaccination is considered to be the most effective way to control the spread of HBV and implementation of the HBV vaccine has led to a significant reduction in viral transmission; however, it remains highly endemic in many areas of the world, particularly in eastern Asia, India, and Pakistan (*3*). Currently, there are six agents approved for the treatment of chronic hepatitis B (CHB) by the US Food and Drug Administration (FDA), including interferon (interferon-alpha (IFN-α) and pegylated interferon-alpha (peg IFN-α)), nucleoside (lamivudine, entecavir, and telbivudine) and nucleotide analogues (adefovir) (*4*). However, their therapeutic effect is not satisfactory with obvious drug resistance and dose-dependent side effects

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(*5*). Therefore, development of novel antiviral drugs and more effective therapies for the treatment of CHB are urgently needed.

Traditional Chinese medicine (TCM) has been widely used in Asia for more than two thousand years. At present, TCM serves as an established segment of the public health system in China and in recent years it has been gaining interest and acceptance as alternative or complementary medicine in Western countries. An estimated 1.5 billion people now use Chinese herbal medicine, an important category of TCM, for the treatment of various diseases including chronic HBV infection worldwide (*6*). In China, it is used as a treatment adjunct or alternative to anti-HBV drugs and accounts for 30% to 50% of total medicine consumption for CHB treatment (*7*). Because of its low cost and low toxicity, about 80% of the patients with CHB in China rely on Chinese herbal medicine (*8*). A number of clinical trials have been performed to assess the therapeutic efficacy and safety of Chinese herbal medicines in CHB treatment. According to the results from meta-analysis of clinical trials, they indicated that: (*i*) Chinese herbal medicines alone may have an equivalent or better effect when compared with interferon or lamivudine in CHB treatment as evidenced by HBeAg and HBsAg sero-conversion as well as HBV DNA clearance; (*ii*) Chinese herbal medicines combined with interferon or lamivudine significantly enhanced the anti-viral activities of these agents; (*iii*) Chinese herbal medicines have a beneficial effect on improving liver function (*7,8*).

Although Chinese herbal medicines are widely used in the clinic in China, their active ingredients are complex and their mechanisms in CHB treatment are not clear, so that at present it is difficult for them to be popular in the world. In recent decades, as the HBV-transfected cell lines and duck HBV (DHBV) model are widely used for the study of anti-HBV drugs *in vitro* and *in vivo*, the anti-viral effects of many Chinese herbal medicines and their active ingredients have been gradually known and confirmed, which will provide evidence for developing novel antiviral drugs. Therefore, in this article, the authors review some Chinese herbal medicines, including Chinese herbal formulas, single herbs and their active ingredients, and Chinese medicine preparations, which are widely reported to have antiviral effects in basic or clinical studies. We hope this review will contribute to an understanding of Chinese herbal medicines as an effective treatment for CHB and provide useful information for the development of more effective antiviral drugs.

2. Traditional Chinese herbal formulas commonly used with anti-HBV activities

Traditional Chinese herbal formulas (or Kampo in Japanese) are a combination of compatible herbs in fixed dosages, most of which come from classical or well-known Chinese textbooks of medicine (*e.g.*, "Shang Han Lun" and "Jin Gui Yao Lue", two classics of traditional medicine edited by Zhang Zhongjing, a well-known Chinese physician during the Han Dynasty) (*9*). Currently, several traditional Chinese herbal formulas, such as Xiao-Chai-Hu-Tang (also called Sho-saiko-to in Japan), have been found to have a potentially beneficial effect for treating CHB (*10*). A brief outline of the antiviral pharmacology of the most commonly used traditional Chinese herbal formulas is presented below (Table 1).

2.1. *Xiao-Chai-Hu-Tang*

Xiao-Chai-Hu-Tang, a famous traditional Chinese herbal formula originally recorded in "Shang Han Lun", has been used to treat liver diseases especially chronic hepatitis for thousands of years in China and Japan. It consists of seven medicinal herbs (*Bupleurum falcatum*, *Scutellaria baicalensis*, *Panax ginseng*, *Zizyphus jujube*, *Pinellia ternate*, *Zingiber officinale*, and *Glycyrrhiza glabra*) (*11*). Much pharmacological research has shown that Xiao-Chai-Hu-Tang has potent anti-inflammation, anti-oxidation, immunomodulation, hepatoprotective, anti-hepatic fibrosis, and antitumor properties (*12-16*). Recently, a lot of basic or clinical studies have been conducted to assess the beneficial effects and safety of Xiao-Chai-Hu-Tang for CHB treatment. Chang *et al.* found that Xiao-Chai-Hu-Tang could inhibit the replication of HBV DNA and decrease the expression of HBeAg in HepG2 2.2.15 cells (*17*). Tajiri *et al.* found that Xiao-Chai-Hu-Tang could promote the clearance of HBeAg in children with chronic HBV infection (*18*). Qin *et al.* gave a systematic review of randomized trials on treatment of CHB using Xiao-Chai-Hu-Tang (*19*). Sixteen randomized trials (involving 1,601 CHB patients) were included in this review. The pooled results showed that Xiao-Chai-Hu-Tang combined with antiviral drugs (*e.g.*, lamivudine and IFN-α) was more effective in serum loss of hepatitis B viral markers and in improving liver function compared to antiviral drugs alone. Moreover, there were no adverse effects reported in the trials regarding Xiao-Chai-Hu-Tang.

2.2. *Xiao-Yao-San*

Xiao-Yao-San is a famous Chinese herbal formula originally recorded in "Tai Ping Hui Min He Ji Ju Fang" (a classical Chinese medicine book of the Song Dynasty). It is a mixture of eight crude herbs (*Bupleurum falcatum*, *Angelica sinensis*, *Paeonia lactiflora*, *Atractylodes lancea*, *Wolfiporia cocos*, *Zingiber officinale*, *Mentha arvensis*, and *Glycyrrhiza uralensis* (*20*). This herbal prescription is reported to possess hepatoprotective, anti-inflammation, anti-oxidation, anti-cancer, and immunomodulation activities, and is commonly used in the clinic to treat functional dyspepsia, postmenopausal women with

Table 1. Traditional Chinese herbal formulas commonly used with anti-HBV activities

Common name	Source	Composition	Biological activity	Evidence of anti-HBV activity	*Ref.*
Xiao-Chai-Hu-Tang	"Shang Han Lun" (the Eastern Han Dynasty (25-220 AD))	Includes 7 herbs: *Bupleurum falcatum*, *Scutellaria baicalensis*, *Panax ginseng*, *Zizyphus jujube*, *Pinellia ternate*, *Zingiber officinale*, *Glycyrrhiza glabra*	Hepatoprotective, anti-hepaticfibrosis, anti-inflammation, anti-oxidation, immunomodulation, anti-tumor	In HepG2 2.2.15 cells: inhibits HBV DNA replication and HBeAg secretion; In patients: (*i*) promotes the clearance of HBeAg; (*ii*) combined with antiviral drugs (*e.g.* lamivudine and IFN-α) exhibits more effectiveness in serum loss of HBV markers and in improving liver function	*11-19*
Xiao-Yao-San	"Tai Ping Hui Min He Ji Ju Fang" (the Song Dynasty (960-1279 AD))	Includes 8 herbs: *Bupleurum falcatum*, *Angelica sinensis*, *Paeonia lactiflora*, *Atractylodes lancea*, *Wolfiporia cocos*, *Zingiber officinale*, *Mentha arvensis*, *Glycyrrhiza uralensis*	Hepatoprotective, anti-inflammation, anti-oxidation, immunomodulation, anticancer	In patients: improves the negative conversion rates of HBV markers (HBeAg and HBV-DNA) and liver function	*20-23*
Long-Dan-Xie-Gan-Tang	"Tai Ping Hui Min He Ji Ju Fang" (the Song Dynasty (960-1279 AD))	Includes 10 herbs: *Gentiana scabra*, *Scutellaria baicalensis*, *Gardenia jasminoides*, *Alisma plantago*, *Plantago asiatica*, *Akebia trifoliate*, *Rhemannia glutinosa*, *Angelica sinensis*, *Bupleurum chinense*, *Glycyrrhiza uralensis*	Anti-inflammation, anti-oxidation, hepatoprotective, immunomodulation, anti-herpetic virus, anti-HBV	In CCl_4-induced hepatic injury rats: improves liver function; In patients: combined with IFN-α improves the negative conversion rates of HBeAg	*24-27*

climacteric symptoms, premenstrual dysphoric disorder, mood stabilizer swings, insomnia, depressive disorders, breast cancer, and so on (*21*). Recently, some studies have shown that Xiao-Yao-San has a potent effect on treating CHB. Xiao-Yao-San could improve the clinical symptoms (*e.g.*, weak, inappetence, and hepatalgia) of patients with CHB. Furthermore, the patients' liver function (ALT and AST) and liver fibrosis indexes, including hyaluronic acid (HA), laminin (LN), pro-collagen III peptide (P-III-P), and collagen type IV (IV-C), were improved significantly after treatment with Xiao-Yao-San (*22*). Furthermore, Xiao-Yao-San combined with adefovir dipivoxil could significantly improve the negative conversion rates of HBeAg and HBV-DNA in the treatment of CHB (*23*). Although Xiao-Yao-San has its unique advantages in treating CHB, a far larger body of literature only exists in Chinese language journals. It is reasonable to publish some well-designed, efficacy-based basic or clinical trials to evaluate the efficacy of Xiao-Yao-San in treating CHB in the future in Englishlanguage journals.

2.3. *Long-Dan-Xie-Gan-Tang*

Long-Dan-Xie-Gan-Tang is a famous Chinese herbal formula which also originally came from "Tai Ping Hui Min He Ji Ju Fang". It was recorded to have inhibitive effects on inflammatory diseases of the liver or gall bladder. There are ten medicinal herbs in Long-Dan-Xie-Gan-Tang including *Gentiana scabra*, *Scutellaria baicalensis*, *Gardenia jasminoides*, *Alisma plantago*, *Plantago asiatica*, *Akebia trifoliate*, *Rhemannia glutinosa*, *Angelica sinensis*, *Bupleurum chinense* and *Glycyrrhiza uralensis*. Recently, much pharmacological research has shown that Long-Dan-Xie-Gan-Tang has potent anti-inflammation, anti-oxidation, immune modulation, anti-herpetic virus, and anti-HBV properties (*24,25*). It is reported that Long-Dan-Xie-Gan-Tang is the most commonly prescribed Chinese herbal formula for subjects with CHB in Taiwan (*25*). It has a hepatoprotective effect on CCl4-induced hepatic injury in rats with the level of serum ALT and AST decreased significantly after treatment with Long-Dan-Xie-Gan-Tang (*26*). In addition, Long-Dan-Xie-Gan-Tang combined with IFN-α could significantly improve the negative conversion rates of HBeAg in the treatment of CHB (*27*).

3. Single Chinese herbs commonly used with anti-HBV activities

In 1982, Thyagarajan *et al.* confirmed *Phyllanthus niruri* with anti-HBV activity for the first time. Since then, the anti-HBV activity of single Chinese medicines has gradually got the attention of researchers. Many single Chinese medicines have been found to have potentially beneficial effects treating CHB. However, because the majority of herbs were administered in combination with other herbs in Chinese herbal formulas, it is not possible to determine exactly which

individual herbs in the formulas have the greatest therapeutic potential in the treatment of CHB. Thus, based upon our review of the related papers and upon our prior knowledge and experience, we have selected some herbs (Table 2) that seem effective and worthy of additional and in-depth study, and we will give them a brief commentary below. Especially these 5 herbs (*Phyllanthus niruri*, *Radix astragali*, *Polygonum cuspidatum*, *Rheum palmatum*, and *Salvia miltiorrhiza*) will be introduced in detail concerning their anti-HBV activities.

3.1. *Phyllanthus niruri*

Phyllanthus niruri (Ye Xia Zhu or Zhen Zhu Cao) is widely distributed in most tropical and subtropical countries of the globe (*e.g.*, China, South Asia, and America) and have long been used as traditional medicines to treat chronic liver disease, as well as a wide number of traditional ailments such as kidney disease, urinary bladder and intestinal infections, jaundice, gonorrhea, frequent menstruation, diabetes, skin ulcers, sores, swelling, and itchiness (*28-30*). It is reported to have many pharmacological effects including antiviral, antibacterial, antihepatotoxic, antihypertensive, and anticancer properties (*31,32*). Many active compounds, such as gallic acid, geraniin, quercetin glucoside, and quercetin rhamnoside, have been identified from *Phyllanthus niruri* (*32*). Since *Phyllanthus niruri* has been used to treat chronic liver disease for thousands of years, a lot of basic or clinical studies have been conducted recently to assess the beneficial effects and safety of *Phyllanthus niruri* for CHB treatment. Lam *et al.* showed that the ethanolic extract of *Phyllanthus niruri* exhibited potent antiviral activity against HBV (*33*). It produced a suppressive effect on HBsAg secretion, HBsAg mRNA expression, and HBV replication in vitro. Liu *et al.* conducted a systematic review of randomized trials on the treatment of CHB using *Phyllanthus niruri* (*34*). Twenty-two randomized trials (n = 1,947) were included in this review. The combined results showed that: (*i*) *Phyllanthus niruri* had a positive effect on clearance of serum HBsAg compared with placebo or no intervention; (*ii*) There was no significant difference on clearance of serum HBsAg, HBeAg and HBV DNA between *Phyllanthus niruri* and IFN; (*iii*) There was a better effect of *Phyllanthus niruri* plus IFN combination

Table 2. Single Chinese herbs commonly used with anti-HBV activities

Common name	Name in Chinese	Major active compounds	Biological activity	Evidence of anti-HBV activity	*Ref.*
Phyllanthus niruri	Ye Xia Zhu, Zhen Zhu Cao	Gallic acid, ellagic acid geraniin, quercetin glucoside, quercetin rhamnoside	Antiviral, antibacterial, antihepatotoxic, antihypertensive, anticancer	In HepG2 2.2.15 cells: inhibits HBsAg secretion, HBsAg mRNA expression, and HBV replication; In patients: combined with IFN exhibits more effectiveness on the clearance of serum HBsAg, HBeAg and HBV DNA	*33,34*
Radix astragali	Huang Qi	Astragaloside, calycosin-7-*O*-beta-D-glucoside, calycosin, formononetin	Immunomodulation, anticancer, anti-fatigue, antiviral	In patients: improves the negative conversion rates of HBeAg and HBV DNA	*35-37*
Polygonum cuspidatum	Hu Zhang	Resveratrol, polydatin	Antiviral, antimicrobial, hepatoprotective, neuroprotective, cardioprotective	In HepG2 2.2.15 cells: inhibits HBeAg secretion and HBV DNA replication	*8,38-40*
Rheum palmatum	Da Huang	Emodin, rhein, sennoside A, chrysophanol	Antiviral, antibacterial	In HepG2 2.2.15 cells: inhibits HBV DNA production and HBsAg secretion; In DHBV models: inhibits HBV DNA production	*38, 41-44*
Salvia miltiorrhiza	Dan Shen	Tanshinone, salvianic acid, protocatechuic aldehyde, cryptotanshinone	Antiviral, antibacterial, hepatoprotective cardioprotective, anti-thrombosis	In patients: improves the negative conversion rates of HBeAg and liver function	*46-48*
Curcuma longa	Jiang Huang	Curcumin, demethoxycurcumin, bisdemethoxycurcumin	Antiviral, anti-inflammation, anti-oxidation, anticancer	In HepG2.2.15 cells: suppresses the secretion of HBsAg, the production of HBV particles and the level of intracellular HBV RNAs	*35,49*
Glossogyne tenuifolia	Lu Jiao Cao	Luteolin-7-*O*-β-D-glucopyranoside, luteolin	Anti-inflammation, antiviral, antipyretic, hepatoprotective	In PLC/PRF/5 cells: inhibits HBsAg secretion	*50*
Arenaria kansuensis	Xue Ling Zhi	Arenarine, tricin, β-sitosterol-3β-D-glucopyranoside, isoscoparin	Antiviral, immunomodulation, hepatoprotective	In HepG2.2.15 cells: inhibits HBsAg and HBeAg secretion	*51*

on clearance of serum HBeAg and HBV DNA than IFN alone; (*iiii*) No serious adverse event was reported.

3.2. *Radix astragali*

Radix astragali (Huang Qi) has been used in China for thousands of years and is one of the most widely prescribed Chinese herbs in many formulas. The major active constituents of *Radix astragali* are saponins and flavonoids, such as astragaloside, calycosin-7-*O*-beta-D-glucoside, calycosin, and formononetin. It is traditionally considered to be a tonic that can improve the functioning of the lungs, adrenal glands, and the gastrointestinal tract, increase metabolism, promote healing, and reduce fatigue (*35*). Currently, some reports have indicated that *Radix astragali* possess many pharmacologic activities including immunomodulatory, anticancer, anti-fatigue, and antiviral activities (*35,36*). Moreover, it can balance serum hormone levels and improve liver function in patients with chronic viral hepatitis (*37*). A clinical evaluation of *Radix astragali* was performed in 208 patients with CHB (*37*). The treatment group (n = 116) was treated with the *Radix astragali* compound (containing *Radix astragali* and adjuvant components), and the control group (n = 92) was treated with regular drugs used for viral hepatitis. The results indicated that negative conversion rates of HBeAg and HBV DNA were significantly higher in the treatment group than in the control group.

3.3. *Polygonum cuspidatum*

Polygonum cuspidatum (Hu Zhang), as an herbaceous perennial plant, is widely distributed in the world and has been used as folk medicine in countries such as China, Japan and Korea for thousands of years. It is frequently prescribed by TCM practitioners for the treatment of hepatitis, cough, jaundice, amenorrhea, leucorrhoea, arthralgia, hyperlipidemia, scalding and bruises, snake bites, and carbuncles, *etc.* (*38*). The major active compounds isolated from this herb include resveratrol, polydatin, and anthraquinones (*e.g.*, emodin and its glycoside) (*39*). Recent pharmacological and clinical studies have indicated that *Polygonum cuspidatum* has antiviral (*e.g.*, HBV and HIV), antimicrobial, hepatoprotective, neuroprotective, and cardioprotective functions (*39,40*). It is also reported that *Polygonum cuspidatum* is widely used for treating CHB. Zhang *et al.* published a review to summarize and critically meta-analyze the results of randomized, controlled, clinical trials of TCM formulations reported from China in 1998-2008 for treatment of CHB. They found that *Polygonum cuspidatum* is ranked in the top five of individual herbs used most frequently in TCM formulations for CHB (*8*). Chang *et al.* found that the water extract of *Polygonum cuspidatum* at higher concentrations (30 μg/mL) could inhibit the expression of HBeAg. Furthermore, the ethanol extract of *Polygonum cuspidatum* could inhibit the production of HBV DNA dose-dependently with an effective minimal dosage (10 μg/mL) (*40*).

3.4. *Rheum palmatum*

Rheum palmatum (Da Huang) is an important Chinese medicinal herb with a long history of over 2,000 years and has been commonly used as an antibacterial or laxative agent in treating gastroenteritic and viral diseases (*38*). About 200 chemical compounds have been isolated or identified in *Rheum palmatum*, among which anthraquinone and its derivatives (*e.g.*, emodin, rhein, sennoside A, and chrysophanol) are considered as the main active ingredients (*41*). Recent pharmacological and clinical studies have indicated that *Rheum palmatum* and its active ingredients have showed activities against some viruses including vesicular stomatitis virus, herpes simplex virus types 1 and 2, parainfluenza, vaccinia virus, human cytomegalovirus and poliovirus (*42*). It is also reported that *Rheum palmatum* could inhibit HBV. Both the aqueous extract and ethanol extract of *Rheum palmatum* demonstrated inhibitory effects on HBV DNA production and HBsAg expression in HepG2.2.15 cells (*42,43*). Furthermore, in DHBV models, the aqueous extract of *Rheum palmatum* showed suppression of plasma HBV DNA levels and HBV DNA polymerase activity (*44*).

3.5. *Salvia miltiorrhiza*

Salvia miltiorrhiza (Dan Shen) was originally recorded in "Shen Nong Ben Cao Jing" (a classical Chinese herbal medicine book of the Dong-Han Dynasty). As a promoting blood circulation and removing blood stasis herb of the nontoxic superior class, it has been widely used for more than 2000 years to prevent and treat various human diseases, such as hepatitis, coronary artery disease, apoplexy, tumor growth and immunological disorders (*45*). Currently, there are numerous pharmaceutical dosage forms (*e.g.*, tablets, capsules, granules, injections, and oral liquids) of Chinese medicine preparations containing *Salvia miltiorrhiza*, such as Fufang Dan Shen tablet, Dan Shen injection, and Fufang Dan Shen dripping pills, which are commercially widely available for use in clinics in China (*38*). According to the pharmacological investigations, the major active constituents of *Salvia miltiorrhiza* can be divided into two groups: the water soluble phenolic acids such as, salvianic acid, protocatechuic aldehyde, rosemarinic acid and salvianolic acid B, and the lipophilic tanshinones such as, tanshinone I, dihydrotanshinone I, cryptotanshinone, tanshinone IIA, tanshinone IIB and hydroxtanshinone (*46*). According to pharmacological and clinical studies,

Salvia miltiorrhiza and its active constituents are not only used in coronary artery disease but also widely used for treating CHB. In a clinical evaluation, 30 patients with CHB were treated with *Salvia miltiorrhiza* (*47*). After 3 months of treatment, the negative conversion rate of HBeAg was 16.7%. A follow up of 3 and 9 months after the end of treatment showed the negative conversion rates of HBeAg were 22.7% and 25.0%, respectively. Ye *et al.* found that different dosages of *Salvia miltiorrhiza* injection (Dan Shen injection, 8 mL, 16 mL, and 24 mL) could improve the clinical symptoms and significantly reduce the level of ALT, total bilirubin (TBIL), and liver fibrosis indexes (pro-collagen type III (PC III), collagen type IV (IV-C) and hyaluronic acid (HA)) in hepatitis B cirrhosis patients. Furthermore, the large dosage (24 mL) of *Salvia miltiorrhiza* injection had the best effect treating the HBV-induced cirrhosis patients, particularly in patients with compensated cirrhosis (*48*).

3.6. *Others*

Except for the above 5 herbs, we will give a brief commentary on some other Chinese herbs commonly used for treating CHB. *Curcuma longa* (Jiang Huang), a rhizomatous herbaceous perennial plant of the ginger family, has been used for treating various liver diseases caused by HBV in Asia for many years with antiviral, anti-inflammation, and anti-oxidation activities (*35,49*). Kim *et al.* found that the aqueous extract of *Curcuma longa* could suppress the secretion of HBsAg, the production of HBV particles and the level of intracellular HBV RNAs in HepG2.2.15 cells (*49*). They also found that the anti-HBV activity of *Curcuma longa* was mediated through enhancing the cellular accumulation of p53 protein by trans-activating the transcription of the p53 gene as well as increasing the stability of p53 protein. *Glossogyne tenuifolia* (Lu Jiao Cao) is a special medicinal plant of the compositae family in the Pescadores Islands. It has been shown to exhibit good anti-inflammatory and antiviral activity, as a traditional antipyretic and hepatoprotective herb used in Chinese medicine. Wu *et al.* found that *Glossogyne tenuifolia* had potent anti-HBV effects on the human hepatocellular carcinoma cell line PLC/PRF/5 (*50*). *Glossogyne tenuifolia* exhibited a dose-dependent inhibition of the release of HBsAg by repressing the expression of HBsAg with an IC_{50} of 0.093 mg/mL. *Arenaria kansuensis* (Xue Ling Zhi) is from the highest elevation for flowering green plants in the world. It belongs to the family Caryophyllaceae and is mainly located in the Qinghai-Tibet Plateau near the permanent snowline around 4,700 to 5,500 meters above sea level. *Arenaria kansuensis* has been shown to exhibit good antiviral and immunomodulation activity. Tang *et al.* found that both the ethanol extract and aqueous extract of *Arenaria kansuensis* could inhibit the release of HBsAg and HBeAg in HepG2.2.15 cells (*51*). Moreover, the aqueous extract of *Arenaria kansuensis* exhibited more obvious anti-HBV activity with lower toxicity than that of the ethanol extract. The maximum inhibition rates of the aqueous extract of *Arenaria kansuensis* on the levels of HBsAg and HBeAg at 96 h were 52.5% and 72.8%, respectively.

4. The active compounds of Chinese medicines commonly used with anti-HBV activities

Although Chinese medicines play an important role in drug discovery and human health, the actual value of them has not been fully recognized worldwide due to their complex components and uncontrollable quality. In recent years, with the developing modernization of TCM and continuing emergence of new theories, methods and techniques, very rapid and significant development has been achieved in the pharmacology of TCM. Many active compounds of Chinese medicines have been found and their activities have been studied. Some active compounds isolated from Chinese herbal medicines have been reported to possess anti-HBV activities. Zuo *et al.* gave a summary of the active compounds of Chinese herbal medicines with anti-HBV activities (*52*). They reported that these anti-HBV active compounds mainly included alkaloids, flavonoids, terpenoids, glycosides, lignans, plant polyphenols, saccharides, and so on. Based upon our review of the related papers, we have selected some active compounds of Chinese herbal medicines with anti-HBV activities (Table 3) that seem effective and worthy of additional and in-depth study in treating CHB, and we will give them a brief commentary below.

4.1. *Alkaloids*

Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms, which can be isolated from many Chinese herbal medicines. Matrine, oxymatrine, sophoridine and sophocarpine are the major bioactive alkaloids extracted from Chinese herbal medicine *Sophora flavescens* (Ku Shen) (*28*). Ye *et al.* found that the aqueous extract of *Sophora flavescens* possessed anti-DHBV activity (*53*). Furthermore, they found the above four alkaloids (matrine, oxymatrine, sophoridine and sophocarpine) were identified in the duck serum, of which oxymatrine, sophoranol and matrine were the effective substances for anti-HBV activity in aqueous extracts of *Sophora flavescens*. Ma *et al.* investigated the anti-HBV activity of the combination of 3TC and either oxymatrine or matrine on HepG2 2.2.15 cells (*54*). They found that the combination of 3TC (30 μg/mL) with oxymatrine (100 μg/mL) or matrine (100 μg/mL) showed significant inhibitory effects on the secretion of HBsAg, HBeAg, and HBV-DNA into culture media, that were higher

Table 3. Typical active compounds of Chinese medicines commonly used with anti-HBV activities

Category	Typical compounds	Source	Biological activity	Evidence of anti-HBV activity	*Ref.*
Alkaloids	Oxymatrine	*Sophora flavescens* (Ku Shen)	Antiviral, antifibrotic, hepatoprotective, immunomodulation	In HepG2.2.15 cells: inhibits the secretion of HBsAg, HBeAg, and HBV-DNA; In patients: combined with lamivudine exhibits higher HBeAg/anti-HBe seroconversion rate	*28,53-58*
Flavonoids	Wogonin	*Scutellaria baicalensis* (Huang Qin)	Antiviral, hepatoprotective	In MS-G2 cells: suppresses HBsAg secretion and HBV DNA production; In HepG2.2.15 cells: inhibits the secretion of HBsAg, HBeAg, and HBV-DNA; In DHBV models: inhibits DHBV DNA polymerase; In human HBV-transgenic mice: inhibits plasma HBsAg levels	*28,60,61*
	Ellagic acid	*Phyllanthus niruri* (Ye Xia Zhu, Zhen Zhu Cao)	Antiviral, immunomodulation	In HepG2 2.2.15 cells: inhibits HBeAg secretion; In HBeAg-producing transgenic mice: blocks immune tolerance caused by HBeAg	*62,63*
Terpenoids	Artesunate	*Artemisia annua* (Qing Hao)	Antiviral, antimalarial, antipyretic, anti-inflammation	In HepG2 2.2.15 cells: (*i*) inhibits HBsAg secretion and HBV DNA production; (*ii*) exhibits a synergic anti-HBV effect combined with lamivudine	*66,67*
Glycoside	Saikosaponin c	*Bupleuri radix* (Chai Hu)	Anti-hepatitis, anti-nephritis, antihepatoma, anti-inflammation, immunomodulation, antibacterial	In HepG2 2.2.15 cells: inhibits HBsAg secretion and HBV DNA production	*68*
	Astragaloside IV	*Radix astragali* (Huang Qi)	Antiviral, anti-oxidation, anti-inflammation, anti-cancer, immunomodulation, regulation of the calcium balance	In HepG2 2.2.15 cells: suppresses the secretion of HBsAg and HBeAg; In DHBV models: reduces serum DHBV DNA levels	*36,41, 69*

than or equivalent to the use of 3TC alone at 100 μg/mL. Chen *et al.* conducted a study to investigate the effect of lamivudine, IFN-α and the combination of lamivudine and oxymatrine on surviving hepatic failure patients with HBV infection (*55*). They found that the HBeAg/anti-HBe seroconversion rate in patients treated with the combination of lamivudine and oxymatrine was lower than that in patients treated with IFN-α, but was higher than that in patients treated with lamivudine alone. Moreover, lamivudine or lamivudine in combination with oxymatrine significantly inhibited the intrahepatic inflammatory activities of subacute or acute-on-chronic hepatic failure survivals. Li *et al.* prepared liposome-encapsulated matrine and studied its anti-HBV effect on HepG2 2.2.15 cells and DHBV models (*56*). They found that liposome-encapsulated matrine could evidently inhibit the replication of hepatitis B virus *in vitro* and *in vivo*, and its anti-HBV effect was better than that of matrine. Nie *et al.* conducted a series of studies to investigate the anti-HBV activities of sophoridine and sophocarpine in HepG2 2.2.15 cells (*57,58*). The results showed that sophoridine could significantly inhibit the secretion of HBsAg, HBeAg, and pre-antigen S1. When the HepG2 2.2.15 cells were treated with 0.001 μmmol/L sophoridine for 9 days, the inhibition rate of the secretion of pre-antigen S1 was 62.20%. Furthermore, they made a comparison between sophocarpine and lamivudine on inhibiting the secretion of HBeAg. The results showed that the inhibition rate of the secretion of HBeAg in the sophocarpine group was higher than that in the lamivudine group. In summary, these alkaloids are much cheaper than INF-α for the treatment of CHB, which makes them attractive therapeutic options and warrants further clinical and basic trials. Moreover, if some other alkaloids possess anti-HBV activity they need further study.

4.2. *Flavonoids*

Flavonoids are a group of plant secondary metabolites with variable phenolic structures and can be found in many Chinese herbal medicines. They are usually divided into seven classes including flavonols, flavones, flavanones, flavononol, flavanols, isoflavones, and anthocyanidins. Some of these flavonoids have been reported to have activities in treatment of various diseases such as heart disease, cancer, and virus infection (*e.g.*, HBV and HCV) as well as potential protective activity against artificially induced-liver damage (*59*).

Wogonin is a flavone derived from the Chinese herbal medicine *Scutellaria baicalensis* (Huang Qin), which has been widely used for treatment of inflammatory and liver diseases for thousands of years in Asia (*28*). In recent years, wogonin has been found to have anti-HBV activity. Huang *et al.* found that

wogonin could suppress HBsAg secretion and HBV DNA production in a HBV transfected liver cell line (MS-G2) without cytotoxicity (*60*). Guo *et al.* found that wogonin effectively suppressed the secretion of HBsAg and HBeAg with an IC_{50} (drug concentration inducing 50% inhibition in HBsAg or HBeAg or HBV DNA release) of 4 μg/mL and reduced HBV DNA levels in a dose-dependent manner in HepG2.2.15 cells (*61*). They also found that in DHBV-infected ducks wogonin dramatically inhibited DHBV DNA polymerase with an IC50 of 0.57 μg/mL, and significantly improved duck liver function in histopathological evaluations. In addition, wogonin significantly reduced plasma HBsAg levels in human HBV-transgenic mice.

Ellagic acid, a flavonoid isolated from Phyllanthus niruri, exhibited a unique anti-HBV function in a HBV infected cell line and in HBeAg transgenic mice. It has been found to effectively block HBeAg secretion in HepG2 2.2.15 cells with an IC50 of 0.07 μg/mL, but does not have any effects on HBV polymerase activity, HBV replication or blockage of HBsAg secretion (*62*). Furthermore, since HBeAg is involved in immune tolerance during HBV infection, ellagic acid might be a new candidate therapeutic against immune tolerance in HBV-infected individuals. It could effectively block the immune tolerance caused by HBeAg in HBeAg-producing transgenic mice (*63*).

Oenanthe javanica or water dropwort (Xi Qin), mainly cultivated in east Asian countries such as China, Korea and Japan, is not only consumed as a spicy vegetable with good amounts of vitamins and great taste, but also as a Chinese herbal medicine widely used in treatment of various diseases including, jaundice, hypertension, polydipsia, and CHB (*64*). Wang *et al.* conducted a study to investigate the antiviral effect of *Oenanthe javanica* flavones on the human hepatoma HepG2.2.15 culture system and DHBV infection (*65*). They reported that *Oenanthe javanica* flavones comprised approximately 2.2% of the whole plant content and were one of the main active ingredients against HBV. *Oenanthe javanica* flavones significantly inhibited HBsAg and HBeAg secretion in HepG2.2.15 cells after 9 days of treatment. Moreover, DHBV-DNA levels decreased significantly after treatment with 0.50 and 1.00 g/kg of Oenanthe javanica flavones in the DHBV model. However, it is worthy of further study to find which *Oenanthe javanica* flavones possess the highest anti-HBV activity.

4.3. *Terpenoids*

Terpenoids are the largest and most widespread class of secondary metabolites. They can be found in all classes of living things especially in Chinese herbal medicines. Terpenoids are a rich reservoir of candidate compounds for drug discovery and they are under investigation for antibacterial, anti-neoplastic, antiviral and other pharmaceutical functions. Recently, some of these terpenoids have been reported to have anti-HBV activities.

Artemisia annua (Qing Hao) has been used as a Chinese herbal medicine to treat fever and malaria in China for thousands of years. Artemisinin is a family of sesquiterpene trioxane lactones derived from *Artemisia annua* and it has been identified as the best medicine with the highest efficiency, the most effective and the lowest toxicity in treating ague, which represents one of the great events in medicine in the latter third of the 20th Century (*66*). In recent years, artemisinin and its derivative artesunate has been reported to have an antiviral effect against HBV. Both artemisinin and artesunate inhibit HBsAg secretion and HBV DNA production in HepG2 2.2.15 cells at concentrations at which host cell viability was not affected, and artesunate had better anti-HBV effects than artemisinin. Artesunate inhibited HBsAg secretion with an IC_{50} of 2.3 μmol/L and reduced the HBV DNA level with an IC_{50} of 0.5 μmol/L. In addition, although the anti-HBV effect of artesunate was not as good as lamivudine which inhibited HBsAg secretion with an IC_{50} of 0.2 μmol/L and reduced the HBV DNA level with an IC_{50} of 0.3 μmol/L, and by combining both agents, a synergic anti-HBV effect could be observed (*67*). This warrants further evaluation of artemisinin and artesunate as antiviral agents against HBV infection.

4.4. *Glycoside*

Glycosides are the major active ingredients isolated from Chinese herbal medicines. They are rich with candidate compounds for drug discovery and possess many pharmaceutical functions including antibacterial, anti-neoplastic, antiviral, and so on. Recently, some of the glycosides have been reported to have anti-HBV activities.

Bupleuri Radix (Chai Hu) is one of the most important traditional Chinese crude drugs for treating hepatitis, malaria and intermittent fever. Saikosaponins, the main active constituents of *Bupleuri Radix*, have been shown to possess various biological activities, specifically anti-hepatitis, anti-nephritis, antihepatoma, anti-inflammation, immunomodulation, and antibacterial effects. Chiang *et al.* conducted a study to evaluate the cytotoxicity and anti-HBV activities of saikosaponins a, c and d. The results showed that, compared with saikosaponins a and d, saikosaponin c showed a significant effect on inhibiting HBsAg secretion and HBV DNA production without cytotoxicity in HepG2 2.2.15 cells (*68*).

Astragaloside IV, a cycloartane-type triterpene glycoside, is one of the major active constituents of *Radix astragali*. It is used as a marker compound for quality control of *Radix astragali* in the Chinese Pharmacopoeia (2005 version), and has various

pharmacological activities including antiviral, anti-oxidation, anti-inflammation, anti-cancer, immunomodulation, regulation of the calcium balance, and so on (*69*). Wang *et al.* conducted a study to investigate the anti-HBV activities of astragaloside IV in HepG2 2.2.15 cells and DHBV-infected ducklings (36). The results showed that astragaloside IV effectively suppressed the secretion of HBV antigens with inhibition rates of 23.6% for HBsAg and 22.9% for HBeAg at100 μg/mL after 9 days of treatment in HepG2 2.2.15 cells. The inhibitory activity of astragaloside IV on the secretion of HBV antigens is more potent than that of 3TC without significant cytotoxicity. Furthermore, in DHBV-infected ducklings, astragaloside IV caused 64.0% inhibition at 120 mg/kg on serum DHBVs after 10 days of treatment and also reduced serum DHBV DNA levels. In summary, these results demonstrated that astragaloside IV possessed potent anti-HBV activity.

In addition, the compound chrysophanol 8-*O*-beta-D-glucoside isolated from *Rheum palmatum* was found to display strong anti-HBV activity. Li *et al.* isolated six anthraquinones from the ethanol extract of *Rheum palmatum* by using reverse phase-high performance liquid chromatography (RP-HPLC) and evaluated their anti-HBV activities (*41*). The results showed that five free anthraquinones showed weak or slightly inhibitory activities against HBV, and the only combined anthraquinone chrysophanol 8-*O*-beta-D-glucoside exhibited significant activity against HBV DNA production with an IC_{50} of 36.98 μg/mL and antigens expression with an IC_{50} value of 237.4 μg/mL for HBsAg and 183.41 μg/mL for HBeAg. Furthermore, they observed that chrysophanol 8-*O*-beta-D-glucoside is a potential inhibitor of HBV-DNA polymerase. Therefore, they concluded that the combined anthraquinone chrysophanol 8-*O*-beta-D-glucoside was the major active compound in the ethanol extract of *Rheum palmatum* and could be a promising candidate for the development of new anti-HBV drugs in the treatment of HBV infection.

5. The Chinese medicine preparations commonly used with anti-HBV activities

Chinese medicine preparations are a form of Chinese medicines that are isolated from single herbs or traditional herbal formulas and that are prepared using modern advanced pharmaceutical technology. There are various dosage forms including injections, tablets, pills, capsules, and liquids. Compared to traditional decoctions, Chinese medicine preparations are safer, more effective, and easier to use. Thus, Chinese medicine preparations are becoming increasingly popular in China and are attracting worldwide attention. Based upon our review of the related papers, we have selected some Chinese medicine preparations with anti-HBV activities (Table 4) which have been approved by the State Food and Drug Administration (FDA) of China and seem effective and worthy of additional and in-depth study, and we will give them a brief commentary below.

5.1. *Silymarin and Silibinin*

Silybum marianum or milk thistle (Shui Fei Ji), a member of the Asteraceae family, is one of the most ancient and extensively used medicinal plants for its beneficial effects on liver and other organs (*38*). Silymarin is a standardized extract from the fruits and seeds of *Silybum marianum* and it is composed of a mixture of several flavonolignans, with the most important being silibinin, isosilibinin, dehydroisosilibinin, silidianin and silichristin. Some studies demonstrate that silymarin possesses powerful antioxidant and hepatoprotective activities. It has beneficial effects on various hepatic disorders, including cirrhosis, fatty liver hepatitis, viral hepatitis, and so on (*70*). Recently, some Chinese medicine preparations containing silymarin (*e.g.*, Silymarin tablet and Silibinin capsule) have been approved for the treatment of hepatic diseases such as CHB in China. Xie *et al.* conducted a study to evaluate

Table 4. Chinese medicine preparations commonly used with anti-HBV activities

Common name	Source	Dosage form	Biological activity	Evidence of anti-HBV activity	*Ref.*
Silymarin	*Silybum marianum* (Shui Fei Ji)	Tablet, capsule	Antioxidant, hepatoprotective, antiviral	In patients: improves liver function	*70,71*
Silibinin	*Silybum marianum* or milk thistle (Shui Fei Ji)	Tablet, capsule	Antioxidant, hepatoprotective, antiviral	In patients: prevents liver necrosis and repairs liver cells	*70,72*
Kushenin	*Sophora flavescens* (Ku Shen)	Injection, tablet, capsule	Antiviral, anti-fibrosis, anti-arrhythmia, anti-inflammation, antibacterial, immunomodulation, enhancing leukocytes	In patients: improves the level of ALT, the negative rate of HBV DNA and HBeAg, and the positive rate of HBeAb	*73,74*
Cinobufacini	*Bufo bufo gargarizans* Cantor	Injection, tablet, oral solution, capsule	Antiviral, anticancer	In HepG2 2.2.15 cells: inhibits the secretion of HBsAg, HBeAg, and HBcrAg; In patients: improves the level of ALT and the negative conversion rates of HBV DNA and HBeAg combined with IFN-α 2b	*75,78,79*

the efficacy of Silymarin tablets on patients with CHB (*71*). After treatment for 12-weeks, ALT and AST levels decreased significantly in the Silymarin tablet group compared to the control group, which indicated that Silymarin tablets might be an effective agent for CHB treatment. Guo *et al.* conducted a study to observe the effect of Silibinin capsules on patients with CHB (*72*). The results showed that the total effective rate and symptom normalization of the Silibinin capsule group improved significantly compared to the control group with ALT, AST and TBIL levels decreased significantly after treatment for three months. These indicated that Silibinin capsules could effectively prevent necrosis of liver cells and repair liver cells in CHB treatment.

5.2. *Kushenin*

Kushenin is a mixed alkaloid of oxymatrine with a little oxysophocarpine, extracted from the root of Chinese herbal medicine *Sophora flavescens*. It has various pharmacological activities including antiviral, anti-fibrosis, anti-arrhythmia anti-inflammation, antibacterial, immunomodulation, enhanced leukocytes, and so on (*73*). Nowadays, many dosage forms containing kushenin, such as injection, tablets, and capsules, have been prepared and approved for treatment of CHB. Yu *et al*. conducted a study to investigate the effect of kushenin on patients with CHB (*74*). In this study, 196 patients were randomly divided into four groups (kushenin group, kushenin + arabinofuranosyl adenine monophosphate (Ara-AMP) group, IFN-α 1b group, and glucose group), and the levels of ALT, AST and viral markers were observed. After treatment for 30 days, the improvement on the level of ALT, the negative rate of HBV DNA and HBeAg, and the positive rate of HBeAb were similar in the kushenin group, kushenin + Ara-AMP group, and IFN-α 1b group, which were better than that of glucose group ($P < 0.05$). After 12 months follow up, the total effective rates were 40.8%, 60.8% and 43.1% in the kushenin group, kushenin + Ara-AMP group, and IFN-α 1b group, respectively. These indicated kushenin might havea better long term efficacy for treating CHB.

5.3. *Cinobufacini*

Cinobufacini (Huachansu), an aqueous extract from the skin and parotid venom glands of the toad (*Bufo bufo gargarizans* Cantor) that contains Chansu which has been widely used in China as an anodyne, cardiotonic, antimicrobial, local anesthetic, and antineoplastic agent for thousands of years (*35,75*). Pharmacological studies demonstrate that the major active constituents of cinobufacini are bufodienolides (which primarily include bufalin, cinobufagin, resibufogenin, bufotalin, and Lumichrome), biogenic amines, alkaloids, peptides, and proteins (*75*). Recently, many dosage forms containing cinobufacini, such as injection, tablets, oral solution, and capsules, have been prepared and approved by the Chinese State Food and Drug Administration (SFDA) (ISO9002) and widely used in clinical cancer and CHB therapy in China (*76*). Our research team has committed to the anti-cancer and anti-HBV activities of cinobufacini for several years. We found that cinobufacini and its active compounds (bufalin and cinobufagin) could induce human hepatocellular carcinoma (HCC) cells apoptosis *via* Fas- and mitochondria-mediated pathways (*75,77*). We also found that after treatment for 6 days in HepG2 2.2.15 cells, cinobufacini at 1 μg/mL effectively inhibited the secretion of HBsAg, HBeAg, and HBcrAg by 29.58, 32.87, and 42.52%, respectively, which was more potent than lamivudine (100 g/mL) (*78*). Furthermore, some clinical studies have indicated that cinobufacini used alone or in combination with other anti-HBV agents (*e.g.*, IFN-α and lamivudine) possesses a significant effect on patients with CHB. Yu *et al.* conducted a study to observe the efficacy of the combination of cinobufacini and IFN-α 2b on patients with CHB (*79*). In this study, 142 patients were randomly divided into two groups (cinobufacini + IFN-α 2b group and IFN-α 2b group). After treatment for 48 weeks, the level of ALT and the negative conversion rates of HBV DNA and HBeAg were improved significantly in the cinobufacini + IFN-α 2b group compared to the IFN-α 2b group ($P < 0.05$). These findings indicated that IFN-α combined with cinobufacini could effectively improve liver function and inhibit HBV DNA replication.

6. Conclusion

Given the significant public health hazard of CHB and the high rates of non-response to interferon and nucleoside antiviral agents, continuous development of agents to treat HBV infections is urgently needed. Over a long period in clinical practice and with progress in basic research, especially advanced and interdisciplinary technology and methodology, the effectiveness and beneficial contribution of Chinese herbal medicines on CHB have been gradually discovered and confirmed, which will provide evidence for development of novel antiviral drugs. Based upon our review of the related papers and upon our prior knowledge and experience, we have selected some Chinese herbal medicines (including Chinese herbal formulas, single herbs and related active compounds, and Chinese medicine preparations) that seem effective and worthy of additional and in-depth study in treating CHB, and we have given them a brief review. According to basic and clinical studies, we conclude that these Chinese herbal medicines exhibit significant anti-HBV activities which improve liver function, and enhance HBeAg and HBsAg sero-conversion rates as well as HBV

DNA clearance rates in HepG2 2.2.15 cells, DHBV models, and patients with CHB. We hope this review will contribute to an understanding of Chinese herbal medicines as an effective treatment for CHB and provide useful information for the development of more effective antiviral drugs. However, more information is needed regarding anti-HBV Chinese herbal medicines, including preparation, standardization, identification of active ingredients, and toxicological evaluation. 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 anti-HBV Chinese medicines.

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References

1. Song PP, Feng XB, Zhang KM, Song TQ, Ma KS, Kokudo N, Dong JH, Yao LN, Tang W. Screening for and surveillance of high-risk patients with HBV-related chronic liver disease: Promoting the early detection of hepatocellular carcinoma in China. Biosci Trends. 2013; 7:1-6.
2. Zhang CY, Zhong YS, Guo LP. Strategies to prevent hepatitis B virus infection in China: Immunization, screening, and standard medical practices. Biosci Trends. 2013; 7:7-12.
3. Chu D, Yang JD, Lok AS, Tran T, Martins EB, Fagan E, Rousseau F, Kim WR. Hepatitis B screening and vaccination practices in asian american primary care. Gut Liver. 2013; 7:450-457.
4. Papatheodoridis GV, Manolakopoulos S, Dusheiko G, Archimandritis AJ. Therapeutic strategies in the management of patients with chronic hepatitis B virus infection. Lancet Infect Dis. 2008; 8:167-178.
5. Lau DT, Bleibel W. Current status of antiviral therapy for hepatitis B. Therap Adv Gastroenterol. 2008; 1:61-75.
6. Dobos GJ, Tan L, Cohen MH, McIntyre M, Bauer R, Li X, Bensoussan A. Are national quality standards for traditional Chinese herbal medicine sufficient? Current governmental regulations for traditional Chinese herbal medicine in certain Western countries and China as the Eastern origin country. Complement Ther Med. 2005; 13:183-190.
7. McCulloch M, Broffman M, Gao J, Colford JM Jr. Chinese herbal medicine and interferon in the treatment of chronic hepatitis B: A meta-analysis of randomized, controlled trials. Am J Public Health. 2002; 92:1619-1628.
8. Zhang L, Wang G, Hou W, Li P, Dulin A, Bonkovsky HL. Contemporary clinical research of traditional Chinese medicines for chronic hepatitis B in China: An analytical review. Hepatology. 2010; 51:690-698.
9. Yu F, Takahashi T, Moriya J, Kawaura K, Yamakawa J, Kusaka K, Itoh T, Morimoto S, Yamaguchi N, Kanda T. Traditional Chinese medicine and Kampo: A review from the distant past for the future. J Int Med Res. 2006; 34:231-239.
10. Chen FP, Kung YY, Chen YC, Jong MS, Chen TJ, Chen FJ, Hwang SJ. Frequency and pattern of Chinese herbal medicine prescriptions for chronic hepatitis in Taiwan. J Ethnopharmacol. 2008; 117:84-91.
11. Zheng N, Dai J, Cao H, Sun S, Fang J, Li Q, Su S, Zhang Y, Qiu M, Huang S. Current understanding on antihepatocarcinoma effects of xiao chai hu tang and its constituents. Evid Based Complement Alternat Med. 2013; 2013:529458.
12. Kusunose M, Qiu B, Cui T, Hamada A, Yoshioka S, Ono M, Miyamura M, Kyotani S, Nishioka Y. Effect of Sho-saiko-to extract on hepatic inflammation and fibrosis in dimethylnitrosamine induced liver injury rats. Biol Pharm Bull. 2002; 25:1417-1421.
13. Shiota G, Maeta Y, Mukoyama T, Yanagidani A, Udagawa A, Oyama K, Yashima K, Kishimoto Y, Nakai Y, Miura T, Ito H, Murawaki Y, Kawasaki H. Effects of Sho-Saiko-to on hepatocarcinogenesis and 8-hydroxy-2'-deoxyguanosine formation. Hepatology. 2002; 35:1125-1133.
14. Huang XX, Yamashiki M, Nakatani K, Nobori T, Mase A. Semi-quantitative analysis of cytokine mRNA expression induced by the herbal medicine Sho-saiko-to (TJ-9) using a Gel Doc system. J Clin Lab Anal. 2001; 15:199-209.
15. Taira Z, Yabe K, Hamaguchi Y, Hirayama K, Kishimoto M, Ishida S, Ueda Y. Effects of Sho-saiko-to extract and its components, baicalin, baicalein, glycyrrhizin and glycyrrhetic acid, on pharmacokinetic behavior of salicylamide in carbon tetrachloride intoxicated rats. Food Chem Toxicol. 2004; 42:803-807.
16. Chen MH, Chen JC, Tsai CC, Wang WC, Chang DC, Lin CC, Hsieh HY. Sho-saiko-to prevents liver fibrosis induced by bile duct ligation in rats. Am J Chin Med. 2004; 32:195-207.
17. Chang JS, Wang KC, Liu HW, Chen MC, Chiang LC, Lin CC. Sho-saiko-to (Xiao-Chai-Hu-Tang) and crude saikosaponins inhibit hepatitis B virus in a stable HBV-producing cell line. Am J Chin Med. 2007; 35:341-351.
18. Tajiri H, Kozaiwa K, Ozaki Y, Miki K, Shimuzu K, Okada S. Effect of sho-saiko-to(xiao-chai-hu-tang) on HBeAg clearance in children with chronic hepatitis B virus infection and with sustained liver disease. Am J Chin Med. 1991; 19:121-129.
19. Qin XK, Li P, Han M, Liu JP. Xiaochaihu Tang for treatment of chronic hepatitis B: A systematic review of randomized trials. Zhong Xi Yi Jie He Xue Bao. 2010; 8:312-320. (in Chinese)
20. Zhang Y, Han M, Liu Z, Wang J, He Q, Liu J. Chinese herbal formula xiao yao san for treatment of depression: A systematic review of randomized controlled trials. Evid Based Complement Alternat Med. 2012; 2012:931636.
21. Yin SH, Wang CC, Cheng TJ, Chang CY, Lin KC, Kan WC, Wang HY, Kao WM, Kuo YL, Chen JC, Li SL, Cheng CH, Chuu JJ. Room-temperature super-extraction system (RTSES) optimizes the anxiolytic- and antidepressant-like behavioural effects of traditional Xiao-Yao-San in mice. Chin Med. 2012; 7:24.
22. Zhan BL, Chen L, Xu SM. The analysis of clinical therapeutic effect on chronic hepatitis B treated with modified Xiao-Yao-San. Journal of practical traditional Chinese medicine. 2013; 29:333-334. (in Chinese)
23. Zhang J. Clinical effect of Xiaoyao Pill auxiliary adefovir dipivoxil on patients with chronic hepatitis B. Practical pharmacy and clinical remedies. 2013; 16:215-217. (in Chinese)

24. Jin Y. A combined use of acupuncture, moxibustion and long dan xie gan tang for treatment of 36 cases of chronic pelvic inflammation. J Tradit Chin Med. 2004; 24:256-258.
25. Chen FP, Kung YY, Chen YC, Jong MS, Chen TJ, Chen FJ, Hwang SJ. Frequency and pattern of Chinese herbal medicine prescriptions for chronic hepatitis in Taiwan. J Ethnopharmacol. 2008; 117:84-91.
26. Shi H, Zhou Y, Zhang FH, Zhang JP, Li JY, Wu MF, Xu HZ, Li Q. Influence of Long-Dan-Xie-Gan-Tang on the liver transfer function in rats treated by CCl_4. Liao Ning Zhong Yi Za Zhi. 2006; 33:1041-1042. (in Chinese)
27. Zhang YX, Li HG, Su YH. The clinical observation about Long-Dan-Xie-Gan-Tang combined with IFN-α for the treatment of chronic hepatitis B. Zhong Yuan Yi Kan. 1997; 24:30-31. (in Chinese)
28. Cui X, Wang Y, Kokudo N, Fang D, Tang W. Traditional Chinese medicine and related active compounds against hepatitis B virus infection. Biosci Trends. 2010; 4:39-47.
29. Amin ZA, Alshawsh MA, Kassim M, Ali HM, Abdulla MA. Gene expression profiling reveals underlying molecular mechanism of hepatoprotective effect of *Phyllanthus niruri* on thioacetamide-induced hepatotoxicity in Sprague Dawley rats. BMC Complement Altern Med. 2013; 13:160.
30. Naik AD, Juvekar AR. Effects of alkaloidal extract of *Phyllanthus niruri* on HIV replication. Indian J Med Sci. 2003; 57:387-393.
31. Lee SH, Jaganath IB, Manikam R, Sekaran SD. Inhibition of Raf-MEK-ERK and hypoxia pathways by Phyllanthus prevents metastasis in human lung (A549) cancer cell line. BMC Complement Altern Med. 2013; 13:271.
32. Bagalkotkar G, Sagineedu SR, Saad MS, Stanslas J. Phytochemicals from *Phyllanthus niruri* Linn. and their pharmacological properties: A review. J Pharm Pharmacol. 2006; 58:1559-1570.
33. Lam WY, Leung KT, Law PT, Lee SM, Chan HL, Fung KP, Ooi VE, Waye MM. Antiviral effect of *Phyllanthus nanus* ethanolic extract against hepatitis B virus (HBV) by expression microarray analysis. J Cell Biochem. 2006; 97:795-812.
34. Liu J, Lin H, McIntosh H. Genus *Phyllanthus* for chronic hepatitis B virus infection: A systematic review. J Viral Hepat. 2001; 8:358-366.
35. Qi F, Li A, Inagaki Y, Gao J, Li J, Kokudo N, Li XK, Tang W. Chinese herbal medicines as adjuvant treatment during chemo- or radio-therapy for cancer. Biosci Trends. 2010; 4:297-307.
36. Wang S, Li J, Huang H, Gao W, Zhuang C, Li B, Zhou P, Kong D. Anti-hepatitis B virus activities of astragaloside IV isolated from *Radix astragali*. Biol Pharm Bull. 2009; 32:132-135.
37. Tang LL, Sheng JF, Xu CH, Liu KZ. Clinical and experimental effectiveness of Astragali compound in the treatment of chronic viral hepatitis B. J Int Med Res. 2009; 37:662-667.
38. The State Pharmacopoeia Commission of the People's Republic of China. Pharmacopoeia of the People's Republic of China 2010. China Medical Science and Technology Press, Beijing, China, 2010.
39. Zhang H, Li C, Kwok ST, Zhang QW, Chan SW. A Review of the pharmacological effects of the dried root of *Polygonum cuspidatum* (Hu Zhang) and its constituents. Evid Based Complement Alternat Med. 2013; 2013:208349.
40. Chang JS, Liu HW, Wang KC, Chen MC, Chiang LC, Hua YC, Lin CC. Ethanol extract of *Polygonum cuspidatum* inhibits hepatitis B virus in a stable HBV-producing cell line. Antiviral Res. 2005; 66:29-34.
41. Li Z, Li LJ, Sun Y, Li J. Identification of natural compounds with anti-hepatitis B virus activity from *Rheum palmatum* L. ethanol extract. Chemotherapy. 2007; 53:320-326.
42. Sun Y, Li LJ, Li J, Li Z. Inhibition of hepatitis B virus replication by *Rheum palmatum* L. ethanol extract in a stable HBV-producing cell line. Virol Sin. 2007; 22:14-20.
43. Kim TG, Kang SY, Jung KK, Kang JH, Lee E, Han HM, Kim SH. Antiviral activities of extracts isolated from *Terminalis chebula* Retz., *Sanguisorba officinalis* L., *Rubus coreanus* Miq. and *Rheum palmatum* L. against hepatitis B virus. Phytother Res. 2001; 15:718-720.
44. Chung TH, Kim JC, Lee CY, Moon MK, Chae SC,Lee IS, Kim SH, Hahn KS, Lee IP. Potential antiviral effects of *Sanguisorba officinalis*, *Terminalis chebula*, *Rubusanus migua*, and *Rheum palmatum* against duck hepatitis B virus (DHBV). Phytother Res. 1997; 11:179-182.
45. Song YH, Liu Q, Lv ZP, Chen YY, Zhou YC, Sun XG. Protection of a polysaccharide from *Salvia miltiorrhiza*, a Chinese medicinal herb, against immunological liver injury in mice. Int J Biol Macromol. 2008; 43:170-175.
46. Cheng HT, Li XL, Li XR, Li YH, Wang LJ, Xue M. Simultaneous quantification of selected compounds from Salvia herbs by HPLC method and their application. Food Chem. 2012; 130:1031-1035.
47. Xiong LL. Therapeutic effect of combined therapy of Salvia Miltiorrhiza and Polyporus Umbellatus Polysaccharide in treating chronic Hepatitis B. Zhong Guo Zhong Xi Yi Jie He Za Zhi. 1993; 13:533-535, 516-517. (in Chinese)
48. Ye F, Liu Y, Qiu G, Zhao Y, Liu M. Clinical study on treatment of cirrhosis by different dosages of salvia injection. Zhong Yao Cai. 2005; 28:850-854. (in Chinese)
49. Kim HJ, Yoo HS, Kim JC, Park CS, Choi MS, Kim M, Choi H, Min JS, Kim YS, Yoon SW, Ahn JK. Antiviral effect of *Curcuma longa* Linn extract against hepatitis B virus replication. J Ethnopharmacol. 2009; 124:189-196.
50. Wu MJ, Weng CY, Ding HY, Wu PJ. Anti-inflammatory and antiviral effects of *Glossogyne tenuifolia*. Life Sci. 2005; 76:1135-1146.
51. Tang QN, Feng M, Gong SJ, Dai ZK, Zhou YZ, Xu Q. The effects of extracts from *Arenaria kansuensis* on the levels of HBsAg and HBeAg in HepG2.2.15 cells. Zhong Yao Cai. 2008; 31:1212-1216. (in Chinese)
52. Zuo GY, Liu SL, Xu GL. The research progress on the in vitro anti-HBV activities of ingredients isolated from medicinal plants over the past 20 years. World Chin J Dig. 2006; 14:1241-1246. (in Chinese)
53. Ye G, Zhu HY, Li ZX, Ma CH, Fan MS, Sun ZL, Huang CG. LC-MS characterization of efficacy substances in serum of experimental animals treated with *Sophora flavescens* extracts. Biomed Chromatogr. 2007; 21:655-660.
54. Ma ZJ, Li Q, Wang JB, Zhao YL, Zhong YW, Bai YF, Wang RL, Li JY, Yang HY, Zeng LN, Pu SB, Liu FF, Xiao DK, Xia XH, Xiao XH. Combining oxymatrine or matrine with lamivudine increased its anti-replication effect against the hepatitis B virus *in vitro*. Evid Based Complement Alternat Med. 2013; 2013:186573.
55. Chen CX, Liu B, Ma Y, Zhou YJ, Pan XN, Zhen RD, Wang QC, Wang MR, He CL, Fu QC, Chen CW. Lamivudine,

interferon-α and oxymatrine treatment for the surviving hepatic failure patients with hepatitis B. Zhonghua Gan Zang Bing Za Zhi. 2009; 17:505-508. (in Chinese)
56. Li CQ, Zhu YT, Zhang FX, Fu LC, Li XH, Cheng Y, Li XY. Anti-HBV effect of liposome-encapsulated matrine *in vitro* and *in vivo*. World J Gastroenterol. 2005; 11:426-428.
57. Nie HM, Chen JJ, Gao YQ, Jin SG, Wang LT. The research on the anti-HBV effect of sophoridine *in vitro*. Beijing Journal of TCM. 2007; 26:678-680. (in Chinese)
58. Nie HM, Wang LT, Chen JJ, Wang R, Jin SG, Gao YQ. Inhibitory effects of sophocarpine on HBsAg and HBeAg *in vitro*. Liao Ning Zhong Yi Za Zhi. 2006; 33:1478-1479. (in Chinese)
59. Xia JF, Gao JJ, Inagaki Y, Kokudo N, Nakata M, Tang W. Flavonoids as potential anti-hepatocellular carcinoma agents: Recent approaches using HepG2 cell line. Drug Discov Ther. 2013; 7:1-8.
60. Huang RL, Chen CC, Huang HL, Chang CG, Chen CF,Chang C, Hsieh MT. Anti-hepatitis B virus effects of wogonin isolated from *Scutellaria baicalensis*. Planta Med. 2000; 66:694-698.
61. Guo QL, Zhao L, You QD, Yang Y, Gu HY, Song GL, Lu N, Xin J. Anti-hepatitis B virus activity of wogonin *in vitro* and *in vivo*. Antiviral Res. 2007; 74:16-24.
62. Shin MS, Kang EH, Lee YI. A flavonoid from medicinal plants blocks hepatitis B virus-e antigen secretion in HBV-infected hepatocytes. Antiviral Res. 2005; 67:163-168.
63. Kang EH, Kown TY, Oh GT, Park WF, Park SI, Park SK, Lee YI. The flavonoid ellagic acid from a medicinal herb inhibits host immune tolerance induced by the hepatitis B virus-e antigen. Antiviral Res. 2006; 72:100-106.
64. Huang ZM, Yang XB, Cao WB, Liu HZ, Xu HD. Modern study and clinic application of *Oenanthe javanica*. Pharm J Chin PLA. 2001; 17: 266-269. (in Chinese)
65. Wang WN, Yang XB, Liu HZ, Huang ZM, Wu GX. Effect of *Oenanthe javanica* flavone on human and duck hepatitis B virus infection. Acta Pharmacol Sin. 2005; 26:587-592.
66. Klayman DL. Qinghaosu (artemisinin): An antimalarial drug from China. Science. 1985; 228:1049-1055.
67. Romero MR, Efferth T, Serrano MA, Castaño B, Macias RI, Briz O, Marin JJ. Effect of artemisinin/artesunate as inhibitors of hepatitis B virus production in an "*in vitro*" replicative system. Antiviral Res. 2005; 68:75-83.
68. Chiang LC, Ng LT, Liu LT, Shieh DE, Lin CC. Cytotoxicity and anti-hepatitis B virus activities of saikosaponins from *Bupleurum* species. Planta Med. 2003; 69:705-709.
69. Ren S, Zhang H, Mu Y, Sun M, Liu P. Pharmacological effects of astragaloside IV: A literature review. J Tradit Chin Med. 2013; 33:413-416.
70. Basiglio CL, Sánchez Pozzi EJ, Mottino AD, Roma MG. Differential effects of silymarin and its active component silibinin on plasma membrane stability and hepatocellular lysis. Chem Biol Interact. 2009; 179:297-303.
71. Xie YM. Evaluation of silymarin in the treatment of chronic hepatitis B. Chinese Journal of New Drugs. 2006; 15:725-727. (in Chinese)
72. Guo JS. Observation on efficacy of Silibinin capsule for chronic hepatitis B. Zhong Guo Gan Zang Bing Za Zhi. 2010; 2:22-24. (in Chinese)
73. Li ZR. The pharmacological and clinical research progress of kushenin. West China Journal of Pharmaceutical Sciences. 2003; 18:435-437. (in Chinese)
74. Yu YY, Wang QH, Zhu LM, Zhang QB, Xu DZ, Guo YB, Wang ZQ, Guo SH, Zhou XQ, Zhang LX. A clinical research on kushenin for the treatment of chronic hepatitis B. Zhonghua Gan Zang Bing Za Zhi. 2002; 10:280-282. (in Chinese)
75. Qi F, Li A, Zhao L, Xu H, Inagaki Y, Wang D, Cui X, Gao B, Kokudo N, Nakata M, Tang W. Cinobufacini, an aqueous extract from *Bufo bufo gargarizans* Cantor, induces apoptosis through a mitochondria-mediated pathway in human hepatocellular carcinoma cells. J Ethnopharmacol. 2010; 128:654-661.
76. Qi F, Li A, Inagaki Y, Kokudo N, Tamura S, Nakata M, Tang W. Antitumor activity of extracts and compounds from the skin of the toad *Bufo bufo gargarizans* Cantor. Int Immunopharmacol. 2011; 11:342-349.
77. Qi F, Inagaki Y, Gao B, Cui X, Xu H, Kokudo N, Li A, Tang W. Bufalin and cinobufagin induce apoptosis of human hepatocellular carcinoma cells *via* Fas- and mitochondria-mediated pathways. Cancer Sci. 2011; 102:951-958.
78. Cui X, Inagaki Y, Xu H, Wang D, Qi F, Kokudo N, Fang D, Tang W. Anti-hepatitis B virus activities of cinobufacini and its active components bufalin and cinobufagin in HepG2.2.15 cells. Biol Pharm Bull. 2010; 33:1728-1732.
79. Yu Q, Xi QH. Observation on the effect of cinobufacini and IFN-α 2b treating chronic hepatitis B. Capital Medicine. 2011; 16:48-49. (in Chinese)

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