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Mexican plants traditionally used for the treatment of type 2 diabetes

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SUMMARY

Although the use of medicinal plants to improve health is a Mexican tradition that dates back to pre-Hispanic times, the use of plants for the treatment of type 2 diabetes is a recent occurrence. Despite receiving their diagnosis from a physician (which may orient them towards a medical point of view), the occurrence of this practice in patients with type 2 diabetes may be explained by the existence of cultural traditions that cause them to seek alternative means of balancing their health that includes plants. However, to avoid potential secondary complications to diabetic patients, it is important that plants used in this context be more closely examined.

Here, we present an example of an ethnopharamacological oriented study, the hypoglicemic effect of the aqueous extract of *Cecropia obtusifolia* was tested in animal models and in type 2 diabetic patients. We discovered that this extract had positive effects on the concentrations of plasma glucose and Hb_{1Ac} levels in patients. Using both animal models and in vitro studies, we discovered that one mechanism that contributed to this effect was the blockade of hepatic glucose output (gluconeogenesis).

RESUMEN

El uso de plantas medicinales para mejorar la salud en México es una tradición desde tiempos prehispánicos, sin embargo el uso de plantas para el tratamiento de la diabetes tipo 2 parece ser de reciente aparición, este fenómeno puede explicarse, en parte, porque los pacientes con diabetes tipo 2 obtienen el diagnostico por medio de médicos y no por curanderos tradicionales, esto hace que una vez diagnosticado, el paciente sea orientado desde una perspectiva medica y debido a la tradición cultural el busque otras formas para equilibrar la salud, en este caso plantas. El estudio de las plantas utilizadas en este contexto es de suma importancia para evitar posibles complicaciones posteriores a los pacientes diabéticos,

Ejemplificamos este tipo de estudios con *Cecropia obtusifolia*, el extracto acuoso de esta especie fue probado con éxito en modelos animales y en pacientes con diabetes tipo 2, mostrando un efecto positivo sobre las concentraciones de glucosa plasmática y sobre los niveles de hemoglobina glucosilada, posteriormente en modelos animales y estudios *in vitro* se pudo confirmar que un mecanismo de acción de esta planta es bloqueando la gluconeogénesis hepática, este tipo de mecanismo de acción coincide con el uso tradicional de la planta que es consumida como agua de uso.

ABREVIATIONS

C.a.	Chlorogenic Acid
Hb_{1Ac}	Glycated Hemoglobin
C. ob.	*Cecropia obtusifolia*.

INTRODUCTION

This chapter focuses on medicinal plants used to treat type 2 diabetes in Mexico. In the first section, we explain the current situation regarding the traditional use of medicinal plants to treat diabetes. Thereafter we describe an example of this kind of research with the plant *Cecropia obtusifolia* Bertol (C. ob.).

Type 2 diabetes is one of the most prevalent health problems in Mexico[1] and common treatment options include a wide variety of both medicinal products and health food plants. [2, 3] In Mexico, at least 306 species from 235 genera and 93 families have been reported for the treatment of diabetes. On a global level, type 2 diabetes is the most common endocrine disorder, and the World Health Organization [4] estimates that by 2030 the number of diabetic people across the world will have increased to 214% of the number of cases in 2000. However, the increase in the prevalence of this disease is predicted to be even greater in Mexico; the number of diabetic patients is set to increase from 2.2 million in 2000 to more than 6 million in 2030 (an increase to 281%).

The term diabetes mellitus is used to refer to a metabolic disorder of multiple etiologies, in which chronic hyperglycemia is caused by defects in either the secretion or action of insulin or alterations to both of these processes. This results in disturbances in carbohydrate, fat and protein metabolism. Type 2 diabetes is caused either predominantly by insulin resistance with a relative deficiency of insulin or predominantly by impaired insulin secretion that may or may not be accompanied by insulin resistance.[5]

The use of medicinal plants

Currently, there are disparities concerning the use of conventional drugs and traditional medicine by the population of Mexican diabetics; as a result of our field work, we have established that in addition to the use of conventional drugs, prescribed by a physician, almost all diabetics that live in rural areas also use medicinal plants.

The use of conventional medicine can be explained by two factors. First, the Mexican health care system covers nearly all of the diabetic population; this means that these patients are enrolled in special groups in which the use of conventional medicine for the treatment of their disease is encouraged. A second reason (as suggested by our field work) is the fact that *diabetes is invisible for traditional medicine*; in other words, the diagnosis of this disease is typically made by a physician (rather than a traditional healer) following laboratory tests that confirm high blood sugar levels.

After the initial diagnosis, because the use of plants is a Mexican tradition, diabetic patients typically start to consume plants in one of three ways: 1) instead of normal medicine, boiled in water and consumed daily, 2) taken in addition to the prescribed medication, or 3) taken in combination with the prescribed medicine on certain days, and this pattern is alternated with sole consumption of either the medicine or the plant (figure 12-1).

Before commencing treatment, typical symptoms of type 2 diabetes include feelings of malaise, frequent urination, excessive thirst, extreme hunger and fatigue. After a visit to the physician, a diagnosis of type 2 diabetes is made and a hypoglycemic agent is generally prescribed. After some time has passed, the patients typically begin to feel better as a result of the improved ability of the body to handle high blood glucose levels. It is often at this time that patients look to supplement their medication with plants. Regardless of whether they consume a hypoglycemic agent or a medicinal plant or nothing at all, patients often tend to feel somewhat better after the initial stage of the acute disease; therefore, to clarify whether the use of medicinal plants has any effect on the disease or whether the patient only feels better as a result of the body compensation to the high plasma glucose levels, studies of the present kind are of considerable importance.

To appreciate the history of medicinal plant use in our country, it should be mentioned that the use of plants to treat diabetes in Mexico can only be traced to the late Ninetieth century.[2] This can be compared to other societies in which traditional medicine is common, such as Indian or Arabian cultures, in which the first mention of the disease can be traced back as far as 2500 years.

The "recent" increase in the use of plants to treat type 2 diabetes is a reflection of the rising number of people that have been diagnosed with the disease in the second half of the Twentieth century; based on our field work we can say that there are several lines of evidence to support the hypothesis that there is a correlation between the number of diagnosed diabetics and the use of medicinal plants to treat the disease, that begin around the middle of the last century: a) diabetic people often try new plant species; sometimes these species have been recommended by other diabetics but oftentimes they sample new plants by themselves; an example of this is that patients may sample plants that have a bitter flavor, as a result of a belief that bitter-tasting plants are more effective against sweet (sugar blood levels) ; b) if one visit a diabetic person for a while, more than a year, you can see that the used plants will change over this time some are discontinued, some not, and new plants will appear in the kitchen.

The increase in the numbers of diabetic patients in Mexico, together with the tradition in the use of medicinal plants to treat any disease in general, results in people using and testing new medicinal plants for this purpose. The goal of any ethnopharmacological study in this field is to provide positive evidence for an effect of the plants on the progress of the disease.

However, there are certain intersections between western medicine and the use of traditional plants; for example, the anti-diabetic drug biguanide metformin was modeled on the natural product guanidine that is derived from the French lilac, *Galega officinalis* L. (a plant that has been used as an anti-diabetic for centuries).[6]

The aforementioned factors give us a perfect framework within which to study the use of plants to treat type 2 diabetes in Mexico. In particular they highlight the importance of finding suitable hypoglycemic agents, regardless of the pharmaceutical preparation; for example, in tea, with quality control, as a phytomedicine or as a novel hypo-

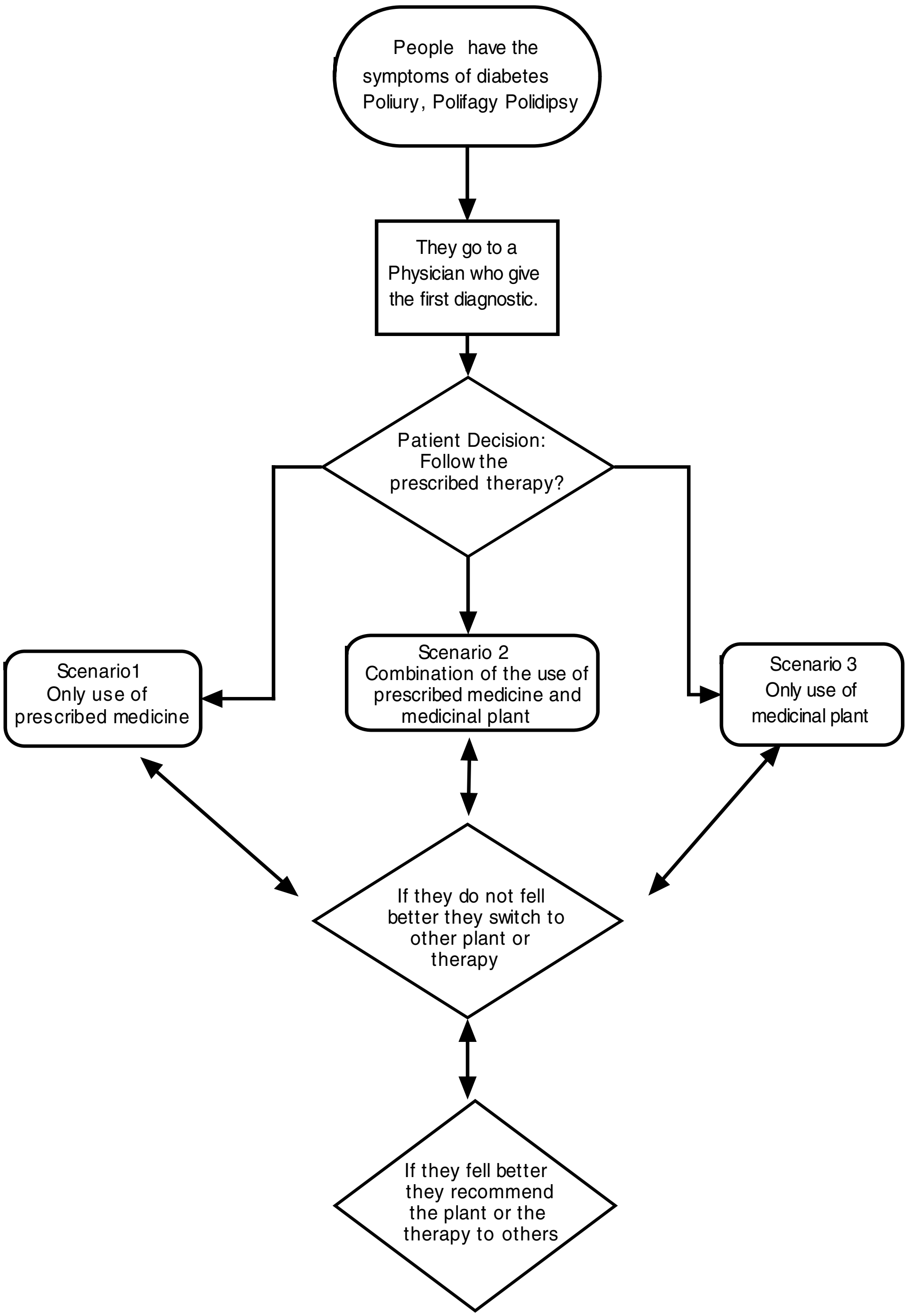

Figure 12-1. Initial therapy for type 2 diabetic patients: three different scenarios.

glycemic agent. Each of these preparations could be used at different scales; however, it must also be borne in mind that many patients will use plants either with or without our intervention.

After the explanation on how we are using medicinal plants, an example of an ethnopharamacological study will be given, from the field work to the possible mechanism of action.

Cecropia obtusifolia bertol (Cecropiaceae)

The use of this plant for the treatment of type 2 diabetes is common in central México, and it is sold in several markets.[3, 7]

Botanical Description

This tree is monopodic, 20 m tall, and grows in secondary vegetation in the tropical rain forest. It has a tall, straight, hollow trunk and a stratified treetop, with few large branches growing horizontally from the trunk. The leaves are attached in a spiral arrangement at the top of the branches; they are simple, peltate or deeply palmate, with a deep green color on the upper face and a grey color on the lower surface. This is a fast-growing pioneer tree that originates from tropical America, and its hollow septate twigs are inhabited by ants. [8]

Distribution

This tree is widespread in Mexico, and it grows along both coasts from Tamaulipas and San Luis Potosi to Tabasco on the Gulf of Mexico and from Sinaloa to Chiapas on the Pacific coast. As it is a weedy species, it would presumably be relatively easy to grow it on a larger scale or to harvest it sustainably by collecting material in the first few years after a *milpa* (corn field) has been given up.

Ethnobotany

Traditionally the dry leaves (15 g) are boiled in water (500 mL), and the resulting infusion is cooled in the pot before being then filtered and drunk as “agua de uso.” The cold infusion is consumed over the course of a day. This use of the plant has been reported by the following Mexican states: Hidalgo, Guerrero, Veracruz, Yucatan, Campeche, Tabasco, Edo. de México, Oaxaca and Chiapas. The traditional names for this preparation include “Guarumbo”, “Chancarro”, “Hormiguillo”, “Chiflon” and “Koochlé”.[3]

Phytochemical Constituents

From the butanolic extract, Andrade-Cetto and Wiedenfeld [9] isolated chlorogenic acid (C.a.) and isoorientin (figure 12- 2: Compounds 1 and 2). These isolated compounds, which are also found in the medicinal tea, were identified as the main constituents of the plant.

Pharmacology

In an acute study that was performed in Streptozotocin diabetic rats and in normal rats, the effects of the water, the butanolic extract, and the isolated compounds were tested. At 60-180 minutes post-administration, statistically significant hypoglycemic effects were observed for each of these samples.[9]

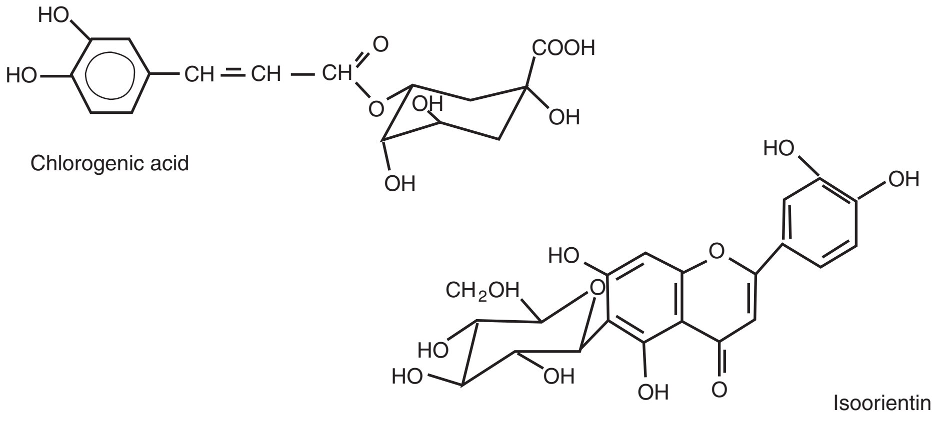

Figure 12-2. Natural products isolated from *Cecropia obtusifolia*.

In a clinical trial that involved twelve type 2 diabetic patients, the aqueous extract of C. ob., was administered (at doses of 13.5 g plant/day/patient in one liter of water) for a period of 32 weeks, after which time the treatment was suspended and the patients were monitored under clinical supervision for a further 34 weeks. Serum glucose, cholesterol, triglyceride and insulin levels were measured every 15 days. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALKP) were measured every month.[10]

Four weeks after commencing administration of the aqueous extract of C. ob., there was a significant hypoglycemic effect, although this was not sustained. The hypoglycemic effect became both significant and sustained ($p = 0.03$) after a period of 18 weeks, and this effect persisted until the end of the first part of the study (32 weeks; figure 12-3). Six weeks after beginning the treatment, there was a significant reduction ($p = 0.05$) in the values of Hb_{A1c} that was sustained until the end of the first part of the study (figure 12-4).

Following suspension of the C. ob. treatment after 32 weeks, the reduced levels of blood glucose persisted for a period of 4 weeks but began to increase thereafter. At the end of the 34 weeks of follow-up study, the patients´ glucose levels were significantly higher than both those that were detected at the end of the treatment period and those that were detected at the beginning of the treatment (basal). After the suspension of the C. ob. treatment, the HbA1c values fluctuate in a similar manner that the glucose levels, first a reduction that persisted for 2 weeks, before it increased again during the final 34 weeks of the study. The levels of Hb_{A1c} at the end of the study were also significantly higher than those that were detected both at the end of the treatment and at the beginning of the study. No changes were observed in any other parameters.

Inhibition of the hepatic glucose output

Previously, chlorogenic acid had been identified as a specific inhibitor of the glucose-6-phosphate translocase component (Gl-6-P translocase) in microsomes of rat liver.[11] To test the hypothesis that the simultaneous targeting of gluconeogenesis and glycogenolysis with an inhibitor of Gl-6-P translocase would result in a reduction of hepatic glucose production, we examined the effects of *Cecropia obtusifolia in vivo* and *in vitro*.

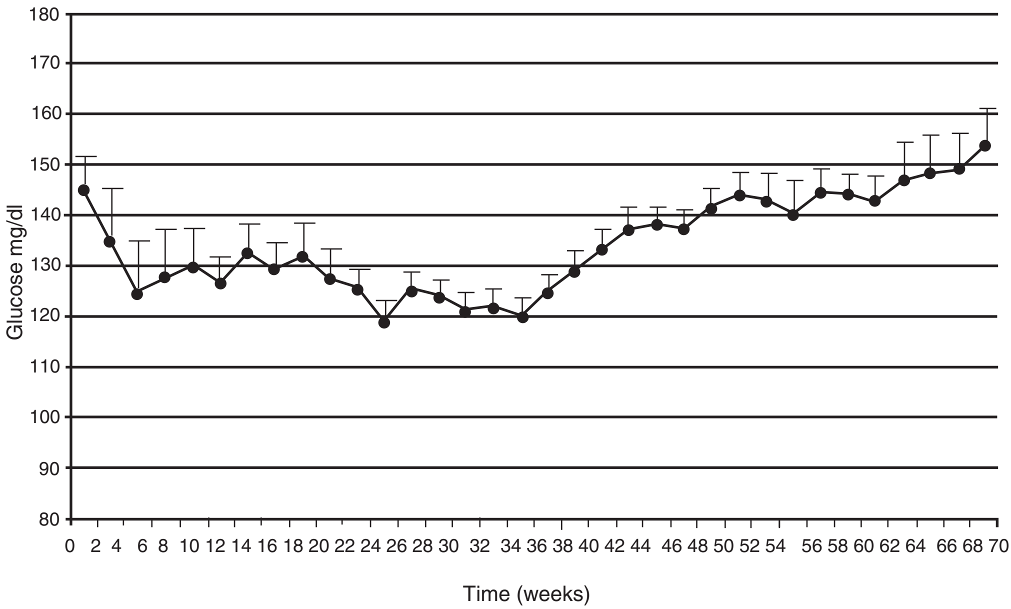

Figure 12-3. Reduction of the glucose values after administration of aqueous leaves extract of *Cecropia obtusifolia* to type 2 diabetic patients. * Statistical significance at $p \leq 0.05$ and ** at $p \leq 0.01$ when compared to T0.

For the in vivo experiments, neonatal diabetic-induced Wistar rats (n5-stz) were used, according to Andrade-Cetto.[12] Pyruvate (2 g/kg) was administered intraperitoneally to 18-hour fasting n5-stz rats that weighed ~250 g. The rats were assigned to one of four groups (n = 11 per group): group one – non diabetic control; group two – diabetic

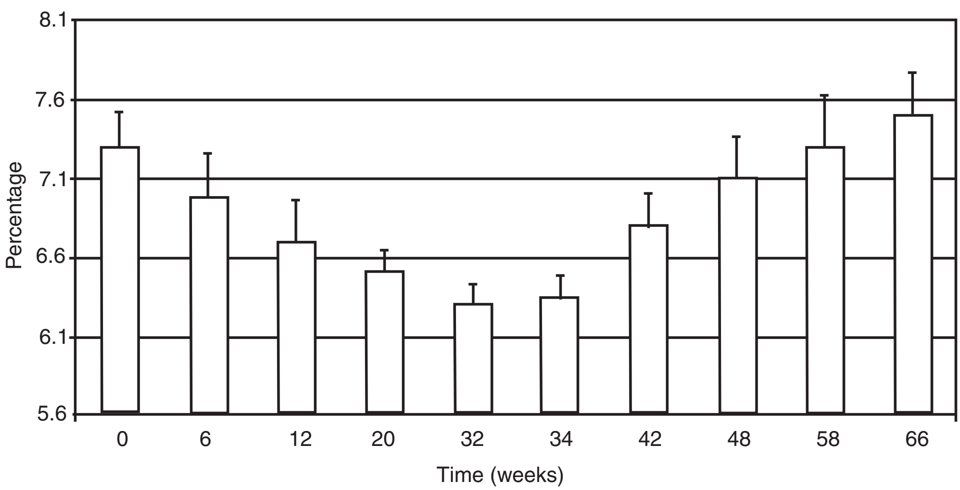

Figure 12-4. Reduction of Hb1Ac values after administration of aqueous leaves extract of *Cecropia obtusifolia* to type 2 diabetic patients. * Statistical significance at $p \leq 0.05$ and ** at $p \leq 0.01$ when compared to T0.

control; group three – chlorogenic acid (Sigma), 5 mg/kg; and group four – *Cecropia Obtusifolia*, 150 mg/kg.

After the administration of pyruvate, glucose was measured at times 0, 60, 90, and 120 min. We demonstrated that between 60 and 120 min post-application, the glucose levels of rats in groups three and four were significantly lower than those in the diabetic control group. As pyruvate (together with citric acid) is the main source of hepatic glucose production after a long period of fasting, we can conclude that C. a. and C. ob. are able to block this pathway.

The above observation was tested *in vitro* using components of the rat hepatic glucose 6-phosphatase system; intact rat lever microsomes were obtained [13] and the enzymatic activity was calculated by an indirect manner that measured the formation of inorganic phosphorus from glucose-6-phosphate.[14] We tested both the plant and the acid at concentrations of 2, 5, 20, 50, 200, 500, 1 000 and 2 000 µg/mL.

To assess the degree of inhibition exerted by glucose-6-phosphatase, we plotted a dose-response curve and reported the results as the IC_{50}. The measured IC_{50}s were 123 µg/mL for cholorogenic acid and 125 µg/mL for *Cecropia obtusifolia*.

In these experiments we obtained fully disrupted microsomes. Glucose 6-phosphatase is an enzyme that hydrolyzes glucose-6-phosphate, which results in the creation of a phosphate group and free glucose. Glucose is then exported from the cell via membrane glucose transporter proteins. This catalysis completes the final steps in gluconeogenesis and glycogenolysis and therefore plays a key role in the homeostatic regulation of blood glucose levels; this is particularly so in the fasting state.

Toxicity

The plant toxicity was tested using two different assays; both the standard version of the Drosophila wing somatic mutation and recombination test (SMART) with basal biotransformation activity, and the variant version with increased cytochrome P-450-dependent bioactivation capacity were used. The exposure concentrations used in these genotoxicity experiments ranged between 0.82 mg/mL and 13.32 mg/mL. The extracts did not produce any genotoxic effects.[15]

A human micronucleus assay in vivo was performed using cultured lymphocytes. These were obtained from six diagnosed type 2 diabetic patients that were treated daily with 13.5 g of the aqueous extract for a period of 32 to 85 days. No statistically significant differences in either cytotoxicity or genotoxicity between the control and diabetic blood samples were observed.[15]

CONCLUSIONS AND PERSPECTIVES

The increased use of medicinal plants by type 2 diabetic patients in Mexico is an established fact. Those individuals currently searching for new plants are mainly diabetic patients living in the rural parts of our country. Although this practice is sometimes associated with traditional healers, on other occasions the patients explore, use and recommend the plants to others without the intervention of a local healer. We can predict that in the coming years the number of medicinal plants used to treat type 2 diabetes will increase. As the use of some plants will likely be discontinued, it can also be predicted that this increase will be followed by a reduction in the number of plants used. Subsequently, a consistent number of plants will be commonly used.

The high prevalence of type 2 diabetes in Mexico, combined with the existence of both rich tropical flora and a variety of indigenous groups that preserve their own traditions, present the perfect conditions within which to search for new phytotherapeutic agents for the treatment of type 2 diabetes. We suggest that the main aim of these studies should be directed towards understanding the mechanisms of action of these plant extracts and to determining the chemical composition of the active ingredients; this will provide a solid base for the development of a plant-based medicine.

Perspective: with this kind of work we must first ensure that the plant-based medicine can work at the clinical level. As the use of traditional medicine is often limited, we must then work to expand the use of the effective plant-based medicine.

Cecropia obtusifolia – conclusion: The chemical composition of the traditionally used aqueous extract is similar to the butanolic extract in that it has two main bioactive compounds: chlorogenic acid and isoorientin.

In clinical trials, we observed a hypoglycemic effect that began after 4 weeks of treatment and became stable after 18 weeks. As this effect was paralleled by changes in the Hb_{1Ac} levels, we assumed that the regular consumption of this plant can control high plasma glucose levels. This observation can be explained by the action of chlorogenic acid, which strongly reduces the production of glucose by the liver in a fasting state.

Perspective: Because the aqueous extracts of the plant produced a hypoglycemic effect and substantially reduced the levels of Hb_{1Ac} in type 2 diabetic patients, but lacked toxicity, we can recommend the use of this extract to treat type 2 diabetes. However, further studies that involve larger numbers of patients are needed to fully clarify the actions of this plant.

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