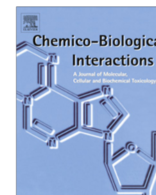
Chemico-Biological Interactions xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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



Nymphayol increases glucose-stimulated insulin secretion by RIN-5F cells and GLUT4-mediated insulin sensitization in type 2 diabetic rat liver

P. Subash-Babu [a], S. Ignacimuthu [b], A.A. Alshatwi [a,*]

[a] Department of Food Sciences and Nutrition, College of Food Sciences and Agriculture, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia
[b] Division of Ethnopharmacology, Entomology Research Institute, Loyola College, Chennai 600 034, Tamil Nadu, India

A R T I C L E I N F O

Article history:
Received 2 June 2014
Received in revised form 10 October 2014
Accepted 2 December 2014
Available online xxxx

Keywords:
Nymphaea stellata
Nymphayol
Streptozotocin
Insulin secretion
Glucose sensitivity
Liver glycogen

A B S T R A C T

Nymphaea stellata (Willd.) has been used in traditional medicine for centuries to treat several illnesses, including diabetes. However, scientific evidence supporting its mechanism of action is lacking. Here, we showed that an *N. stellata* flower chloroform extract (*NSFCExt*) has significant plasma glucose lowering ability. Furthermore, an active compound was identified and purified by column chromatography, and the structure of this compound, nymphayol, was determined by X-ray crystallographic analysis. Nymphayol was tested for its effects on insulin secretion by RIN-5F cells cultured in low or high glucose medium; we found that nymphayol treatment improved glucose-stimulated insulin secretion *in vitro*. Additionally, insulin sensitization and glucose uptake were increased in L6 myotubes. Nymphayol was administered to type 2 diabetic male Wistar rats at several doses (5, 10 or 20 mg/kg/day) for 45 days. After nymphayol administration, the plasma glucose concentration was significantly ($p \leqslant 0.05$) lower (60.33%) than in control diabetic rats, and the plasma insulin level increased in a dose-dependent manner. In addition, the cellular insulin response was analyzed in type 2 diabetic rats; oral administration of nymphayol increased IRS1 phosphorylation and GLUT4 protein expression in liver and muscle. Nymphayol significantly ($p \leqslant 0.05$) restored the levels of HbA1c, hepatic glycogen and hepatic glucose-metabolizing enzyme (hexokinase, glucose-6-phosphate dehydrogenase, glucose-6-phosphatase, fructose-1, 6-bisphosphatase, glycogen synthase and glycogen phosphorylase) activity in diabetic rats. The administration of glibenclamide, a reference drug (600 μg/kg), also produced a significant ($p \leqslant 0.05$) reduction in blood glucose in STZ-nicotinamide induced diabetic rats. The results suggest that nymphayol may be a useful therapy for diabetes because it stimulates insulin secretion and promotes glucose absorption.

© 2014 Published by Elsevier Ireland Ltd.

1. Introduction

Diabetes mellitus (DM) is a common endocrine disorder that is characterized by hyperglycemia and results from absent or inadequate pancreatic insulin secretion with or without concurrent impairment of insulin activity [1]. This condition affects approximately 150 million people worldwide, and its incidence is expected to double during the next 20 years [2]. Epidemiological studies and clinical trials strongly support the notion that insulin deficiency causes hyperglycemia, and persistent hyperglycemia is associated with coronary artery disease, cerebrovascular disease, renal failure, blindness, limb amputation, neurological complications and premature death [3]. Cardiovascular disease and stroke are also common complications of DM. In diabetic patients, the blood glucose concentration is controlled by multiple injection of insulin, a hormone that principally regulates glucose metabolism. Oral hypoglycemic agents, such as sulfonylureas, biguanidines,

Abbreviations: DM, diabetes mellitus; STZ, streptozotocin; NA, nicotinamide; NSFCExt, *Nymphaea stellata* flower chloroform extract; XRD, X-ray crystallography; IRS1, insulin receptor substrate-1; GLUT4, glucose transporter-4; G6PDH, glucose-6-phosphate dehydrogenase; G6Pase, glucose-6-phosphatase; F-1,6-Pase, fructose 1,6-bisphosphatase; RPMI, Roswell park memorial institute medium; DCFH-DA, 2,7-dichlorofluorescin diacetate; ROS, reactive oxygen species; CPCSEA, committee for the purpose of control and supervision of experiments on animals; DMSO, dimethylsulfoxide; HbA1C, glycosylated hemoglobin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ACP, acid phosphatase; ALP, alkaline phosphatase; TEM, transmission electron microscope.

* Corresponding author at: Molecular Biology Research Lab, Department of Food Sciences and Nutrition, College of Food Sciences and Agriculture, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia. Tel.: +966 552563043, +966 14677122 (O).
E-mail address: subashbabu80@hotmail.com (A.A. Alshatwi).

http://dx.doi.org/10.1016/j.cbi.2014.12.011
0009-2797/© 2014 Published by Elsevier Ireland Ltd.

thiazolidinediones and α-glucosidase inhibitors, are often used to treat type 2 diabetes. When therapy with oral hypoglycemic agents is ineffective, insulin can also be used to treat type 2 diabetes [4].

Chemical ligands that induce insulin secretion and β-cell regeneration may be useful as new therapeutic agents for both type 1 and type 2 diabetes [5]. Worldwide, many indigenous medications have been used by local practitioners for the treatment of DM [6]. However, only a few have received scientific or medical scrutiny, also World Health Organization has recommended that these traditional plant treatments undergo further evaluation [7]. A botanical substitute for insulin seems unlikely, but traditional treatments may provide valuable clues toward the development of new oral hypoglycemic agents and simple dietary adjuncts. Natural products are sources of bioactive metabolites, including useful chemical ligands. Worldwide, over 1200 species of plants have been recorded as traditional medicines for diabetes [8]. Some of these plants have been evaluated in laboratories, and in a number of cases, their efficacy has been confirmed, e.g., *Panax ginseng* (ginseng), *Momardica charantia* (bitter melon), *Opuntia cactus* (cactus), *Tecomastans* (trompeta), and *Syzygium cumini* (jambolan) [9]. The specific chemical constituents of these plants, such as flavonoids, polyphenolic compounds, polysaccharides, alkaloids, triterpenoids, sterols and xanthones, are thought to be responsible for the hypoglycemic effects, and they may be related to increased insulin release and increased glucose metabolism [10].

In an attempt to obtain new compounds that effectively reduce blood glucose levels via insulin secretion stimulation, we isolated a novel steroid, 17-(hexan-2-yl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-3-ol, from *Nymphaea stellata* Willd. flowers by bioassay-guided fractionation and named the compound nymphayol. In this context, treatment of STZ-induced diabetic animals with steroid (Charantin), well known phytoprinciples isolated from *Momordica charantia* prevented hyperglycemia through reduced oxidative stress and restored beta -cell function [11]. Also previously we have reported the isolation of nymphayol and its antihyperglycemic activity in streptozotocin-induced diabetic rats [12]. However, no evidence exists about its effects on insulin secretion or insulin sensitization or about the mechanism responsible. The aim of this study was therefore to examine the effect of nymphayol on the stimulation of insulin secretion, glucose uptake and glucose metabolism in type 2 diabetic rats.

2. Materials and methods

2.1. Chemicals and biochemical measurements

Streptozotocin and nicotinamide were purchased from Sigma Chemical Co., St. Louis, MO, USA. All other chemicals were of analytical grade. RIN-5F and L6 myotube cell culture materials included RPMI-1640 (AG Biochrom, Germany), fetal bovine serum (FBS) (Hiclone, Germany) and streptomycin (AG Biochrom, Germany). All spectrophotometric measurements were performed with a UV2010 spectrophotometer (Hitachi, Germany).

2.2. Plant collection and identification

Fresh *N. stellata* (Willd.) flowers were collected from Thiruvallur district, Tamil Nadu, India. The species was identified and authenticated by Dr. D. Narasimhan, taxonomist, Department of Botany, Madras Christian College, Chennai, and the voucher specimen (MPC-186) was deposited at the Department herbarium, Loyola College, Chennai, Tamil Nadu, India.

2.3. Extraction and screening for antihyperglycemic effects

Shade-dried and coarsely powdered *N. stellata* flowers (3 kg) were sequentially extracted with hexane, chloroform, ethyl acetate and methanol at room temperature for 48 h. All extracts were filtered and concentrated under reduced pressure with a rotary evaporator to obtain completely dry extracts. The remaining powder residue was soaked in H_2O for 24 h, filtered, and concentrated to obtain an aqueous extract. The yields of the *N. stellata* flower crude extracts were 12 g hexane extract, 45 g chloroform extract, 29 g ethyl acetate extract, 60 g methanol extract and 24 g aqueous extract. All the extracts were tested for antihyperglycemic effects [12].

2.4. Bioassay-guided fractionation of active crude extract

The active *N. stellata* flower chloroform extract (*NSFCExt*) (25 g) was fractionated on a silica gel column (Acme's silica gel, 100–200 mesh size, 750 g, 3.5 i.d. × 60 cm) and successively eluted with stepwise gradients of hexane:chloroform followed by hexane:ethyl acetate (100%, 70%, 50%, 30%, 20%, 10%, 5%, and 0% hexane). Seventy-four fractions (150 ml each) were collected; each fraction was spotted on a precoated silica gel 60 F_{254}, 0.25 mm-thick TLC plate (Merck) and eluted in hexane:ethyl acetate (4:1), and fractions with similar Rf values were pooled. Finally, 17 major fractions were obtained, and a crystal (30:70 hexane:ethyl acetate) was obtained from fraction 12 (4.8 g). The fractions and crystallized compound were assayed for their effects on plasma glucose levels in STZ-induced diabetic rats [12].

2.5. X-ray crystallography

2.5.1. Crystal data

$(C_{25}H_{42}0)_2{\cdot}H_2O$, *Mr*735.19, monoclinic, space group $P2_1$ (No. 4), $a = 9.618(5)$, $b = 7.518(5)$, $c = 37.491(5)$ Å, $\beta = 94.483(5)^{\circ}$, $V =$ Q2 $2703(2)$ Å^3, $Z = 2$, $Dc = 0.903$ Mg/m^3, $F(000) = 820$, $\mu(M_0K\alpha) = 0.054$ mm^{-1}, crystal size = 0.3 × 0.1 × 0.1 nm.

2.5.2. Data collection and reduction

A crystal of suitable size was inspected for single crystallinity using a LEICA DMLSP polarizing microscope and mounted on a Kappa Apex2 CCD diffractometer equipped with graphite monochromatic Mo-Kα radiation ($\lambda = 0.71073$ Å). The unit cell parameters were obtained using reflections scanned from three different zones of the reciprocal lattice. The intensity data were collected using ω and φ scans with frame widths of 0.5°. The frame integration and data reduction were performed using Brukker SAINT-Plus (Version 7.06a) software [13]. Multiscan absorption corrections were applied to the data using SADABS (Brukeraxs) software [14].

2.5.3. Structure solution and refinement

The structure was solved using SIR92 and refined using SHELXL-97 [15] programs. All the non-hydrogen atoms were refined with anisotropic displacement parameters. All the hydrogens could be located in the difference Fourier map. However, they were relocated at chemically meaningful positions and were given riding model refinement. For the hydrogens of tertiary CH_3 groups, C–H was fixed at 0.96 Å with $U_{iso} = 1.2\ U_{eq}$ of parent carbon. For the primary CH group, C–H = 0.98 Å, and $U_{iso} = 1.2\ U_{eq}$ of the parent carbon. The hydroxyl hydrogen (OH) was fixed at 0.82 Å, and the C–O–H angle was tetrahedral. The structure was full matrix least squares refined with 5337 unique reflections. The waiting scheme employed was $w^{-1} = (\sigma^2\,(F_0^2) + 0.20\,P^2)$, where $P = (F_0^2 + 2F_c{}^2)/3$. The refinement continued until shift/esd converged to 0. The goodness of fit factor for refinement was 1.105. The highest residual electron density peak was 0.404 e/Å^3. The crystal diffracted poorly at higher

UNCORRECTED PROOF

Please cite this article in press as: P. Subash-Babu et al., Nymphayol increases glucose-stimulated insulin secretion by RIN-5F cells and GLUT4-mediated insulin sensitization in type 2 diabetic rat liver, Chemico-Biological Interactions (2014), http://dx.doi.org/10.1016/j.cbi.2014.12.011

Download English Version:

https://daneshyari.com/en/article/5847969

Download Persian Version:

https://daneshyari.com/article/5847969

Daneshyari.com