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Characterization and pharmacodynamic properties of *Arnica montana* complex

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ABSTRACT

A dark brown polymeric complex was isolated from flowering parts of medicinal plant *Arnica montana* L. by hot alkaline extraction followed by neutralization and multi-step extractions with organic solvents. It was recovered in 5.7% yield, on GPC showed two peaks of molecular mass of 9 and 3.5 kDa. The compositional analyses of *Arnica* complex revealed the presence of carbohydrates (26%), uronic acids (12%), phenolics (1.25 mM or 213 mg of GAE/1 g), and low protein content (~1%). The carbohydrate moiety was rich mainly in rhamnogalacturonan and arabinogalactan. The antitussive tests showed the reduction of the cough efforts by *Arnica* complex, however, its total antitussive effect was lower compared with that of codeine, the strongest antitussive agent. The bronchodilatory activity of *Arnica* complex was similar to salbutamol, a classic antiasthmatic drug, and was confirmed by significantly decreased values of specific airways resistance *in vivo* and by considerably attenuated the amplitude of acetylcholine and histamine-induced contractions in vitro. *Arnica* complex did not show any cytotoxic effect on mouse fibroblast cultures and human lung cells, up to the dose of 500 μg/mL.

1. Introduction

Arnica montana L. (Asteraceae) is an herbaceous perennial plant growing on mountains to alpine meadows, pastures and in light forests, up to the alpine level. *Arnica* roots and flowers have been used for thousands of years in the traditional medicine for many health purposes [1]. The extract of root had been used externally to treat of bruises and sprains, rheumatic pain, phlebitis, inflammation of the skin, and as homeopathic preparations for stimulation of immune system [2,3]. In veterinary phytotherapy, *A. montana* is strongly recommended topically, for the treatment of acute inflammations of tendons and joints, but also for cleaning and treatment of wounds of skin and mucous membranes, eczema, skin inflammations (tinctures, fluids) and ointments [4].

Individual parts of *A. montana* plant contain various constituents, *e.g.* sesquiterpene lactones, thymol derivates, phenolic acids or flavonoids, possessing several biological activities such as antiinflammatory, cytoprotective, antioxidant and tissue regenerative, among others [5–7]. Further antitumour and immunomodulating activities were successfully tested [8]. The roots of plant contain relatively high amout of thymol derivatives, which are used as fungicides and preservatives and may be responsible for the antiinflammatory effect [9]. *Arnica* also contains the toxin helenalin, which is responsible for some side effects, *e.g.* skin irritation and especially severe gastroenteritis and internal bleeding of the digestive tract, if large amounts of the plant are eaten [10]. Thus, *Arnica* extracts or decoctions are not recommended for internal administration, although some homeopathic practitioners claim, that a diluted solution of it can be taken by mouth, to treat low-grade fever, cold, bronchitis, seasickness, inflammation of the throat and epilepsy [11].

Many plants belong to *Asteraceae* family, including *A. montana*, are also known as being rich in biologically active acidic polysaccharides and their glycoconjugates. Recently, *in vitro* anticoagulant activity of some acidic glycoconjugates of *Asteraceae* family on human blood plasma has been reported [12]. Moreover, the

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Step		Phase	Mass [g]	Yield [%]
Starting dry plant material		dry mass	505.64	100
Extraction with 0.1 M NaOH		dry extract	105.78	20.9
Extraction with organic solvents	heptane	organic phase	0.06	91.5
	diethyl ether	organic phase	0.45	
	chloroform	organic phase	0.11	
	chloroform/methanol (3:2)	organic phase	1.36	
		water residue	96.81	
Etching with methanol		methanol phase	45.21	53.1
		solid fraction	51.45	
Dialysis of solid fraction (yield on NaOH fraction)			28.88	56.1
Yield (on dry mass of starting material)				**5.7**

Fig. 1. Isolation steps of *A. montana* complex.

antioxidant effect on the glycoconjugate isolated from *A. montana* has been observed [13]. The bronchodilatory effects of glycoconjugates rich in phenolics have been recently studied [14,15]. The ability of different acidic polysaccharides and their glycoconjugates to create protective layer on mucous membranes was experimentally confirmed [16]. Covering of mucous membrane by a carbohydrate layer is one of the supposed mechanisms of antitussive action of many glycoconjugate complexes [17,18].

In the present work the chemical characterization and pharmacokinetic properties of the glycoconjugate complex isolated from flowering parts of *A. montana* have been studied. The influence of *Arnica* complex on the cough and the reactivity of airways smooth muscle was tested *in vitro* and *in vivo* conditions. Studies were performed in connectivity with the verification of a possible cytotoxic effect of *Arnica* complex.

2. Material and methods

2.1. Plant material and chemicals

Dry flowering parts of the medicinal plant *A. montana* L. (*Chamomilla Doronicum Arnica* Desf. or *Doronicum montana* Lam.) were purchased from the local market. The identity of the plant was certified by K. D. Kromer and J. Kochanowska from Botanical Garden of Wrocław University, Wrocław, Poland and a voucher specimen (No. 002355) has been deposited in the Botanical Garden of Wrocław University.

Acetylcholine, histamine, citric acid, codeine (codeinium dihydrogenphosphoricum) and salbutamol were purchased from Sigma Aldrich (Lambda Life, Slovakia). *A. montana* complex, salbutamol and codeine were dissolved in water for application while acetylcholine, histamine and citric acid in 0.9% saline. All other chemicals were of analytical grade and purchased from POCh (Gliwice, Poland) and from Sigma Aldrich (Poznań, Poland).

2.2. Isolation of Arnica complex

The isolation of *Arnica* complex (AM) from dried flowering parts was performed according previously described procedure [12].

2.3. General methods

Solutions were concentrated under the reduced pressure at bath temperature not exceeding 40 °C. Carbohydrate, phenolic and protein contents in *Arnica* complex were estimated by the phenol–sulfuric acid method, Folin–Ciocalteu and Lowry assays, respectively [19–21].The uronic acids content was determined by *m*-hydroxybiphenyl reagent [22]. Samples were 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 Focus ITQ 700 chromatograph coupled with ion trap detector, Thermo Scientific, equipped with a Rtx-225 column (0.25 mm × 30 m), the temperature program of 110–125 (2 °C/min) 165 °C (20 °C/min) and flow rate of helium 1 mL/min [23]. All colorimetric assays were measured using Cecil CE 2021 spectrophotometer. The molecular mass distribution pattern of *Arnica* complex (AM) was performed by GPC column calibrated by dextran standards.

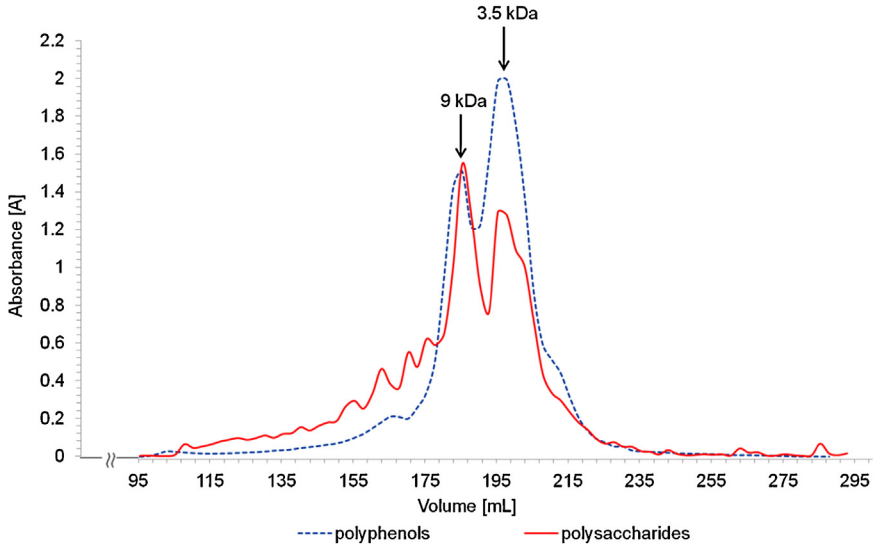

Fig. 2. GPC of *A. montana* complex on Sephacryl S-100 HR column.