

Ethnopharmacological Studies of Two Mayan Medicinal Plants Used in the Treatment of Type 2 Diabetes

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Abstract

The Mayan diabetic population of Chikinzonot Yucatan, Mexico, uses diverse medicinal plants to treat their disease. With the help of a mathematical tool, the Disease-Consensus Index (DCI), we identified *Cecropia peltata* (Cp) and *Malmea depressa* (Md) as the most widely used plants. Both plants result in a hypoglycemic effect in animal models and we found that the administration of both plants can improve glycemic control by blocking the hepatic glucose output, especially in the fasting state. These data support the traditional use of the plants as “agua de uso”, an infusion of the plant consumed over the course of a day.

INTRODUCTION

Diabetes mellitus is defined as an elevated blood glucose level associated with absent or inadequate pancreatic insulin secretion, which may occur with or without impairment of insulin signaling. Type 2 diabetes is characterized by tissue resistance to the action of insulin combined with a relative deficiency in insulin secretion. A given individual may exhibit either increased insulin resistance or increased beta-cell deficiency, and these abnormalities may be mild or severe. Although insulin is produced by beta cells in these patients, production is inadequate to overcome insulin resistance; therefore, blood glucose rises. 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 (American Diabetes Association, 2011).

Ethnopharmacology can be defined as the study of traditionally used, biologically active natural products with the aim of understanding their therapeutic actions (Andrade-Cetto and Heinrich, 2011).

The traditional use of medicinal plants among the Mexican population is well-established, and the use of 306 species for the treatment of type 2 diabetes has been previously reported (Andrade-Cetto and Heinrich, 2005). In the Yucatec Maya community of Chikidnzonot, the plants *Cecropia peltata* L. (*Cecropiaceae*) and *Malmea depressa* (Baill) R.E. Fries (*Annonaceae*) are traditionally used in the control of type 2 diabetes.

MATERIALS AND METHODS

The present article summarizes the previous works by our group on the selection of medicinal plants in Chikindzonot, Yucatan, Mexico, used by diabetic people, the hypoglycemic effect of the selected plants, and their proposed mechanism of action.

Plant Selection

With the aim of selecting species to treat a single chronic disease, Andrade-Cetto et al. (2006) developed the Disease-Consensus Index (DCI). For the application of the index, a questionnaire was formulated with answers that can be evaluated in a binary manner: 0 for no and 1 for yes. The questions must include personal knowledge of a specific species to treat the disease (see below). The index is a comparison based on mathematical aspects (limit theory), the ideal answers of informant reports (Cc), and the

ideal answers for each species (Vx).

The DCI is calculated as follows: where

- (x) is any species.
- (mVxi) is the sum of the individual values obtained for one species within the community; it evaluates knowledge and mentions.
- (mVx) is the statistical mean of the individual values for one species; it evaluates knowledge.
- (Cc) is the correlation coefficient defined as the maximal number of informants who refer to a species; it evaluates mentions.
- (Pm^{0.1}) is the compensation factor and analyzes the dispersion for one species considering the mode of preparation and parts used.

The following formula is used to calculate the DCI:

$$DCI = \left(\sum_{i=1}^{\infty} V_{xi} \right) / Cc \cdot m Vx \cdot Pm^{-0.1} \quad (1)$$

Acute Hypoglycemic Effect

1. *Cecropia peltata*. The hypoglycemic effect of Cp was demonstrated on neonatal induced diabetic rats (n5-Stz). The diabetic animals were classified into 8 groups (1-8) with 11 rats each: Group 1, non-diabetic control; Group 2, diabetic control; Group 3, glibenclamide (3 mg/kg); Group 4, glibenclamide (5 mg/kg); Group 5, aqueous extract (20 mg/kg); Group 6, aqueous extract (200 mg/kg); Group 7, butanolic extract (27 mg/kg); and Group 8, butanolic extract (60 mg/kg). The extracts were dissolved in 1.5 ml of physiological NaCl-solution and administered orally by a cannula (Andrade-Cetto et al., 2007).

2. *Malmea depressa*. The acute effect of *Malmea depressa* was demonstrated on streptozotocin-induced diabetic rats. The diabetic animals were classified into 8 groups (1-8) with 11 rats each: Group 1, non-diabetic control, 1.5 ml of physiological NaCl-solution (vehicle); Group 2, diabetic control, 1.5 ml of physiological NaCl-solution (vehicle); Group 3, standard oral hypoglycemic agent glibenclamide (3 mg/kg) in the same vehicle; Group 4, the hypoglycemic agent metformin (14 mg/kg); Groups 5 and 6, water extract (40 mg/kg) and water extract (80 mg/kg), respectively; Group 7, ethanolic extract (112 mg/kg); and Group 8, butanolic extract (80 mg/kg). The extracts were dissolved in 1.5 ml of physiological NaCl-solution and administered orally by a cannula (Andrade-Cetto et al., 2005).

3. Collection of Blood and Determination of Blood Glucose. Blood samples were taken from the tail vein before oral administration of the extracts or vehicle and at 0, 60, 120, and 180 min following administration. Thirty-two microliters of blood were used for each assay. The glucose concentration was measured in plasma serum with Reflotron equipment and confirmed by Accutrend GC and Accu-check compact measurement systems (Roche). The data were statistically analyzed by the ANOVA Tukey test. The plasma glucose levels were expressed as the mean (S.E.M.).

4. In Vivo Pyruvate Load Test. N5-Stz rats, weighing approximately 250 g, were fasted for 18 h. Subsequently, the drug extracts were administered orally followed 15 min later by the IP administration of 2 g/kg of pyruvate (Sigma 2256). The rats were assigned to one of seven groups (n=11 per group): Group 1, non-diabetic control; Group 2, diabetic control; Group 3, chlorogenic acid (CA, Sigma C3878) (5 mg/kg); Group 4, metformin (Me, 0.012 mg/kg); Group 5, Md extract (80 mg/kg); and Group 6, Cp butanolic extract (150 mg/kg). In all the groups, glucose was measured at 0, 30, 60, 90, and 120 min after the IP injections.

5. Glucose-6-Phosphatase Activity. The in vivo results were confirmed in vitro using components of the rat hepatic glucose 6-phosphatase system. Intact rat liver microsomes were obtained according to Arion et al. (1997), and the enzymatic activity was calculated

by measuring inorganic phosphorus formation from glucose-6-phosphate. We tested Md ethanolic extracts and Cp butanolic extracts at concentrations of 2, 5, 20, 50, 200, 500, 1,000, and 2,000 µg/ml. Chlorogenic acid was used at 1 mM to assess enzyme inhibition.

RESULTS AND DISCUSSION

Plant Selection

As a result of the application of the DCI, we confirmed that type 2 diabetes is an important health problem among the Yucatec-Mayan community of Chikindzonot. Informants share similar concepts and perceptions of the disease. In terms of how people identify the illness, 91% label it as diabetes, and only 9% describe it as “Orina dulce” (sweet urine) or the Mayan equivalent “Chu-juk’uis”. Many of the informants associate the onset of their problem (diabetes) with different psychological problems they faced at a certain point in the past. Seventeen species of plants were identified as current treatments of the illness, according to the results obtained using the DCI. The most common species used were *Cecropia peltata* L. (score: 0.74) and *Malmea depressa* (Baill.) R.E. Fr. (0.59) (Andrade-Cetto et al., 2006).

Cecropia peltata

Cecropia peltata (traditional names: “Hormiguillo” and X’kooch”) is a monopodic tree that grows up to 20 m high in the form of secondary vegetation in the tropical forest. Traditionally, the dry leaves (mean 15 g) are boiled in 1 L of water, and the infusion is consumed throughout the day. Additionally, the plant is sold at the Merida Market in Yucatan Mexico for the treatment of diabetes. The active components of the plant are the phenolic compounds chlorogenic acid and isoorientin.

The drug glibenclamide produces a hypoglycemic effect in a dose-dependent manner, while the aqueous extract of *Cecropia peltata* has hypoglycemic activity only at high doses; the butanolic extract shows a hypoglycemic effect in a dose-dependent manner when compared to the diabetic group (Fig. 1).

Malmea depressa

Malmea depressa (traditional names: ‘Elemuy’, ‘Sufricaya’ and ‘Nazareno Prieto’) is a tree that grows up to 10 m high and is associated with disturbed areas in the tropical rainforest of southeast Mexico and Guatemala. The main physical features of the tree are a tall straight trunk, flowers with fleshy oval-shaped yellowish petals, and a plain, light gray external cortex. Traditionally, the dry root (mean 20 g) is boiled in 1 L of water, and the infusion is consumed throughout the day. We have isolated the following components from the root: A) 2-Hydroxy-3,4,5-trimethoxy-1-(2’,4’-hydroxy-3’-dihydroxy) butyl-benzene; B) 2-Hydroxy-3,4,5-trimethoxy-1-(2’,3’,4’-hydroxy) butyl-benzene and C) 3-(3-hydroxy-2,4,5-trimethoxyphenyl) propane-1,2 diol. The extracts showed significant hypoglycemic effects. The water extract, at doses of 40 and 80 mg/kg bw, showed a significant reduction ($p < 0.01$) of plasma glucose levels. The ethanolic (112 mg/kg) and the butanolic (80 mg/kg) extracts also led to a significant decrease in plasma glucose levels compared with the control. Glibenclamide (3 mg/kg) and metformin (14 mg/kg) produced a significant decrease in plasma glucose levels. These results indicate that there is no significant difference between the tested plant preparations in comparison to glibenclamide and metformin, which are standard hypoglycemic drugs (Table 1).

In Vivo Pyruvate Load Test

Between 30 and 90 min after pyruvate administration, glucose levels in the non-diabetic control group were significantly higher than at time 0 and returned to baseline by 120 min after pyruvate administration. In the diabetic control group, glucose levels increased beginning at 30 min and did not return to baseline. After 60 min, chlorogenic acid inhibited the glucose peak in the diabetic rats relative to the baseline. The metformin

control group also showed more pronounced inhibition of the peak at 90 min compared to the diabetic control. Both plant extracts and CA were able to block this increase in blood glucose levels starting at 60 min, while the effect of metformin was observed until 120 min (Table 2).

Glucose-6-Phosphatase Activity

To assess the degree of inhibition of glucose-6-phosphate hydrolysis, we plotted a dose-response curve and reported the results as the IC_{50} . The measured IC_{50} s are: Cp, butanolic extract: 152; Md, ethanolic extract: 267.62; and chlorogenic acid: 354. All of the extracts tested from both plants showed an IC_{50} within the same order of magnitude.

CONCLUSIONS

Type 2 diabetes is an important health problem among the Mayan population of Chikindzonot Yucatan. Among the plants used by the diabetic people to treat the disease, *Malmea depressa* and *Cecropia peltata* are the most widely used species. Each plant has an acute hypoglycemic effect on animal models.

Proposed Mechanism of Action

The liver plays a key role in maintaining blood glucose levels during fasting by converting stored glycogen to glucose (glycogenolysis) and by synthesizing glucose from lactate and amino acids (gluconeogenesis) (Guyton and Hall, 2006). In the postabsorptive state, approximately 85% of endogenous glucose production occurs in the liver, and the remaining amount occurs in the kidney (Ekberg et al., 1999).

In type 2 diabetic subjects with mild to moderate fasting hyperglycemia (140-200 mg/dl), basal hepatic glucose production is increased by approximately 0.5 mg/kg/min. Consequently, during the overnight sleeping hours (2200 to 0800 h), the liver of an 80-kg diabetic individual with modest fasting hyperglycemia adds an additional 35 g of glucose to the systemic circulation (DeFronzo and Mandarino, 2009).

Because pyruvate (together with citric acid) is the main source of hepatic glucose production after a long fasting period, we can conclude that CA, Cp, and Md are able to block this pathway. This fact supports the traditional use of both plants by the Mayans to treat type 2 diabetes.

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Tables

Table 1. Effect of the oral administration of extracts of *M. depressa* root on plasma glucose concentrations in diabetic rats. The values represent the mean \pm SEM compared with the control group at various time intervals.

Treatments	Blood glucose levels (mg/dl) \pm standard error			
	T0	T60	T120	T180
Control (+) no diabetic (vehicle)	131 \pm 6 _a	143 \pm 2 _a	137 \pm 3 _a	132 \pm 5 _a
Control (-) (vehicle)	410 \pm 10 _a ¹	408 \pm 25 _a ¹	413 \pm 19 _a ¹	442 \pm 12 _a ¹
Glibenclamide (3 mg/kg bw)	418 \pm 11 _a ¹	349 \pm 26 _a ¹	317 \pm 28 _b ²	318 \pm 19 _b ²
Metformin (14.16 mg/kg bw)	390 \pm 20 _a ¹	308 \pm 19 _a ¹	260 \pm 16 _b ²	239 \pm 23 _b ²
<i>M. depressa</i> aqueous extract (40 mg/kg bw)	411 \pm 11 _a ¹	344 \pm 16 _a ¹	268 \pm 21 _b ²	296 \pm 28 _b ²
<i>M. depressa</i> aqueous extract (80 mg/kg bw)	408 \pm 17 _a ¹	328 \pm 7 _a ¹	329 \pm 14 _a ¹	306 \pm 17 _b ²
<i>M. depressa</i> ethanolic extract (113 mg/kg bw)	407 \pm 12 _a ¹	324 \pm 14 _a ¹	277 \pm 14 _b ²	248 \pm 11 _b ²
<i>M. depressa</i> butanolic extract (80 mg/kg bw)	397 \pm 17 _a ¹	347 \pm 17 _a ¹	313 \pm 20 _b ²	316 \pm 12 _b ²

Table 2. The pyruvate tolerance test on n5-STZ diabetic rats.

Groups	Gl	Gl	Gl	Gl	Gl
	(mg/dl) T0	(mg/dl) T30	(mg/dl) T60	(mg/dl) T90	(mg/dl) T120
1: ND control	115 \pm 1	142 \pm 8 ^a	134 \pm 6 ^a	125 \pm 3 ^a	114 \pm 3
2: D Control	144 \pm 7 ³	186 \pm 5 ^{c,3}	221 \pm 8 ^{c,3}	236 \pm 13 ^{c,3}	278 \pm 22 ^{c,3}
3: D + CA	142 \pm 6	168 \pm 4 ^{c,1}	154 \pm 7 ¹	155 \pm 6 ¹	151 \pm 7 ¹
4: D + Me	148 \pm 3	169 \pm 8 ^a	187 \pm 14 ^c	172 \pm 13 ^{b,1}	155 \pm 8 ³
5: D + Md 80 mg/kg	141 \pm 3	149 \pm 4 ³	150 \pm 3 ^{b,3}	146 \pm 4 ³	146 \pm 4 ³
6: D + Cp 150 mg/kg	145 \pm 4	157 \pm 12	148 \pm 14 ^{b,3}	129 \pm 6 ^{c,3}	137 \pm 7 ^{c,3}

The values represent the mean \pm SEM. Superscript letters in the same row indicate significant differences compared with time 0. Superscript numbers in the same column indicate significant differences from the control group (diabetic control group compared with non-diabetic control). "A,1" shows significance at $p < 0.05$, "b,2" shows significance at $p < 0.01$ and "c,3" shows significance at least at $p < 0.001$. Gl, glucose; ND, non-diabetic; D, diabetic; CA, chlorogenic acid; Me, metformin; Md, *Malmea depressa* ethanolic extract; Cp, *Cecropia peltata* butanolic extract.

Figures

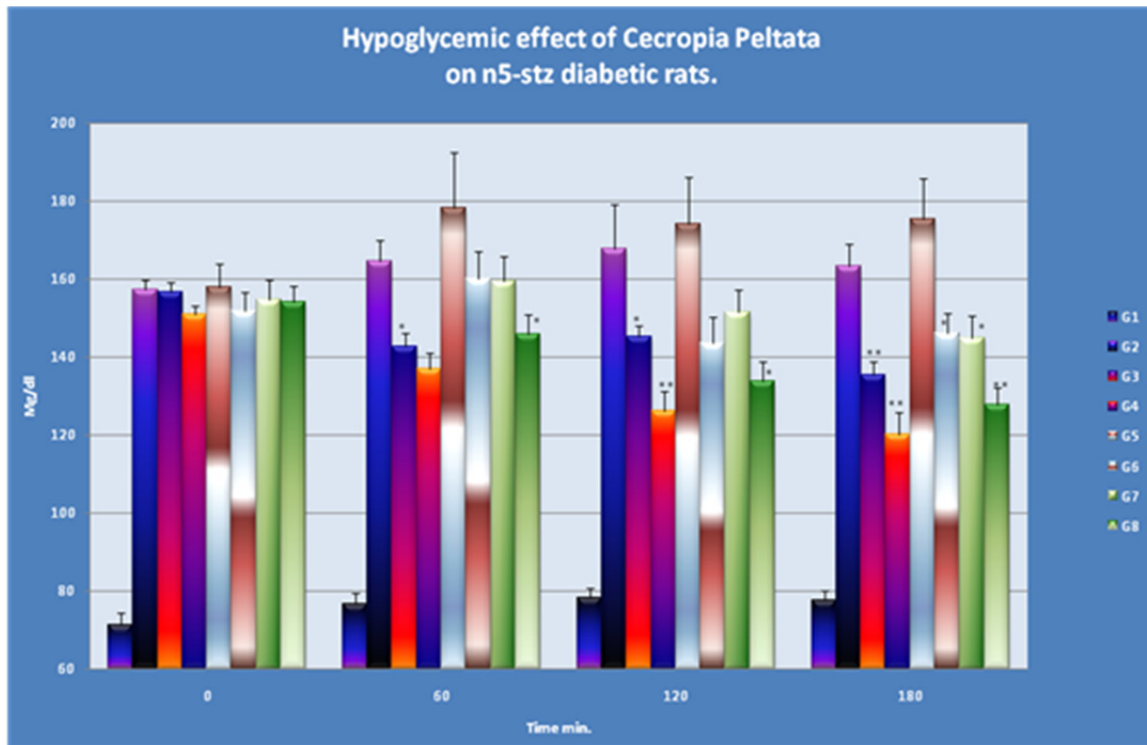


Fig. 1. Hypoglycemic effect of *C. peltata* on n5-stz diabetic rats. G1, non-diabetic control; G2, diabetic control; G3, glibenclamide 3 mg/kg; G4, glibenclamide 5 mg/kg; G5, Ae-P 20 mg/kg; G6, Ae-P 200 mg/kg; G7, Be-P 27 mg/kg; and G8, Be-P 60 mg/kg. * $p < 0.05$, ** $p < 0.005$.