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Chemical characterization of *Centaurium erythrea* L. and its effects on carbohydrate and lipid metabolism in experimental diabetes

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ABSTRACT

Ethnopharmacological relevance: *Centaurium erythrea* L. fam. Gentianaceae (CE) has been traditionally used for centuries in folk medicine of Balkans as a bitter medicinal herb for digestive complications and for treating febrile conditions and diabetes. The aim of this study was to gain insight into the chemical composition and underlying biochemical mechanism of action of the antihyperglycemic and antilipidemic activities of the dry extract of *Centaurium erythrea* L., wildly growing and traditionally used medicinal plant in the Republic of Macedonia.

Materials and methods: An ultrasonic methanol maceration of the aerial parts of the dried plant was performed and the extract was freeze-dried. HPLC–DAD–ESI–MS^n was carried out on 150 mm × 4.6 mm, 5 μm RP-18 Eclipse XDB column, at 40 °C. Mobile phase: water with 1% formic acid (A) and methanol (B) with linear gradient starting with 10% B was used to reach 15% at 5 min, 40% B at 25 min, 55% of B at 50 min and 100% at 60 min, with flow rate of 0.4 mL min^{-1}. Normal and streptozotocin (STZ) hyperglycemic Wistar rats were used for assessment of the antihyperglycemic and antilipidemic activity by measurement of the key carbohydrate-related enzymes and substrates, as well as lipid state of the organism.

Results: HPLC–DAD–ESI–MS^n analyses revealed presence of four different secoiridoids, seven flavonoid glycosides and seven xanthones in the freeze-dried extract of CE representing 53%, 25% and 22% of all compounds, respectively. The short-term (12 days) treatment of the STZ-diabetic rats with CE-extracts resulted in a 74% reduction of the produced hyperglycemia, which is only 6% less than the reduction caused by glibeclamide (GLB, positive control). The CE-extract had a significant impact on the hepatic carbohydrate metabolism enhancing the direct synthesis of glycogen, normalizing phosphorylase *a* activity and reducing the activity of glucose-6-phosphatase, which further causes reduction in production of blood glucose level. The long-term (45 days) treatment showed that the HbA1c in CE-treated group of animals was even lower than in the GLB-treated groups. The antilipidemic assessment of the CE-extract revealed decrease of total cholesterol, triglycerides, HDL and LDL level in the blood of the normal and STZ-hyperglycemic rats.

Conclusion: The obtained results indicate that treatment with CE extract in STZ-diabetic rats regulates the elevated level of blood glucose and carbohydrate-related disturbances slightly better than the effect of glibenclamide. There was also regulation of the serum lipid status in diabetic rats. Identified groups of bitter compounds in the extract (flavonoides, iridoids and xanthones) probably have influence on the expressed antihyperglycaemic effect.

1. Introduction

Diabetes mellitus is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both (Baquer et al., 1998). Nature has been a source of medicinal treatments for thousands of years, and plant-based systems continue to play an essential role in the primary health care of 80% of the world's underdeveloped and developing countries (King et al., 1998).

Centaurium erythraea (CE) is a species of flowering plant in the gentian family known by the common names Common centaury and European centaury. This centaury is a widespread plant in Europe and parts of western Asia and northern Africa. In the folk

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medicine of Balkan's cultures it is known as “crven kantarion” or “kichica” and it has been used as a medicinal herb for over 2000 years for its bitterness as an amarum and digestive and also for treating febrile conditions, diabetes, hepatitis and gout (Iordanov, 1970; Tucakov, 1990). It is also known as a hypothensive, antispasmotic, sedative and diuretic plant (Mounsif et al., 2000).

The decoction of the whole plant has been used to relieve indigestion and to treat jaundice, wounds and sores, urine retention, colic and diabetes mellitus (Hager, 2006). Furthermore, infusion of the aerial parts is used to reduce blood pressure, gastrointestinal tract smooth muscle spasms, inflammation and edema (Valentão et al., 2001; Loizzo et al., 2008). There are published data for its antipyretic and antiinflammatory effects in experimental animals (Hager, 2006). The protective effect of CE on free radicals (lipid and protein peroxides) and on antioxidant enzyme activities in diabetes and its complication are also reported (Sefi et al., 2011).

To our knowledge, no biochemical investigation had been carried out on the effect of *Centaurium erythraea* on key hepatic glycolytic and gluconeogenic enzymes and substrates, as well as on lipid state of the organism in diabetic rats. For more comprehensive assessment of the antihyperglycemic effect of the administered extract, along with the immediate and short-term, long-term blood glucose regulation and HbA1C should be also determined. Alterations in lipid metabolism during diabetic disorder are well known. Monitoring the lipid status could contribute to a more holistic impression of the antidiabetic effects of the plant.

The chemical composition of this plant has been studied and several groups of components were reported such as iridoids, or more precisely secoiridoids, secoiridoid alkaloids, xanthones, triterpenes, flavonoids and organic acids (Kumarasamy et al., 2003; Valentão et al., 2003; Hager, 2006). All these data on the phytochemical composition and biological activities imply the need for further detailed chemical characterization of the Centauri extract and correlation to its proclaimed activities.

Taking the above into consideration, the aim of this study was to gain insight into the chemical composition and underlying biochemical mechanism of action of the antidiabetic activities of *Centaurium erythrea* L., wildly growing and traditionally used medicinal plant in the Republic of Macedonia.

2. Materials and methods

2.1. Plant material

Aerial parts of wild, flowering plants were collected in July, at Baba Mountain in the region of city Bitola, Republic of Macedonia. The material was air dried, packed in paper bags and kept in a dark and cool place until analysis. Plant was identified by Dr. Gjoshe Stefkov and was verified as *Centaurium erythrea* L. fam. Lamiaceae and voucher specimens (No. CE7/05) were deposited at the Herbarium at the Department of Pharmaceutical Botany, Institute of Pharmacognosy, Faculty of Pharmacy, Skopje.

2.2. Preparation of plant extracts

Extraction of the milled aerial parts of the dried plant was performed by maceration in methanol in ratio 1:10=drug (g):solvent (mL) by continuous stirring, 24 h at room temperature and then, after filtration, plant material was extracted with additional 100 mL of methanol with ultrasonic agitation (ultrasonic bath 50/60 Hz, ULTRASONS-H, JP Selecta, Spain) at room temperature for 45 min. By employing this extraction we assume that a broad spectrum of chemical compounds were extracted, thus covering the wide range of components enclosed in various traditional herbal preparations from Centaury. Then, both filtered portions were combined and methanol evaporated under low pressure (150 mBar; 40 °C) on a rotavapor (Buchi Rotavapor R-215, Vacuum controller V-850, Vacuum Pump V-700, from Buchi, Switzerland). The residue was frozen at −80 °C and freeze-dried (Labconco, FreeZone 2.5 L Freeze Dry System, USA). The obtained freeze-dried extract was weighted for calculating the extraction yield. Before the LC/DAD/MS^n analysis, an appropriate amount of freeze-dried extract was dissolved in methanol to obtain 10 mg mL^{-1} samples for analysis, which were filtered through 0.45 μm, (Millipore, Milex-HV 13 mm, France) before injection.

2.3. Reagents and authentic samples

The reagents used were of highest purity (> 99.95%) and were purchased from Merck (Darmstadt, Germany). Authentic samples of luteolin, rutin, catechin, gallic acid and verbascoside were the products of Extrasynthese (Genay, France) and Merck (Darmstadt, Germany).

2.4. LC/DAD/ESI–MS^n analysis

Chromatographic separation was carried out on a 150 mm × 4.6 mm, 5 μm RP-18 Eclipse XDB column (Agilent Technologies, Waldbronn, Germany), protected with the corresponding guard column at 40 °C. The mobile phase consisted of two solvents: water–formic acid (1%) (A) and methanol (B). A linear gradient starting with 10% B was installed to reach 15% at 5 min, 40% B at 25 min, 55% B at 50 min and 100% B at 60 min. The flow rate was 0.4 mL min^{-1} and the injection volume 10 μL. The HPLC system was equipped with an Agilent 1100 series diode array and a mass detector in series (Agilent Technologies, Waldbronn, Germany). It consisted of a G1312A binary pump, a G1313A autosampler, a G1322A degasser and a G1315B photo-diode array detector, controlled by ChemStation software (Agilent, v.08.03).

Spectral data from all peaks were accumulated in range 190–400 nm and chromatograms were recorded at 280 nm for secoiridoids and xanthones and at 350 nm for flavonoid glycosides.

The mass detector was an Agilent G2445A Ion-Trap Mass Spectrometer equipped with an electron spray ionization (ESI) system and controlled by the LCMSD software (Agilent, v.4.1.). Nitrogen was used as a nebulizing gas at pressure of 65 psi and the flow rate was adjusted to 11 L min^{-1}. The heated capillary and the voltage were maintained at 350 °C and 4 kV, respectively. MS data were acquired in the negative ionization mode. The full scan covered the mass range from *m/z* 100 to 1200. Collision-induced fragmentation experiments were performed in the ion trap using helium as a collision gas, with voltage ramping cycle from 0.3 up to 2 V. Maximum accumulation time of the ion trap and the numbers of MS repetitions to obtain the MS average spectra were set at 300 ms and 5, respectively. Classical nomenclature for glycoconjugates was adopted to designate the fragment ions (Domon and Costello, 1988).

2.5. Qualitative and quantitative analysis

Phenolic compounds were identified by their UV and mass spectra recorded with a diode array detector and an ion-trap mass spectrometer, respectively. They were quantified using peak areas from UV-DAD chromatograms of each peak measured at the corresponding wavelength where an absorption maximum is exhibited. Secoiridoids and xanthones were quantified using catechin as external standard at 280 nm, whereas flavonoid glycosides were quantified and expressed as rutin (quercetin-3-*O*-rutinoside) equivalent at 350 nm. Also, all components were quantified using gallic acid as external standard and expressed as gallic acid equivalents at 280 nm.