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# Hepatoprotective and inhibiting HBV effects of polysaccharides from roots of *Sophora flavescens*

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ABSTRACT

Roots of *Sophora flavescens* is an important herbal medicine for treatment of HBV and hepatic carcinoma in China. Alkaloids in the root were well known for exhibiting good hepato-protective and anti-HBV effects. However, polysaccharides as main components in the root remained unknown. In the studies, we investigated the chemical features and hepatoprotective effects of *Sophora flavescens* polysaccharides (SFP-100 and its active fractions) with ConA-induced hepatitis mice, human liver LO2 cells and HepG2.2.15 cells. The results showed that SFP-100 was composed of arabinose, glucose, galactose and galacturonic acid, SFP-100-A mainly contained glucose. SFP-100-B and SFP-100-C were acidic polysaccharides. SFP-100 significantly decreased hepatocytes apoptosis, inhibited the infiltration of neutrophils and macrophages into liver, and improved the production of IFN-γ and IL-6 of splenocytes in ConA-induced hepatitis mice. SFP-100 and its two sugar fractions increased LO2 cell proliferation and reduced cell apoptosis induced by ConA. SFP-100, SFP-100-A and SFP-100-C remarkedly inhibited the secretion of HBsAg and HBeAg by HepG2.2.15 cells.These results suggested *Sophora flavescens* polysaccharides exerts significant hepato-protective and anti-HBV roles, and further is used for treatment of immune-mediated liver disease in the future.



## 1. Introduction

The dried roots of *Sophora flavescens* Ait (named Kushen in China), is a Chinese medicinal herb used for the treatment of gastrointestinal hemorrhage, skin diseases, pyretic stranguria and viral hepatitis.The aqueous-extract of the herb used in different herbal formulations is commonly known for treatment of liver disorders. It enhances liver functions, reduces hepatotoxicity, anti-tumor and protects liver against hepatitis B infections and liver fibrosis [1,2]. Alkaloids and flavonoids are well-characterized components in kushen. Matrine, oxymatrine and total alkaloids have been developed treatment drugs for hepatic carcinoma, hepatitis B infections and liver fibrosis in China [3]. Matrine inhibited human hepatocellular carcinoma (HepG2) cells proliferation primarily via up-regulating expression of Bax, down-regulating expression of the alpha fetal protein (AFP), proliferating cell nuclear antigen (PCNA) and Bcl-2 [4]. Oxymatrine exhibited the therapeutic effect on the hepatic steatosis in high fructose diet induced fatty liver rats is partly due to down-regulating Srebf1 and up-regulating Pparα mediated metabolic pathways simultaneously [5]. Kurarinol is a flavonoid isolated from roots of Sophora flavescens. kurarinol dose-dependently provoked HepG2, Huh-7 and H22 HCC cell apoptosis with undetectable toxic impacts on the host [6].

*Abbreviations:* Ara, arabinose; CE, capillary electrophoresis; ConA, concanavalin A; DCs, dendritic cells; DMEM, Dulbecco's modified eagle medium; DMSO, dimethyl-sulfoxide; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; HBV, hepatitis B virus; Glc, glucose; Gal, galactose; GalA, galacturonic acid; HE, hematoxylin–eosin; HPGPC, high press gel permeation chromatography; IFN-γ, interferon-gamma; β, interleukin-1β; IL-12, interleukin-12; JNK, c-Jun N-terminal kinase; MTT, thiazolyl tetrazolium colourimetry; TFA, trifluoroacetic acid; TNF-α, tumor necrosis factor α; VEGF, vascular endothelial growth factor.

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However, the polysaccharides as main components in the aqueous-extract of *Sophora flavescens* are not still known less. Previously, Bai et al. reported that a polysaccharide (SFPW1) from the herb possessed antitumor and immunomodulating activities. SFPW1 effectively inhibits tumor growth in H22 tumor-bearing mice and promote splenocyte proliferation. SFPW1 also strengthened peritoneal macrophages to devour H22 tumor cells and stimulated macrophages to produce NO [7]. Zhang et al. isolated four acid heteropolysaccharides (SF1, SF2, SF3 and SF4) with the average molecular weights of 400.9, 98.6, 99.3 and 42.7 kDa, respectively. SF4 showed the most significant scavenging activity on $O_2\cdot$, ABTS and DPPH radicals, while SF3 posses the most scavenging activity on •OH *in vitro*. SF1–SF4 significantly stimulated NO production in macrophages and promoted splenocyte proliferation [8].

In this study, our aim was preliminary to investigate hepatoprotective and immunolo-regulative effect of *Sophora flavescens* polysaccharides and its fractions *in vivo* and *in vitro*. According to these results of the present study, we hope to find an active component and its mechanism for treatment of immune liver damages and HBV in future.

## 2. Methods and materials

### 2.1. Plant materials

Dried root of *Sophora flavescens* produced from Beijing in China were purchased from Beijing Tong-Ren-Tang Pharmacy. A voucher specimen (number 59) was preserved in the Laboratory of Natural Products Chemistry of Beijing Institute of Pharmacology and Toxicology.

### 2.2. Chemical, biochemical, cell line and animals

The standard monosaccharides and a series of dextrans were purchased from Sigma, Glycyrrhizin capsule was made from Beijing Kawin Co. Ltd, RPMI 1640 and fetal calf serum was purchased from Gibco. DEAE-cellulose was purchased from Shanghai Reagent Company.

The L-O2 cell line (human liver cell) was obtained from Prof. Qian LI (Beijing Institute of Pharmacology and Toxicology). The cells were cultured in RPMI 1640, supplemented with 10% fetal calf serum and 1% penicillin/streptomycin at 37 °C in 5% $CO_2$.

Female Balb/c mice (18–20 g) were used in this study. These mice were supplied by the Animal Center of the Academy of Military Medical Sciences (SCXK JUN 2012-001) in Beijing. These mice were bred with free access to standard diet and water *ad libitum* under standard environment condition (22–25 °C, humidity 50–70%, 12 h light and 12 h dark cycle). All animal procedures were performed according to the Guide for the Care and Use of Laboratory Animal of the National Institute of Health and Guide of the Animal Welfare Act.

### 2.3. Preparation of Sophora flavescens polysaccharides and its fractions

The root of *Sophora flavescens* (1 kg) was pulverized and decocted with water (15 L) for 1 h. The mixture was centrifugated and the residue was repetitive operated with same condition. The supernatant was combined and concentrated at reduced pressure to 1L, and 3 L 95% ethanol was incorporated the supernatant to precipitate polysaccharides for 48 h. The precipitation was dissolved, dialyzed against distilled water for 48 h and lyophillizated to obtain total polysaccharide (SFP-100).

SFP-100 (1 g) was dissolved in distilled water and injected into DEAE-cellulose column (φ6.5 cm × 35 cm), then was eluted with $H_2O$, 0.25 and 0.5 mol/L $NaHCO_3$ sequentially at a flow rate of 1.0 mL/min. Three sugar fractions were obtained and named SFP-100-A ($H_2O$), SFP-100-B (0.25 mol/L $NaHCO_3$) and SFP-100-C (0.5 mol/L NaHCO3) respectively. These fractions were dialyzed against distilled water and lyophillizated.

### 2.4. Physico-chemical properties analysis of SFP-100 and its fractions

The sugar content of SFP-100 and three fractions were determined by phenol-sulfuric acid method [9]. Their molecular weight distribution were evaluate by high performance gel permeation chromatography (HPGPC, Waters Delta 600, equipped with a TSK-gel G-3000swxl column, column temperature 35 °C, refractive index detector). The mobile phase was 0.1 mol/L $Na_2SO_4$, and flow rate was 0.5 mL/min [10]. The standard curve was established using a series of dextrans with different molecular weight.

The monosaccharide compositions of SFP-100 and its three fractions were determined by capillary electrophoresis (CE, Beckman P/ACE MDQ capillary electrophoresis instrument) [11]. Firstly, the sample (5 mg) was hydrolyzed with 2 mol/L TFA at 120 °C for 2 h, and TFA was removed at 45 °C under reduced pressure with methanol. 1 mL standard mixture of monosaccharides (10 mmol/L) or the hydrolyzed sample was mixed with 0.6 mL of 0.5 mol/L PMP and reacted for 30 min under 70 °C water bath, then the mixture was cooled and neutralized with 0.6 mL of 0.3 mol/L HCl. The resulting solution was extracted with chloroform three times, and the aqueous layer was filtered and performed CE analysis. The electrolyte system consisted of 75 mmol/L $Na_2B_4O_7$ (pH = 10.05) with direct DAD monitoring at 245 nm.

### 2.5. Establishment of conA-induced hepatitis model and treatment of SFP-100

We examined the protective effects of SFP-100 on ConA-induced hepatitis in mice. Animals were randomly divided into four groups (n = 11 each): (1) Control group that received water (i.g); (2) ConA model group that received ConA alone (10 mg/kg, dissolved in saline, i.v); (3) ConA (10 mg/kg, i.v.) + SFP-100 (500 mg/kg, dissolved in water, i.g) group; (4) ConA (10 mg/kg, i.v) + glycyrrhizin capsules (50 mg/kg, dissolved in water, i.g) group. Mice were injected ConA two times each week (interval of three days) and received SFP-100 one time every day from treated ConA until end of the test (10 days).

Before one day of the test end, the mice were injected ConA and administrated SFP-100, and were fasted overnight. The mice were sacrificed by dislocation. The liver, thymus and kidney of mice were selected, weighed and accounted their weight indexes. The liver and kidney samples of mice were fixed in 10% formalin, dehydrated, embedded, stained with hematoxylin and eosin and examined under a light microscope. The spleen of mice was excised for determining immune indexes.

### 2.6. Splenocyte proliferation and cytokines assay

The spleen of ConA-induced hepatitis mice was excised under aseptic conditions in essential medium (DMEM, containing 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin), and passed through a steel mesh to obtain a homogeneous cell suspension. The erythrocytes were lysed with Tris-$NH_4Cl$ buffer. The spleen cells were washed three times with the essential medium, and resuspended in the medium and counted cell numbers with a haemocytometer (cell viability >95%). Splenocyte proliferation was assayed by MTT methods. Briefly, 100 μL splenocytes were seeded into three wells of a 96-well plate at $5 \times 10^6$ cell/mL, then 100 μL Con A (final concentration 4 μg/mL), LPS (final concentration 15 μg/mL), or medium were added. The plates were incubated at 37 °C in a humid atmosphere with 5% $CO_2$. After 48 h,