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Antitussive activity of a glucuronoxylan from *Rudbeckia fulgida* compared to the potency of two polysaccharide complexes from the same herb

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An alkali-extracted low-molecular glucuronoxylan and two water-extractable polysaccharide complexes isolated from various parts of *Rudbeckia fulgida* were tested for antitussive activity on mechanically induced cough in nonanaesthetized cats. Glucuronoxylan consisted of a (1 → 4)-linked β-D-xylopyranosyl backbone with about 18% of 4-0-methyl-D-glucuronic acid attached to 0–2 of the chain xylose residues. The polysaccharide complexes differed from each the other regarding the in qualitative and quantitative composition of the sugar components. It was found that peroral administration of all the compounds led to a significant suppression of the cough reflex without negative influence on expectoration. Glucuronoxylan and the complex from the aerial parts of the herb exhibited much higher antitussive activity than the complex from the roots which did not contain any uronic acid component. Their activity (48.2% and 46,5%, respectively) highly surpassed the activity of the complex from the roots (23.5%) as well as that of the peripherally acting drugs dropropizine (28.3%) and prenoxdiazine (24.7%).

1. Introduction

The problems emerging from the treatment of catarrhs of the respiratory system by conventional, centrally acting antitussive agents, such as codeine and codeine-like compounds, are well known [1]. In recent years much effort has been made to create drugs which exhibit minimum side effects on the organism. Many scientists have directed their research towards isolation and identification of compounds from plants. Our ongoing research program includes the search for potentially active herbal polysaccharides, aiming at the investigation of structure-activity relationships. In our previous works we described the cough-suppressing activity of polysaccharides isolated from *Althaea officinalis* L. [2, 3] and *Malva mauritiana* L. [4]. Recently we have found that also a water-extractable polysaccharide complex from the aerial parts of the medicinal plant *Rudbeckia fulgida* exhibits high antitussive activity [5, 6]. Bukovský et al. [7] reported on significant immunostimulating activity of the aqueous-ethanolic extracts from the roots of some *Rudbeckia* species. Motivated by these findings, we continued the exploration of *Rudbeckia fulgida* for further potentially active polysaccharides and isolated a glucuronoxylan [8] and from the roots a water-extractable polysaccharide complex.

The present work provides the results of antitussive activity tests with these compounds in confrontation with the potency of the polysaccharide complex from the aerial parts of the herb and that of the comparative drugs used in clinical practice.

2. Investigations and results

Peroral administration of the polysaccharide complex isolated from the roots of *Rudbeckia* (RR) brought about a mild antitussive efficiency with a statistically significant decrease in the number of cough efforts (NE) only from the laryngopharyngeal part of the airways. The number of cough efforts from the tracheobronchial region and the intensity of the cough attacks in expirium (IME^{+}) and inspirium (IME^{-}) were not significantly affected (Fig. 1).

The results of the antitussive activity tests performed with the complex of polysaccharides isolated from the aerial parts of *Rudbeckia* (RL), and the glucuronoxylan (RX) showed that both compounds suppressed the cough reflex

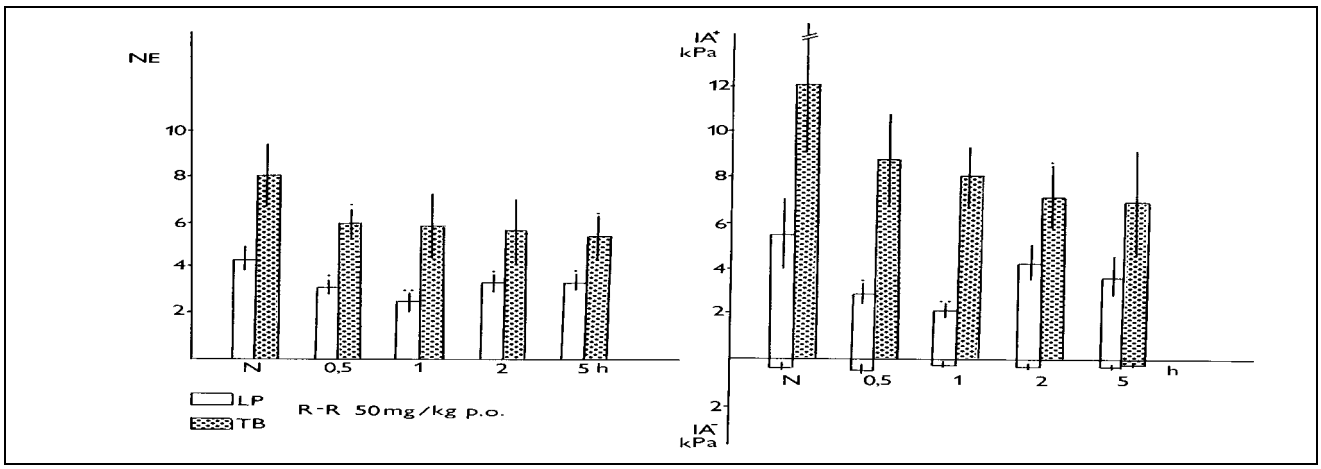

Fig. 1: Changes in the number of cough efforts (NE) and intensity of cough attack in expirium (IA^{+}) and in inspirium (IA^{-}) from laryngopharyngeal (LP) and tracheobronchial (TB) areas after peroral administration of the polysaccharide complex (RR) in the dose 50 mg/kg b.w. N = normal values of cough parameters. The columns represents the mean values of cough parameters, the range denotes standard error of means, 5% significance is marked with 1 dot, 1% with 2 dots

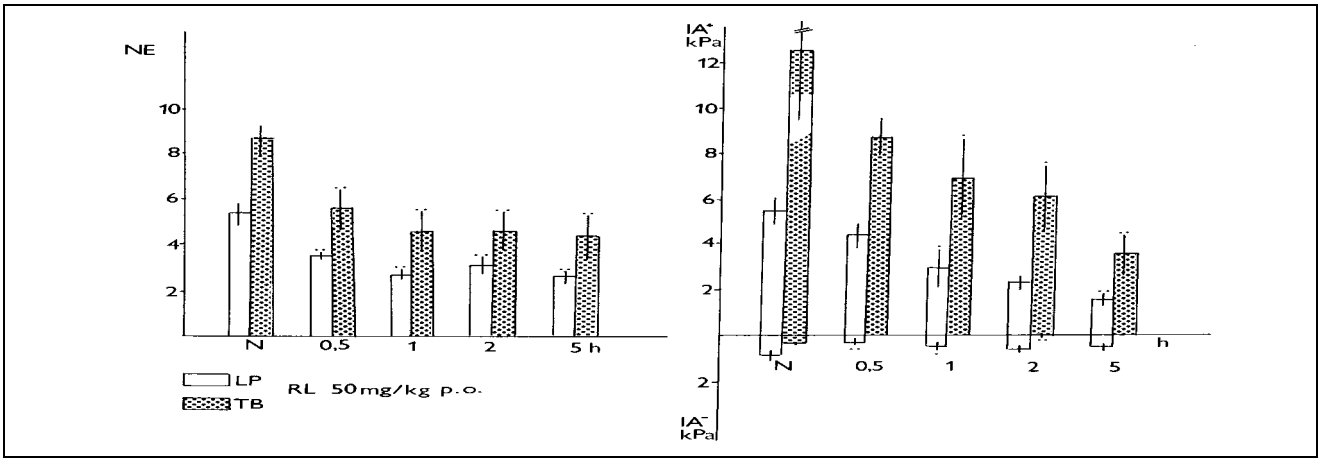

Fig. 2: Changes in the number of cough efforts (NE) and intensity of cough attack in expirium (IA^+) and in inspirium (IA^-) from laryngopharyngeal (LP) and tracheobronchial (TB) areas after peroral administration of the polysaccharide complex (RL) in the dose 50 mg/kg b.w.

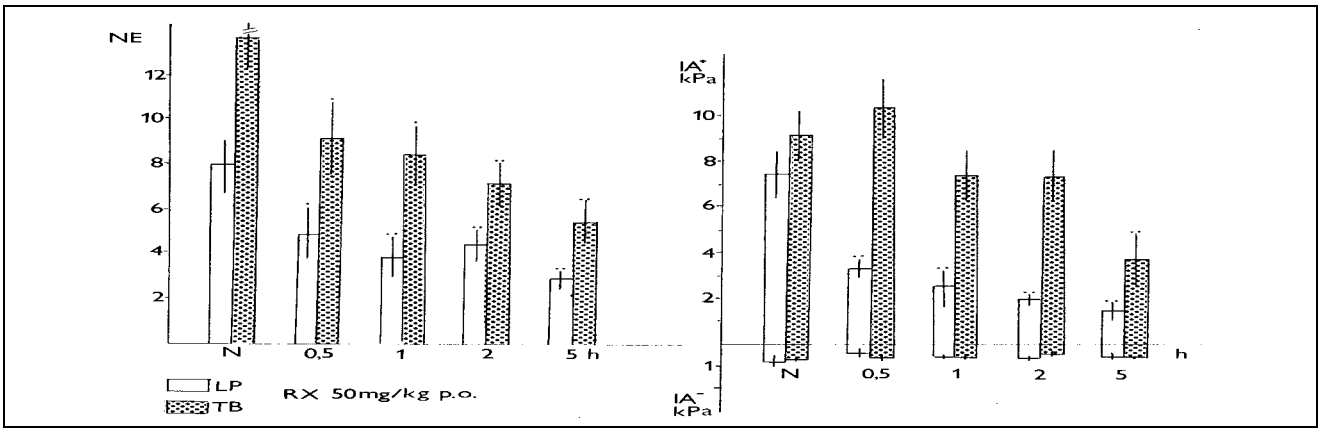

Fig. 3: Changes in the number of cough efforts (NE) and intensity of cough attack in expirium (IA^+) and in inspirium (IA^-) from laryngopharyngeal (LP) and tracheobronchial (TB) areas after peroral administration of glucuronoxylan (RX) in the dose 50 mg/kg b.w.

in cats. Administration of RL resulted in a statistically significant decrease in the number of cough efforts. The intensity of expiration cough attacks (IME^+) was significantly decreased in 1, 2, 5 h after RL administration (Fig. 2).

Glucuronoxylan administered perorally evoked a statistically significant decrease in the number of cough efforts and also in the intensity of cough attacks in expirium from the laryngopharyngeal part of the airways (Fig. 3.). Administration of glucuronoxylan caused a noticeable decrease in the parameters, which characterized the cough-suppressing activity, except for the intensity of maximum cough effort from the tracheobronchial part in expirium and inspirium (Fig. 4). This finding is advantageous from the point of view of expectoration.

The ability of RR, RL and glucuronoxylan to suppress the cough parameters was compared to antitussive drugs commonly used in clinical practice. The results are shown in Fig. 5. As shown in Fig. 5, the antitussive activity of both RL (46.5%) and glucuronoxylan (48.2%) was higher than that of RR (23.5%). It is evident from the experiment that the antitussive efficiency of RA and glucuronoxylan was lower than that of the most frequently used opioid antitussive codeine (61.8%), but higher than that of the non-narcotic antitussives dropropizine (28.3%) and prenoxdiazine (24.7%).

3. Discussion

Coughing is one of the main symptoms of airway illness. When coughing ceases to fulfil its physiological function, it becomes a pathological reflex burdening the organism of the patient. Then it is necessary to suppress or at least to keep this reflex on a reasonable level. Opioid antitussives are widely used in the treatment of catarrhs of the airways. Because of their side effects, such as depression of the respiratory centre, increase of the viscosity of the mucus, decrease of the expectoration, hypotension and obstipation [1], it is necessary to find new drugs with antitussive effects but less adverse effects. Under our experimental conditions, plant polysaccharides isolated from

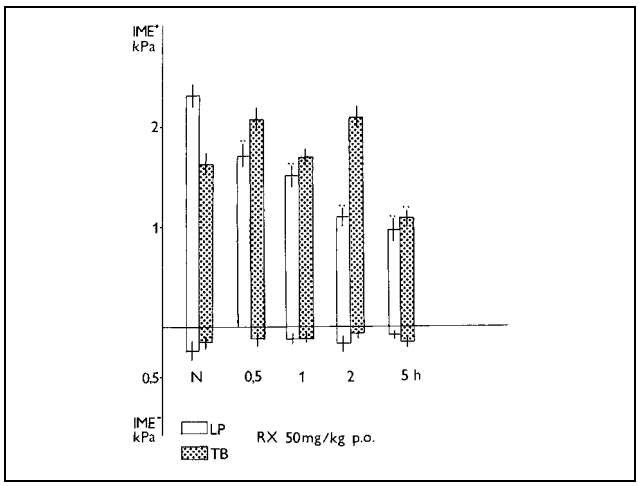

Fig. 4: The effect of glucuronoxylan (RX) on the intensity of maximum expiratory (IME^+) and inspiratory (IME^-) cough effort

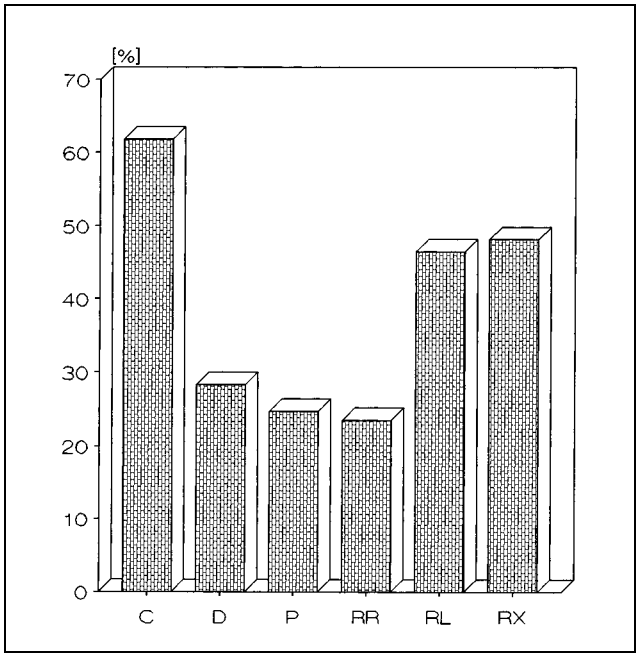

Fig. 5: Antitussive activity of the polysaccharide complexes RR, RL and RX in comparison to those of the commercial drugs (C: codeine, D: dropropizine, P: prenoxdiazine)

Rudbeckia fulgida showed high antitussive activity. A polysaccharide complex isolated from the aerial parts of *Rudbeckia* (RL) significantly decreased the number of cough efforts from both tracheobronchial and laryngopharyngeal parts of the airways, while the polysaccharide complex isolated from the roots (RR) influenced only a number of cough efforts from the laryngopharyngeal part. The differences in cough suppressing effects of RR and RL may be explained by their different sugar composition (see Table).

The significant antitussive action of RL and glucuronoxylan may be explained by a protective effect on sensitive nerve endings. A comparison of cough parameters from TB and LP regions revealed the different abilities to influence the mechanism regulating the quality and quantity of coughing. The tests confirmed our earlier finding [9] that compounds with dominant peripheral mechanisms, reduce the frequency of coughing, however, they have much less influence on its amplitude. The frequency of coughing depends probably on the condition of the cough receptors, while the amplitude is determined by the conditions of the cough centre.

In order to recognise the importance of the observed antitussive activity of these polysaccharides, we performed comparative experiments with some drugs generally used in clinical practice, i.e. codeine, dropropizine and prenoxdiazine. The activity of RL and glucuronoxylan was lower than that of the most frequently used opioid antitussive drug codeine, but higher than that of the non-narcotic antitussives dropropizine and prenoxdiazine. From a clinical point of view is considered that tested plant compounds suppressed the cough reflex but promote the expectoration.

Table: Sugar composition of the polysaccharide complexes RL and RR

Sugar	RL (mol%)	RR (mol%)
Rhamnose	14.8	2.0
Arabinose	10.6	3.0
Xylose	5.9	–
Glucose	33.3	7.8
Galactose	34.2	17.2
Mannose	1.2	–
Fructose	–	70.0
Uronic acid	+	–

In conclusion, the results of our study show that the polysaccharide complexes and glucuronoxylan isolated from the aerial parts of *Rudbeckia fulgida* can surpass the antitussive effect of non-narcotic antitussives. The statistically significant cough-suppressing effect and the absence of negative side effects of these plant compounds might be advantageous in clinical practice.

4. Experimental

The plants were collected in the Garden of the Faculty of Pharmacy, Comenius University, Bratislava in 1996. Though the polysaccharide complex from the aerial parts has already been described [5], we made a new isolation from this collection to exclude any effect of different growth conditions. Polysaccharides were hydrolyzed with 2 M trifluoroacetic acid at 120 °C

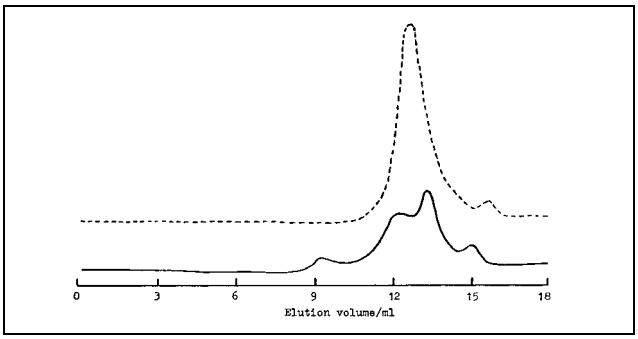

Fig. 6: HPGPC elution pattern of the polysaccharide complex from the aerial parts of *Rudbeckia fulgida* (RL). —— RI response (carbohydrate), – – – UV response (protein)

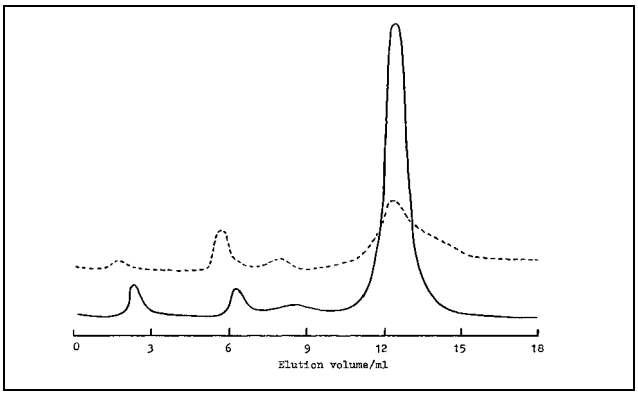

Fig. 7: HPGPC elution pattern of the polysaccharide complex from roots of *Rudbeckia fulgida* (RR). —— RI response (carbohydrate), – – – UV response (protein)

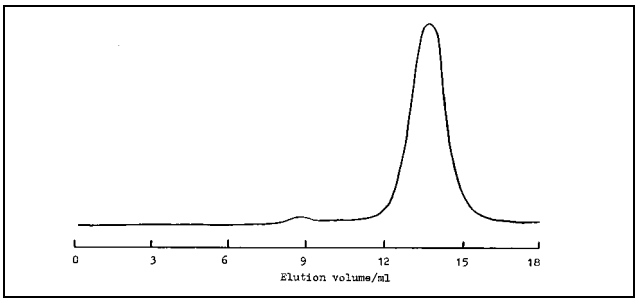

Fig. 8: HPGPC elution pattern of glucuronoxylan from *Rudbeckia fulgida* (RX). —— RI response (carbohydrate)

for 2 h. Descending paper chromatography of the hydrolyzates was performed on Whatman No. 1 paper in the solvent systems ethyl acetate/pyridine/water (8 : 2 : 1) and ethyl acetate/acetic acid/water (18 : 7 : 8), the sugars being detected with anilinium hydrogen phthalate.

Quantitative sugar composition was determined in the form of alditol acetates on a Hewlett-Packard 5890 Series II gas chromatograph equipped with a PAS-1701 column (0.32 mm × 25 m) at the temperature program of 110–125 (2 °C/min) – 165 °C (20 °C/min) and flow rate of hydrogen 20 ml/min.

High-performance gel-permeation chromatography (HPGPC) was performed using a commercial instrument (Laboratorní přístroje, Prague, Czech Republic) equipped with two Tessek Separon HEMA BIO-100 exclusion columns (8 mm × 250 mm) and aqueous 0.1 M-$NaNO_3$ as solvent (0.4 ml/min). The eluate was monitored by RI (carbohydrates) and UV (proteins) detectors.

Proteins were determined by the method of Lowry et al. [10], using bovine serum albumin as standard.

4.1. Isolation of the polysaccharide complexes from aerial parts (RL) and roots (RR) of the medicinal plant

The air-dried, methanol-pretreated aerial parts (50 g) and dry cut roots (50 g), respectively, were macerated in cold water (2.5 l) for 48 h at room temperature. After filtration of the plant residue and centrifugation of the extract, the concentrated supernatants were treated with 4 volumes of ethanol. The precipitate was collected by centrifugation, washed with aqueous ethanol (70 vol.%), suspended in water, exhaustively dialyzed, and freeze-dried. The brownish product (RL) and the yellowish product (RR) were obtained in 1.4% and 2.6% yields, respectively (dry herb basis) and contained besides carbohydrates also 23.9% and 8% proteins and 14.8% and 10% ash, respectively. The sugar compositions of the polysaccharide complexes is presented in the Table. The presence of uronic acids in RL was shown on paper chromatography only (based on comparison with the D-glucuronic acid and D-galacturonic acid standards) as quantitative determinations gave distorted results due to the accompanying non-carbohydrate material. As can be seen from the Table, uronic acid was not detected in the RR complex. While in RL of the neutral sugar components glucose and galactose dominated, in RR the predominating sugar was fructose. We have shown in a separate work [11] that this sugar was a building unit of a low-molecular homopolysaccharide component, a (2 → 1)-β-D-fructofuranan of the inulin type, isolated from the complex in fractionation steps. On HPGPC both complexes showed molecular heterogeneity and displayed also UV absorption (Figs. 6, 7) in accordance with the above determined proteins. The HPGPC record of RR showed one dominant band in the region of low-molecular mass (pullulan standards), representing the fructofuranan, and two small bands in the high-molecular mass region. On the other hand, the HPGPC profile of RL, together with its sugar composition, suggested that the complex consists of more polysaccharide species, one of which, a (1 → 6)-α-D-glucan, has already been described [5].

4.2. Isolation of (4-0-methyl-α-D-glucurono)-D-xylan (RX)

The plant residue after extraction of RL was air-dried (100 g) and extracted with 0.5 M-NaOH (2 l) for 24 h at room temperature. The residue was separated by filtration and the supernatant after centrifugation was treated with 4 volumes of ethanol. The precipitate was collected by centrifugation, suspended in water, exhaustively dialyzed, and freeze-dried to give a brownish product in 4.5% yield. This product was purified by washing with 80% aqueous ethanol acidified with HCl (5 vol.%) and subsequently fractionated on a DEAE-Sephadex A-50 column by successive irrigation with water and 0.25 M and 0.5 M ammonium carbonate solutions. The respective fractions were dialyzed and freeze-dried. The dominant, non-dialyzable 0.25 M carbonate fraction (3.7 g) was subjected to gel filtration on a Biogel P-2 column to yield a white product (3.5 g) homogeneous by free-boundary electrophoresis and HPGPC (Fig. 8). Structure determination of this polysaccharide [8], showed that the polysaccharide consisted of a (1 → 4)-linked β-D-xylopyranosyl backbone with about 18% of 4-0-methyl-D-glucuronic acid attached to 0–2 of the xylose residue. The uronic acid units were separated and distributed regularly along the xylan chain, i.e. approximately each sixth D-xylose chain unit bore a 4-0-methyl-D-glucuronic acid residue.

4.3. Antitussive activity tests

The experiments were performed on adult nonanaesthetized cats of both sexes weighing 1500–2500 g (10 in each set). After several days quarantine, a tracheal cannula was surgically implanted into the animals. This enabled a mechanical stimulation of airways and the recording of the side tracheal pressure. The mucous membranes of the laryngopharyngeal (LP) and tracheobronchial (TB) areas were stimulated consecutively five times by an 0.35 mm diameter nylon fibre. The cough parameters, i.e. the number of efforts (NE), intensity of cough attacks in expirium (IA^+) and inspirium (IA^-), cough frequency (NE/min), and intensity of maximal cough effort in expirium (IME^+) and inspirium (IME^-), were evaluated on the basis of the pressure values recorded on a Biograph 12-03 electromanometer. The water solution of the tested compounds RR, RL and RX were administrated perorally in a dose of 50 mg/kg b.w. The values of cough parameters obtained prior to administration of the compounds represented the normal value (N). The effect of drugs was monitored in time intervals 0.5, 1, 2, and 5 h. For comparative purposes commercial products generally used in clinical practice to treat coughing, i.e. prenoxdiazine (P), dropropizine (D), and codeine (C) were tested along with the polysaccharides. Statistical evaluation of the results was carried out by the method of Wilcoxon and Wilcox [12]. The doses of the individual comparative drugs used herein, i.e. P = 30 mg/kg b.w., D = 100 mg/kg b.w., C = 10 mg/kg b.w., represented the amounts which exhibited the highest antitussive effect in earlier experiments.

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