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ALPHA-GLUCOSIDASE INHIBITING ACTIVITY OF FIVE MEXICAN PLANTS USED IN THE TREATMENT OF TYPE 2 DIABETES.

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Abstract

Type 2 diabetes is a public health problem that is characterized by tissue insulin resistance combined with a relative deficiency in insulin secretion. Postprandial hyperglycemia is an independent risk factor for cardiovascular disease, stroke and mortality. The alpha-glucosidase inhibitors lower postprandial blood glucose concentrations, act as competitive inhibitors and exhibit a high affinity for alpha-glucosidases, blocking the enzymatic reaction. In maltose-loaded rats, and in in vitro assay of glucosidasesisolated from rat gut, the present study the effect on tests the α-glucosidase activity of five extracts of Mexican plants: *Smilax moranensis* M. Martens y Galeotti, *Tournefortia hirsutissima* L., *Rhizophora mangle* L., *Cecropia obtusifolia* Bertol, and *Bromelia plumieri* (E. Morren.) L.B. Sm. All of the tested plants showed hypoglycemic activity after 90 minutes. Furthermore, Cecropia Obtusifolia, Bromelia plumieri and Rhizophora mangle showed in vitro an inhibitory effect over the enzyme.

Keywords: Type 2 diabetes, hypoglycemic agent, medicinal plant, alpha-glucosidase inhibitors.

Abbreviations: Smilax moranensis M. Martens y Galeotti, (Sm), Tournefortia hirsutissima L. (Th), Rhizophora mangle L. (Rm), Cecropia obtusifolia Bertol (Co) Bromelia plumieri (E. Morren.) L.B. Sm. (Bp), STZ-Na, Streptozotocin-nicotinamide; T2D, Type 2 diabetes.

Introduction

Diabetes mellitus is the most common endocrine disease. It comprises a heterogeneous group of hyperglycemic disorders characterized by high serum-glucose concentration and disturbances of carbohydrate and lipid metabolism. It is defined as an elevated blood-glucose level associated with absent or inadequate pancreatic insulin secretion, which may occur with or without the impairment of insulin signaling. Type 2 diabetes (T2D) is characterized by tissue resistance to insulin combined with a relative deficiency in insulin secretion. A given individual may exhibit either increased insulin resistance or increased β-cell deficiency, and these abnormalities may be mild or severe. Although the β-cells in these patients produce insulin, their production is inadequate to overcome insulin resistance and their blood glucose level therefore increases. Impaired insulin signaling also affects fat metabolism, resulting in increased free fatty acid flux, elevated triglyceride levels and reciprocally low levels of high-density lipoprotein (HDL). The long-term complications of diabetes include retinopathy with a potential loss of vision, nephropathy leading to renal failure, peripheral neuropathy with a risk of foot ulcers, amputations, and Charcot joints (autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction) [1].

According to the International Diabetes Federation (IDF) [2], more than 382 million people worldwide were affected by T2D in 2013; this numbers will increase to 592 million in 2035. A large number are unknowingly progressing towards complications. Consequently, the disease is a major cause of mortality, providing a worrying indication of the future impact of diabetes as a major threat to global development. In 2013, the IDF placed Mexico'as the country with the 6th highest number of people living with diabetes. The IDF estimates 8.7 million people in the country are diabetic, but in 2012 the Mexican health services report found that 9.2% (6.4 million) of the Mexican adult population has been diagnosed with diabetes, and this number could be double the reported number because many patients do not know their condition and have not been diagnosed. This fact would mean that in 2012 at least 12.8 million people were living with diabetes in Mexico. [3].

Postprandial hyperglycemia is an independent risk factor for cardiovascular disease, stroke and mortality; it initiates a cascade of pro-atherogenic and pro-thrombotic events. It has been shown that a rapid rise in glucose level increases the activity of low-grade inflammation. Furthermore, a direct correlation between oxidative stress measured by urinary 8-iso PGF2α excretion and mean amplitude of glucose excursion in circadian blood-glucose profile measurement was demonstrated. On the other hand, it may also have harmful effects on β-cells (glucotoxicity) and has been shown to deteriorate insulin sensitivity of the musculature [4].

The alpha-glucosidase inhibitors (AGIs) lower postprandial blood glucose concentrations, act as competitive inhibitors and exhibit a high affinity for alpha-glucosidases, blocking the enzymatic reaction particularly because of their nitrogen component. Thus, AGIs must be present at the site of enzymatic action at the same time as the carbohydrates; this action retards glucose entry into the systemic circulation. Three AGIs are now in therapeutic use worldwide: Acarbose, isolated from culture filtrates of actinoplanes, was first described by Schmidt in 1977 and introduced onto the market in 1990; Miglitol, a semisynthetic derivative of 1-deoxynojirimycin, from Bacillus and Streptomyces sp., and Voglibose, isolated from validamycin A, a product of Streptomyces hygroscopicus. Acarbose has efficacy against various alpha-glucosidases, glucoamylase, followed by sucrase, maltase and dextranase. It also inhibits alpha-amylase, but has no effect on beta-glucosidases, such as lactase [4]. In a recent review [5] of Mexican plants with inhibitory activity on α-glucosidases, 38 plants (growing in Mexico) were reported to have activity, of which six have led to the isolation of new inhibitors of α-glucosidases, and only three were conducted on plants grown in Mexico. Thus it is necessary to continue the evaluation of plants growing in Mexico.

In the present work, we tested the activity of α-glucosidases from five extracts of Mexican plants: the ethanoic extract of the Smilax moranensis M. Martens y Galeotti, (Sm) root; the butanolic extract of the Tournefortia hirsutissima L. (Th) stem; the water extract of the Rhizophora mangle L. (Rm) cortex; the cortex sap of Cecropia obtusifolia Bertol (Co); and the ethanol water extract of Bromelia plumieri (E. Morren.) L.B. Sm. (Bp) leaves. All of these plants have been tested previously [6, 7, 8, and 9] for their hypoglycemic activity and the type of extract and tested doses for this study were selected from those works.

The aims of this study were to evaluate the activity of extracts from five Mexican plants in maltose-loaded hyperglycemic STZ-NA rats and to evaluate the effects of the extracts on the activity of α-glucosidases isolated from rat gut.

Methods

Extracts

The plant extracts were prepared as previously described and tested using the following doses: Sm 80 mg/kg; Th 80 mg/kg; Rm 56 mg/kg; Co 280 mg/kg; and Bp 300 mg/kg. The type of extract and the part of the plant used to make the extract were also as previously described.

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 Guide for the Care and Use of Laboratory Animals [10].

Induction of experimental diabetes

Experimental diabetes was induced as described by Masiello [11]. The rats were fasted overnight and injected intraperitoneally with 150 mg/kg nicotinamide (NA) (Sigma, N3376) 15 min before an intravenous injection of 65 mg/kg streptozotocin (STZ) in citrate buffer (Sigma, S0130).

Maltose Tolerance Test

For the oral maltose tolerance test (OMTT), the animals were classified into 8 groups (1-8) of eleven rats each. Group 1 was the no hyperglycemic control group (NHCG) and received 1.5 ml of physiological NaCl-solution (vehicle). Group 2, the hyperglycemic control group (HCG), also received 1.5 ml of physiological NaCl-solution. Group 3 was the positive control group and received acarbose (3 mg/kg), whereas Groups 4-8 received the extracts of the five plants Sm, Th, Rm, Co and Bp. Test samples, control drug or vehicle were given orally to 8-hr-fasted rats, five min before the administration of maltose 3 g/kg diluted in 1.5 ml of saline (NaCl 0.9%). The extracts were also dissolved in 1.5 ml of physiological NaCl-solution and administered by gastroesophageal gavage to ensure that the liquid reached the digestive tract of the rat. All groups were fed Purina Rodent Laboratory Chow 5001.

Blood samples were obtained from the tail vein according to procedures outlined in the Institutional Animal Care and Use Committee Guideline 9 (3/10/99) [12]. During the first experiment, blood was collected five minutes before the oral administration of extracts or vehicle (T0) and at times T30, T60 and T90 min thereafter. The glucose concentration was measured by an Accutrend GC ® (Cobas).

Crude extract of the small intestine

Crude extract of the rat intestine was prepared according to the method described in [13] with modifications. The small intestine from 6 Wistar rats was dissected and washed two times with physiologic solution (NaCl, 9%) and once more with 0.1 M potassium phosphate buffer (pH 7) with 5 mM EDTA. From the washed small intestine, the mucosa was scraped and homogenized in the same potassium phosphate buffer and then centrifuged at 21,000 g for one hour. The precipitate was resuspended and incubated for 30 min in 0.1 M potassium phosphate buffer (pH 7) containing 1% Triton X-100 before being centrifuged at 100,000 x g for 90 min. The supernatant was dialyzed against 0.01 M potassium phosphate buffer (pH 7.0) for 24 h. The dialysate was lyophilized and stored at -20°C until needed.

In vitro glucosidase assay

Enzyme activity was measured following the procedure previously described in [14] with slight modifications. Activity was quantified by the amount of p-nitrophenol released from p-nitrophenyl-alpha-D-glucopyranoside. The assay contained 0.1 M sodium phosphate buffer (pH 6.8) with 2 mM p-4-nitrophenol glucopyranoside (p-NPGP) and 0.1 U of alpha-glucosidase from crude extract of the small intestine as well as experimental extract or control (Acarbose, Bayer) at concentrations ranging from 0.2 µg/ml to 2,000 µg/ml in a volume of 1 ml. The reaction was tracked for 480 sec and readings were acquired every 15 sec at 405 nm wavelength with a Beckman Coulter spectrophotometer model DU-640.

Data analysis

The data were analyzed by one-way ANOVA followed by Tukey's test. Plasma glucose levels are expressed as the mean ± S.E.M.

Results

Maltose Tolerance test

The results are presented in table 1,30 minutes after the maltose load both control groups, normal and hyperglycemic, showed elevated glucose levels compared with time 0. The values remained high until 90 minutes. In addition, the hyperglycemic control was significantly different compared with the normal control at all of the time points. In the acarbose group, as expected, the glucose levels were

not modified at time 30 and thereafter. After the maltose load, *extracts of Smilax moranensis*, *Tournefortia hirsutissima*, *Cecropia obtusifolia* and *Bromelia plumieri* did not suppress the hyperglycemic peak but the plant extracts did display a hypoglycemic effect at the 90 min time point.

In vitro glucosidase assay

The percent of inhibition is presented in table 2. As expected, Acarbose inhibited 50% of the enzyme activity at a concentration of 11.1 µg/ml. None of the plant extracts reached 50% inhibition at the maximum testable concentration, however, Cecropia Obtusifolia inhibited 45% of the enzyme activity at 500 µg/ml, Bromelia plumieri inhibited 30% at 2000 µg/ml, Rhizophora mangle inhibited 17% at 1000 µg/ml, Smilax moranensis inhibited 7% at 500 µg/ml and Tournefortia hirsutissima inhibited 6% at 1000 µg/ml.

Discussion

In traditional medicine, the plant extracts tested here are used as an infusion, which diabetic individuals drink over the course of the day. This form of consumption, would not explain the hypoglycemic effect of a plant as an inhibitor of the alpha-glucosidases, because the individuals do not drink the infusion in the fasting state just after the meal. For this reason, one cannot expect that all plants produce an inhibitory effect on the alpha-glucosidases; however, the tested plant extracts produced a hypoglycemic effect after 90 minutes. This observation is in agreement with previous results. Furthermore, Cecropia Obtusifolia and Bromelia plumieri are at least partially able to inhibit the enzyme. It is possible that the concentration of the active compound is low; this observation does not explain the hypoglycemic effect, but it can contribute in a synergistic way to better glucose control in the diabetic patient.

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Table 1. Glucose levels. The values represent the mean ± SEM. In the same column: [a] indicates statistically significant differences compared with the diabetic control group; [c] indicates statistically significant differences compared with the control group. In the same row: [b] indicates statistically significant differences compared with time 0; $p < 0.05$, n=11.

	Glucose [mg/dl]			
Group/Time (min)	T0	T30	T60	T90
Normal control	111±1	181±9[b]	175±4[b]	157±4[b]
Hyperglycemic control	173±3[c]	292±3[b, c]	291±13[b, c]	241±12 [b, c]
Hyperglycemic + acarbose	174±3	182±4[a]	173±4[a]	170±3[a]
***Smilax moranensis* 80 mg/kg**	172±1	202±4[a,b]	196±6[a,b]	176±4[a]
***Tournefortia hirsutissima* 80 mg/kg**	167±1	205±2[a,b]	187±4[a,b]	172±3[a]
***Rhizophora mangle* 56 mg/kg**	171±2	202±2[a,b]	193±4[a,b]	185±5[a,b]
***Cecropia obtusifolia* 280 mg/kg**	166±2	226±7[a,b]	195±9[a,b]	178±4[a]
***Bromelia plumieri* 300 mg/kg**	170±2	211±4[a,b]	195±5[a,b]	170±2[a]

Table 2. In vitro enzyme assay results.

Treatment	Concentration (µg/ml)	Inhibition (%)
Acarbose	11.1	50
Smilax moranensis	500	7
Tournefortia hirsutisima	1000	6
Rhizophora mangle	1000	17
Cecropia Obtusifolia	500	45
Bromelia plumieri	2000	30