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Antitussive activity of the *Schisandra chinensis* fruit polysaccharide (SCFP-1) in guinea pigs models

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ABSTRACT

Ethnopharmacological relevance: Schisandra chinensis (Turcz.) Baill. (*S. chinensis*), locally known as "Wu-weizi", has been used in the treatment of chronic cough as prescription medications of Traditional Chinese Medicine for thousands of years. However, the components of antitussive activity of *S. chinensis* and the mechanism are poorly understood.

Aim of the study: This study aims to investigate the antitussive activity of polysaccharides extracted from *S. chinensis*.

Materials and methods: S. chinensis fruit polysaccharide-1 (SCFP-1) was extracted by 95% ethanol and distilled water successively, and then the water extraction was isolated with chromatographic columns. The preliminary characterization of SCFP-1 was analyzed by gel permeation chromatography (GPC), gas chromatography–mass spectrometry (GC–MS) and some other recognized chemical methods. Antitussive potential of SCFP-1 was estimated at dose of 250, 500, and 1000 mg/kg respectively by peroral administration in a guinea pigs model with cough hypersensitivity induced by cigarette smoke (Chronic cough model) or acute cough guinea model induced by citric acid (Acute cough model). Also, the time-dependent antitussive effect of SCFP-1 were evaluated with acute cough model, and compared with codeine.

Results: The molecular of SCFP-1 was 3.18×10^4 Da, mainly being composed of glucose and arabinose (66.5% and 29.4%, respectively). Peroral administration of SCFP-1 at 250, 500, and 1000 mg/kg showed remarkable suppressive effects respectively on cough in both of chronic cough model and acute cough model. Meanwhile, inflammatory cell in BALF and some typical characteristics of nonspecific airway inflammation in animals exposed to CS was significantly attenuated after pretreatment with SCFP-1. The cough suppression of SCFP-1 (500 mg/kg) stablly lasted during the whole 5 h of time-dependent experiment, while no positive effect was observed after 300 min of oral administration of codeine.

Conclusions: SCFP-1 is one of the antitussive components of *S. chinensis*.

1. Introduction

Cough is the most common complaint in respiratory clinics (Whitburn et al., 2011; Adekoya, 2010), which can be divided into acute cough, sub-acute cough and chronic cough. Chronic cough is defined as a cough lasting for at least 8 weeks (Pavord and Chung, 2008). The common causes of chronic cough include upper airway cough syndrome (UACS), eosinophilic bronchitis (EB), cough variant asthma (CVA), and gastroesophageal reflux related cough (GERC). The patients with those common causes are usually responsive well to treatment according to cough guideline (Lai, 2008). Unfortunately, 10–46% of chronic cough patients revealed no convincing etiology during the initial workup, while cough remained refractory (Chung and Pavord, 2008). Morice et al. defined this kind of chronic cough as cough hypersensitivity syndrome (CHS) (Morice, 2010). CHS is characterized by cough reflux hypersensitivity, which can be evaluated by cough challenge test with protussive agents such as capsaicin or ally isothiocyanate (Morice, 2011).

The pathogenesis of CHS is related with the activation of

Abbreviations: S. chinensis, *Schisandra chinensis* (Turcz.) Baill.; SCFP, *Schisandra chinensis* fruit polysaccharide; EWE, ethanol-water extract; GPC, gel permeation chromatography; CS, cigarette smoke; LD, low-dose group; MD, middle-dose group; HD, high-dose group; BALF, bronchoalveolar lavage fluid; H&E, hematoxylin and eosin; Rha, Rhamnose; Fuc, Fucose; Ara, Arabinose; Man, Mannose; Gal, Galactose; Glu, Glucose; Rib, Ribose; Gal acid, Galacturonic acid; Xyl, Xylose; Tol, Talose; Myo, Myo-inositol; Glu acid, Glucuronic acid

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transient receptor potential vaniloid 1 (TRPV1), transient receptor potential ankyrin 1 (TRPA1) in airway (Groneberg et al., 2004; Birrell et al., 2009; Andrè et al., 2009), airway inflammation (Jatakanon et al.,1999; Cho et al., 2003), immune dysfunction (Birring et al., 2003; Mund et al., 2005; Grace et al., 2012), submucosa injury mediated by oxidative stress (Koskela and Purokivi, 2013; Rahman and MacNee, 2000) and mucus hypersecretion (Niimi et al., 2005; Thai et al., 2008; Jackson, 2001) etc. Currently available drugs are ineffective for patients with CHS. *Schisandra chinensis* (Turcz.) Baill. (*S. chinensis*) has been used to treat chronic cough in Traditional Chinese Medicine for thousands of years (Halstead et al., 2007; Wang et al., 2008). It is officially listed in the Chinese Pharmacopoeia as a tonic and sedative and 'Shen Nong Ben Cao Jing' book (year 1596, 2697 BCE) as a superior drug to treat asthma and cough (especially chronic cough) (Panossian and Wikman, 2008). It has been considered as indispensable in the prescription medications for the treatment of chronic cough in China and Russian States (Kim et al., 2014). Moreover, *S. chinensis* is with high safety and is officially listed as health food by State Food and Drug Administration (SFDA).

A phytochemical analysis revealed that *S. chinensis* extract mainly contains lignans, polysaccharides, volatile oils, triterpenes and organic acids (Yin, 2012; Cheng et al., 2014). There are many reports on the pharmacological and molecular mechanisms of *S. chinensis* extract on liverdamage (Cheng et al., 2013), cardiovascular diseases (Chun et al., 2014), neurasthenia (Lin et al., 2011), neoplasms (Kim et al., 2015), diabetes (An et al., 2014), sleeplessness (Zeng et al., 2014), etc. Suen reported that *S. chinensis* extract could increase respiratory frequency and volume of respiration on rabbits and dogs (Suen, 1959). Moreover, schizandrae fructus could lower airway hyper-responsiveness (AHR) to methacholine, antigen-specific immunoglobulin E (IgE) level, and immune cell infiltration in mice with asthma (Hyungwoo et al., 2014). However, the activity of *S. chinensis* on cough is poorly understood. Our preliminary study indicated that the ethanol extract (EE) and ethanol-water extract (EWE) of *S. chinensis* could decrease cough frequency and inhibit airway inflammation in a guinea pig model with cough hypersensitivity induced by cigarette smoke exposure (chronic cough model), and lignans may be the active components of EE (Zhong et al., 2015). The major components of *S. chinensis* EWE is polysaccharides (Chen et al., 2012), which present a wide range of pharmacological effects, including hepatoprotective, antitumor, antioxidant, immunoregulatory and anti-diabetic effects (Table 1). Chung and Widdicombe argued that the antioxidant and immunoregulatory activities may be related with the treatment of chronic cough (Chung and Widdicombe, 2009). So far many plants polysaccharides were proved to be with remarkable antitussive activities (Saraswathy et al., 2004), such as polysaccharides from *Glycyrrhiza glabra* (Nosalova et al., 2013a), *Terminalia chebula* (Nosalova et al., 2013b), and so on (Chattopadhyay et al., 2011; Kardošová et al., 1997; Sutovská et al., 2012). Thus, we hypothesized that *S. chinensis* polysaccharides may also have promising antitussive activities.

In the present study, a kind of *S. chinensis* fruit polysaccharide (SCFP-1) was isolated from *S. chinensis* fruits. Its chemical composition and physiochemical characteristics were determined. The suppressive activities of SCFP-1 on cough were assessed in chronic cough model and guinea pig models with acute cough induced by citric acid.

2. Methods and materials

2.1. Materials and reagents

S. chinensis fruits were collected in Anshan County, Liaoning Province, China in November 2014, and identified according to the Pharmacopoeia of the People's Republic of China (Specimen No. 20141123S). They were dried at 60°C for 24 h and ground into powder.

Diethylaminoethyl (DEAE)-cellulose and Sephadex G-75 were purchased from Pharmacia (Sweden). Polysaccharide standards were purchased from Sigma Chemical Co. (USA). Filtered cigarettes (Hongmei, 12 mg tar and 1.2 mg nicotine per cigarette) were from Guangdong Tobacco Industrial Co., Ltd. (China). Codeine tablets (30 mg per tablet, China National Pharmaceutical Industry Co. Ltd, China) were provided by The First Affiliated Hospital of Guangzhou Medical University, China. Deionized water was prepared using a Millipore water purification system. The other reagents were of analytical grade and purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China).

Table 1
Molecular weights, sugar composition and relative molar ratio of polysaccharides extracted from different parts of *S. chinensis*.

Parts	Mw. (Da)	Sugar composition and relative molar ratio	Activity	Ref.
fruit	76000	Rha, Fuc, Ara, Man, Gal, Glu, Gal acid, 1.2:0.5:0.9:1.6:1:3.3:0.3	–	(Tong et al., 2012)
	20400	Rha, Fuc, Man, Gal, Glu, Gal acid, 1.6:1.4:0.4:1:2.8:0.1		
	5300	Rha, Fuc, Gal, Glu, 1.8:0.6:1:4.2		
fruit	841000	Rha, Fuc, Rib, Ara, Xyl, Tol, Man, Glu, Myo 1.5:2.9:4.6:1.4:1.3:0.8:0.2:0.2:1.1:0.1	hepatoprotective	(Chyau et al., 2014)
leaf	127000	Man, Glu, Glu acid, 5.6:3.3:1	antitumor	(Xu et al., 2012)
fruit	–	–	antitumor	(Liu et al., 2014)
fruit	103300	Ara, Xyl, Glu, Gal acid, 2.2:1.5:5.1:0.6	antioxidant	(Sheng et al., 2011)
	61600	Ara, Xyl, Glu, Gal acid,1.4:1.8:3.3:3.6		
	34700	Ara, Xyl, Glu, Gal acid,1.6:0.9:3.2:4.1		
fruit	33500	Ara, Xyl, Man, Glu, Glu acid, 2.3:1.5:1:3.2: nd	antioxidant	(Gao and Chen, 2011)
	86400	Ara, Xyl, Man, Glu, Glu acid, 1.8:1.3:1:4.1: nd		
	42500	Ara, Xyl, Man, Glu, Glu acid, 1.2:0.4:1:3.5:3.7		
fruit	7700	–	immunoregulatory	(Chen et al., 2012)
fruit	3400	Man, Glu, Gal,1:11.38:3.55	antitumor	(Zhao et al., 2013a)
fruit	68000	Rha, Ara, Xyl, Glu, Gal, Man, –	anti-diabetic	(Zhao et al., 2013b)
	350000	–		
	3000000	–		

Rhamnose (Rha), Fucose (Fuc), Arabinose (Ara), Mannose (Man), Galactose (Gal), Glucose (Glu), Ribose (Rib), Galacturonic acid (Gal acid), Xylose (Xyl), Talose (Tol), Myo-inositol (Myo), Glucuronic acid (Glu acid)