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Alfa-glucosidase-inhibiting activity of some Mexican plants used in the treatment of type 2 diabetes

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Abstract

Type 2 diabetes is an endocrine disease, which accounts for 9% of deaths worldwide. The aim of oral therapy is to reach normoglycemia to prevent later complications. Among glucose-lowering medications, α-glucosidase inhibitors delay the absorption of ingested carbohydrates, reducing the postprandial glucose and insulin peaks. In the present study, we tested the butanolic extracts of four Mexican plants with respect to their α-glucosidase inhibition activity, without excluding other possible mechanisms of action. The plants *Cecropia obtusifolia* Bertol., *Equisetum myriochaetum* Schlecht & Cham, *Acosmium panamense* (Benth.) Yacolev and *Malmea depressa* (Baill) R.E. Fries are used in traditional medicine to treat type 2 diabetes. In previous studies, we have demonstrated these plants' hypoglycemic activity and determined the phytochemical composition of their extracts. Our results in n-STZ diabetic rats loaded with maltose showed that *Malmea* and *Acosmium* extracts decreased plasma glucose significantly from 30 min on resembling the effect of acarbose. *Cecropia* extract produced the highest reduction of plasma glucose, and at 90 min, the glucose level was lower than the fasting level, which suggests another mechanism of action. *Equisetum* did not exert any effect. *In vitro* assays of α-glucosidase activity showed an IC_{50} of 14 μg/ml for *Cecropia*, 21 μg/ml for *Malmea*, and 109 μg/ml for *Acosmium*, which were lower than that of acarbose (128 μg/ml). *Equisetum* did not show any significant effect on this assay, either. These results contribute to understand the mechanism of action of these plants on glucose metabolism.

Keywords: Medicinal plants; Type 2 diabetes; α-Glucosidase inhibitors; n-STZ diabetic rat

1. Introduction

It is well known that diabetes mellitus is the most common endocrine disorder. According to the World Health Organization (WHO, 2006), the prevalence of the disease will grow from 171 million in 2000 to 366 million people affected in 2030, which amount to an increase of 144% over the next 30 years.

Deaths related to diabetes are estimated at about 9% of global mortality. Overall direct health care cost of diabetes range from 2.5 to 15% of annual health care budgets, depending on local diabetes prevalence and treatments available (WHO, 2006). In México, type 2 diabetes is one of the two main causes of mortality among the population (SSA, 2006).

The term diabetes mellitus describes a metabolic disorder of multiple etiologies that is characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The causes of type 2 diabetes are either a predominant insulin resistance with a relative insulin deficiency or a predominant insulin secretory defect with or without insulin resistance (WHO, 1999).

The aim of oral therapy in type 2 diabetes is to reach normoglycemia to prevent later complications (retinopathy, nephropathy, neuropathy and microangiopathy). Near normal or improved glycemic control (ADA goals: preprandial plasma glucose of 90–130 mg/dl and peak postprandial plasma glucose <180 mg/dl; ADA, 2006) has been shown to significantly

Abbreviations: AGIs, α-glucosidase inhibitors; BW, body weight; 4-NPGP, 4-nitrophenol glucopyranoside.

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diminish the risk of long-term complications (Florence and Yeager, 1999).

α-Glucosidase inhibitors (AGIs) are among the available glucose-lowering medications. The α-glucosidase enzyme is located in the brush border of the small intestine and is required for the breakdown of carbohydrates to absorbable monosaccharides. The AGIs delay, but do not prevent, the absorption of ingested carbohydrates, reducing the postprandial glucose and insulin peaks (Stuart et al., 2004).

In recent years, several researchers have focused on the AGIs of medicinal plants (Abesundara et al., 2004; Önal et al., 2005; Yuhao et al., 2005, among others). A recent review of hypoglycemic compounds mentions the following plants with AGI activity: *Rhizoma polygonati* Odorati (Lilliaceae), *Syzygium malaccense* L. Merrill &, L.M. Perry (Myrtaceae), *Lobelia chinensis* LOUR (Campanulaceae), *Nicandra physalodes* L. (Solanaceae), *Origanum majorana* L. (Lamiaceae), *Lactuca indica* L. (Compositae), *Cuscuta reflexa* Roxb (Convovulaceae) and *Hyssopus officinalis* L. (Lamiaceae). As a conclusion, the authors mention that flavonoids and polyphenols, as well as their sugar derivates, are found to be effective inhibitors of α-glucosidase (Jung et al., 2006).

2. Objective

The aim of the present study was to test the α-glucosidase inhibitory effect of four Mexican plants, with previously reported hypoglycemic effects, to contribute to the understanding of their mechanisms of action.

3. Plant background

3.1. Ethnobotany

The plants studied are used by the Mexican population for the treatment of type 2 diabetes as oral infusions drink during the day. These plants have already been studied and have shown an acute hypoglycemic effect. A review can be found in Andrade-Cetto and Heinrich (2005). Herein, a short summary of the already published data for each plant is presented.

3.2. Cecropia obtusifolia Bertol. (Cecropiaceae)

People use the leaves of the plant. The acute hypoglycemic effect of the butanolic and aqueous extracts prepared from the leaves has been demonstrated in streptozotocin diabetic rats. From these extracts, chlorogenic acid and isoorientin were isolated as the main constituents. We have also demonstrated that both extracts have the same phytochemical composition, though in the butanolic extract we found higher concentrations of the compounds (Andrade-Cetto and Wiedenfeld, 2001). The chronic hypoglycemic effect of the aqueous extract has been demonstrated in type 2 diabetic patients (Revilla-Monsalve et al., 2007).

3.3. Equisetum myriochaetum Schlecht & Cham (Equisetaceae)

Traditionally, the aerial part of the plant is used. Its acute hypoglycemic activity was demonstrated in streptozotocin diabetic rats. The following constituents have been isolated from the aerial part: kaempferol-3-*O*-sophoroside, kaempferol-3,7-di-*O*-ß-glucoside, caffeoyl-methylate-4-ß-glucopuranoside and kaempferol-3-*O*-sophoroside-4′-*O*-ß-glucoside (Andrade-Cetto et al., 2000; Wiedenfeld et al., 2000). On this plant we also demonstrate that the aqueous extract has the same phytochemical composition as the butanolic extract. The acute hypoglycemic effect was also proven on type 2 diabetic patients (Revilla-Monsalve et al., 2002).

3.4. Acosmium panamense (Benth.) Yacolev (Fabaceae)

People use the bark of the plant. From the butanolic extract of the bark, the following compounds have been isolated as the main constituents: caffeic acid and three pyrones: desmethylyangonine and its $O^{4'}$- mono- as well as di-(1–6) glucosides (Wiedenfeld and Andrade-Cetto, 2003). The acute hypoglycemic effect of this extract was demonstrated in streptozotocin diabetic rats as was the phytochemical equivalence between the aqueous and the butanolic extracts (Andrade-Cetto and Wiedenfeld, 2004).

3.5. Malmea depressa (Baill) R.E. Fries. (Annonaceae)

Traditionally, the root of the tree is used. The butanolic extract of the roots yields two phenyl butane derivatives as the main compounds: 2-hydroxy-3,4,5-trimethoxy-1-(2′,4′-hydroxy-3′-dihydroxy)butyl-benzene and 2-hydroxy-3,4,5-trimethoxy-1-(2′,3′,4′-hydroxy)butyl-benzene. The acute hypoglycemic effect of this extract has also been demonstrated on streptozotocin diabetic rats (Andrade-Cetto et al., 2005).

The plants studied here were previously investigated by our group (see above). In these works, the ethnopharmacological aspects were clearly outlined for each plant, the hypoglycemic effect was demonstrated, and the phytochemical composition has been established. Because we proved for these plants that the aqueous extracts (equivalent to the medicinal tee) have the same phytochemical composition as the butanolic extracts (equivalent to the medicinal tee), but in a higher concentration, we only tested the butanolic extract at one concentration in this work. The dose-dependent hypoglycemic response of these extracts had already been established, so we only use the maximum dose previously published by us, to assure an ethnopharmacological mean.

As we noted above, the objective of this work was to test one of the possible mechanisms of action of the plants.

4. Materials and methods

4.1. Plant extracts

The butanolic extracts as already described (Andrade-Cetto and Wiedenfeld, 2001), were prepared from the same plant

Table 1
Effects of the oral administration of plant extracts and control drugs on the plasma glucose concentration of maltose-loaded n-STZ diabetic rats

Treatments	Plasma glucose levels (mg/dl)			
Time (minutes)	T0	T30	T60	T90
Non-diabetic control	71 ± 3	101 ± 5[a]	100 ± 4[a]	101 ± 5[a]
Diabetic control	154 ± 7[1]	241 ± 12[b,1]	212 ± 16[b,1]	193 ± 15[b,1]
Acarbose (3 mg/kg bw)	150 ± 6	179 ± 11[b,2]	169 ± 6[b,2]	158 ± 6[a,2]
Repaglinide (4 mg/kg bw)	146 ± 2	250 ± 12[b,1]	243 ± 9[b,1]	226 ± 9[b,1]
Glibenclamide (3 mg/kg bw)	158 ± 4	207 ± 11[b,1]	188 ± 11[b,1]	161 ± 9[a,1]
Equisetum myriochaetum extract (96 mg/kg bw)	142 ± 5	238 ± 19[b,1]	208 ± 20[b,1]	184 ± 18[b,1]
Cecropia obtusifolia extract (96 mg/kg bw)	144 ± 4	151 ± 4[a,2]	141 ± 6[a,2]	129 ± 6[b,2]
Malmea depressa extract (96 mg/kg bw)	153 ± 7	174 ± 10[a,2]	172 ± 6[b,2]	164 ± 5[a,1]
Acosmium panamense extract (100 mg/kg bw)	144 ± 4	177 ± 8[b,2]	175 ± 8[b,1]	169 ± 8[b,1]

Different superscript letters (a and b) in the same row indicate statistical differences ($p < 0.05$) as compared to time 0. Different superscript numbers (1 and 2) in the same column, indicate statistical differences compared to a diabetic control group (the diabetic control group is compared to a non-diabetic control). Values are expressed as mean ± S.E., $n = 11$.

material described in our previous works and compared to the previous extracts by DAD and HPLC to ensure that the reported compounds were present in the extracts used here.

4.2. α-Glucosidase inhibitory activity in vitro

The α-glucosidase inhibitory activity was determined according to Matsui et al. (1996) with slight modifications, by measuring the release of 4-nitrophenol from 4-nitrophenyl α-D-glucopyranoside (4-NPGP). The assay media contained 0.1 M sodium phosphate buffer, pH 6.8, 2 mM 4-NPGP, 0.1 U α-glucosidase (from yeast) and plant extract or control drug in the range of 0.2–2000 μg/ml assay media, in a total volume of 1 ml. The assay was started by addition of 4-NPGP and the change in absorbance at 405 nm was followed with a Beckman Coulter spectrophotometer model DU-640. IC_{50} values were calculated by the graphic method.

4.3. Animals and induction of experimental diabetes

Five-day-old Wistar rats (weighing 10–12 g) received 90 mg/kg i.p. of streptozotocin (STZ; Sigma, no. 242-646-8) in 0.1 M acetate buffer, pH 4.5. A non-diabetic control group received only the buffer i.p. At 4 weeks of age, the rats were separated from their mothers and kept with free access to food and water in an air-conditioned room (23 °C with 55% humidity) under a 12 h light:dark cycle, at the Bioterium of the Faculty of Sciences, UNAM. The animals used in this study were housed, cared for, and used in accordance with the Official Mexican Regulation on Technical Specifications in the Production, Maintenance and Use of Laboratory Animals (NOM-062-ZOO-1999), an approved by the Technical council of our Faculty.

After 12 weeks of streptozotocin injection, diabetes was identified by polydipsia, polyuria, and by fasting plasma glucose levels. Male and female rats with glucose levels >145 mg/dl were included in the study.

According to Portha et al. (2001) and Verspohl (2002), the n-STZ model is adequate for testing type 2 diabetes. A single dose of STZ given to neonatal rats induces beta-cell injury, which is followed by limited regeneration (short-term normalization of glycemia). At 6–15 weeks of age, an impaired glucose disposal rate and significant beta-cell secretory dysfunction (type 2 diabetes) is observed (Verspohl, 2002).

4.4. Plant extracts administration to maltose-loaded rats

The diabetic animals were divided into eight groups (numbered 2–9) of eleven rats each. Group 1 consisted of eleven non-diabetic control rats that received a physiological NaCl solution (vehicle); group 2 (diabetic control) also received a physiological NaCl solution (vehicle). Rats in groups 3–5 were diabetic animals treated with standard oral hypoglycemic agents: group 3 received acarbose (3 mg/kg bw), group 4 received Repaglinide (4 mg/kg bw) and group 5 received glibenclamide (3 mg/kg bw) in the same vehicle. Rats of groups 6–9 were diabetic animals treated with plant extracts: group 6 received *Equisetum myriochaetum* (96 mg/kg bw), group 7 received *Cecropia obtusifolia* (96 mg/kg bw), group 8 received *Malmea depressa* (96 mg/kg bw) and group 9 received *Acosmium panamense* (100 mg/kg bw).

Plant extracts and control drugs were suspended in 1.5 ml of physiological NaCl solution and administered orally by a cannula to 8 h-fasted rats 5 min before the oral administration of maltose (3 g/kg). Control groups (1 and 2) were given the same volume of vehicle before the maltose solution.

4.5. Collection of blood and blood glucose determination

Blood samples were obtained from the tail vein (according to the Institutional Animal Care and Use Committee Guideline 9, 3/10/99, IACUC, 1999) 5 min before the oral administration of the extracts or the vehicle (T0) and at times 30, 60 and 90 min thereafter.

Glucose concentration was measured in plasma with a Reflotron machine and confirmed by Accutrend GC and Accu-check compact machines (Roche). Thirty-two microliters of blood were used for each assay.

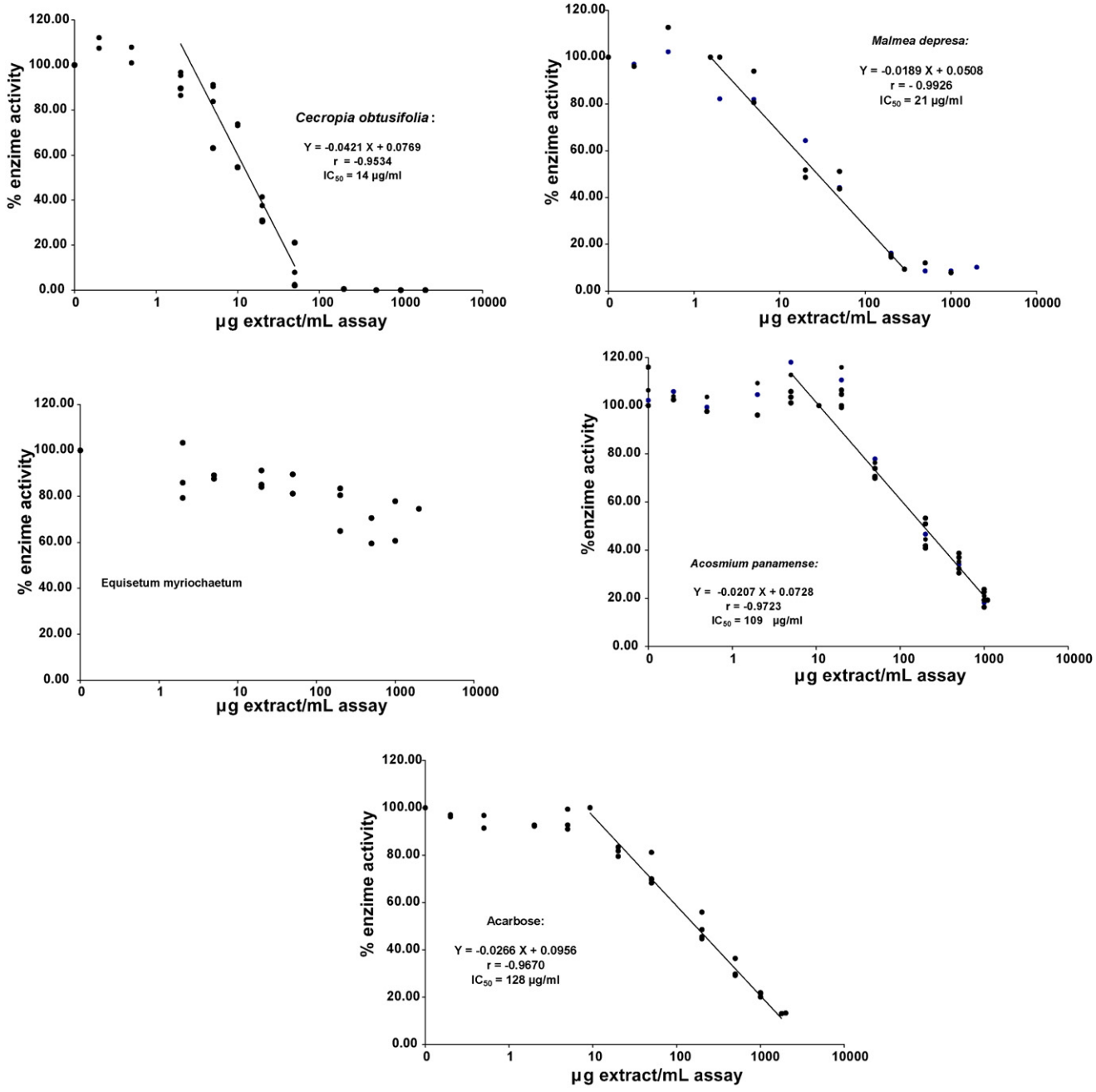

Fig. 1. Comparison of the inhibitory concentration IC_{50} of the plant extracts and acarbose over yeast alpha-glucosidase activity.

4.6. Statistical analysis

Plasma glucose levels of the different group and times were statistically analyzed by one-way ANOVA followed by Tukey's test. Plasma glucose levels are expressed as the mean ± standard error.

5. Results

5.1. Phytochemical composition of extracts

The DAD-HPLC spectra of each of the extracts tested here from *Equisetum*, *Cecropia*, *Malmea and Acosmium* (data not shown) demonstrated that the originally reported compounds were present.

5.2. Effects of the control drugs on maltose-loaded rats

The glucose levels of the diabetic control group (n-STZ) were statistically increased compared to the non-diabetic control group (Table 1). According to the data presented in Table 1, after 30 min of the maltose load, of the 3 control drugs assayed, only acarbose reduced plasma glucose concentration significantly when compared with the diabetic control group. This reduction was observed until 90 min while glibenclamide and repaglinide did not show a significant effect at 90 min compared with the

diabetic control. However, when the T90 of the glibenclamide group was compared to its own T0, a significant reduction was observed.

5.3. Effect of the extracts on maltose-loaded rats

The data presented in Table 1 shows that the group receiving the extract of *Equisetum* exhibited a glucose tolerance curve very similar to that of the diabetic control group, indicating that this extract does not affect maltose hydrolysis and/or glucose absorption. *Malmea* and *Acosmium* extracts, in contrast, decreased plasma glucose significantly from 30 min on resembling the acarbose effect. *Cecropia* extract exerted the highest reduction in plasma glucose, such that at 90 min, glucose level was lower than the fasting level.

5.4. Effects of the extracts on α-glucosidase activity in vitro

As can be observed in Fig. 1, the *in vitro* assay of α-glucosidase confirmed the results observed in the animal model. *Cecropia* (IC_{50} = 14 μg/ml) exerted the most powerful inhibitory activity, closely followed by *Malmea* (IC_{50} = 21 μg/ml), with *Acosmium* (IC_{50} = 109 μg/ml) being less effective. *Cecropia* and *Malmea* extracts exhibited a higher inhibitory activity than acarbose (IC_{50} = 128 μg/ml), which exerts an effect similar to *Acosmium* extract under our assay conditions. Equisetum did not show a significant effect at the assayed concentrations.

6. Discussion

Without excluding any other possible mechanism of action, the aim of the present study was to establish the potential α-glucosidase inhibitory activity of plant extracts previously reported as hypoglycemic. Initially, we tested three different commercial hypoglycemic drugs on the animal model. All three of these have well known and different mechanisms of action. According to our results, only acarbose showed a significant reduction in plasma glucose 30 min after an oral load of maltose, while neither glibenclamide nor repaglinide showed any effect. This result indicates that, in the n-STZ model loaded with maltose, a reduction in the plasma glucose curve is mainly due to the inhibition of intestinal glucosidase(s), and it can be assumed that glucose absorption at the gut level is inhibited.

The plant extracts tested in the present report were similar to the ones previously reported as hypoglycemic, as shown by their DAD-HPLC profile (data not shown). We can assume that one or more different compounds present in the extracts play an important role in the α-glucosidase inhibition. However, the plants could have other mechanism of action besides the α-glucosidase inhibitory activity. *Cecropia* has been shown to reduce fasting glucose levels in type 2 diabetic patients after chronic administration (Revilla-Monsalve et al., 2007). This could be the reason why in the present study, after 90 min of maltose loading, the extract reduced glucose levels below the initial fasting concentration.

Furthermore, the nature of some of the compounds found in these extracts (phenolics, flavonoids and their glycosides) are in accordance with those mentioned by Jung et al. (2006) as being effective inhibitors of α-glucosidases.

From the plants tested, *Equisetum* did not show any significant effect in the animal model or in the *in vitro* assay, while *Cecropia*, *Acosmium* and *Malmea* produced both a reduction in plasma glucose in the animal model and an inhibition of α-glucosidase *in vitro* in a degree similar to or greater than acarbose. There was a good correlation between the results with the animal model and the *in vitro* assay of α-glucosidase, with *Cecropia* exerting the greatest effect on both parameters.

Based on the results presented here, we can say that, besides any other possible mechanism of action, *Cecropia obtusifolia*, *Acosmium panamense* and *Malmea depressa* exert and inhibitory effect on α-glucosidases, with *Cecropia obtusifolia* being the most effective. With these results, we can further support the traditional use of the plants based on their inhibitory activity on glucose absorption at the level of the gut.

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