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Original Article

Phytochemical composition and chronic hypoglycemic effect of *Rhizophora mangle* cortex on STZ-NA-induced diabetic rats

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ABSTRACT

Type 2 diabetes is a major health problem in Mexico, as it is in other countries, is a chronic condition that develops when the body cannot produce enough insulin or cannot use it appropriately. Both insulin deficiency and insulin resistance lead to high blood glucose levels. In Mexico, people with diabetes are known to use the decoction of red mangrove (*Rhizophora mangle* L., Rhizophoraceae) bark to control blood glucose levels. Therefore, in this study, we sought to investigate the chronic hypoglycemic and hypolipidemic effects of *R. mangle*; we also elucidate some of the major phytochemical compounds of *R. mangle*. To analyze the hypoglycemic and hypolipidemic effects, we used rats with streptozotocin–nicotinamide-induced hyperglycemia; the rats were classified into four groups (six rats each), based on the treatment given, as follows: group 1, non-hyperglycemic control; group 2, hyperglycemic control; group 3, glibenclamide (5 mg/kg body weight); and group 4, *Rhizophora* ethanol–water extract (90 mg/kg). The extract or glibenclamide was orally administered, dissolved in 1.5 ml of physiological NaCl-solution, twice a day (in the morning and in the evening) over a period of 42 days. The methanolic extract was used to elucidate the main compounds present in *R. mangle* via conventional phytochemical methods, such as TLC, HLPC, UPLC–DAD–MS, and NMR. The following compounds were detected: cinchonains Ia and Ib, catechin-3-*O*-rhamnopyranoside, epicatechin, lyoniside, and nudiposide. The daily administration of *Rhizophora* ethanol–water extract, similar to the traditional usage to control type 2 diabetes, was shown to exert chronic hypoglycemic and hypolipidemic effects. This effect may be associated whit the constituents in the extract. These findings suggest that *R. mangle* and its constituents could be potentially used to treat type 2 diabetes.

Introduction

Diabetes is a chronic condition that occurs when the body cannot produce enough insulin or cannot use it appropriately. In type 2 diabetes (T2D), the body can produce insulin but becomes resistant to it, causing ineffectiveness of insulin. Consequently, insulin levels may become insufficient, and thus insulin resistance and insulin deficiency result in high blood glucose levels (IDF, 2015) and (ADA, 2015). Individuals with T2D suffer from insulin resistance and usually relative, rather than absolute, insulin deficiency. However, at least initially, and often throughout their lifetime, these individuals may not require insulin treatment to survive.

In 2015, the IDF estimated that 415 million people are living with diabetes worldwide; Mexico stands sixth among the top ten countries, with 11.5 million people (IDF, 2015). Diabetes-associated complications, such as cardiovascular disease, blindness, kidney failure, and lower-limb amputation, are a major cause of disability, low quality of life, and premature death.

Among the World Health Organization list of essential drugs used for the treatment of diabetes, metformin (a biguanide) and gliclazide (a sulfonylurea) are well-established medications and they should be available and easily accessible (according to need), to all patients with T2D (IDF, 2015). It is of significance that metformin was originally isolated from the French lilac (*Galega officinalis*) (Witters, 2001).

Plants have been used for medicinal purposes in Mexico since pre-Hispanic times. The high prevalence of T2D among Mexicans, associated to vulnerable economic stability, and the fact that people trust the effectiveness of medicinal plants have led to the increased use of plants to treat T2D. These factors have made it essential to

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study the pharmacological and phytochemical properties of plants with hypoglycemic properties in Mexico.

Rhizophora mangle L., Rhizophoraceae (Andrade-Cetto and Heinrich, 2005), traditionally known as "mangrove" or "red mangrove," is widely used for the treatment of diabetes in Mexico. It is a 25-m-tall tree that grows in mangroves and distributed along the Pacific and Gulf Coasts of Mexico. It has a tall, straight trunk with abundant roots, a round treetop with sympodial branching, bitter-red wood, and cortex (Pennington and Sarukhán, 1998).

The anti-hyperglycemic effect of the plant was previously reported by Alarcon-Aguilara et al. (1998), they test the effect of 28 plants in rabbits under a glucose tolerance test, the results obtained from the variance analysis showed that *R. mangle* significantly decreased the hyperglycemic peak by 16.1%.

In a previous study performed by our group, the ethnobotanical relevance and the acute hypoglycemic effect of *R. mangle* were reported (Andrade-Cetto and Mares, 2012), in that study we confirm that the dose of 90 mg/kg has the better hypoglycemic effect, this dose is the traditional used dose multiplied by 10. In the present study, we aimed to examine the chronic hypoglycemic effect of the ethanolic extract of the bark of *R. mangle* in streptozotocin–nicotinamide (STZ-NA)-induced diabetic rats; we also evaluated the lipid profile and glycated hemoglobin after chronic administration. In addition, we sought to characterize the major phytochemical compounds present in the plant cortex.

Materials and methods

Plant extracts

Based on the results of the previous study in which the water and ethanol–water extracts (EW) were tested (Andrade-Cetto and Mares, 2012), we selected the ethanol–water extract which is similar to the traditional used infusion and presented better activity (Fig. 1). New botanical samples of *Rhizophora mangle* L., Rhizophoraceae, were collected with the help of informants in Manialtepec, Oaxaca Mexico, the original plant was deposited at the IMSS, Herbarium in Mexico City with the voucher number IMMSM15816. The extract to be used in pharmacological tests was prepared as previously described; in brief; a 50 g sample of the plant material was added to 500 ml of an ethanol–water mixture (1:1), it was then heated at 40 °C for 4 h before being filtered for three times. This was followed by elimination of the solvent under reduced pressure in a Büchi rotary evaporator. The yield of the extract ethanol–water (1:1) was 14.75 g.

For the phytochemical identification of the main compounds of the cortex; the methanolic extract (ME) was prepared using 200 g of plant material through Soxhlet extraction. Defatting with hexane (24 h) followed by methanol (MeOH) extraction (48 h), and the resulting extract evaporated under reduced pressure until it reached dryness producing 15 g of ME.

Compounds isolation

HPLC–DAD–MS analysis was performed to confirm that the ethanol–water extract used in the pharmacological testing has a similar phytochemical profile than the water and methanolic extracts (Fig. 1), but the last one was more accessible for the isolation process.

A sample of ME (3 g) was dissolved in MeOH and partitioned with hexane to yield a hexane soluble fraction (HSF; 50 mg), a MeOH-soluble fraction (MSF, 2.90 g), and a red precipitate (RP; 28 mg).

The MSF was subjected to column chromatography (CC) on 360 g of silica gel (70–230 mesh, Merck) starting with hexane 100% (400 ml), increasing the polarity with EtOAc using a mixture of hexane/EtOAc as eluent, until 100% (500 ml), and subsequently with MeOH until 100% (500 ml). This process led to fourteen primary fractions (MSF1–MSF14). Fraction MSF8 (400 mg) was subjected to silica gel CC eluted with EtOAc/MeOH (10:0–0:10), this process led to five subfractions (MSF 8.1–MSF8.5). Preparative thin layer chromatography (TLC) (Macherey & Nagel, 0.25 mm) of fraction MSF8.2 (20 mg) using EtOAc/MeOH/H_2O, 7:2:1, as eluent resulted in the isolation of a mixture of **1** and **2** (10 mg). Preparative TLC (EtOAc/MeOH/H_2O, 7:2:1) of fraction MSF8.3 (80 mg) resulted in the isolation of **3** (23.7 mg) and **4** (13 mg). FSM8.4 was resolved by HPLC (Nucleosil 250 × 10 mm i.d., 5 μm, C18, Macherey & Nagel); using a gradient of MeCN/H_2O starting with 20/80 to 70/30 during 17 min (3 ml/min; 250 and 280 nm UV-det.) to obtain 10 mg of a mixture of **5** and **6** with an Rt 10.5 min.

An efficient method based on HPLC–DAD–MS technique was used for identifying the isolated compounds from the methanolic extract corresponding to (**1**–**6**) in the water and ethanol–water extract. The components were separated on a Kinetex HPLC/UPLC XB-C18 column (50 × 2.1 mm i.d., 2.6 μm) at 25 °C. The mobile phase consisted of a water gradient (containing 0.1% FA) (A) and acetonitrile (B). The following gradient elution program was used: 1% B during 0.5 min, 1–35% B 0.5–15 min, 35–100% B 15–18 min, 100–1% B 18–20 min at a flow rate of 0.2 ml min^{-1}, the injection volumen was 3 μl. Majority compounds of the tradicional decoction and the ethanol–water extract were identified and are shown in Fig. 1.

General experimental procedures

NMR spectra including HSQC, HMBC, COSY, and TOCSY were recorded in a Varian Inova spectrometer at 500 (^{1}H) and 125 MHz (^{13}C) or a JEOL-ECA at 300 (^{1}H) and 75 MHz (^{13}C); chemical shifts were recorded as δ values. HRESIMS were recorded on a Thermo Scientific LTQ Orbitrap XL hybrid FTMS (Fourier transform mass spectrometer). Data were collected in both positive and negative ionization modes via a liquid chromatographic/autosampler system that consisted of an Acquity UPLC system. Profile HPLC–DAD–MS were performed using an Agilent 1200 Infinity system equipped with a G1312-95006 Binary pump, G1329-90012 Autosampler, controlled by Agilent ChemStation software, coupled to a Waters diode array detector (DAD) and a Squire 6000 Bruker ESI-MS in negative mode ion polarity.

Analytical and preparative HPLC analyses were performed in an Agilent 1260 Infinity system equipped with a G1311B Quaternary pump, G1367E Autosampler, G1315C DAD VL+ and controlled by Agilent ChemStation software. For analytical and semipreparative HPLC, Macherey-Nagel (Nucleosil C_{18}, 250 × 4.6 mm i.d., 5 μm), Macherey-Nagel (Nucleosil C_{18}, 250 × 10 mm i.d., 5 μm) columns, respectively, were used. Column chromatography (CC) was carried out on silica gel (70–230 mesh, Merck). Thin-layer chromatography analysis was carried out on silica gel 60 F_{254} plates (Macherey & Nagel) using ceric sulfate (10%) solution in H_2SO_4 as color reagent.

Experimental animals

Eight-week-old Wistar rats weighing 200–250 g were obtained from the Bioterium of the Science School, UNAM, and were acclimated with free access to food and water for at least one week in an air-conditioned room (25 °C with 55% humidity) on a 12 h light–dark cycle prior to performing the experiments. The animals were handled according to the National Institute of Health, USA (Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011). Experimental diabetes was induced

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