Journal of Ethnopharmacology 202 (2017) 172–183

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Centaurium erythraea methanol extract protects red blood cells from oxidative damage in streptozotocin-induced diabetic rats

Miloš Đorđević[a], Mirjana Mihailović[a], Jelena Arambašić Jovanović[a], Nevena Grdović[a], Aleksandra Uskoković[a], Anja Tolić[a], Marija Sinadinović[a], Jovana Rajić[a], Danijela Mišić[b], Branislav Šiler[b], Goran Poznanović[a], Melita Vidaković[a], Svetlana Dinić[a,*]

[a] *Department of Molecular Biology, Institute for Biological Research, University of Belgrade, Bulevar despota Stefana 142, 11060 Belgrade, Serbia*
[b] *Department of Plant Physiology, Institute for Biological Research, University of Belgrade, Bulevar despota Stefana 142, 11060 Belgrade, Serbia*

ARTICLE INFO

Keywords:
Diabetes mellitus
Red blood cells
Hyperglycemia
Oxidative stress
Antioxidant activity
Centaurium erythraea

ABSTRACT

Ethnopharmacological relevance: *Centaurium erythraea* Rafn (CE) is a traditional medicinal herb in Serbia with antidiabetic, digestive, antipyretic and antiflatulent effects

Aim of the study: To investigate the potential protective effects of the methanol extract of the aerial parts of CE against glyco-oxidative stress in red blood cells (RBCs) in rats with experimentally induced diabetes.

Material and methods: Diabetes was induced in Wistar rats by intraperitoneal (i.p.) injection of multiple low-dose streptozotocin (STZ) (40 mg/kg, for five consecutive days), with the 1st day after the last STZ injection taken as the day of diabetes onset. The methanol extract of CE (100 mg/kg) was administered orally and daily, two weeks before the first STZ injection, during the 5-day treatment with STZ, and for four weeks after the STZ injections (pre-treated group) or for four weeks after diabetes onset (post-treated group). The effect of CE extract administration on the redox status of RBCs was evaluated by assessing lipid peroxidation, the ratio of reduced/oxidized glutathione (GSH/GSSG), the level of S-glutathionylated proteins (GSSP) and the enzymatic activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) in RBCs four weeks after diabetes onset. The major biochemical parameters of diabetes, protein glycation/glycosylation of erythrocytes and parameters which correlate with their aggregation and deformability were also evaluated.

Results: Daily application of CE extract to STZ-induced diabetic rats provided important antidiabetic effects, observed in both pre-treated and post-treated groups of diabetic rats as elevated serum insulin concentration, reduction of blood glucose and glycated hemoglobin concentrations and an improved lipid profile. Antioxidant effects of CE extract were detected in RBCs of diabetic rats and observed as decreased lipid peroxidation and ameliorated oxidative damage as a result of increased SOD, CAT and GR activities, an improved GSH/GSSG ratio and reduced GSSP levels. Moreover, the CE extract protected RBC proteins from hyperglycemia-induced damage by reducing non-enzymatic glycation and enzymatic glycosylation processes. CE extract was more effective when applied before diabetes induction (pre-treated group).

Conclusions: The results of this study show that the *Centaurium erythraea* methanol extract protects RBCs in diabetic animals from oxidative damage. They provide additional support for the application of this traditionally used plant in diabetes management.

Abbreviations: GlyHb, glycated hemoglobin; TAG, triacylglycerol; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CE, *Centaurium erythraea* Rafn; STZ, streptozotocin; GSH, glutathione; GSSG, oxidized glutathione; GSSP, S-glutathionylated proteins; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; GR, glutathione reductase; RBCs, red blood cells; ROS, reactive oxygen species; AGEs, advanced glycation end products; CML, N(ε)-(carboxymethyl)lysine; α2 M, α_2-macroglobulin; UHPLC-qqqMS, ultra-high performance liquid chromatography-triple quadrupole mass spectrometry; TBARS, thiobarbituric acid reactive substances

* Corresponding author.
E-mail addresses: milos.djordjevic@ibiss.bg.ac.rs (M. Đorđević), mista@ibiss.bg.ac.rs (M. Mihailović), jelena.arambasic@ibiss.bg.ac.rs (J. Arambašić Jovanović), nevenag@ibiss.bg.ac.rs (N. Grdović), auskokovic@ibiss.bg.ac.rs (A. Uskoković), anja.tolic@ibiss.bg.ac.rs (A. Tolić), marija.sinadinovic@ibiss.bg.ac.rs (M. Sinadinović), jovana.rajic@ibiss.bg.ac.rs (J. Rajić), dmisic@ibiss.bg.ac.rs (D. Mišić), branislav.siler@ibiss.bg.ac.rs (B. Šiler), goranpoz@ibiss.bg.ac.rs (G. Poznanović), melita@ibiss.bg.ac.rs (M. Vidaković), sdinic@ibiss.bg.ac.rs (S. Dinić).

http://dx.doi.org/10.1016/j.jep.2017.03.016
Received 28 December 2016; Received in revised form 21 February 2017; Accepted 12 March 2017
Available online 16 March 2017

1. Introduction

Diabetes mellitus is one of four priority non-infectious diseases affecting a large segment of the population. The defining attribute of diabetes is hyperglycemia, which is preceded by impaired insulin secretion and/or action. Over time, poorly or uncontrolled hyperglycemia can increase the risk of heart disease and stroke, development of microvascular complications such as peripheral neuropathy, retinopathy, nephropathy and other pathologies (Beckman et al., 2002). Among the different pathogenic factors that lead to diabetes and its complications oxidative stress stands as a major contributor (Maiese et al., 2007). Chronic hyperglycemia results in increased production of free radicals, especially reactive oxygen species (ROS) *via* glucose autoxidation (Maritim et al., 2003), increased flux of glucose through the polyol pathway (Chung et al., 2003), increased production of advanced glycation end products (AGEs) (Li et al., 2007), overactivity of the hexosamine pathway (Brownlee, 2001) and increased protein kinase C (PKC) activation (Noh and King, 2007).

The oxidants generated by hyperglycemia impair oxygen delivery by affecting the oxygen delivery system represented by RBCs (Tu et al., 2015). As circulatory cells with a primary function in transportation of respiratory gasses, RBCs are one of the first cells to be affected by increased level of free radicals because of the high intracellular concentration of oxygen and iron (Fe^{2+}) in hemoglobin (Bryszewska et al., 1995). As an oxygen shuttle, RBCs perform their essential task while exposed to a wide range of environments on each vascular circuit (Sivilotti, 2004). To cope with the deleterious effect of oxidative stress RBCs are supplied with a very efficient antioxidant defense system, comprised of enzymatic (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR)), and non-enzymatic antioxidants (reduced glutathione (GSH), ascorbic acid, α-tocopherol). In hyperglycemia-associated oxidative stress conditions, ROS-induced damage of RBCs results in abnormalities in their function, morphology and metabolism (Dallak and Jaliah, 2010). Oxidative stress induces changes in RBCs membrane fluidity and inactivates membrane-bound receptors and enzymes (Halliwell and Gutteridge, 1986), causes an increase in lipid peroxidation (Rohn et al., 1998), oxidation of glutathione (GSH) and protein sulphydryl group (Telci et al., 2000). Chronic exposure to high concentrations of glucose in plasma causes a series of compositional changes in RBC lipids and proteins which impair erythrocyte deformability and increase rouleaux formation/erythrocyte aggregation (Singh and Shin, 2009). Impaired RBC deformability reduces the flow of RBCs in the microcirculation, which in turn causes lowered oxygen delivery to the target tissues resulting in tissue damage (Mohanty et al., 2014). Furthermore, the ROS present in RBCs can be transferred to other cells which come into contact with RBCs, thus inducing and spreading tissue damage and inflammation (Huertas et al., 2013). Hyperglycemia promotes oxidative binding of glucose to a number of proteins, including hemoglobin, which leads to impairment of protein structure and function. Glycated hemoglobin (GlyHb) is more susceptible to oxidation and proteolytic degradation than non-glycated Hb (Sen et al., 2005). In turn, the increased release of heme and free iron in association with free radicals further enhances oxidative stress in RBCs and their fragility. Glycation of Hb increases blood viscosity and contributes to endothelial inflammation and vascular dysfunction (Saleh, 2015).

Maintenance of the equilibrium between free radical production and ROS neutralization by the antioxidative system is the principal cellular mechanism responsible for preventing the accumulation of oxidative stress-induced molecular and cellular damage. Antioxidants are expected to prevent or alleviate oxidative stress and diabetes-related complications by inhibiting the formation and scavenging free radicals or by enhancing the capability of the endogenous antioxidant system (Bajaj and Khan, 2012). Many plants contain polyphenols with powerful antioxidant activities (Maqsood et al., 2014). Since polyphenols are products of plant secondary metabolism and cannot be synthesized by humans, consumption of diets rich in plant polyphenols represents a promising approach in the preventive and supplementary treatment of diabetes and its complications. In this respect, special attention should be focused on plants used in traditional medicine. The plant species, common or European centaury (*Centaurium erythraea* Rafn (CE)) belonging to the Gentianaceae family, is a recognized medicinal herb. The traditional use of CE is well documented in the pharmacopoeias of 23 different countries (Hatjimanoli and Debelmas, 1977). In Serbian traditional medicine it is used for treating flatulence, stomach ulcers, laryngitis, digestive disorders, diabetes, for improving appetite, and as an antipyretic and cold remedy (Jarić et al., 2015; Zlatković et al., 2014). Results from *in vitro* and *in vivo* studies with extracts and isolated constituents support the traditional use of CE. It has been demonstrated that CE possess antioxidant (Valentao et al., 2001) and antidiabetic activities (Hamza et al., 2010, 2011).

To our knowledge, the effect of CE on the redox status of RBCs has not been explored. Bearing in mind the consequences of erythrocyte injury in diabetes, we investigated whether the CE extract could protect RBCs from glyco-oxidative damage. The parameters which correlate with RBC aggregation and deformability and affect blood flow properties were also evaluated. The results of this study provide additional support for the traditional use of this plant in folk medicine for diabetes management.

2. Material and methods

2.1. Plant material

Centaurium erythraea Rafn (CE), locally known as "kičica", was collected in 2010 from the locality Andrijevica (Montenegro; 42° 44′ 26′′ N, 19° 48′ 12′′ E). Plants were authenticated in the field by the authors. Plant material for the herbarium was deposited in the Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", University of Belgrade, Serbia. Plant name was verified with The Plant List (www.theplanlist.org), accessed on December 10th, 2016.

2.2. Preparation of the methanol extract

The above ground (aerial) parts of the plants were harvested, air-dried and stored in paper bags until use. The dried tissue was ground into a fine powder using liquid nitrogen. The plant material was extracted overnight at room temperature with 96% methanol (w:v=1:5). Following sonication for 20 min, the samples were filtered through Whatman No. 1 filter paper. For the biological assays, the methanol extract was evaporated in a vacuum evaporator (Eppendorf Concentrator 5301, Germany) at 30–45 °C until dry. For UHPLC-qqqMS analysis, dry extracts were diluted in 96% methanol (w:v=1:1000) filtered through 0.2 µm cellulose filters (Agilent Technologies, CA, USA) and stored at 4 °C until use.

2.3. Determination of the total phenolic and flavonoid contents

Total phenolic content in the methanol extract was determined according to Singleton and Rossi (1965). The dry extract dissolved in 96% methanol (0.2 mL) was mixed with 1 mL of 10% Folin-Ciocalteu reagent for 4 min, followed by the addition of 0.8 mL of 7.5% sodium carbonate, and incubation at room temperature for 2 h. The absorbance was read at 765 nm. Gallic acid was used as a reference standard. The results were expressed as mg gallic acid equivalents (mg GAE)/g extract.

The total flavonoid content was determined by the method described by Djeridane et al. (2006). The dry extract dissolved in 96% methanol (1 mL) was mixed with 1 mL of 2% aluminium trichloride ($AlCl_3$) methanol solution. The reaction mixture was incubated at room temperature for 1 h and the absorbance was measured at 430 nm.