Revista Brasileira de Farmacognosia 25 (2015) 145–151

Revista Brasileira de Farmacognosia
BRAZILIAN JOURNAL OF PHARMACOGNOSY
www.sbfgnosia.org.br/revista

Original Article

Stevia rebaudiana loaded titanium oxide nanomaterials as an antidiabetic agent in rats

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ARTICLE INFO

Article history:
Received 25 July 2014
Accepted 4 March 2015
Available online 30 March 2015

Keywords:
Diabetes
Stevia rebaudiana
Nanomaterials
Hypoglycemic
Antihyperlipidemic

ABSTRACT

Stevia rebaudiana (Bertoni) Bertoni, Asteraceae, is a plant with hypoglycemic and antihyperlipidemic properties. *S. rebaudiana* (SrB) has become a lead candidate for the treatment of the diabetes mellitus. However, chronic administrations of *S. rebaudiana* are required to cause the normoglycemic effect. Importantly, nanomaterials in general and titanium dioxide (TiO_2) in particular have become effective tools for drug delivery. In this work, we obtained TiO_2 nanomaterials with SrB at different concentrations (10, 20 and 30 μM) by sol–gel method. After this nanomaterials were characterized by Fourier transform infrared spectroscopy and transmission electron microscopy. Where it was demonstrated, the presence of the *S. rebaudiana* in TiO_2 nanomaterials, which were observed as hemispherical agglomerated particles of different sizes. The nanomaterials were evaluated in male rats whose diabetes mellitus-phenotype was induced by alloxan (200 mg/kg, *i.p.*). The co-administration of TiO_2-SrB (20 and 30 μM) induced a significant and permanent decrease in the glucose concentration since 4 h, until 30 days post-administration. Likewise, the concentrations of insulin, glycosylated hemoglobin, cholesterol, and triacylglycerides showed a significant recovery to basal levels. The major finding of the study was that the TiO_2-SrB (20 and 30 μM) has a potent and prolonged activity antidiabetic. TiO_2 can be considered like an appropriated vehicle in the continuous freeing of active substances to treat of diabetes mellitus.

Introduction

Traditional medicine principally derived from plants and currently represents a therapeutic potential for Diabetes mellitus (DM) (Laitiff et al., 2010; Malviya et al., 2010).

Stevia rebaudiana (Bertoni) Bertoni (SrB), also known as "sweet herb," is a shrubby perennial plant belonging to the Asteraceae family (Soejarto et al., 1982). SrB sheets containing approximately 4–15% stevioside, which have been isolated and characterized by chemical and spectral studies (Starratt et al., 2002).

SrB has various pharmacological properties including antioxidant activity, antihypertensive, antihyperlipidemic and cardiovascular protector (Chan et al., 1998). Recent studies have shown that the SrB stimulates insulin secretion from pancreatic β-cells and cause normolipidemia and normoglycemia in diabetic rats (Raskovic et al., 2004; Chen et al., 2005). In this sense, SrB provides evidence of his traditional use in the control of DM. However, it has been demonstrated that are required continuous administrations of SrB to achieve normoglycemic effect (Curry and Roberts, 2008). One way to address this problem is, to develop a controlled release system of SrB, in order to reduce the dose and make available SrB in the target sites and keep its prolonged activity. A variety of systems have been developed and used, including liposomes, micelles, dendrimers, and copolymers.

Currently, nanomaterials of titanium dioxide (TiO_2) have attracted attention as delivery potential systems (Barb et al., 2004). The TiO_2 nanomaterials are chemically inert, possess hydrophilic features and its synthesis isn't complicated, in addition, these present high mechanical strength and low toxicity (Son et al., 2007). Recently the nanomaterials have been implanted in the amygdala of rats with epilepsy and obtained optimal results, and found that the porous TiO_2 matrix release their contents in a controlled manner

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http://dx.doi.org/10.1016/j.bjp.2015.03.004
0102-695X/

for a period approximately of 500 h (h) (López et al., 2010a,b). Here, we performed the synthesis of TiO_2 nanomaterials from solutions of titanium *n*-butoxide and the aqueous extract of SrB concentrate obtained from the dried leaves of the plant. This with the purpose of using it as a controlled release system SrB extract. For the above, this study aimed to evaluate the antidiabetic activity of intraperitoneal injection of TiO_2-SrB nanomaterials in a diabetic rat model.

Materials and methods

Preparation of SrB-extract

Stevia rebaudiana (Bertoni) Bertoni, Asteraceae, was grown on campus grounds of the Benemérita Universidad Autónoma de Puebla (BUAP), México. Dr. Albino Moreno-Rodríguez confirmed the identification, additionally; a sample was deposited in the botanical garden of the BUAP (file 21-09/14). Leaves SrB (10 g) washed and dried in an Elisa-550 oven for 24 h. Then it pulverized and macerated with a solution of ethanol and water (with a ratio of 80:20). The solution obtained from the mashing process is filtered and the SrB aqueous extract is placed on a rotary evaporator of the ESEVE-402-2 brand to eliminate to 80% of solvent (water and alcohol) under reduced pressure. Thus we obtain the SrB concentrated extract to 80 wt.

Preparation of TiO_2-SrB nanomaterials by sol–gel method

The homogeneous solutions were prepared separately with different concentrations of the SrB concentrated extract (10, 20 and 30 μM). Each homogeneous solution containing 150 ml of anhydrous 1-butanol (99.8%, Sigma–Aldrich), 10 ml of deionized water and 0.5 g of polyvinylpyrrolidone (with an average molecular weight of 40,000, Sigma–Aldrich) and the required concentration of the SrB concentrated extract (10, 20 and 30 μM).

For each homogeneous solution was added 21.5 ml of titanium *n*-butoxide (97%, Sigma–Aldrich) in a reflux system at 70 °C with constant agitation. The final solution with properties of gel was immersed in a container with ice for 15 min at 3 °C. The solvent was removed on a rotary evaporator at 50 °C under vacuum conditions to finally obtain the TiO_2-SrB nanomaterials (López et al., 2010a,b).

Characterized of TiO_2-SrB nanomaterials

Infrared spectroscopy (FTIR)

The TiO_2-SrB nanomaterials were mixed with KBr (5 wt %) and pressed in transparent wafers. Fourier transform infrared spectroscopy was recorded using a Perkin-Elmer 1600 spectrophotometer (Perkin-Elmer, Shelton, CT) in the 4000–400 cm^{-1} range, and 32 scans were run for each measurement.

Scanning electron microscopy (SEM)

The particle size was measured using conventional Scanning electron microscopy (SEM, Zeiss; Carl Zeiss, Oberkochen, Germany), operated at 100 kV, with entry goniometer at the side and 0.4 nm point-to-point resolution, and attached to a CCD camera (MegaVision, Santa Barbara, CA).

Animals

Adult male Long Evans rats (230–250 g) were obtained from Bioterio "Claude Bernard" BUAP. Animals were individually housed in an environment with controlled temperature, humidity, and light conditions (12 h light: 12 h dark cycle), with free access to food and water. All procedures described in this study were performed in accordance to the Mexican Law of Animal Treatment and Protection Guidelines (NOM-062-ZOO-1999) and the Research Committee uses of laboratory animals of the BUAP (VIEP-3450-2013).

Induction of diabetes in rats

The animals were injected with alloxan dissolved in 0.1 M citrate buffer, pH 4.5 at a dose 200 mg/kg body weight (*i.p.*). The alloxan dose was selected based on various reports (Raskovic et al., 2004; Szkudelski, 2001). Three day after of the administration of alloxan, blood samples were taken and blood glucose levels were determined. Animals that had higher concentrations of glucose (150 mg/dl) were considered diabetic.

SrB-TiO_2 administration protocols

In the experiment a total of forty rats were used, which were divided in five groups ($n = 8$ per group).

Group 1: normal rats treated with TiO_2 nanomaterials (vehicle group)
Group 2: diabetic group treated with TiO_2 nanomaterials
Group 3: diabetic rats with TiO_2-SrB nanomaterials to 10 μM
Group 4: diabetic rats with TiO_2-SrB nanomaterials to 20 μM
Group 5: diabetic rats with TiO_2-SrB nanomaterials to 30 μM

The nanomaterials were injected by *i.p.* The route in all animals depending of treatment.

Subsequently, both the empty TiO_2 nanomaterials (1 g/kg in sterile water at 37 °C) and SrB (10, 20 and 30 μM) (1 g/kg in sterile water at 37 °C) were administered, respectively.

Method for determination of plasma blood glucose level

To evaluate the plasmatic concentration of glucose with respect at course of time in the animals injected with SrB nanomaterials, the animals were deprived of the food for 5 h before the determination. The blood glucose levels were determined at 0, 4, 8 and 24 as well as at 5, 10, 15 and 30 days after administration of each treatments. In the experiment the blood glucose level of the animals were estimated by Glucose Oxidase–Peroxidase Enzymatic Method using a digital glucometer (ACCU-CHEK brand active). Blood from the tail vein was collected.

Evaluation of the effect of TiO_2-SrB nanomaterials on hyperglycemia and hyperlipidemia induced by alloxan

At 31 days post-injection of TiO_2 and TiO_2-SrB, the animals of each experimental group were sacrificed by dislocation and by cardiac puncture. The blood samples were collected to measure glucose, insulin, glycated hemoglobin, cholesterol and triacylglycerides. Collected samples were centrifuged at $2 \times g$ for 5 min. Serum determinations of glucose, cholesterol and triacylglycerides were developed by enzymatic colorimetric assay, following the protocols respectively labeled according the BioSystem kits and analyzed in a semi-automated spectrophotometer (Bayer RA-50). Plasma insulin concentration was determined by an ELISA immunoassay (Diagnóstica Internacional), and antibody-antigen complex was determined at 415 nm in a Stat fax 2600 plate reader (WinerLab group). The glycated hemoglobin measuring was carried out by immunofluorescence method, following the protocol according the i-Chroma kit and it was analyzed in a detector of the same brand.

Statistical analysis

The data were expressed as mean ± standard error (SE) for all experiments. Statistical analysis was developed through of ANOVA