

HYPOGLYCEMIC EFFECT OF *Smilax moranensis* ROOT ON N5-STZ DIABETIC RATS.

Adolfo Andrade-Cetto^{1*}

¹ *Laboratorio de Etnofarmacología, Departamento de Biología Celular, Facultad de Ciencias, Universidad Nacional Autónoma de México, Coyoacán 04510, D.F. México.*

*Corresponding author: Dr. Adolfo Andrade-Cetto Address: Apartado Postal 70-359, Coyoacan México DF, aac@ciencias.unam.mx.

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Abbreviations: WE, Water Extract; EE, Ethanolic Extract; STZ, Streptozotocin, DER, Drug Extraction Ratio.

Summary

Diabetes is a fast-growing epidemic among the native Mexican population; among them are the Chatinos who live in the southwestern Mexican state of Oaxaca. The Chatinos use a decoction of the *Smilax moranensis* root to control the disease. In the present study, we confirm that a water and an ethanol/water extract of the *Smilax* root produces a dose-dependent hypoglycemic effect in n5 streptozotocin diabetic rats. We also show that the main constituents of these extracts are flavonoids.

Introduction

According to the World Health Organization [1], more than 220 million people worldwide have diabetes type 2. In Mexico, it is the primary cause of general mortality in the adult population [2].

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 and therefore blood glucose rises. Impaired insulin signaling also affects fat metabolism, resulting in increased free fatty acid flux and elevated triglyceride levels as well as reciprocally low levels of high-density lipoprotein (HDL) [3].

The traditional use of medicinal plants among the Mexican population is well-established, and the use of 306 species for the treatment of diabetes type 2 has been previously reported [4].

Smilax moranensis M. Martens & Galeotti, Family Smilacaceae is a woody vine with stems that are covered with spikes. The leathery upper leaves have un-toothed blades with three to nine large veins, and the plant has yellow-green flowers that are followed by clusters of black berries. The *Smilax moranensis* plant root has been used in Mexico since the sixteenth century as a diuretic [5]. Our own studies report that the Chatino ethnic group in the southwestern Mexican state of Oaxaca uses a decoction of this root to treat diabetes.

The aim of the current study was to examine the acute hypoglycemic effects of water and ethanol:water extracts of the Smilax plant root in streptozotocin (N5-Stz) induced diabetic rats. We also sought to analyze the main constituents of those extracts.

Methods

According to previous studies in our lab [6, 7, 8] and work done by others [9, 10], we performed an ethnopharmacological study in accordance with ethnobotanical, phytochemical and pharmacological methodologies.

Ethnobotany

The traditional use of the Smilax moranensis plant has been documented in the community of Santos Reyes Nopala in Oaxaca, Mexico. These studies were based on [11] direct interviews conducted with diabetic people regarding their personal use of medicinal plants.

Plant Materials

The plant root was collected in its natural habitats in 2007 and 2009, through the help of local diabetics and community healers. The root was botanically determined, and voucher specimens were deposited at our Herbarium in Mexico City (ETNOF 183, and ETNOF 1193).

Preparation of the extracts and detection of compounds

Two different types of plant extracts were prepared from stem samples:

1) To obtain an aqueous extract, similar to traditional methods, 15 g of coarsely fragmented root were placed into a 1 L flask with 500 ml of distilled boiling water for 10 minutes. The extract was filtered through a sieve and a fine nylon mesh and then lyophilized, resulting in 1.51 g of dry extract (WE), (Drug Extraction Ratio, DER native 10:1. [12].

2) The ethanolic extract (EE), was prepared from the root material. First, 100 g of material was macerated at 30° C for 2 hours. Then, the extract was dried with a rotary evaporator, resulting in 2.84 g of extract, DER native 35:1. The EE was used for phytochemical detection of the main components by standard TLC methods. The EE was also analyzed by application to a 100 x 2 cm Polygoprep 60-30 C18 flash-column (Macherey & Nagel, Düren, Germany) and elution with H₂O/MeOH/ACN 70:15:15 at a flow rate of 4 ml/min monitored by DAD-HPLC (Beckman System Gold with 32 Karat software).

Animals and Induction of experimental diabetes

Two-month-old Wistar rats (weighing 150–180 g) received an i.p. injection of 90 mg/kg. of STZ (Sigma, No. 242-646-8) in acetate buffer (0.1 M, pH 4.5). Non-diabetic control rats received only buffer via ip injection.

At 4 weeks of age, rats were separated from their mothers and acclimatized with free access to food and water in an air-conditioned room (23° C with 55% humidity), under a 12 h light: dark cycle (Bioterium of the Science School, UNAM). The animal handling was in accordance with the Federal Government legislation on animal care.

After 12 weeks, diabetes was identified by polydipsia, polyuria and by measuring fasting plasma glucose levels. Male and female rats with glucose levels >150 mg/dl were included in the study.

Experimental Groups

The diabetic animals were classified into 8 groups (1-8), with eleven rats in each group. Group 1 non-diabetic control rats received 1.5 ml of a physiologically NaCl solution (Vehicle). Group 2 diabetic controls also received 1.5 ml of a physiological NaCl-solution (vehicle). Group 3 rats were treated with the standard oral hypoglycemic agent Glibenclamide (5 mg/kg) in the same vehicle. Group 4 rats were treated with the standard oral hypoglycemic agent Metformin (12 mg/kg bodyweight) in the same vehicle. Groups 5 and 6 received WE (2 mg/kg) and WE (200 mg/kg) respectively, whereas Groups 7 and 8 received EE (8 mg/kg) and EE (80 mg/kg), respectively. The extracts were re-dissolved in 1.5 ml of a physiologically relevant NaCl solution and administered orally by a cannula.

Collection of blood and determination of blood glucose

Blood samples were obtained from the tail vein according to previously described methods [13]. Samples were collected before the oral administration of the extracts or the vehicle (T0) and at times T60, T120 and T180 (minutes) thereafter. Thirty-two microliters of blood were used for each assay, and the serum glucose concentration was measured with Reflotron equipment and confirmed by Accutrend GC and Accu-chek compact equipment (Roche).

Statistical analysis

The data were statistically analyzed by one-way ANOVA followed by Tukey's test. Plasma glucose levels are expressed as the mean \pm SEM.

Results

As a result of direct interviews with diabetic residents and community healers, we confirmed the use of the *Smilax moranensis* plant to treat type 2 diabetes in the Chatino population of southwestern Mexico. The plant is locally known by its traditional name, Cocolmecatl. In traditional preparation methods, local people unearth the root of the plant, cut it and leave it to dry in the shade for one or two days. They then prepare an infusion with approximately 15 g to 20 g of dry material and drink the infusion throughout the day.

Through the use of TLC, we observed that the main constituents of both extracts (WE and EE) are flavonoids (data not shown). HPLC-DAD analysis indicated the presence of a principal component, with three additional components present in smaller amounts (Fig. 1).

Our results show that the control group of the rat model used in this study (n5-stz) exhibits higher glucose levels compared to the non-diabetic control group. The administration of two different standard hypoglycemic agents to the diabetic rats produced a hypoglycemic effect after 60 minutes. This effect is statistically significant when comparing the diabetic control group and T0 for each group and the observed effect is maintained until 180 min. The administration of *Smilax* root water extracts at a dose of 20 mg/kg produces an effect after 120 min, while a higher dose of 200 mg/kg produces an effect after 60 min. These results are statistically significant when compared to the control group and T0 for each group. The administration of *Smilax* root ethanolic extracts produced a hypoglycemic effect after 60 min for both doses. However, the higher doses show an effect at 60, 120 and 180 min when compared to T0 of the same group, and the effect is sustained and statistically significant.

Discussion

The Results presented herein confirm the traditional use of *Smilax moranensis* root to treat type 2 diabetes. The plant produces a dose-dependent hypoglycemic effect, which may be partially explained by the compounds detected in this study. However, further studies are needed to clarify the mechanism of action of these compounds.

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Table 1 Effect of oral administration of extracts of *S. moranensis* root on plasma glucose concentration in n5-stz diabetic rats.

Treatments	Plasma glucose levels (mg/dl) \pm standard error			
	To	T60	T120	T180
Control (+) non-diabetic (vehicle)	112 \pm 2	114 \pm 3	119 \pm 2	119 \pm 3
Control (-) (vehicle)	171 \pm 2 ²	172 \pm 3 ²	168 \pm 2 ²	167 \pm 2 ²
Glibenclamide (5 mg/kg)	172 \pm 5	157 \pm 6 _a ¹	149 \pm 5 _b ²	141 \pm 5 _b ²
Metformin (12 mg/kg)	170 \pm 3	153 \pm 3 _b ¹	156 \pm 4 _a ²	155 \pm 3 _b ²
<i>S. moranensis</i> WE extract (20 mg/kg)	167 \pm 4	161 \pm 8	150 \pm 5 _b ¹	148 \pm 6 _b ¹
<i>S. moranensis</i> WE extract (200 mg/kg)	168 \pm 5	147 \pm 5 _b ²	143 \pm 6 _b ²	148 \pm 9 _b ²
<i>S. moranensis</i> EE extract (8 mg/kg)	169 \pm 5	159 \pm 5 ¹	151 \pm 5 _b ²	153 \pm 6 _a ¹
<i>S. moranensis</i> EE extract (80 mg/kg)	171 \pm 4	163 \pm 4 _a ¹	158 \pm 5 _a ²	155 \pm 5 _b ²

The values represent the mean \pm SEM as compared with control time intervals. ^{1, 2} indicate statistical significance as compared to the diabetic control group (or non-diabetic group in the case of the diabetic control) with $p < 0.05$ and $p < 0.01$ respectively. _{a, b} indicate statistical significance as compared to T0 of the same group with $p < 0.05$ and $p < 0.01$, respectively.

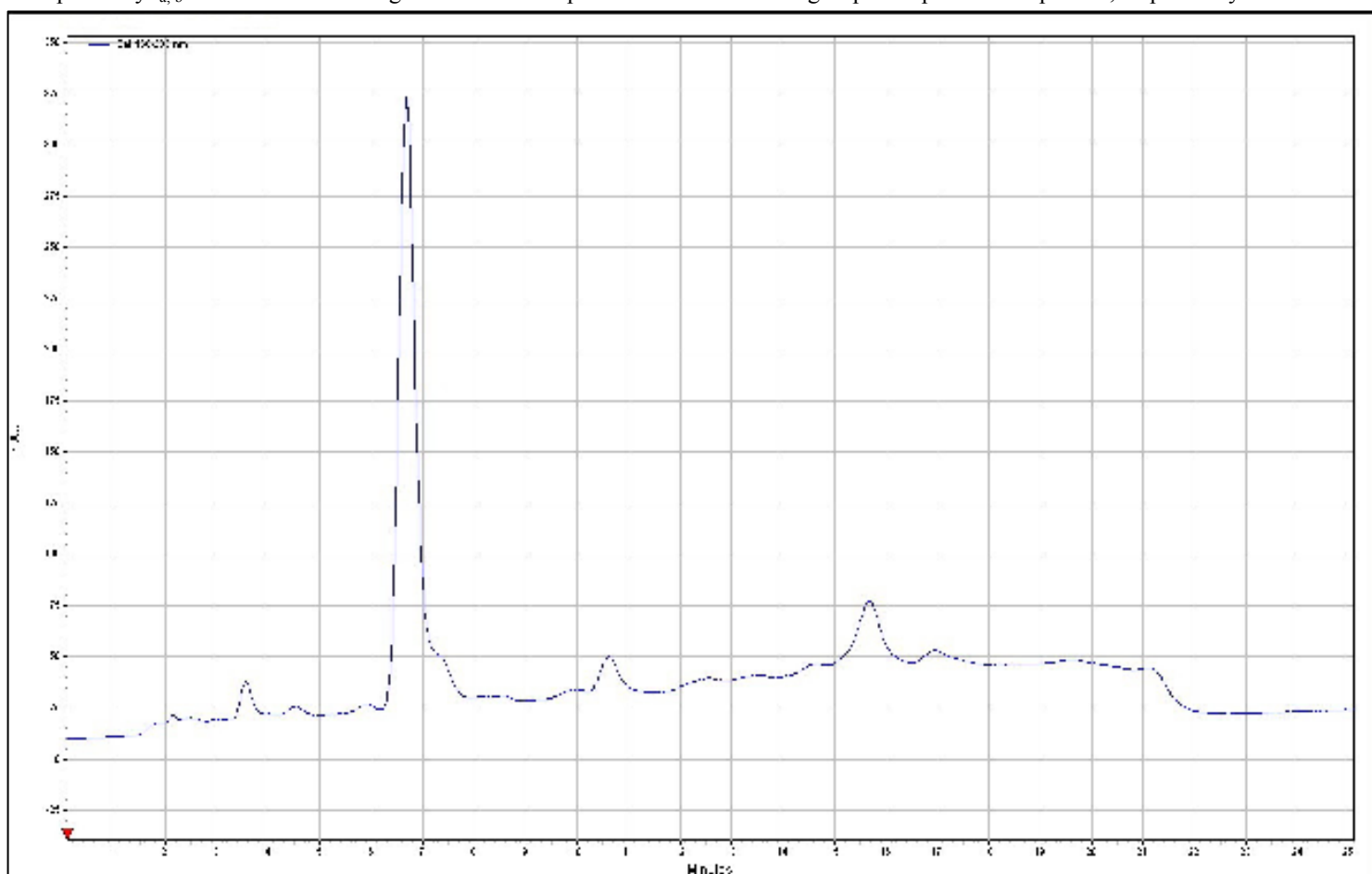


Figure 1. HPLC-UV at 254 nm, of the Ethanolic extract of *Smilax moranensis*. Y axis absorbance, x axis time in minutes.

References

- 1) World Health Organization, Web page; <http://www.who.org>. (Accessed: Jan, 2011).
- 2) SSA, Ministry of Health Mexican Government. México, Web page; www.ssa.gob.mx. . (In Spanish). (Accessed: Jan, 2011).
- 3) American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care*; 2011 61(1,suppl): 62-69.
- 4) Andrade-Cetto A. Heinrich M. Mexican plants with hypoglycaemic effect used in the treatment of diabetes. *J Ethnopharmacol* 2005; 99: 325–248.
- 5) Argueta A. ed. Atlas of the traditional Mexican medicinal plants, National Indigenous Institute, Mexico, 1994: 81.
- 6) Andrade-Cetto A. H. Wiedenfeld. Hypoglycemic effect of *Cecropia obtusifolia* on streptozotocin diabetic rats. *J Ethnopharmacol* 2001; 78: 145-149.
- 7) Andrade-Cetto A. Revilla-Monsalve MC. Wiedenfeld H. Hypoglycemic effect of *Tournefortia hirsutissima* on n-Streptozotocin Diabetic Rats. . *J Ethnopharmacol* 2007; 112: 96-100.
- 8) Andrade-Cetto A. Martinez-Zurita E. Soto-Constantino A. *et al.* Chronic Hypoglycemic effect of *Malmea depressa* root on n-Streptozotocin Diabetic Rats. *J Ethnopharmacol* 2008; 116: 358–362.
- 9) Holmstedt B., Historical perspective and future of Ethnopharmacology. *J Ethnopharmacol* 1991; 32: 7-24.
- 10) Etkin N. Elisabetsky E. Seeking a transdisciplinary and culturally germane science: The future of Ethnopharmacology. *J Ethnopharmacol* 2005; 100: 23-26.
- 11) Andrade-Cetto A., Becerra-Jiménez J. Martinez-Zurita E. *et al.* Disease-Consensus Index as a tool of selecting potential hypoglycemic plants in Chikindzonot, Yucatan, México. *J Ethnopharmacol* 2006; 107: 199–204.
- 12) Gaedcke F. Steinhoff B. Herbal Medicinal Products. Medpharm CRC, Germany, 2003: 177.
- 13) Institutional Animal Care and Use Committee, Guideline of Selected Techniques for Rat and Mouse Blood Collection, (1999) Guideline 9, USA, 6.