

## Short Communication

# Inhibition effects on HBV replication by hydrophobic extracts from *Ferula ferulaeoides* (Steud.) Korov

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**Hepatitis B virus (HBV) infection is a worldwide public health problem, which can lead to life threatening chronic hepatitis, liver cirrhosis and cancer. Despite this, treatments for chronic HBV infection are still very limited. In our investigation herbal medicines as antiviral agents, FF, the lipid soluble fraction of *Ferula ferulaeoides*, which has been used as a traditional Chinese medicine, showed a significant inhibitory effect against HBV in HBV-producing cell line HepG2.2.15. In the experiment, FF reduced the HBsAg level and HBV replication by 87% and 36%, respectively. The results suggest the lipophilic fraction of root extracts from *F. ferulaeoides* could serve as a effective natural constituents of herbs for the search of novel anti-HBV agents.**

**Key words:** Hepatitis B Virus, anti-HBV, HBsAg, *Ferula ferulaeoides*.

## INTRODUCTION

Although efforts to prevent and control HBV with vaccines have met with increasing levels of success, there are still nearly two billion people worldwide with serologic evidence of past or present HBV infection and more than 350 million chronically infected by HBV (The World Health Report, 1997). Hepatitis B virus (HBV) persistence infection can cause chronic hepatitis, which often leads to liver cirrhosis and even hepatocellular carcinoma. Although prophylactic HBV vaccines have been effective, current treatment for chronic HBV infected patient remains limited. Contemporary clinical treatment for chronic HBV infection relies on two kinds of antivirals: interferon and nucleotide/nucleoside. Conventional and pegylated interferons act on the immune system to enhance host antiviral capacity, whereas reverse transcriptase inhibitor (NRTI), nucleotide/nucleoside

analogue, targets viral polymerase and inhibit the replication of HBV DNA (Ganem and Prince, 2004). However, limitations exist for both interferon and NRTI for the treatment of HBV. Interferon shows a low response rate (<30%) and is poorly tolerated in a substantial number of patients. NRTI often encounters the occurrence of antiviral resistance during long term treatment (Zoulim, 2004; Perrillo, 2009; Lok and Chotiyaputta, 2009). Thus, discovery of more effective antivirals agents is urgently needed.

HBV belongs to a group of hepatotropic, enveloped animal DNA viruses known as hepadnaviridae. HBV virion contains a circular, partially double-stranded genome which encodes four open reading frames (ORF), S/pre-S, core/pre-core, P and X. These ORFs encode three envelope proteins, core and E proteins, polymerase and HBV X transactivator, respectively (Seeger and Mason, 2000; Bouchard and Schneider, 2004). During HBV infection, envelope proteins and E protein are continuously secreted from infected cells, which are serologic markers of HBV infection and termed as HBsAg and HBeAg, respectively. The replication of HBV DNA is unique in the utilization of a pregenomic RNA as a template for reverse transcription carried out by viral polymerase. Progeny HBV virions are secreted from

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**Abbreviations:** FF, The lipid soluble fraction of *Ferula ferulaeoides*; HBV, hepatitis B virus; ORF, open reading frames; DMSO, dimethyl sulfoxide; SD, standard deviation; RT-PCR, real-time polymerase chain reaction.

infected cells and can be measured quantitatively by real-time PCR. The genus *Ferula* (Apiaceae) includes about 150 species grown in a vast geographical region ranging from Central Asia to the Mediterranean region (Mozaffarian, 1996), and 26 species distribute in China (She and Watson, 2005). Different parts of extract for plant of *Ferula* have a reputation in the treatment of various diseases such as neurological disorders, inflammations, dysentery, digestive disorders, rheumatism, headache, arthritis and dizziness (Tamemoto et al., 2001). Constituents from genus *Ferula* were summarized many biological effects including antiinflammatory, cytotoxicity and P-gp inhibitory, cancer chemopreventive, antibacterial, and antileishmanial activities (Nazari and Iranshahi, 2011). In addition, activities of influenza A (H<sub>1</sub>N<sub>1</sub>) antiviral from plants of the genus *Ferula* have also been reported (Lee et al., 2009). In our primary bioassay screening, dichloromethane extracts from *F. ferulaeoides* (Steud.) Korov, showed appreciable inhibition against HBV. With further study using an HBV-producing cell model HepG2.2.15, the extract possessed antiviral effect on reducing HBsAg expression and HBV DNA replication.

## MATERIAL AND METHODS

### Extraction and isolation of the herbal sample

Roots of *Ferula ferulaeoides* used in the experiment were collected in suburban of Shihezi City, Xinjiang province. The plant was identified by Dr. Hui-Qin Xie of the Department of Plant Protection, Agricultural School of Shihezi University, China, and the voucher specimen (XHQ-FF-1) was kept in the herbarium of the Department of Plant Protection. Air-dried and powdered roots (2 kg) were extracted with 95% ethanol and yielded 1 L extract after the ethanol was evaporated. The extract was suspended in 2 L of distilled water and then partitioned with dichloromethane (1 L × 4). The solvent was evaporated under vacuum at 50°C to yield FF (400 g). In the virological experiment the sample, FF, was solved in 1% dimethyl sulfoxide (DMSO) for tests.

### Cell culture in virological experiments

The HepG2.2.15 cell line which harbors two copies of the HBV genome integrated in the chromosome in a head-to-tail form supports HBsAg and HBeAg production as well as viral replication. The cells were cultured at 37°C under 5% CO<sub>2</sub> in Dulbecco's modified Eagle's medium supplemented with 20% fetal bovine serum, 100 U/ml penicillin, 100 mg/ml streptomycin, 2 mM L-Glutamine, and 250 g/ml G418.

### Detection of HBsAg and HBeAg

HepG2.2.15 cells were cultured in 96-well plate (10<sup>4</sup> cells/well). The sample FF was added into the medium to a final concentration. Wells containing only medium were conducted as Controls. Similarly, the cells with 1% DMSO added at the same volume of the sample in maintenance media are conducted as DMSO Controls. Supernatants of culture suspension were collected 4 days after the treatment. HBsAg and HBeAg in the supernatants were measured using ELISA kits (Kehua Inc., Shanghai, China) according to the

manufacturer's instruction and were represented as absorbance values at A450, using an ELISA reader (Molecular Devices).

### Real-time PCR for quantitating HBV DNA

HepG2.2.15 cells were cultured to a 10<sup>4</sup> cells/well. The sample was added into the test wells to a final concentration of 0.1 µg/µL. Control wells containing 1% DMSO in media was setup. After 4 days incubation, the supernatants were collected. The concentration of HBV DNA were quantified by RT-PCR using a commercially available HBV RT Detection Kit (PG BIOTECH, Shenzhen, China). RT-PCR was performed on the Stratagene MX3000P Detection System (Agilent Inc., USA) using following PCR conditions: 50°C for 2 min for uracil N-glycosylase incubation, 95°C for 10 min for Hotstart Taq DNA polymerase activation, followed by 40 cycles of 95°C for 5s (denaturation), 60°C for 30 s (annealing and extension).

### Statistical analysis

Statistical analysis was performed using the Sigmaplot software. Data from three separate experiments are presented as means ± standard deviation (SD). Student's t-test was performed to determine the difference between groups with P<0.05 considered significant.

## RESULTS AND DISCUSSION

### Effects of sample FF on HBsAg

HepG2.2.15 cells were cultured and the concentration of HBsAg and HBeAg in the supernatant was measured. Treated with the sample FF, the OD450 value of HBsAg vastly decreased as low as 0.237 ± 0.016, which indicated the HBsAg level reduced by 87% comparing with that of DMSO (1.408 ± 0.098); while the HBeAg level increase slightly. (Sample FF shows 1.769 ± 0.057, while the DMSO Control shows 1.399 ± 0.084) (Figure 1).

### Effects of sample FF on reducing HBV DNA

To investigate the mechanism of the inhibition, the tissue culture supernatants were collected and purified, and the HBV DNA in the virions was quantified with the HBV-specific real-time polymerase chain reaction (RT-PCR). As shown in Figure 2, the treatment with FF resulted in 36% reduction of HBV DNA/virion in the supernatants (P < 0.05). *F. ferulaeoides* is a traditional ethnic medicine, and its gum obtained from the aerial parts has been used for detoxication, and for anti-inflammatory helminthicide, anti-convulsion, anti-epileptic, and antifertility treatments. In this study, the hydrophobic extract of roots from the plant was found to exhibit a significant inhibition of HBsAg production and HBV replication. It is noteworthy that the drug had little inhibitory effect on the HBeAg production. The HepG2.2.15 cell used in this study contains HBV genome integrated in the cell chromosome. It is possible that in might have the

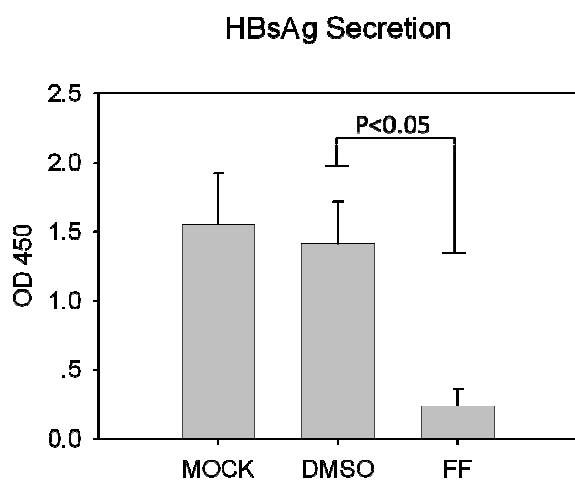


Figure 1. Effect of FF on the production of HBsAg.

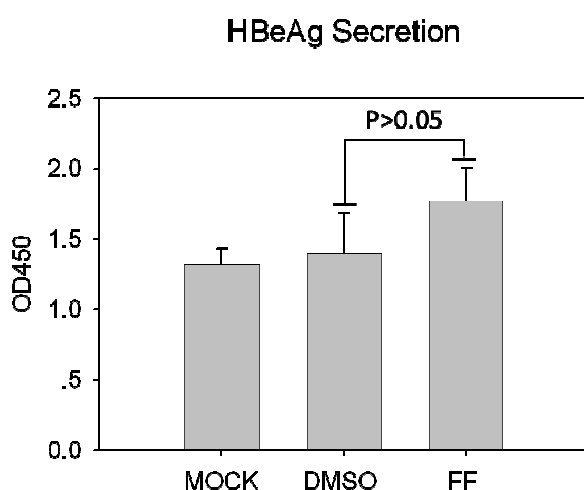


Figure 2. Effect of FF on the production of HBeAg.

this cellular context, HBeAg expression is therefore not inhibited by the drug. Biological activities are almost attributed to sesquiterpene coumarin constituents from studied *Ferula* species (Nazari, and Iranshahi, 2011), however, the species of *F. ferulaeoides* existed a little difference in smell – there was no such disgusted smell like common *Ferula* species. This plant have ever been isolated and identified a series of sesquiterpene coumarin derivatives (Nagatsu et al., 2002), there was no biological activity investigation on those compounds. A recent research on this species reported two novel sesquiterpenoids (Hu et al., 2010). This suggested that more need on chemical constituents especially on pharmacology should be carried out on *F. ferulaeoides*. The finding we reported suggests that this traditional drug

potential to act against HBV infection. It is encouraging to further isolate and identify the chemical in the fraction FF which is responsible for the antiviral effect shown in this study. Furthermore, investigation for the mechanism of inhibition by this drug on HBsAg and HBV replication will probably give new insight for the improvement of the antiviral activity.

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