PHARMACOLOGICAL STUDY

The antitussive activity of polysaccharides from *Althaea officinalis* L., var. *Robusta*, *Arctium lappa* L., var. *Herkules*, and *Prunus persica* L., Batsch

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Abstract

Background: **The therapy of pathological type of cough presents serious medical problem.**
Objectives: **The aim of experiments was to investigate polysaccacharide influence on experimentally induced cough.** ***Methods:*** **The purified and/or modified polysaccharides from the flowers and plants, characterized by chemical composition and molecular properties were subjected to tests for antitussive activity on cough, induced mechanically in conscious cats of both sexes.**
Results: **The results revealed that the tested polysaccharides exhibited statistically significant cough-suppressing activity, which was noticeably higher than that of the non-narcotic drug used in clinical practice to treat coughing. The most expressive antitussive activity was observed with the polysaccharide from marsh mallow, containing the highest proportion of the uronic acid constituent. Negative influence of the tested compounds on expectoration was negligible when compared to that of codeine.**
Conclusion: **Antitussive activity of various plant polysaccharides was confirmed. These results allow ranging them among prospective antitussive agents** ***(Tab. 2, Fig. 6, Ref. 15)*** **Full Text (Free, PDF)** ***www.bmj.sk.***
Key words: polysaccharides, ***Althaea officinalis, Arctium lappa,*** **peach-tree gum, antitussive activity.**

The cough, a powerful physiological protective and clearing mechanism of the airways, results from the stimulation of airway sensory receptors by a variety of inhaled substances and released mediators or mechanical irritation of nerve endings, whose afferent impulses activate a brainstem cough center (Dicpinigaitis and Gayle, 2003). Cough is also the most common symptom of respiratory tract disorders. Pharmacological modulation of pathological cough is still unsatisfactory despite of the intensive research resulting in large number of publications on experimental results. Opioid antitussives are still widely used in treatment of dry cough during catarrhs of the respiratory system. However, their use brings about side effects such as increase of the mucus viscosity, decrease of expectoration, hypotension or constipation (Braga and Allegra, 1991). Moreover, long-term treatment with opiate agonists may lead to drug dependence. Therefore, in recent years much effort has been made to search for natural active plant components with diminished adverse effects. Plant polysaccharides are ranked among compounds, which shield and protect the nerve endings and alleviate pain. The calming and protective effect is apparent also in inflamed mucous membranes of upper airways, leading to reduction of reflexive irritation that induces cough. In our previous works (Nosalova et al, 1992, 1993, Kardosova et al, 1997) we investigated the effect of extracts, mucilages, and structurally defined polysaccharides from medicinal plants on mechanically induced cough in conscious cats. The results, revealing noticeable antitussive activities, in most cases higher than the activity of reference non-narcotic drugs, encouraged us to carry on with such a research and widen the spectrum of natural agents, potential substitutes

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Acknowledgements: This work was supported by the research Grant No. 2/3162/23 from the Slovak Grant Agency VEGA and Grant No. 38/ /2004 offered by Comenius University in Bratislava.

for synthetic drugs. Now, we present the isolation, characterization, and antitussive activity of polysaccharides from marsh mallow, burdock, and peach-tree gum exudates. The choice of the polysaccharide sources used herein was motivated by the long recognized curing effects of these medicinal plants in the relevant field and in the case of the peach gum polysaccharide we expected a soothing effect on the mucous membrane due to its uronic acid content.

Materials and methods

Plant material

The flowers of marsh mallow (*Althaea officinalis L.*, var. *Robusta, Malvaceae*) were obtained from the Center for Cultivation of Medicinal Plants, Faculty of Medicine, J. E. Purkyně University, Brno, Czech Republic, where a voucher specimen is deposited. The leaves of burdock (*Arctium lappa L.*, var. *Herkules, Asteraceae*) were a gift from CPN, a workshop producing polysaccharides, Ústí nad Orlicí, Czech Republic. This variety is included in the List of registered plant varieties in Slovakia and Czech Republic (Zvachova and Miskolcziova, 2001). The gum exudate from peach tree (*Prunus persica L.* Batsch) was collected in an orchard near Bratislava, Slovakia.

General methods

Descending paper chromatography was performed on Whatman No.1 with systems: S1, EtOAc-pyridine-water (8:2:1); S2, EtOAc-acetic acid-formic acid-water (18:3:1:4), the sugars being detected with aniline hydrogen phthalate. TLC was effected on Silica Gel 60 plates (Merck, Darmstadt, Germany) with 1-propanol-methanol-water (2:1:1). After spraying the plates with 20 % ammonium sulfate, the spots were visualized by charring. Nitrogen content was determined with elemental analyzer Fisons instrument EA1108 and ash content gravimetrically by combustion at 850—900 °C. Polysaccharides were hydrolyzed with 2 M trifluoroacetic acid at 100 °C for 20 h. Sugars in the hydrolyzates were converted into alditol trifluoroacetate derivatives and analyzed by GLC on a Hewlett-Packard 5890 Series II using a PAS-1701 column (0.32 mm 25 m) at a temperature program of 110–125 °C (2 °C min^{-1}) -165 °C (20 °C min^{-1}) and a flow rate of hydrogen 20 ml min^{-1}. The uronic acid content was determined potentiometrically using a Radiometer PHM 64 (Denmark) equipped with a GK 2401 C combined electrode. High-performance gel--permeation chromatography (HPGPC) was performed on Labio Prague Biospher 300 and 1000 exclusion columns (8 x 250 mm) using a commercial instrument (Laboratorní přístroje, Prague, Czech Republic) and 0.1 M $NaNO_3$ as eluent (0.4 ml min^{-1}). RI and UV detectors monitored the eluate and the columns were calibrated with pullulan standards P5 – P800 (Shodex Standard, PS-82, Macherey-Nagel GmbH, Germany). ^{13}C and ^{1}H NMR spectra of the aldobiouronic acid were recorded in deuterated water at 25 °C on an FT NMR Bruker AVANCE DPX 300 spectrometer (^{1}H at 300.13 MHz and ^{13}C at 75.46 MHz) equipped with selective unit and gradient enhanced spectroscopy kit (GRASP) for generation of z-gradients up to 50 Gauss cm^{-1} in a 5 mm inverse probe kit. Chemical shifts of signals were referenced to internal acetone (2.225 and 31.07 ppm for ^{1}H and ^{13}C, respectively).

Isolation of the polysaccharide (ILK) from the flowers of marsh mallow (Althaea officinalis L., var. Robusta)

The dry flowers, cut into small pieces, (100 g) were defatted with $CHCl_3$-MeOH (2:1 v/v; 500 ml) at 60 °C for 2 h, followed by removal of pigments from the residue with MeOH-H_2O (4:1 v/v; 500 ml) at 70 °C for 2 h. The air-dried residue was extracted with distilled water (5000 ml) under stirring for 4 h at room temperature. After filtration through a nylon cloth and centrifugation (5000 g), the filtrate was poured into 3 vol of acidified EtOH (1 % HCl). The precipitate was washed with 70 % aqueous ethanol, dissolved in water, the insoluble part was removed by centrifugation and the supernatant was exhaustively dialyzed (MW-cut off 1214 kDa) against distilled water. The retentate was freeze-died to give the polysaccharide (ILK).

Isolation of the polysaccharide (LL) from the leaves of burdock (Arctium lappa L., var. Herkules)

Dry leaves cut into small pieces were preextracted with 50 % aqueous EtOH (5 v/w) under stirring for 2 h at room temperature. The air-dried plant residue was macerated in distilled water (50 v/w) under stirring for 24 h at room temperature, filtered through a nylon clothe and sintered glass filter S2. The filtrate was precipitated with 4 vol EtOH, washed with 80 % aqueous EtOH containing1 % HCl, followed by 80 % aqueous EtOH, dissolved in distilled water, and dialyzed to constant conductivity of the dialyzate. The retentate was freeze-dried to give the polysaccharide (LL).

Isolation of the peach gum polysaccharide (BG) and preparation of the degraded polysaccharide (DPS)

The hard, light-brown peach gum was dissolved in 25 vol-distilled water, the insoluble part was filtered through a nylon cloth and discarded. The filtrate was centrifuged (5 000 g) and precipitated with 3 vol EtOH containing 1 % HCl. The precipitate was washed with 70 % aqueous EtOH, dissolved in distilled water and exhaustively dialyzed. The crude polysaccharide (BG) was recovered from the retentate by freeze-drying.

BG (10 g) was modified by hydrolysis with 0.5 M H_2SO_4 (50 ml) for 30 min at 100 °C. After cooling, neutralization with 0.5 M NaOH and filtration, the solution was exhaustively dialyzed against distilled water and the nondialyzable retentate was freeze-dried. This product was further purified on a column (2.2 cm x 150 cm) of Biogel P-2 (Bio-Rad, USA) by water elution and the degraded polysaccharide DPS (3.8 g) was recovered from the void volume eluate.

Animals

Adult cats of both sexes weighing 1500–2500 g (10 in each set) were used in the experiments. The experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (N.I.H. Publication No. 85–23) and with approval

Tab. 1. Characteristic data of polysaccharides ILK, LL, BG, and DPS.

Characteristics	Polysaccharides			
	ILK	LL	BG	DPS
Ash (%)	5.8	7.2	1.3	–
Protein (%)	15.5	21.7	–	–
Uronic acid(%)	46.2[a]	–	7.0[b]	26.3[c]
	Neutral sugar composition (mol %)			
Ribose	–	1.9	-	–
Fucose	–	4.6	-	–
Arabinose	12.4	8.8	43.2	–
Xylose	2.6	3.9	20.2	5.9
Mannose	2.6	15.9	2.7	41.5
Glucose	5.8	28.2	–	–
Galactose	20.2	22.0	33.9	52.6
Rhamnose	56.4	14.7	–	–

[a]galacturonic acid; [b]mixture of glucuronic and 4-O-methyl-glucuronic acids; [c]glucuronic acid
Protein content (%) was obtained by multiplying the nitrogen content by the coefficient 6.25.

of the Ethics Committee of Jessenius Faculty of Medicine, Comenius University and Faculty Hospital in Martin (Slovakia).

Antitussive activity assay

Adult cats were during several days quarantine adapted to our conditions. Subsequently, a chronic endotracheal cannula was surgically implanted under general anesthesia, followed by standard surgical care of the wound during seven days. The cannula enabled mechanical stimulation of airways and the recording of the side tracheal pressure. The mucous membranes of the tracheobronchial (TB) and laryngopharyngeal (LP) areas were stimulated consecutively five times with a 0.35 mm diameter nylon fiber. This step of experiment was realized on conscious animals to eliminate possible influence of anesthetics on cough reflex. The cough-related parameters, i.e. the number of efforts (NE), intensity of cough attack in expiration (IA^+) and inspiration (IA^-), cough frequency (NE min^{-1}), and intensity of maximum efforts in expiration (IME^+) and inspiration (IME^-) were evaluated on the basis of the pressure changes values recorded on a Biograph 12-03 electromanometer. The parameters marked on figures as N (control) represented the cough values obtained after stimulation of the airways in the absence of the tested compounds. The water solution of the tested compound was administered perorally in a dose of 50 mg kg^{-1} b.w. and 100 mg kg^{-1} b.w, respectively. For comparative purpose commercial drugs generally used in clinical practice to treat cough, i.e. dropropizine (D), guaifenesin (G), and codeine (C), were tested along with the polysaccharides under the same conditions. The doses of D=100 mg kg^{-1} b.w., G=100 mg kg^{-1} b.w., and C=10 mg kg^{-1} b.w., used herein, represented the amounts, which in our earlier experiments exhibited the highest antitussive activity. The effect of drugs was monitored in time intervals 0.5, 1, 2, and 5 h. Statistical evaluation of the results was carried out by the method of Wilcoxon and Wilcox (1964).

Results

Characterization of polysaccharides

Water extraction of the defatted flowers of **A. officinalis,** followed by ethanol precipitation, gave a brown crude product in 3.7 % yield per weight of dry plant material. The nondialyzable retentate (ILK) contained besides carbohydrates also protein and inorganic material (Tab. 1). The carbohydrate moiety consisted of six neutral sugar components of which rhamnose represented as much as 56.4 mol %. The proportions of galactose (20.2 mol %) and arabinose (12.1 mol %) were also noticeable. The content of uronic acid in the polysaccharide (ILK) was 46.2 % and the mobility of this component on paper chromatography in the system S2 was identical with that of the standard D-galacturonic acid.

The molecular properties of ILK could not be characterized due to its limited solubility in water. Nevertheless, the sugar composition with dominating rhamnose and galacturonic acid pointed to a high similarity with the acidic heteropolysaccharide isolated from the flowers of *Malva mauritiana L.*, which had a branched structure with the backbone formed by alternating sequences of 2- and 4-linked rhamnogalacturonan-type, while the side chains consisted mainly of galactose and arabinose units (Capek et al, 1997).

From the burdock leaves, pretreated with aqueous ethanol, a crude polysaccharide was isolated by water extraction, followed by ethanol precipitation. Subsequent purification of the precipitate by exhaustive dialysis gave the polysaccharide (LL) in 0.9 % yield per weight of dry leaves. Table 1 show that this polysaccharide consisted of 8 neutral sugar components, indicating a complex of different polysaccharide species. Table 2 shows the molecular-weight diversity from about 240 kDa to less than 2 kDa. The dominant sugar components were glucose and galactose, followed by mannose, rhamnose, and arabinose.

Tab. 2. Relative molecular-mass distribution pattern of polysaccharides LL and DPS obtained by HPGPC on pullulan-calibrated GM Biospher columns.

Polysaccharide	Mw (Da)	Mw/Mn	Area (%)
LL	238 000	1.21	65.7
	14 000	1.19	14.8
	6 300	1.18	11.8
	<2 000	–	7.7
DPS	15 650	1.09	89.4
	2 200	1.19	10.6

Attempts to fractionate this complex polysaccharide by ion exchange and gel-permeation chromatography have not led to any distinct fractions with regard to the sugar composition. Differences were detected only in their relative proportions (results not shown). In our earlier works (Kardosova, 1992, Kardosova and Capek, 1994) we described a glucan and a rhamnoarabinogalactan isolated by water extraction from the leaves of a medicinal plant *Plantago lanceolata L., var. libor.* A complex polysaccharide was obtained also by water extraction of the green parts of other medicinal plant *Rudbeckia fulgida, var. sullivantii* (Boynton et Beadle) and was shown to comprise glucans, arabinogalactans, and xylans (Kardosova et al, 1997). Watanabe et al (1991) isolated from burdock pectic substances, hemicelluloses, and cellulose and Kato and Watanabe (1993) a branched xyloglucan containing also galactose and fucose.

With regard to our earlier positive results with acidic plant polysaccharides in antitussive activity tests, it seemed useful to include in the comparative studies also 2 acidic polysaccharides isolated from peach-tree gum exudates. The native polysaccharide (BG) was obtained from the freeze-dried gum solution by ethanol precipitation in 65 % yield. Its sugar composition (Tab. 1) shows the dominance of arabinose, followed by galactose and xylose. The uronic acid content accounted for 7 % of the polysaccharide. The gross structural features of this high-molecular-weight acid heteropolysaccharide (Mw=1100 kDa) were described years ago in our work (Rosik et al, 1966). The main chain was shown to consist mainly of 1,6- and 1,3-linked galactose residues, while arabinose, xylose and uronic acids were attached to the main chain residues, as confirmed by fragments released during graded acid hydrolysis.

The native gum polysaccharide (BG) was modified by partial acid hydrolysis to remove the branchings and get a polysaccharide (DPS) of much lower molecular weight (ca.15 kDa) (Tab. 2). As seen from Table 1, galactose and mannose were the main neutral sugar components resisting partial acid hydrolysis and, thus, pointing to their positions in the backbone in accordance with the results of Rosik et al (1966). The acid sugar component was proved to be D-glucuronic acid and accounted for as much as 26 % of the polysaccharide. The aldobiouronic acid, produced on acid hydrolysis of DPS and recovered from the hydrolysis products by preparative paper chromatography in the system S2 was shown to be composed of galactose and glucuronic acid. Its ^{13}C NMR spectrum was identical with that of the standard 6-O-ß-D-glucuronopyranosyl-D-galactose (Kardosova et al, 1986), evidencing the attachment of the glucuronic acid to chain galactose units.

Antitussive activity of polysaccharides

The acidic polysaccharide ILK from the flowers of marsh mallow, administered to cats perorally in the dose of 50 mg kg^{-1} body weight, significantly decreased the number of cough efforts (NE) and intensity of cough attacks (IA^+ and IA^-) from both tracheobronchial (TB) and laryngopharyngeal (LP) irritated areas (Figs 1 and 3). The first statistically significant drop of NE 30 min indicated prompt onset of the effect from both TB and LP regions after administration of the polysaccharide. The suppressive effect lasted throughout the 5-h experiment. The parameters of intensity of cough attacks during inspiration (IA^-) from both TB and LP areas and during expiration (IA^+) from TB region were reduced also 30 min. after administration of the compound and the values remained reduced during the whole ex-

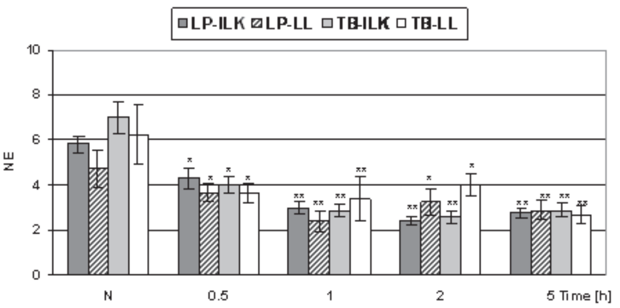

Fig. 1. Effect of polysaccharides from the flowers of Althaea officinalis (ILK) and leaves of Arctium lappa (LL) administered perorally in 50 mg kg^{-1} b.w. dose and 100 mg kg^{-1} b.w. dose, respectively, to conscious cats on number of cough efforts (NE) from laryngopharyngeal (LP) and tracheobronchial (TB) areas of airways. N is the control value of cough parameter, error bars indicate ±SEM, * $p<0.05$, ** $p<0.01$.

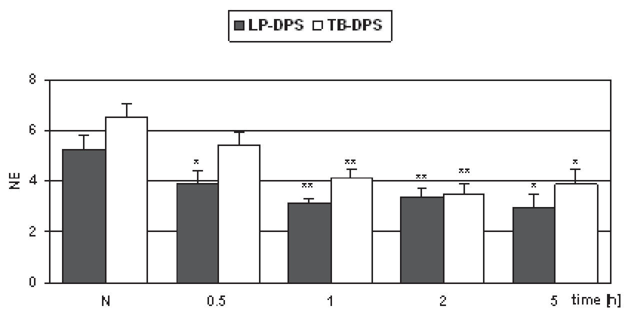

Fig. 2. Effect of the polysaccharide from peach-tree gum (DPS) administered perorally in 50 mg kg^{-1} b.w. dose to conscious cats on number of cough efforts (NE) from laryngopharyngeal (LP) and tracheobronchial (TB) areas of airways. For other symbols see Figure 1.

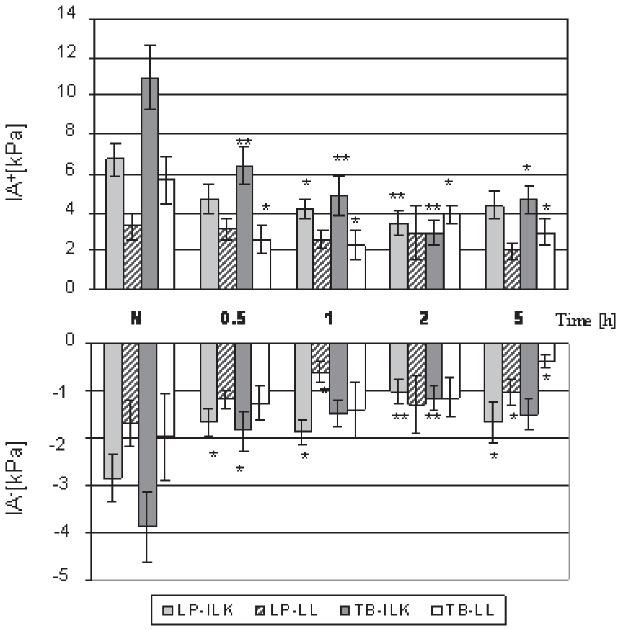

Fig. 3. Effect of ILK and LL on intensity of cough attacks during expiration (IA^+) and inspiration (IA^-) from laryngopharyngeal (LP) and tracheobronchial (TB) areas. For other symbols see Figure 1.

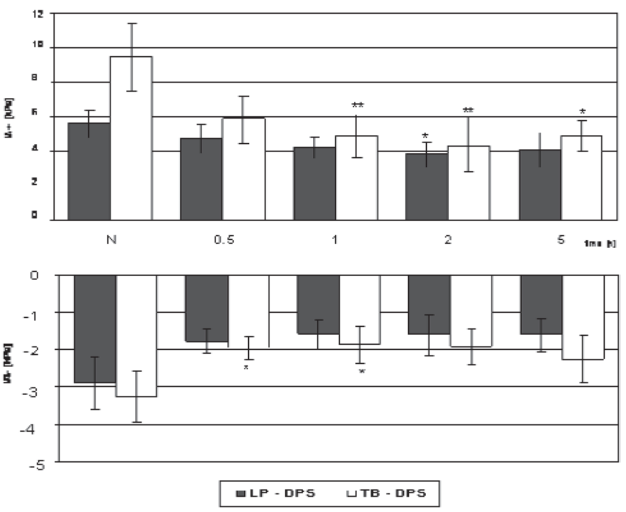

Fig. 4. Effect of DPS on intensity of cough attacks from LP and TB areas during expiration (IA^+) and inspiration (IA^-). For other symbols see Figure 1.

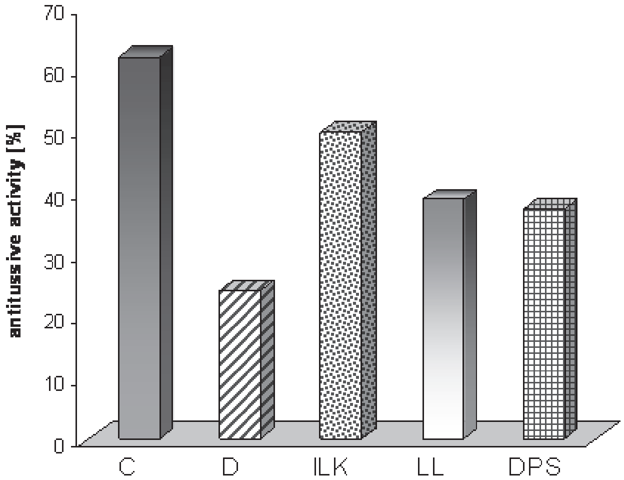

Fig. 5. Antitussive activity of polysaccharides ILK, LL and DPS in comparison to reference drugs (C – codeine, D – dropropizine) on mechanically induced cough in conscious cats.

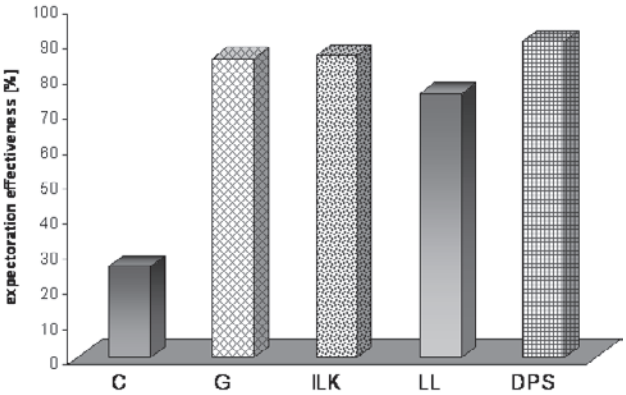

Fig. 6. Comparison of the effect of the tested polysaccharides (ILK, LL, DPS) and the reference drugs (C – codeine, G – guaifenesin) on expectoration, as indicated by the intensity values of maximal expiratory (IME^+) and inspiratory (IME^-) cough efforts.

periment. The decrease of IA^- from LP area was observed later, i.e. 1 h after administration of the polysaccharide. The other parameters followed i.e. intensity of maximum cough efforts in expiration (IME^+) and inspiration (IME^-) were not affected, therefore, graphical illustration is not presented.

In tests with the polysaccharide LL from burdock leaves, administration of 50 mg kg^{-1} b.w. did not result in statistically significant values of the parameters followed. Therefore, the dose was increased to 100 mg/kg b.w., which brought about a statistically significant decrease in the values of the main parameters, i.e. number of efforts from both TB and LP areas and intensity of expiratory cough attacks from TB region (Figs 1 and 3). Like in the case of the polysaccharide ILK, the onset of the effect was prompt. The decrease of the values of the above-mentioned parameters was observed as early as 30 min after administration of the agent. The intensity of inspiratory cough attacks was not reduced significantly and maximal cough inspiratory and expiratory efforts were found to be unchanged.

However, when applying this higher dose, relatively high incidence of side effects, especially nausea and sedation lasting longer than 24 h, was observed with more than 50 % of the experimental animals.

In spite of the fact, that the native, high-molecular-weight polysaccharide BG from peach gum was found ineffective in cough inhibition (not shown), the degraded polysaccharide DPS, prepared by modification of the native peach-gum polysaccharide, possessed an antitussive activity. This effect was associated with the influence on the number of efforts from both TB and LP regions and the intensity of expiratory cough attacks from TB region (Figs 2 and 4). However, the expiratory cough attacks from LP region and the intensity of attacks during inspiration were not affected significantly. Likewise, the values of intensity of maximal cough efforts were not influenced.

To evaluate the importance of the observed effects of the tested polysaccharides on cough parameters, comparative tests with commonly used narcotic (codeine) and non-narcotic (dropropizine) drugs to treat cough were carried out at the same conditions. The results of antitussive activities are presented on Figure 5. It can be seen that the activities of the 3 tested compounds were in the range of 49.9 % to 36.6 % that are lower than that of codeine (61.8 %) but significantly higher than the activity of non-narcotic dropropizine (28.3 %).

The other important factor of each agent antitussive activity is the influence on expectoration. Expectorants alleviate cough discomfort by increasing sputum volume and decreasing its viscosity, thereby promoting effective cough. Therefore, it is important that a cough suppressant has no negative impact on expectoration. It is known that the very efficient antitussive agent codeine brings about a change for the worse in expectoration, which is reflected in reduction of the intensity of maximal expiratory (IME^+) and inspiratory (IME^-) cough efforts. We demonstrated the decrease of these parameters by 74 % when codeine was administered to cats in the dose 10 mg kg^{-1} b.w. (Fig. 6). On the other hand, guaifenesin represents a cough depressant with no negative impact on expectoration (Dicpinigaitis and Gayle, 2003). In our tests, application of guaifenesin to cats in the dose of 100 mg kg^{-1} b.w. reduced the IME^+ and IME^- values only by 15 %. Comparison of these data with those of the tested polysaccharides reveals that the influence of the studied compounds on expectoration was negligible (Fig. 6). The only exception was the polysaccharide LL from burdock leaves, reducing expectoration by 25 %. LL was also the only polysaccharide, which caused side effects in cats, i.e. nausea and sedation. In this respect, a negative role of proteins, present in the polysaccharide mixture in a rather high proportion (Tab. 1), and accompanying color components cannot be excluded.

Discussion

The present study has demonstrated that polysaccharides from the flowers of marsh mallow, burdock leaves, and peach-tree gum exudates possess antitussive activity. This ability was found to be higher than that of the peripherally acting non-narcotic drug dropropizine, frequently prescribed in clinical practice for cough treatment. The highest activity was observed with ILK, the polysaccharide having the highest proportion of uronic acid in its molecule. When considering the generally accepted principle of the action of plant polysaccharides, i.e. their ability to protect the cough receptors by a layer against irritation by exogenous and endogenous tussigens, then the mucilaginous nature of ILK might partly explain its impressive cough-inhibiting property. Other alternative, which could participate on the cough depression after plant polysaccharides administration are their mucoactive attributes. They could be connected with following:

a) Increased production of the thin phlegm. Likewise, this change of the mucus volume by polysaccharides is induced through vago-vagal reflexive mechanism similarly as followed by saponins.

b) The property of carbohydrate compounds to cover sensitive nerve endings in epipharynx. The result of these processes is decreased cough reflex through "gate control".

c) Increased saliva production. Irwin et al (1993) assumed that stimulation of the saliva production by sugar components soothed the oropharynx and associated swallowing may also interfere with the cough reflex and lead to cough reduction.

For future implication in clinical practice it is noticeable that this polysaccharide has no negative influence on expectoration. The effect was negligible as that of guaifenesin (15 %), known for its expectorant property. In this respect the polysaccharide from burdock leaves was the only one that suppressed expectoration by about 25 %.

Adverse effects were not observed during the experiments, except of the burdock polysaccharide, exhibiting nausea and sedation when administered in the higher dose (100 mg kg^{-1} b.w.). However, these side effects were much less serious than those reported to occur during the treatment with codeine.

It can be concluded that the tested polysaccharides, enlarging the group of natural compounds with high cough-suppressing activity and rare side effects, can be ranged among prospective antitussive agents.

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Received June 29, 2006.
Accepted December 12, 2006.