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Antitussive and bronchodilatory effects of *Lythrum salicaria* polysaccharide-polyphenolic conjugate

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ABSTRACT

A high molecular mass polysaccharide-polyphenolic conjugate has been isolated from flowering parts of *Lythrum salicaria* by hot alkaline extraction. Its chemical analysis revealed 74% of carbohydrates and 17% of phenolics. Compositional analysis of carbohydrate part showed a high GalA content (49%), Rha (25%), Gal (13%) and Ara (9%) residues, and indicated thus rhamnogalacturonan associated with arabinogalactan in *Lythrum* conjugate. Antitussive activity tests, performed in three doses of *Lythrum* conjugate – 25, 50 and 75 mg/kg of animal body weight, showed the reduction of the number of cough efforts even 5 h after administration. However, their antitussive effects were lower in comparison with that of codeine, the strongest narcotic antitussive agent. The tests evaluating the influence of different doses on airways smooth muscle reactivity revealed more significant effect of *Lythrum* conjugate in comparison with that of salbutamol, a commercial bronchodilator used in a clinical practice. Measurements of specific airway resistance pointed at both, the dose-dependent bronchodilatory activity and possible participation of bronchodilation on antitussive effect of *Lythrum* conjugate. This study represents the first sight into pharmacodynamic properties of *Lythrum* polysaccharide-polyphenolic glycoconjugate.

1. Introduction

Lythrum salicaria L. (Purple loosestrife) is an herbaceous perennial plant belonging to the Lythraceae family. It is widespread mainly in Europe, Asia, Africa, North America and Australia. This plant is characteristic by rich reddish purple flowers, which are flowering throughout the summer, and by length of stems which can reach 100–200 cm. *L. salicaria* is an invasive and competitive plant, easily adapted to the environment, however, the most frequent growing area are wetlands, i.e. wet meadows, along rivers, periphery of fishponds, gravel deposits, fen bogs, etc. In term of economy, rich abundance of *L. salicaria* plants in the above mentioned territories, provides a large source of nectar from their flowers for honey-bees. Although, *L. salicaria* is considered nowadays as an invasive weed, it has a long tradition in the folk medicine as a medicinal herb. Flowering parts and roots of this plant were used as drugs in the traditional medicine for their astringent, styptic, antibiotic, hypoglycaemic or vulnerary effects. As an astringent herb it was employed mainly in the treatment of diarrhea and dysentery. The drug was used on a large scale as styptic agent to treat internal bleeding, nosebleeds as well as excessive menstruation bleeding. *L. salicaria* drug was used as well to treat various sores, wounds, eczema, etc. [1–3]. Latter scientific investigations of *L. salicaria* herbs revealed antimicrobial, anti-inflammatory, antioxidant, anti-nociceptive, hypo- and hyperglycaemic activities of its extracts [4–6]. Lately, anticoagulant and procoagulant activities of *L. salicaria* polyphenolic-polysaccharide conjugates were estimated and experimentally confirmed the influence of the components of this drug on haemostasis [7–9]. Relatively a large scale of biological effects of *L. salicaria* drug is due to a wide range of active compounds (alkaloids, glycosides, tanstuffs, flavonoids, pectins, mucilages, etc.) occurring in different plant organs.

The aim of this paper was to broaden knowledge about the chemical characterization of *L. salicaria* polyphenolic-polysaccharide conjugate and to examine the possible ability of this conjugate to modulate chemically induced cough reflex and airway smooth muscle activity in the test system – guinea pigs.

2. Materials and methods

2.1. Plant material and animals

Flowering parts of *L. salicaria* (L.) were collected in Lower Silesia (Proboszczow), Poland. A voucher specimen has been deposited in

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the Botanical Garden of Wrocław University, Wrocław, Poland (No. 005291).

Healthy awaken male Trik strain guinea-pig, weighing 200–350 g were used in experimental procedure. The animals were obtained from the animal breeding facility VELAZ, Prague, Czech Republic, and located in faculty animal house for one week of quarantine, food and water were available *ad libitum* with a standard air conditioning system. During subsequent several days, animals were daily placed into the bodyplethysmograph box to achieve 60 min time interval of undisturbed breathing. All experiments were approved by Institutional Ethics Committee of the Jessenius Faculty of Medicine, Comenius University in Martin, Slovakia, registered in Institutional Review Board/Institutional Ethic Board Office (IRB 00005636), complied with Slovakian and European Community regulations for the use of laboratory animals and follow the criteria of experimental animal's well fare.

Codeine (*codeinium dihydrogenphosphoricum*), citric acid and salbutamol were obtained from Sigma–Aldrich (Lambda life, Slovakia). Citric acid was dissolved in saline, codeine and salbutamol in water for injection.

2.2. Isolation of Lythrum glycoconjugate

The isolation procedure of *L. salicaria* glycoconjugate (**Ls**) was in detail described by Pawlaczyk et al. [7,10]. Briefly, dried flowering parts of the plant were minced and suspended in 0.1 M NaOH at room temperature for 24 h, and refluxed for 6 h at 97 °C. The non-extracted part was removed by centrifugation (1850 × *g*; 20 min) and the supernatant was neutralized by 1 M HCl, it was concentrated to a lower volume and gradually extracted twice with hexane (water:hexane 1:1, v/v) for 6 h at 69 °C, diethyl ether (water:diethyl ether 1:1) for 6 h at 34 °C, chloroform (water:chloroform 1:1, v/v) for 6 h at 61 °C, and with similar proportions of chloroform and ethanol mixture (chloroform:ethanol 3:2, v/v) for 6 h, at 70 °C. The organic extracts were discarded and the water fraction was evaporated to a paste and treated with methanol at room temperature. The soluble part was filtered off and the residue was dissolved in distilled water and exhaustively dialyzed against distilled water, and freeze-dried to give a crude *Lythrum* glycoconjugate (**Ls**).

2.3. General methods

The glycoconjugate was hydrolyzed with 2 M trifluoroacetic acid for 1 h at 120 °C and the quantitative determination of the neutral sugars was carried out in the form of their trifluoroacetates by gas chromatography on a Hewlett-Packard Model 5890 Series II chromatograph equipped with a PAS-1701 column (0.32 mm × 25 m), the temperature program of 110–125 (2 °C/min)–165 °C (20 °C/min) and flow rate of hydrogen 20 mL/min [11]. The uronic acid content was determined with m-hydroxybiphenyl reagent [12]. The total carbohydrate content in the samples was estimated by the phenol–sulfuric acid assay [13]. The content of phenolics was measured by Folin–Ciocalteu assay, using gallic acid as a standard, and the result was expressed as gallic acid equivalents [14]. The protein content was determined by the Lowry method [15]. Elemental analysis was performed with EA 1108 apparatus (FISONS Instruments, East Grinstead, UK). Protein content was calculated from the nitrogen content (% $N \times 6.25$).

The colorimetric assays were measured using Specol 11 spectrophotometer, Carl Zeiss Jena. Infrared spectra were obtained on a NICOLET Magna 750 spectrometer with DTGS detector and OMNIC 3.2 software, where 128 scans were recorded with 4 cm^{-1} resolution. NMR spectra of glycoconjugates were recorded in D_2O at 60 °C on Varian 400 MR spectrometer on direct 5 mm PFG AutoX probe. A sample of the glycoconjugate was twice freeze-dried from D_2O before measurements. For ^1H and ^{13}C NMR spectra, the chemical shifts were referenced to the internal standard – acetone (δ 2.217 and 31.07, respectively). For the assignment of the signals 1D and 2D NMR techniques were used.

2.4. Method of citric acid-induced cough reflex

The method of chemically-induced cough was used for assessing the cough reflex [16–18]. Awaken guinea pigs were individually placed in a bodypletysmograph box (HSE type 855, Hugo Sachs Elektronik, Germany) and were exposed to citric acid aerosol in concentration 0.3 M for 2 min. The citric acid aerosol was generated by a jet nebulizer (PARI jet nebulizer, Paul Ritzau, Pari-Werk GmbH, Germany, output 5 L/s, particles mass median diameter 1.2 μm) and delivered to the head chamber of the body plethysmograph. The following methods for detection of cough were used to distinguish the cough efforts from sneezing and movements: (i) the changes of the expiratory airflow interrupting the basic respiratory pattern during cough effort were measured by pneumotachograph connected to the head chamber of bodyplethysmograph and (ii) the typical cough reflex movements and sounds were recognized by two independent trained observers.

The number of coughs was evaluated on the basis of sudden enhancement of expiratory flow accompanied by a typical cough movement and sound during 3 min inhalations of the tussigen. The cough response was measured before administration of any agents (baseline measurement; *N* value in graphs) and then after their application in confirmed time intervals (60, 120 and 300 min). Minimal time difference between two measurements was 2 h to prevent cough receptors adaptation on that kind of irritation. The tested glycoconjugates were administered in the doses of 25, 50 and 75 mg/kg bw and the peroral dose of control antitussive agent codeine (10 mg/kg bw) was selected according to previous experiments [16–18].

2.5. The airway smooth muscle reactivity, in vivo conditions

The airway smooth muscle reactivity was expressed as values of specific airway resistance (sRaw) calculated by the method of Pennock et al. [19]. This non-invasive plethysmograph technique is commonly used for evaluation of bronchoactive substances effect [20]. Conscious adult male TRIK strain guinea pigs were individually placed in a double chambers bodyplethysmograph box for laboratory animals (HSE type 855, Hugo Sachs Elektronik, Germany) consisting of head and body chambers. The nasal airflow is registered in the head chamber, and the thoracic airflow in the body chamber. The value of specific airway resistance is proportional to phase difference between nasal and thoracic respiratory airflow, which means the bigger phase difference the higher value specific airway resistance and also more significant degree of bronchoconstriction.

The values of sRaw were measured consecutively after the citric acid exposure and the cough response registration during 1 min interval. Their intensity prior to administration of the polysaccharides and the control bronchodilating drug salbutamol was considered as baseline (*N* value in graphs). The next values were measured 60, 120 and 300 min time intervals. Between the cough response recording and measurements of airways specific resistance was an interval of minimum 5 min. During intervals, fresh air was insufflating into the nasal chamber. The dose of salbutamol (10 mg/kg bw, intraperitoneally) was selected according to our previous results [21].

2.6. Statistics

Student's *t*-test was used for the statistical analysis of the obtained results. Data are presented as mean ± standard error of the