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Echinacea complex – chemical view and anti-asthmatic profile

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ABSTRACT

Ethnopharmacological relevance: *Echinacea purpurea* (L.) Moench is one of the mostly used herbs in the traditional medicine for the treatment of respiratory diseases. Modern interest in *Echinacea* is directed to its immunomodulatory activity. Recent studies have shown that secretion of asthma-related cytokines in the bronchial epithelial cells can be reversed by *Echinacea* preparations.

Aim of the study: To examine the pharmacodynamics profile of *Echinacea* active principles, a complex has been isolated from its flowers by alkaline extraction and has been tested using an animal model of allergic asthma.

Material and methods: The structural features of *Echinacea purpurea* complex was determined using chemical and spectroscopic methods. Allergic inflammation of the airways was induced by repetitive exposure of guinea pigs to ovalbumin. *Echinacea* complex was then administered 14 days in 50 mg/kg b. w. daily dose perorally. Bronchodilatory effect was verified as decrease in the specific airway resistance (sRaw) *in vivo* and by reduced contraction amplitude (mN) of tracheal and pulmonary smooth muscle to cumulative concentrations of acetylcholine and histamine *in vitro*. The impact on mucociliary clearance evaluated measurement of ciliary beat frequency (CBF) *in vitro* using LabVIEW™ Software. Anti-inflammatory effect of *Echinacea* complex was verified by changes in exhaled NO levels and by Bio-Plex® assay of Th2 cytokine concentrations (IL-4, IL-5, IL-13 and TNF-alpha) in serum and bronchoalveolar lavage fluid (BALF).

Results: Chemical and spectroscopic studies confirmed the presence of carbohydrates, phenolic compounds and proteins, as well as the dominance of rhamnogalacturonan and arabinogalactan moieties in *Echinacea* complex. The significant decrease in sRaw values and suppressed histamine and acetylcholine-induced contractile amplitude of isolated airways smooth muscle that were similar to effects of control drug salbutamol confirmed *Echinacea* complex bronchodilatory activity. The anti-inflammatory effect was comparable with that of control agent budesonide and was verified as significantly reduced exhaled NO levels and concentration of Th2 cytokines in serum and BALF. The values of CBF were changed only insignificantly on long-term administration of *Echinacea* complex suggested its minimal negative impact on mucociliary clearance.

Conclusion: Pharmacodynamic studies have confirmed significant bronchodilatory and anti-inflammatory effects of *Echinacea* complex that was similar to effects of classic synthetic drugs. Thus, results provide a scientific basis for the application of this herb in traditional medicine as a supplementary treatment of allergic disorders of the airways, such as asthma.

Abbreviations: Ep, *Echinacea* complex; sRaw, specific airway resistance; CBF, ciliary beat frequency; NO, nitrogen oxide; BALF, bronchoalveolar lavage fluid; Th2 cytokine, T helper cell cytokine; IL-1-β, IL-4, IL-5, IL-6, IL-10, IL-13, interleukins 1, 4, 5, 6, 10, 13; TNF-α, tumor necrosis factor alpha; NMR, nuclear magnetic resonaces; AC, Citric acid; HCl, hydrochloric acid; TFA, trifluoroacetic acid; HPLC, high-performance liquid chromatography; *UV–vis*, ultraviolet–visible; NaCl, sodium chloride; DTGS detector, deuterated-triglycine sulfate detector; D2O, deuterium oxide; HSQC, heteronuclear single quantum correlation experiment; OVA+, ovalbumin-sensitized animals; i.p., intraperitoneally; Sal LT, salbutamol long term; Bud LT, budenoside long term; Ep LT, Echinacea complex long term; $Al(OH)_3$, aluminum hydroxide; eNO, exhaled nitric oxide; ASM, airway smooth muscle; ppb, particle per billion; ROI, region of interest; FFT, Fourier transform algorithm; GalA, galacturonic acid; *O*Me, *O*-methyl; βGal, β-linked galactose; Araf, arabinofuranose; AHR, airway hyperreactivity; iNOS, inducible NO-synthase; Gal, galactose; Ara, arabinose; Rha, rhamnose; Xyl, xylose; Glc, glucose; Man, mannose; Fuc, fucose; GalA, galacturonic acid

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1. Introduction

Echinacea purpurea (L.) Moench (Purple coneflower or coneflower) is a perennial plant of Asteraceae family. Nowadays, it is regarded as the medicinal and as well ornamental plant. Several *Echinacea* species have been used in the traditional medicine to treat infections, cold, cough, bronchitis, inflammations, etc. (Barrett, 2003; Goel et al., 2004; Wagner et al., 1999). *Echinacea* is one of the most used plants in herbal medicine and in dietary supplements (Percival, 2000). Modern interest in *Echinacea* is directed to its immunomodulatory activity, in particular in the prevention and treatment of the common cold, cough, bronchitis, and other respiratory infections. Phytoconstituents of *Echinacea* species are still the subject of chemical and pharmacological research in order to identify their active ingredients, however, the description of all constituents is still not completed. A great deal of clinical trials was performed to verify the efficacy of *Echinacea* constituents isolated by different procedures/solvents from various *Echinacea* species to describe their active principles. Four main groups of constituents are considered to be active in *Echinacea* species-alkylamides, glycoproteins, phenylpropanoids and polysaccharides. It is believed that these compounds are responsible for immune-stimulating, anti-inflammatory and anticoagulant activities (Pawlaczyk et al., 2009; Spelman et al., 2009).

Concerning polymeric compounds, the modulation of immune system was reported by *Echinacea* polysaccharides, i.e. enhancing production of TNF-α, IL-6, IL-10, and IL-1-β, adjuvant effects on human T-cell cytokine responses, increasing production of reactive oxygen intermediates, etc. (Roesler et al., 1991; Steinmuller et al., 1993; Currier and Miller, 2000; Currier et al., 2002; Fonseca et al., 2014). Furthermore, it was found that active polysaccharides did not stimulate B cells. Allergic asthma is a chronic obstructive disease of the lower airways, characterized by airway inflammation, reversible airflow obstruction, mucus hypersecretion, and airway hyperreactivity (Nakagome and Nagata, 2011). Many herbs have shown interesting results in various target specific biological activities such as bronchodilation, mast cell stabilization, immunomodulatory, anti-inflammatory and inhibition of mediators such as leukotrienes and cytokines, in the treatment of asthma (Mali and Dhake, 2011). Recent studies have shown, that expression of asthma-related cytokine genes and product secretions in the bronchial epithelial cells can be reversed by *Echinacea* preparations (Barrett, 2003).

From the literature it is evident, that phytoconstituents of *E. purpurea* are still subject of chemical and pharmacological attention. This fact inspired us to evaluate pharmacodynamic profile of *E. purpurea* complex using an animal model of allergic asthma. This model can mimic the pathological symptoms common in humans suffered from allergic bronchial asthma, for example airway hyperreactivity and inflammatory changes in small diameter bronchioles (Franova et al., 2013).

2. Material and methods

2.1. Plant material and chemicals

Air-dried flowers of medicinal plant *E. purpurea* (L.) Moench were purchased from a local market in Wroclaw, Poland. The identity of the plant was certified by Prof. K. D. Kromer and J. Kochanowska from Botanical Garden of Wrocław University, Poland and a voucher specimen (No. 011493) has been deposited in the Botanical Garden of Wrocław University, Poland. Citric acid (AC) p.a., histamine, acetylcholine, methacholine, salbutamol, aluminium hydroxide, budesonide and chicken ovalbumine purchased from Sigma Aldrich (Lambda Life, Slovakia). Budesonide was prepared as suspension in 1% TWEEN 80 (in 0.9% saline) according to manufacturer's instruction. All other above-mentioned drugs were dissolved in 0.9% saline.

2.2. Isolation of Echinacea complex

The isolation of *E. purpurea* complex was made according to already described procedure (Pawlaczyk et al., 2009). Shortly, flowering parts were minced and suspended in 0.1 M sodium hydroxide at room temperature for 24 h and refluxed for 6 h. The rest of plant was removed by centrifugation. The supernatant was neutralized by 1 M HCl, concentrated to a lower volume and gradually extracted with hexane (1:1, v/v), diethyl ether (1:1, v/v), chloroform (1:1, v/v), and chloroform and ethanol mixture (3:2, v/v). Organic extracts were discarded while the water fraction was evaporated to a paste and treated with methanol at room temperature. The soluble methanolic part was filtered off and the dark residue was solubilized in deionized water, dialyzed and freeze-dried to give a dark brown *Echinacea* complex.

2.3. General methods

Concentration of solutions was performed under reduced pressure at bath temperature not exceeding 40 °C. The content of carbohydrate, phenolic and protein was estimated by the phenol–sulfuric acid, Folin–Ciocalteu and Lowry assays, respectively (Dubois et al., 1956; Lowry et al., 1951; Singleton et al., 1999) and the uronic acid content was determined by m-hydroxybiphenyl reagent (Blumenkrantz and Asboe-Hansen, 1973). Sample was hydrolyzed with 2 M TFA for 1 h at 120 °C and the quantitative determination of the neutral sugars was carried out in the form of their alditol acetates (Englyst and Cummings, 1984), by gaschromatography on a Trace GC Ultra coupled with ITQ 900 (Thermo Scientific, USA) equipped with a Restek RT-2330-NB column (0.32 mm × 105 m), the temperature program of 80 °C (12 min)–160 °C (8 °C/min)–250 °C (4 °C/min, 25 min at 250 °C)–265 °C (20 °C/min, 10 min at 265 °C) and the flow rate of helium was 1 mL/min. Molecular mass determination of a sample was performed with HPLC Shimadzu apparatus (Vienna, Austria) equipped with a differential refractometer RID-6A and a UV–vis detector SPD-10AV using the column HEMA-BIO 1000 (8 mm × 250 mm) of particle size 10 μm (Tessek, Prague, Czech Republic). As a mobile phase 0.02 M phosphate buffer pH 7.2 containing 0.1 M NaCl was used at a flow rate 0.8 mL/min. A set of dextran standards was used for of the column (Gearing Scientific, Polymer Lab., Hertfordshire, UK). The colorimetric assays were measured using UV-VIS 1800 spectrophotometer (Shimadzu, Japan). Fourier-transform infrared (FT-IR) 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 conjugate were recorded in D2O at 60 °C on Varian 400 NMR spectrometer on direct 5 mm PFG AutoX probe. Sample was twice freeze-dried from D_2O before measurements. For ^1H and ^{13}C NMR spectra, chemical shifts were referenced to internal standard – acetone (δ 2.22 and 31.07, respectively). For the assignment of signals one-dimensional (^1H NMR) and two-dimensional Heteronuclear Single Quantum Correlation experiment (HSQC) were used.

2.4. Animals

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