

ANTITUSSIVE ACTIVITY OF EXTRACTS FROM FALLOPIA SACHALINENSIS

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Abstract

Giant knotweed (*Fallopia sachalinensis*) and related plants are commonly used in Chinese and Japanese folk medicine. Bioactive natural products derived from them are believed to possess a variety of biologic activities. We have analyzed two polysaccharide fractions from leaves of *Fallopia sachalinensis*. We have focused on its activities to experimentally induced cough reflex and the changes of specific airway resistance. We have compared the observed antitussive activity with cough suppressing activity of codeine („positive“ control) and effect acquired after application of water for injection („negative“ control) using conscious male guinea pigs individually placed in a double chambers bodyplethysmograph box. Peroral administration of both polysaccharides significantly inhibited the number of coughs induced by citric acid in guinea pigs and have not significantly changed the values of specific airway resistance. Moreover, the substances in experimental animals have not provoked any notable adverse events. The biological activity observed in derivatives of *Fallopia sachalinensis* provides a scientific basis for the use of the plant in traditional medicines.

Key terms: *Fallopia sachalinensis*, pectic polysaccharides, antitussive activity, cough reflex

INTRODUCTION

Giant knotweed (*Fallopia* or *Reynoutria sachalinensis*) is a known invasive plant species native to East Asia, belonging to the Polygonaceae family. Bioactivity of derivatives of these species of plants as well as its use in traditional Chinese and Japanese medicines was already described in literature (1,2). Extracts from the leaves of *Fallopia sachalinensis* were already investigated for potential utilizations. (3) The content of neutral carbohydrate components of pectic polysaccharides and hemicelluloses was 37.7 %. Uronic acid (UA) represented 10.1% and the rest (52.2%) was comprised by ash, protein, lignin and -cellulose. A sequential extraction of the extractives-free leaves revealed the presence of pectic polysaccharides of the homogalacturonan and rhamnogalacturonan types.

Bioactive properties of pectins are well known and widely used. (4) The effects of polysaccharides extracted from other various plants on experimentally induced cough reflex in experimental animals (conscious guinea pigs or cats) were widely studied and described in previous works (5,6,7,8).

The aim of this presented work was to assess antitussive activity of two different polysaccharide fractions extracted from the leaves of *Fallopia sachalinensis* in two different doses and compare it with cough suppressing activity of codeine.

MATERIAL AND METHODS

Plant material

Leaves of giant knotweed (*Fallopia sachalinensis*) were collected in April 2007 in Český Krumlov (Czech Republic) and kindly provided by Dr. N. Vrchotová (Institute of Landscape Ecology, AS CR). The leaves were dried at laboratory temperature and ground to a fine powder.

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Extraction of polysaccharides:

Extractive substances were removed from a fine powder of air dried leaves by Soxhlet extraction with chloroform/ethanol (65 : 35) for 6 h and subsequently with methanol for 3.5 h to remove the main extractive compounds. The resulting extractive-free leaves of *F. sachalinensis* (FS, 90 g) were sequentially extracted using distilled water (1 100 mL) for 2 h at 60°C and than 14 h at laboratory temperature. The suspension was centrifuged at 12 000 min⁻¹ for 7 min. The supernatant was concentrated, poured into four volumes of ethanol and the precipitate was separated by filtration, subjected to dialysis, and the retentate was lyophilized (FS-1; 1.34 g). The dry residue (75.6 g) was extracted with acetate buffer pH 4.8 (800 mL at 70 °C) for 2.5 h and then the suspension was centrifuged and extract was concentrated. The concentrated extract was filtered on a S4 filter, than neutralized to pH 7.1 and precipitated with ethanol. The precipitate was separated by filtration, subjected to dialysis (cellulose membrane, MWCO, 1 kg mol⁻¹, Serva), and the retentate was lyophilized yielding fraction FS-2 (2.15 g).

Antitussive activity

Animals:

Healthy adult male guinea pigs, weighing 200-350 g, supplied by Department of Experimental Pharmacology, Slovak Academy of Science, Dobra Voda, Slovakia, were kept in the animal house with food and water *ad libitum* with standard air-conditioning system. Animals were kept at least one week in quarantine before starting experiment. Each polysaccharide samples as well as control agents ("positive" codeine and "negative" vehicle) were tested on individual group of 8 guinea pigs.

The experimental protocols were approved by the institutional Ethics Committee of Jessenius Faculty of Medicine, Comenius University in Martin and complied with Slovakian and European Community regulations for use of laboratory animals. This is in accordance with the revised Declaration of Helsinki from 1983 and follows the criteria of experimental animals welfare.

Assessment of chemically induced cough and airways defence reflexes

Conscious guinea pigs were placed in a double-chamber bodyplethysmograph box (HSE type 855, Hugo Sachs Elektronik, Germany) and restricted so that the head protruded into nasal chamber and the neck was sealed with a soft diaphragm. The cough reflex was induced by an citric acid aerosol in concentration of 0.3 M, generated by a jet nebulizer (PARI jet nebulizer, Paul Ritzau, Pari-Werk GmbH, Germany, output 51 s⁻¹, particle mass median diameter 1.2 µm) and delivered into the head chamber of the pletysmograph for 3 minutes. Cough efforts, defined as sudden enhancements of expiratory air flow associated with typical motion and sound observed by trained personnel were registered over this time interval (9). The airway smooth muscle reactivity *in vivo* was expressed as specific airway resistance calculated according to Pennock et al. by time difference between pressure changes in both parts of bodyplethysmograph during normal breathing pattern (10). Cough efforts as well as airway resistances were registered before administration and subsequently 30, 60, 120 and 300 minutes after application of the compound. Minimum 2 hours interval between two measurements was set to prevent adaptation of cough receptors as well as general adaptation of guinea pigs to this kind of irritation.

All tested substances (vehicle, codeine as well as both polysaccharides) we applied orally. Vehicle (water for injection) was given in the dose of 1 ml.kg⁻¹ body weight and codeine in the dose of 10 mg.kg⁻¹ body weight. Both fractions of pectin polysaccharides were dosed 50 mg.kg⁻¹ and 75 mg.kg⁻¹ body weight respectively, in order to assess basic dose dependent antitussive activity differences.

Statistical analysis

For statistical analysis Student t-test was used. Data are represented as means ± standard errors of the mean (± S.E.M.) and p<0.05 was considered as threshold for statistical

significance. Significance levels of $p < 0.05$, $p > 0.01$ and $p < 0.001$ are shown by one, two or three asterisks, respectively.

RESULTS

Extraction of polysaccharides

The leaves of giant knotweed were repeatedly treated with chloroform/ethanol and subsequently with methanol to remove the main extractive compounds. The drug residue was extracted with hot water and subsequently from the dry residue with acetate buffer. Polysaccharides FS-1 and FS-2 were obtained by precipitation of the water extracts. The amount of material recovered in the FS-1 fraction represented 1.47 % and in the FS-2 fraction 2.37 % of total cell wall material (FS). The analytical data of both fractions are summarized in Table 1. Fractions differed in the content of UA, composition of the neutral sugars and content of protein and total phenolics. In comparison to the FS-2, the hot water extracted FS-1 showed small portion of protein and a significantly higher content of total phenolics. Analysis of the hydrolyzates revealed the galacturonic acid to be the main acidic sugar component in these fractions, which is characteristic for pectin. The high content of arabinose and galactose residues in FS-1 as well as the presence of UA (29%) indicates presence of arabinogalactans associated with galacturonan core. On hydrolysis fraction FS-2 contained very high portions of UA (~68 %). Galactose, arabinose and rhamnose were the predominant neutral sugars and the other sugars were detected only in low amounts (Table 1). These results suggest that the FS-2 is pectic polysaccharide and contains less neutral sugars than this of FS-1. Polysaccharide fraction FS-1 showed one peak at M_w 51 000 with broad molecular-mass distribution pattern with degree of polydispersity $P = 1.48$. On the contrary, fraction FS-2 was homogeneous ($P = 1.06$) and on HPLC showed one symmetrical peak with M_w 168 000.

Table 1. Analytical data of polysaccharide fractions from extractive-free leaves of *Fallopia sachalinensis*; TC – total carbohydrates content, NC – neutral carbohydrates, UA – uronic acid content, P – protein content, TP – total phenolic content, EC_{50} the lowest mass ratios of the sample (mg) to DPPH* (mg) needed to scavenge the 50% of initial DPPH* and $\pm$ standard deviation, Gal – Galactose, Glc – Glucose, Man – Mannose, Ara – Arabinose, Xyl – Xylose, Rha – Rhamnose.

	TC (%)	UA (%)	NC (%)	P (%)	TP (%)	EC_{50}
FS-1	65.9 $\pm$ 1.4	29.2 $\pm$ 1.2	36.7 $\pm$ 2.1	5.6 $\pm$ 0.09	18.9 $\pm$ 1.6	0.85
FS-2	67.9 $\pm$ 1.3	57.9 $\pm$ 2.6	10.0 $\pm$ 1.8	–	7.1 $\pm$ 0.6	1.1
	Neutral sugar composition (rel. mol %)					
	Ga	Glc	Man	Ara	Xyl	Rha
FS-1	28	31	8	24	6	2
FS-2	29	18	2	32	6	12

Antitussive activity

Both polysaccharide fractions of *Fallopia sachalinensis* leaves (sample FS-1 and sample FS-2) were tested for cough-suppressing activity using double-chamber bodyplethysmography. The antitussive effect was evaluated on the citric-acid induced cough efforts and airways smooth muscle reactivity *in vivo* in adult healthy conscious guinea pigs after oral application of two different doses 50 mg.kg⁻¹ and 75 mg.kg⁻¹ body weight of both samples (FS-1, FS-2) respectively, in order to assess the impact of dose escalation on suppression of cough reflex and specific airway resistance.

Polysaccharide fraction marked as FS-1 showed significant suppression of chemically induced cough efforts already after 30 minutes after oral application in dose of 50 mg.kg⁻¹ body weight (Fig. 1). Subsequently in next time intervals cough-suppressing activity was more pronounced and maximal antitussive effect was achieved 120 minutes after oral application of sample FS-1. 300 minutes after oral application of sample FS-1 we observed decline in suppression of number of cough efforts, but still within the range of statistical significance comparing to baseline values. Dose increase of polysaccharide FS-1 up to 75 mg.kg⁻¹ body weight brought slightly increased suppression of the number cough efforts comparing to application of the lower dose (Fig. 1).

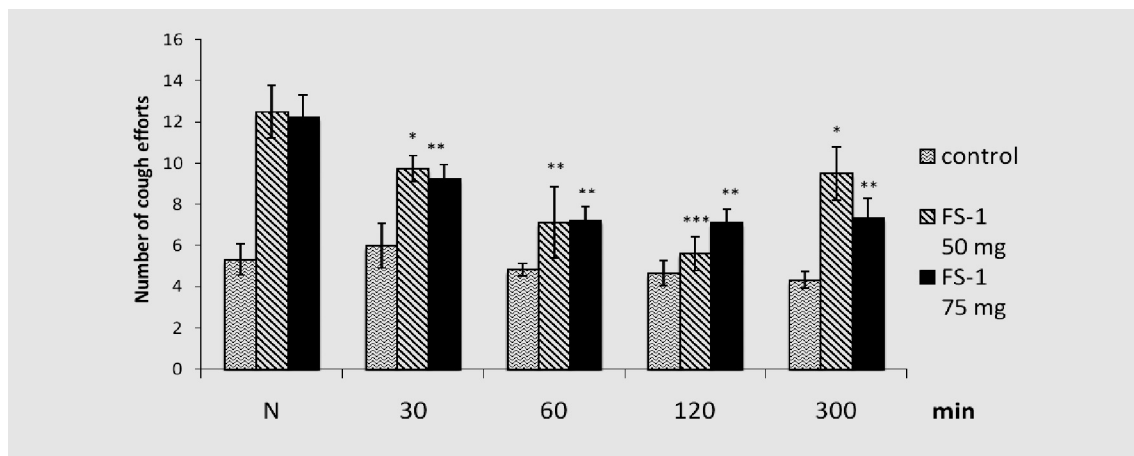


Fig. 1. Number of citric acid induced cough efforts after oral application of polysaccharide fraction FS-1 from *Fallopia sachalinensis* in a dose of 50 mg.kg⁻¹ b.w. and 75 mg.kg⁻¹ b.w. First column represents control group (water for injection). Axis x - represents time intervals of the chemical stimulation of the airways and axis y - represents changes of the number of the cough efforts. N - cough values recorded before application of FS-1. The columns are represented as mean values of number of cough efforts, the range denotes standard error of means $\pm$ S.E.M. The significance $p < 0.05$ is marked by one asterisk, $p < 0.01$ by two asterisks and $p < 0.001$ by three asterisks.

Polysaccharide fraction marked as FS-2 in dose of 50 mg.kg⁻¹ body weight showed significant suppression of chemically induced cough efforts also already after 30 minutes after oral application (Fig. 2). However, the highest suppressive effect was achieved already in 60

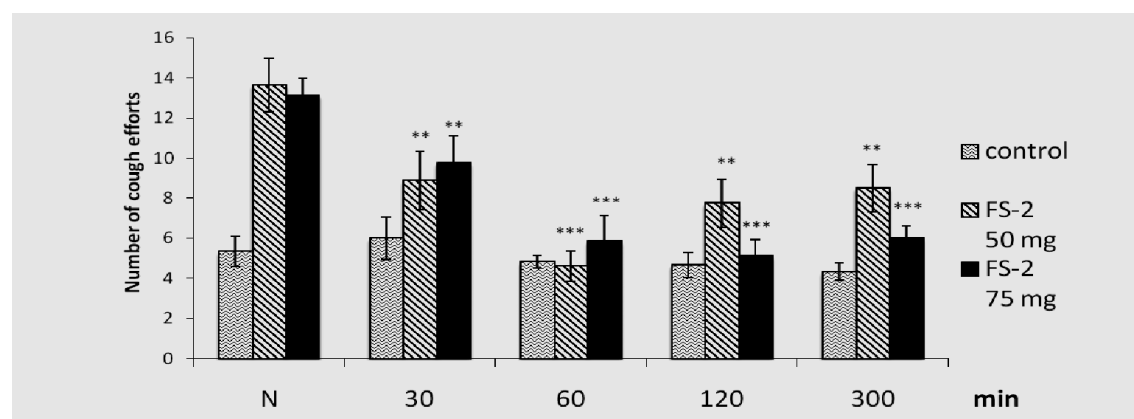


Fig. 2. The changes of the cough efforts after oral application of polysaccharide fraction FS-2 from *Fallopia sachalinensis* in a dose of 50 mg.kg⁻¹ b.w. and 75 mg.kg⁻¹ b.w. First column represents control group (water for injection). N - cough values recorded before application of sample marked as FS-2.

minutes after application of the sample FS-2. The statistically significant suppression of the number of the cough efforts were observed during the whole experiment. Dose increase of polysaccharide FS-2 up to 75 mg.kg⁻¹ body weight showed more significant antitussive effect in 60, 120 and 300 min intervals after oral application of this sample comparing to the lower dose (Fig. 2).

Comparison of cough suppressing activities of both FS-1 and FS-2 on the number of cough efforts revealed quantitatively higher cough suppression after application of the higher dose (75 mg.kg⁻¹ b.w.). Direct comparison of the cough suppressive activity of the samples FS-1 and FS-2 to centrally acting and in clinical condition the most active the cough suppressive agent codeine (administered orally in the dose of 10 mg.kg⁻¹ b. w.) we found that sample FS-2 was comparable or even more active in suppression the number of cough efforts as codeine in some time intervals (Fig. 3).

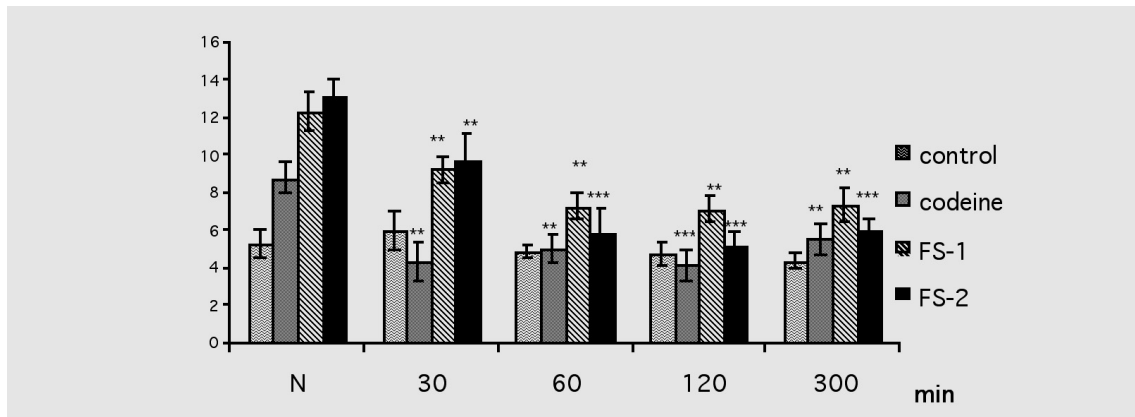


Fig. 3. The comparison of the cough suppressive activities of the samples FS-1 and FS-2 extracted from the *Fallopia sachalinensis* leaves and administered in a dose of 75 mg.kg⁻¹ b.w. orally and codeine phosphate (positive control – administered in a dose 10 mg.kg⁻¹ orally). First column represents control group (negative control – water for injection).

Neither of polysaccharide fractions (FS-1 as well as FS-2) significantly alter specific airway smooth muscle reactivity, measured as specific airways resistance (sRaw), after oral application, regardless the time interval or dose administered (50 mg.kg⁻¹ body weight or 75 mg.kg⁻¹ body weight) (Fig. 4).

DISCUSSION

The analysis of *in vivo* antitussive activities of polysaccharides FS-1 and FS-2 found significant reduction of number of cough efforts chemically induced by citric acid in adult healthy awaken guinea pigs. This cough-suppressing effect was confirmed in both polysaccharide fractions administered orally in doses of 50 mg.kg⁻¹ body weight as well as 75 mg.kg⁻¹ body weight, respectively without provoking any notable undesirable reactions. The analysis of *in vivo* airway smooth muscle reactivity showed no effect on specific airway resistance in both fractions, regardless of the dose. Increase in dose led to prolongation and increase of suppressive activities. Intensity of antitussive effect was numerically higher after administration of FS-2 when comparing to FS-1, especially after oral application in the dose of 75 mg.kg⁻¹ body weight. Antitussive activity, measured as suppression of number of cough efforts, of polysaccharide sample FS-2 was found to be comparable or even bigger as codeine, until now the most effective and in clinical practice most frequently used cough suppressing agent. Results of our experiments confirmed the statistically significant sup-

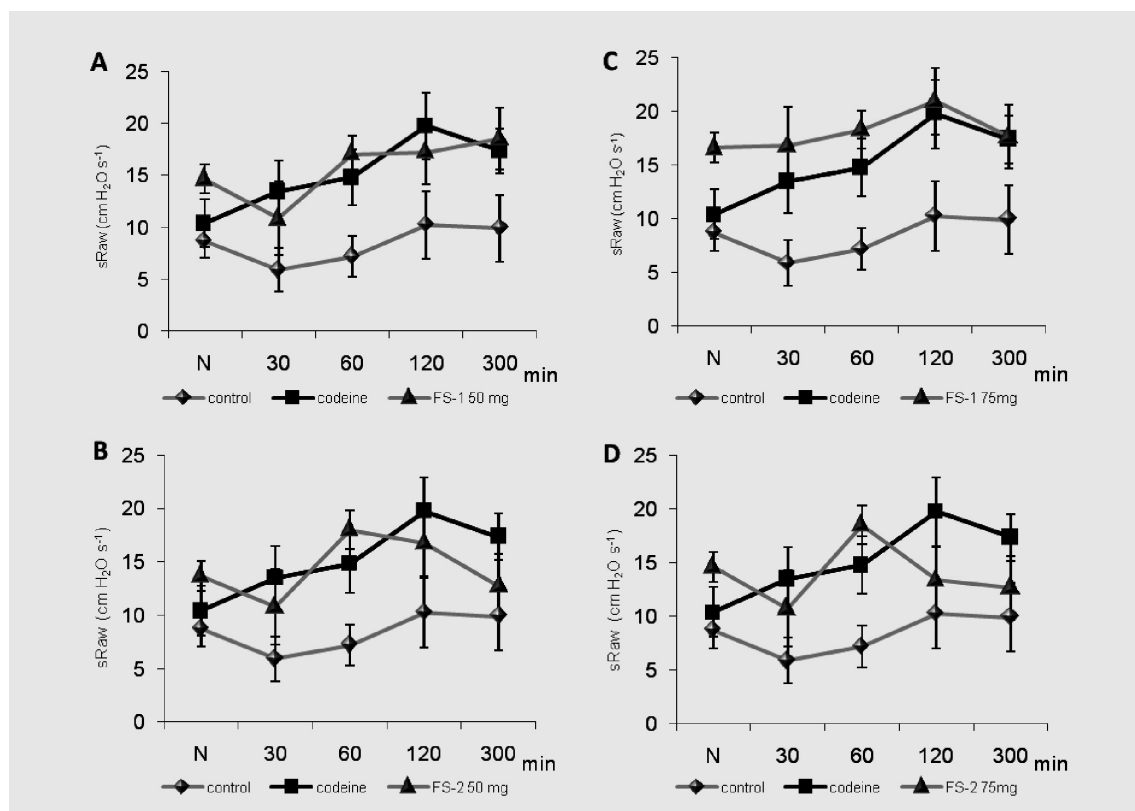


Fig. 4. The influence of polysaccharide samples FS-1 and FS-2 administered orally in the doses of 50 mg kg⁻¹ and 75 mg kg⁻¹ body weight, respectively, compared to control (water for injection, negative control) and codeine (positive control - administered in a dose 10 mg kg⁻¹ b.w.) on specific airways resistances (sRaw) in healthy adult con-

pressive effect on number of cough efforts induced chemically by citric acid *in vivo* conditions in healthy adult male awoken guinea pigs in both polysaccharide samples FS-1 as well as FS-2. We tried to address the question: what mechanism could take part in antitussive bioactivity of these molecules. Analysis of *in vivo* airway smooth muscle reactivity showed no alteration of specific airway resistances in both tested samples after oral application. Bronchodilatation thus probably does not play a role in the mechanism of the cough suppressive activity of followed samples. Nevertheless, we have found differences in cough suppressive activities when comparing sample FS-1 to sample FS-2. Analysis of both samples revealed structural differences which could drive the different antitussive properties of both polysaccharides (Table 1). Higher molecular weight as well as higher content of uronic acid in the sample FS-2 comparing to sample FS-1 could play a role in more pronounced cough suppressive effect observed after oral application of FS-2. In fact earlier studies of antitussive activities of various polysaccharides revealed increase of antitussive effect with increased content of uronic acid (7). The part of this difference could be due to higher molecular weight of FS-2. Polysaccharides, mainly pectins, as present in sample FS-2, with high molecular size are not completely absorbed after oral application and stay longer in contact with the mucous terminals of the epipharyngeal nerve. This interaction could cause the decrease of sensitivity of cough receptors to chemical irritation (in our case citric acid) of upper respiratory tract, and thus indirectly lead to cough suppression (11,12,13). It was described that some polysaccharides administered in the form of highly viscose solutions

formed gels on the mucous surface and thus preventing irritation of the airways and contributing to cough suppressing activities as well (14). Contribution to the overall antitussive properties could have also known fact that some polysaccharides increase production of saliva with subsequent activation of swallow reflex interfering with cough reflex and suppressing it.

We assume that various combinations of those mechanisms can contribute to observed cough suppressing activity of polysaccharides derived from the leaves of *Fallopia sachalinensis*.

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