Journal of Ethnopharmacology 152 (2014) 599–602

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Ethnopharmacological communication

Walnut leaf extract inhibits PTP1B and enhances glucose-uptake *in vitro*

Anna Pitschmann, Martin Zehl, Atanas G. Atanasov, Verena M. Dirsch, Elke Heiss, Sabine Glasl *

Department of Pharmacognosy, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria

ARTICLE INFO

Article history:
Received 29 November 2013
Received in revised form
3 February 2014
Accepted 10 February 2014
Available online 15 February 2014

Keywords:
Juglans regia
Diabetes mellitus
Glucose uptake
C2C12 myocytes
PTP1B inhibition
PPARγ agonism

ABSTRACT

Ethnopharmacological relevance: Walnut, *Juglans regia* L. (Juglandaceae), is one of the medicinal plants used to treat diabetic symptoms in Austrian folk medicine. The air-dried green leaves are either used as aqueous decoctions or liquor preparations and are consumed on a daily basis. We investigated the hypoglycemic effect of a methanolic *Juglans regia* leaf extract on glucose uptake, protein tyrosine phosphatase 1B (PTP1B) inhibition and peroxisome proliferator-activated receptor gamma (PPARγ) activation.

Material and methods: Hypoglycemic activity was assessed by glucose-uptake in C2C12 myocytes, inhibition of PTP1B and activation of PPARγ. Phytochemical characterization of the extract was carried out by LC–MS and GC–MS.

Results: Methanolic *Juglans regia* leaf extract enhanced the glucose uptake rate in C2C12 myocytes at concentrations of 25 μg/mL compared to untreated cells. This activity may partly be explained by the inhibition of PTP1B but not PPARγ agonism. LC–MS analyses revealed chlorogenic acid (**1**), 3-p-coumaroylquinic acid (**2**), a trihydroxynaphthalene-hexoside (**3**), as well as eight flavonoids (**4–11**) as main phenolic constituents in the active extract.

Conclusions: The finding that *Juglans regia* leaf extract enhances glucose uptake and inhibits PTP1B provides an *in vitro*-based rationale for the traditional use of walnut leaf preparations against elevated blood-glucose levels.

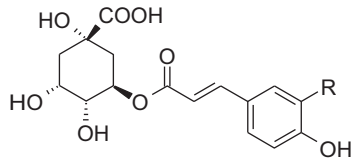
1. Introduction

Diabetes mellitus is a metabolic disorder characterized by high blood glucose levels (hyperglycemia) resulting from defects in the body's ability to produce or respond to insulin. Long-term hyperglycemia is associated with severe micro- and macrovascular damage, whose prevention is the main aim of hypoglycemic pharmacotherapy (Fowler, 2007). Due to – partly counterproductive – side effects of current clinical treatment and the continuously rising number of patients (Wild et al., 2004), new, more efficient therapeutic principles are needed. Inhibitors of protein–tyrosine phosphatase 1B (PTP1B), a cytosolic enzyme, not only increase cellular response to insulin, but also elevate leptin signaling and are therefore a promising strategy for the treatment of diabetes mellitus and obesity (Thareja et al., 2012).

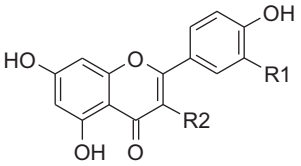
In search of new lead compounds, traditional medicines proved to constitute a valuable source (Patwardhan and Mashelkar, 2009). In this regard, *Juglans regia* L. (Juglandaceae), the common walnut, represents an interesting candidate for scientific investigations, since it is used in different traditional medicines for a variety of indications (Taha and Al-wadaan, 2011). In Austrian folk medicine, water decoctions and liquor preparations of the foliage are known as traditional remedy against diabetic symptoms (Gerlach, 2007). The positive effect of Juglandis folium (walnut leaf) extracts on blood glucose levels and lipid profiles has been scientifically proven by numerous animal studies (Asgary et al., 2008; Mohammadi et al., 2011) as well as by a recent human clinical trial (Hosseini et al., 2014). The activity was attributed to the antioxidant capacity of the polyphenols present in walnut leaves (Amaral et al., 2004; Pereira et al., 2007). The mechanism of action, however, remained speculative. The present study aims to fill this gap by evaluating the influence of *Juglans regia* leaf extract in *in vitro* functional (glucose uptake) and target-oriented (PTP1B and PPARγ) models related to insulin sensitivity. Phytochemical characterization ensures similar qualitative extract composition as in previous studies.

* Corresponding author. Tel.: +43 1 4277 55207; fax: +43 1 4277 9552.
E-mail address: sabine.glasl@univie.ac.at (S. Glasl).

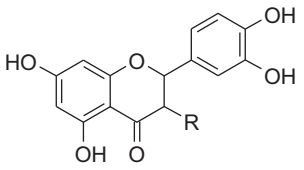
http://dx.doi.org/10.1016/j.jep.2014.02.017
0378-8741



1 R = OH
2 R = H



5 R1 = OH, R2 = O-galactose
6 R1 = OH, R2 = O-pentose
7 R1 = OH, R2 = O-arabinose
8 R1 = OH, R2 = O-L-arabinose
9 R1 = OH, R2 = O-L-rhamnose
10 R1 = H, R2 = O-L-arabinose
11 R1 = H, R2= O-pentose



4 R = O-pentose

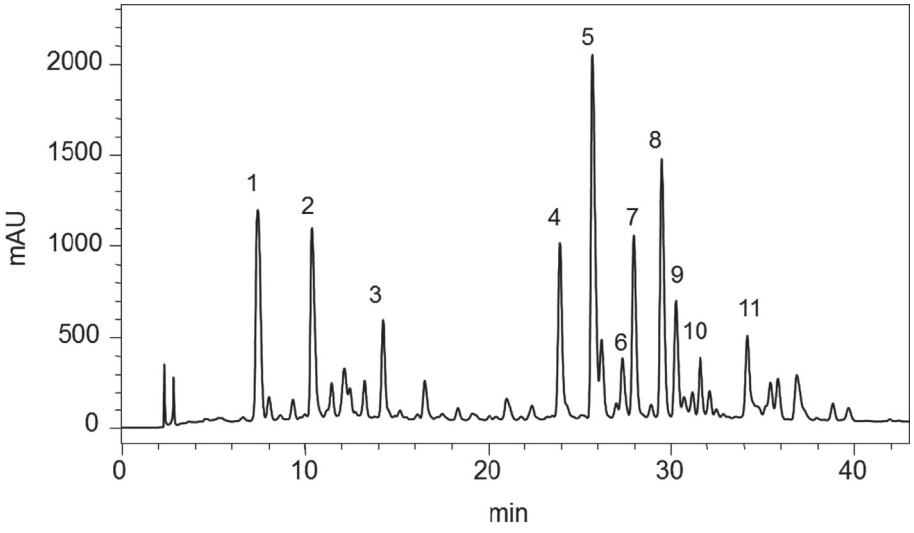


Fig. 1. HPLC chromatogram (300 nm) of JR: (**1**) chlorogenic acid, (**2**) 3-p-coumaroylquinic acid, (**3**) trihydroxynaphthalene-hexoside, (**4**) dihydroquercetin–pentoside, (**5**) hyperoside, (**6**) quercetin–pentoside, (**7**) avicularin, (**8**) quercetin–L-arabinoside, (**9**) quercetin–L-rhamnoside, (**10**) kaempferol–L-arabinoside, and (**11**) kaempferol–pentoside.

2. Material and methods

2.1. Chemicals

(S)-(+)-2-butanol with a purity of 99% was purchased from Sigma Aldrich (St. Louis, MO, USA). Reference substances for GC and HPLC were of HPLC quality and purchased from Carl Roth (Karlsruhe, Germany). Solvents used for extraction were of analytical grade; those used for HPLC were of gradient grade and obtained from VWR (West Chester, PA, USA).

2.2. Plant material

Dried, cut *Juglans regia* leaf material was obtained from Kottas Pharma GmbH (Vienna, Austria). The lot number of the material was 10240212. Prior to extraction, the leaves were pulverized with a basic electric grinder and stored at room temperature, protected from light.

2.3. Extraction and extract-purification

Pulverized *Juglans regia* leaves were extracted three times with methanol, using an ASE 200 system from Dionex (Sunnyvale, CA, USA). Extractions were carried out at 40 °C under a pressure of 10 MPa with an acetone tube cleaning step between every cycle. Resulting liquid extracts were combined and evaporated to dryness under reduced pressure at 40 °C. The drug to extract ratio was 4–6:1.

For chlorophyll depletion, the crude extract was re-dissolved in CH_2Cl_2 and partitioned with equal volumes of H_2O/MeOH (1:1). The upper layer was separated and evaporated to dryness to yield a chlorophyll free extract (JR).

2.4. Cell culture, assessment of glucose uptake rate and PTP1B inhibition assay

Settings were used as previously in (Heiss et al., 2012) and can also be found in the supplementary information.