



Antidiabetic effects of *Justicia spicigera* Schltdl (Acanthaceae)

Rolffy Ortiz-Andrade^{a,*}, Angel Cabañas-Wuan^a, Víctor E. Arana-Argáez^a,
Angel Josabad Alonso-Castro^{b,c}, Rocio Zapata-Bustos^d, Luis A. Salazar-Olivo^d, Fabiola Domínguez^e,
Marco Chávez^{f,g}, Candy Carranza-Álvarez^h, Alejandro García-Carrancá^{c,i}

^a Facultad de Química, Universidad Autónoma de Yucatán, Mérida, Yucatán, México

^b Facultad de Química, Universidad Nacional Autónoma de México, D.F., México

^c Unidad de Investigación Biomédica en Cáncer, Instituto Nacional de Cancerología, Secretaría de Salud, México, D.F., México

^d División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica, San Luis Potosí, SLP, México

^e Centro de Investigación Biomédica de Oriente, IMSS, Metepec, Puebla, México

^f Unidad de Investigación Médica en Farmacología de Productos Naturales, Pediatría, CMN Siglo XXI, IMSS, D.F., México

^g Unidad de Investigación en Enfermedades Neurológicas, Especialidades, CMN Siglo XXI, IMSS, D.F., México

^h Unidad Académica Multidisciplinaria Zona Huasteca, Universidad Autónoma de San Luis Potosí, Ciudad Valles, San Luis Potosí, México

ⁱ Unidad de Investigación Biomédica en Cáncer, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, D.F., México

ARTICLE INFO

Article history:

Received 1 May 2012

Received in revised form

19 June 2012

Accepted 25 June 2012

Available online 20 July 2012

Keywords:

Justicia spicigera

Glucose uptake

Cytotoxic

Antioxidant

Diabetes

ABSTRACT

Ethnopharmacological importance: *Justicia spicigera* is a plant species used for the Teenak (Huesteca Potosina) and Mayan (Yucatan peninsula) indigenous for the empirical treatment of diabetes, infections and as stimulant.

Aim of the study: To evaluate the cytotoxicity, antioxidant and antidiabetic properties of *J. spicigera*.

Materials and methods: The effects of ethanolic extracts of *J. spicigera* (JSE) on the glucose uptake in insulin-sensitive and insulin-resistant murine 3T3-F442A and human subcutaneous adipocytes was evaluated. The antioxidant activities of the extract of JSE was determined by ABTS and DPPH methods. Additionally, it was evaluated the antidiabetic properties of JSE on T2DM model.

Results: JSE stimulated 2-NBDG uptake by insulin-sensitive and insulin-resistant human and murine adipocytes in a concentration-dependent manner with higher potency than rosiglitazone 1 mM. JSE showed antioxidant effects *in vitro* and induced glucose lowering effects in normoglycemic and STZ-induced diabetic rats.

Conclusion: The antidiabetic effects of administration of *J. spicigera* are related to the stimulation of glucose uptake in both insulin-sensitive and insulin-resistant murine and human adipocytes and this evidence justify its empirical use in Traditional Medicine. In addition, *J. spicigera* exerts glucose lowering effects in normoglycemic and STZ-induced diabetic rats.

© 2012 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Type 2 diabetes (T2DM), the cause of about 5% of all deaths globally each year, affects more than 220 million people worldwide and it is expected to rise to more than double in 2030 (World Health Organization (WHO), 2011). T2DM has expensive implications for health systems in Mexico (Arredondo and de Icaza, 2009) and other countries. The lack of awareness of this disease combined with insufficient access to health services can lead to complications such as atherosclerosis, cardiac dysfunction, retinopathy, neuropathy, nephropathy and blindness (World Health Organization (WHO), 2011). Therefore, there is a worldwide interest for new drugs that can help to reverse disease progression.

The use of medicinal plants is a common practice among Mexican diabetics for the empirical treatment of diabetes (Argáez-López et al., 2003). *J. spicigera* (JS) Schltdl (Acanthaceae) is an evergreen shrub with tubular orange flowers that grows in hot climates, native from México to South America.

JS is widely distributed in the Yucatan peninsula and is known as lool chak, ts'i'its and yichkaan; in Huasteca Potosina (San Luis Potosí, Mexico) it is commonly known as muicle or muite. This species of *Justicia* is described as erect or scandent perennial herbs or subshrubs. Leaves present cystoliths and are petiolate with a leaf margin that is usually entire. Inflorescences are in spikes or panicles cimas, and the species rarely has solitary, terminal, or axillary flowers. The bracts and bracteoles are usually conspicuous and imbricate. This can be easily recognized by their bilabial corolla, with a posterior lip that is generally two-lobed, an anterior lip that is three lobed, two stamens, a capsule with four seeds, and a basal sterile portion (Graham, 1990; Braz et al., 2002; Arellano-Rodríguez et al., 2003).

* Corresponding author. Tel.: +52 999 9225711.

E-mail address: rolffy@uady.mx (R. Ortiz-Andrade).

Traditionally, in the Yucatan peninsula, the leaves are used as an infusion and is indicated for the treatment of chronic headaches, hypertension and epilepsy, so as for the treatment of ailments related to the digestive system, such as stomach pain, diarrhea, dysentery as well as for constipation. In addition to this, is used as decoction of the leaves or branches (and sometimes the flower) for cases of skin diseases such as erysipelas (skin infection caused by the itch mite), syphilis, tumors, or difficult-cure pimples. It's also indicated for use in fever, kidney infection, anemia, so as anti-inflammatory, for dizziness and sleep, and for some respiratory ailments such as cough, bronchitis and constipation. There are also reports that both the infusion of leaves, as decoction of aerial parts are used in Mexican traditional medicine are widely used for the empirical treatment of diabetes (Graham, 1990; Braz et al., 2002; Arellano-Rodríguez et al., 2003; Meckes et al., 2004; Vega-Avila et al., 2009; Andrade-Cetto and Heinrich, 2005; Johnson et al., 2006). The indigenous Teenek from Huasteca Potosina (San Luis Potosi, Mexico) make a tea by boiling 20 g of JS leaves with 1 l of water. The tea is taken 3 times a day before every meal (personal communication). However, there is no scientific evidence about the antidiabetic properties of JS.

Previous phytochemical studies with ethanol extracts of *J. spicigera* leaves shown the isolation of the flavone kaempferol-3,7- bisrhamnoside (kaempferitrin; KM) (Euler and Alam, 1982; Domínguez et al., 1990). KM has showed antidiabetic properties *in vitro* and *in vivo* assays (De Sousa et al., 2004; Tzeng et al., 2009; Jorge et al., 2004; Vishnu-Prasad et al., 2009).

2. Materials and methods

2.1. Reagents, cell lines and chemicals

Murine 3T3-F442A preadipocytes and adult cat serum were a gift from Dr. W. Kuri-Harcuch (CINVESTAV, México). Human normal subcutaneous preadipocytes were obtained as previously described (Herrera-Herrera et al., 2009). Dulbecco's modified Eagle medium (DMEM), Leibovitz L15 medium and fetal bovine serum (FBS) were purchase from GIBCO BRL (Grand Island, NY, USA) whereas calf serum was from HyClone (Logan, UT, USA). Human Tumor Necrosis Factor alpha (TNF- α) and 2-[N-(7-nitro-benz-2-oxa-1, 3-diazol-4-yl) amino]-2-deoxy-D-glucose (2-NBDG) were obtained from Peprotech (London, UK) to Invitrogen (Carlsbad, CA, USA), respectively. Rosiglitazone (RGZ), from Cayman Chem. (Ann Arbor, MI, USA), was 98% purity according to the manufacturer. Kaempferitrin (KM) obtained from ChromaDex (Laguna Hills, CA, USA) was 98% purity according to the manufacturer. All other chemicals were from Sigma.

ABTS (2,2-azinobis-(3-ethyl-benzothiazoline)-6-sulfonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl) and the water soluble analogue of vitamin E (Trolox; 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchase from Sigma (St Louis, MO, USA).

As positive controls in pharmacological evaluations were used Glibenclamide (Silanes Laboratories, México City, México), Repaglinide (Prandin[®], Sanfer Laboratories, México City, México) and Saxagliptin (Onglyza[®], AstraZeneca/Bristol-Myers Squibb, México City, México) which were solubilized in saline (0.9% NaCl solution).

2.2. Plant material and extraction

Samples of *J. spicigera* collected in Ciudad Valles, San, Luis Potosí, México (21°58'43.90"N and 98°58'31.44"W) on August 2010 were identified by a specialist (J. Garcia-Perez) and preserved at the herbarium Isidro Palacios of Instituto de Investigación de Zonas

Desérticas, Universidad Autónoma de San Luis Potosí (SLPM) for future reference with the voucher number 46450.

Dried leaves of *J. spicigera* (40 g) were extracted with ethanol using a Soxtherm apparatus (Soxtherm automatic, Gerhardt, Germany) for 3 h. The extract (JSE) was filtered and evaporated under vacuum and then lyophilized (Free Zone 2.5 Labconco, USA). The extract was dissolved in methanol and filtered through a 0.45 μ m nylon membrane filter (Gelman Science) before use. For bioassays, extract was dissolved in DMSO and preserved at room temperature protected from light until use on cell cultures.

2.3. Quantitation of kaempferitrin in *Justicia spicigera*

The detection and quantification of KM in JSE were performed by reversed-phase HPLC method in a waters 2795 (Waters Corp., Milford, MA, USA) instrument equipped with quaternary pump, autosampler and a 996 photodiode array detector, and Waters Millenium 32 software for peak identification and integration. Kromasil column C-18 with a 150 $\times$ 4.6 mm i.d 0.45 μ m (Metachem Technologies Inc) with injection volume 5 μ l, flow 0.4 ml/min. The mobile phase was acetic acid (HPLC grade Merck Dormstadt, Germany) 2% in water and the stationary phase was acetonitrile (Merck, Dormstadt, Germany HPLC grade), gradient % B 10 to 70 in 20 min. Retention times and UV spectra of KM was compared to those of high-purity commercial standards. The calibration plot was obtained in the range of concentrations 124 to 496 μ g/ml. The concentration of kaempferitrin in the extracts was calculated from calibration plot. Maximum of absorbance was 260 nm with retention time of 10.54 min.

2.4. Cell viability assay

Murine and human preadipocytes were seeded as described previously in 96-well microplates (Alonso-Castro et al., 2011). After 24 h of incubation, ethanol extract of *J. spicigera* (JSE) at concentrations ranging from 0.1 to 250 μ g/ml were added to the cells. After 48 h, MTT assay was carried out (Alonso-Castro et al., 2011). The optical density (O.D.) was measured at 590 nm in an ELISA reader (Biorad Laboratories, CA, USA) using wells without cells as a blank. The viability of treated cells was estimated from the relative growth as follows:

$$\text{relative viability} = \frac{\text{control O.D.} - \text{sample O.D.}}{\text{control O.D.}} \times 100$$

2.5. 2-NBDG uptake assay

Murine 3T3-F442A preadipocytes were differentiated into adipocytes with DMEM containing 10% FBS, 5 μ g/ml insulin and 1 μ M D-biotin, whereas human preadipocytes were differentiated into human adipocytes with L15 containing 5% FBS, insulin 5 μ g/ml, RGZ 1 μ M, dexamethasone 100 nM, tri-iodothyronine 0.2 nM, and 3-iso-butyl-1-methylxanthine 25 μ M. Murine 3T3-F442A or human adipocytes were seeded on 96-well fluorescence plates and incubated for 60 min with PBS containing BSA 1 mg/ml and 80 μ M of the fluorescent glucose analog 2-NBDG (Alonso-Castro et al., 2011) in the presence of different concentrations of ethanol extract of JSE (1 to 50 μ g/ml). Control cultures were treated with insulin 100 nM or RGZ 10 μ M. JSE effects on 2-NBDG incorporation were also evaluated on 3T3-F442A and human adipocytes pre-incubated with TNF- α 10 ng/ml for 7 day to induce insulin-resistance (Hotamisligil et al., 1994). After incubation, free 2-NBDG was washed out from cultures and fluorescence retained in cell monolayers was measured with a Tecan-GENios fluorescence microplate reader (Tecan, Salzburg, Austria). Values of 2-NBDG incorporation in the absence of insulin

Download English Version:

<https://daneshyari.com/en/article/5838328>

Download Persian Version:

<https://daneshyari.com/article/5838328>

[Daneshyari.com](https://daneshyari.com)