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ScienceDirect
Journal of Ethnopharmacology 111 (2007) 636–640
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Hypoglycemic effect of *Cecropia obtusifolia* Bertol aqueous extracts on type 2 diabetic patients

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Received 27 April 2006; received in revised form 8 January 2007; accepted 12 January 2007
Available online 18 January 2007

Abstract

Cecropia obtusifolia is widely used in Mexican traditional medicine due to its reputed hypoglycemic effect. During a period of 32 weeks, aqueous extracts of the leaves of this plant were administrated daily to 12 recently diagnosed type 2 diabetic patients, controlled only with diet and exercise. Serum glucose, cholesterol, triglyceride and insulin levels were determined every 15 days; $HbA_{1c,}$ ALT, AST and ALKP were measured every month. A significant reduction of glucose was detected after 4 weeks of administration but the reduction was significant and sustained after 18 weeks of administration. The HbA_{1c} was also significantly reduced after 6 weeks of treatment. No significant changes on cholesterol, triglycerides ALT, AST, ALKP or insulin could be detected. No collateral effects were observed. After suspending the administration of the extract, and a follow-up of 34 weeks, the glucose and HbA_{1c} levels increased reaching levels higher than the basal ones. It can be concluded that the aqueous extracts of *Cecropia obtusifolia* have a significant hypoglycemic effect with no adverse effects and that the mechanism of action is not brought about by stimulating the insulin secretion. The results support the fact that the extracts of *Cecropia obtusifolia* have a great potential to be further developed into a phytomedicine.

Keywords: *Cecropia obtusifolia*; Cecropiaceae; Hypoglycemic agent; Type 2 diabetes treatment

1. Introduction

Type 2 diabetes (DM2) is one of the primary threats to human health due to its increasing prevalence, chronic course and disabling complications. According to the Word Health Organization (2005) there were 150 million people over 20 years of age living with diabetes in 2000 and they project that by 2025 there will be 300 million people living with this condition. The increase is expected to be 42% in developed countries and 70% in developing countries (Word Health Organization, 2005; King et al., 1998). México will probably occupy the seventh place.

One of the principal objectives when treating patients with DM2 is to control glucose levels. Presently, there is an arsenal of synthetic hypoglycemic drugs available; however, these drugs normally cause side effects prompting the patients to stop taking the medication and DM2 progresses with further acute and chronic complications and even death. For this reason, a phytomedicine capable of treating the disease at early stages, but with fewer side effects and less expensive, will be of great help to the diabetic patients specially due to the extended belief that natural treatments cause less harm to the organism.

México has a long-standing tradition of using hypoglycemic plants to treat DM2 and according to ethnopharmacological studies, there are 306 documented plant species from 235 genera and 93 families used for the treatment of this disease (Andrade-Cetto and Heinrich, 2005).

Cecropia obtusifolia Bertol. (Cecropiaceae) has shown analgesic and anti-inflammatory as well as antihypertensive, muscle relaxant and CNS depressant effects but the most important and best known is the hypoglycemic effect (Pérez et

Abbreviations: DM2, type 2 diabetes; CNS, central nervous system; BMI, body mass index; HbA1c, glycosilated haemoglobin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALKP, alkaline phosphatase

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doi:10.1016/j.jep.2007.01.014

al., 1984; Roman-Ramos et al., 1991; Andrade-Cetto and Wiedenfeld, 2001; Pérez Guerrero et al., 2001). Commonly called "Guarumbo", "Chancarro" and "Hormiguillo", *Cecropia obtusifolia* is widely used by the Mexican traditional healers for the treatment of type 2 diabetes. Previous ethnobotanical studies have reported that a mean of 12–15 g of the dry leaves boiled in 1 l of water must be consumed several times per day as "agua de uso" (water which is normally consumed throughout the day) (Andrade-Cetto, 1999). In an assay-guided isolation performed by our group, the hypoglycemic activity on streptozotocin diabetic rats was demonstrated. Two main compounds were detected in the active fraction, chlorogenic acid and isoorientin that were isolated and tested (Andrade-Cetto and Wiedenfeld, 2001).

The aim of this particular study was to investigate the hypoglycemic effect of the aqueous extract of the leaves of *Cecropia obtusifolia* administered to recently diagnosed type 2 diabetic patients, to determine the biosecurity of its administration and to contribute to the elucidation of the possible mechanism of action.

2. Materials and methods

2.1. Plant material

Cecropia obtusifolia Bertol. (Cecropiaceae) was collected in the Mexican state of Hidalgo in November 2003. The specimens were identified and voucher specimens were deposited at the IMSS Herbarium with the numbers IMSS 14695 and IMSS 14697.

2.2. Extract preparations

The leaves of *Cecropia obtusifolia* were dried at 40 °C, milled and stored. The aqueous extract of the leaves was prepared, as recommended by the traditional healers, by boiling a single dose of 13.5 g of dried and milled leaves of *Cecropia obtusifolia* for 5 min in 1 l of water, allowing it to cool for 10 min (described by Andrade-Cetto and Wiedenfeld, 2001). The aqueous extracts were monitored by HPLC to determine their chemical constituents.

2.3. Patients

Twelve recently diagnosed (2 ± 0.8 years) type 2 diabetic patients, 8 women and 4 men, with an average age of 48 ± 4 years, all controlled with diet and exercise, participated in the study. The patients were non-smokers who did not consume alcohol. None of the patients had ever taken any hypoglycemic drug. After a clear explanation of the study the patients signed an informed consent. The research followed the guidelines of the Declaration of Helsinki and Tokyo and was approved by the Ethic Committee of the National Health Research Coordination.

Before administering the *Cecropia obtusifolia* extract, each patient's body mass index was calculated (BMI = kg/m^2) and blood samples were obtained for basal biochemical values. Blood samples were obtained again every 15 days during the 32 weeks that comprised the first part of the study. In every visit a blood drop was obtained from his or her fingertip in order to determine glucose level with a Bayer Elite glucometer. The test is based on the glucose oxidase method. The HbA_{1c} values were evaluated monthly from a blood drop with a Bayer DCA 2000 Analyzer, Bayer Diagnostics.

Serum glucose, cholesterol, triglyceride and insulin levels were determined every 15 days. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALKP) were determined every month. Body weight and blood pressure was measured every 15 days.

The patients were instructed to prepare the *Cecropia* extract daily, by boiling a single dose of 13.5 g of the dried and milled leaves (previously packed and handed to the patients) for 5 min in 1 l of water, allowing it to cool for 10 min. The patients were also instructed to drink one glass of the filtered extract before each meal and the rest throughout the day.

After 32 weeks the daily administration of the *Cecropia obtusifolia* extract was suspended and the patients continued their glucose control for additional 34 weeks, with the same diet and exercise routine from the first part of the study. During the second 34-week period, the same testing procedures were performed.

2.4. Calibration curves

For the standard solution of chlorogenic acid, an authentic sample (Sigma C3878) was used. For isoorientin (not available reference compound) the calibration was done by the reference method (internal standard method). Quercentin (Sigma Q015) was found to be a suitable compound with a detection ratio of 1:1. Calibration curves were constructed for each compound with dilutions of 1, 0.5, 0.25, 0.125, 0.0625, 0.031, 0.016 and 0.008 mg/ml.

2.5. Serum determinations

Blood samples were obtained from the antecubital vein of the patients with vacutainer tubes. Serum samples were obtained by centrifugation and analyzed with the Vitros® DT 60 II (Johnson Medical) using the following methods: glucose (mg/dl) was determined by the glucose oxidase technique (Caraway, 1987); triglycerides (mg/dl) were processed by separation with lipoproteic carriers hydrolyzed by lipase; ascorbic acid residues were eliminated with ascorbate oxidase (Tietz, 1987); cholesterol (mg/dl) was processed by the dissociation of the lipoprotein carriers and, after hydrolysis of the cholesterol esters, the free cholesterol was oxidized with cholesterol oxidase. Apartate aminotransferase AST (U/L) and alanine aminotransferase ALT (U/L) were determined by an enzyme-coupled oxidation of NADH to NAD^+. The rate of oxidation is monitored by reflectance spectrophotometry and the value is the ALT or AST activity. Alkaline phosphatase ALKP (U/L) determination is based on alkaline phosphatase catalyzing the hydrolysis of *p*-nitrophenyl phosphate to *p*-nitrophenol. Insulin (μU/ml) was measured by radioimmunoassay (Bio-Cis International®). Glycated hemoglobin (%) was measured with the Bayer DCA 2000®

by inhibition of latex agglutination with mouse monoclonal antibodies (Knowless et al., 1986).

2.6. Statistical analysis

Data were analyzed with an ANOVA test, followed by a Fisher LSD post hoc analysis.

3. Results

3.1. Plant extraction and administrated compounds

From 13.5 g of the dried leaves of *Cecropia obtusifolia* (which would yield one single dose for one patient), 1.35 g (mean) of plant extract was obtained leading to a drug-extract-ratio (DER) of 10:1 (Gaedcke and Steinhoff, 2003). Each of those 13.5 g plant portions contained 2.91 mg of chlorogenic acid and 2.4 mg of isoorientin.

3.2. Demographic, anthropometric and biochemical parameters

The demographic and anthropometric parameters of the patients are shown in Table 1. The BMI values calculated for each patient showed that all the patients were overweight; blood pressure measures showed that none of them was hypertense.

No alterations of the patients' blood pressure or a significant change of their body weight could be detected during the study. There were no adverse effects reported by the patients due to the intake of the extract. It was well tolerated and there were no complaints about its taste or smell.

Table 1
Demographic and anthropometric parameters of type 2 diabetic patients

Demographic and anthropometric parameters	Mean	S.D.
Age (years)	48	4
Diagnosis of diabetes	2	0.8
Body weight (kg)	74.2	5.2
Size (m)	1.60	0.07
BMI (kg/m^2)	28.98	1.5
Systolic pressure (mmHg) (110–140)	126	11.6
Diastolic pressure (mmHg) (70–90)	85	8.5

The data show the recent diagnosis of the patients included in the study and the overweight demonstrated by the BMI

The aqueous extract of *Cecropia obtusifolia* showed a significant but not sustained hypoglycemic effect 4 weeks after starting administration. The hypoglycemic effect became significant and sustained ($p = 0.03$) after 18 weeks and continued until the end of the first part of the study (32 weeks) (Fig. 1). The values of HbA_{1c} showed a significant ($p = 0.05$) reduction 6 weeks after the start of the treatment (Fig. 2) and was sustained until the end of the first part of the study.

The cholesterol, triglyceride and insulin levels showed no significant changes with the administration of the *Cecropia obtusifolia* extract or after its suspension. No significant changes in the levels of AST, ALT and ALKP were detected during the 66 weeks that the study lasted. Table 2 shows the mean values and standard error of the concentrations of glucose, cholesterol, triglycerides, insulin, HbA_{1c}, ALT, AST and ALKP.

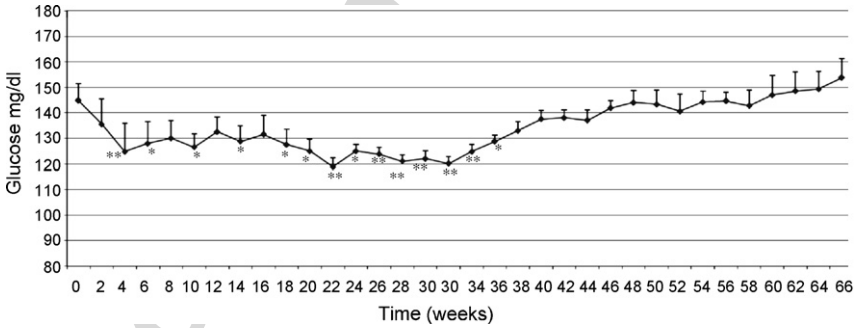

Fig. 1. Reduction of the glucose values after administration of aqueous leaves extract of *Cecropia obtusifolia* to type 2 diabetic patients. The symbol (*) shows statistical significance at $p \leq 0.05$ and the symbol (**) shows statistical significance at $p \leq 0.01$ when compared to T0.

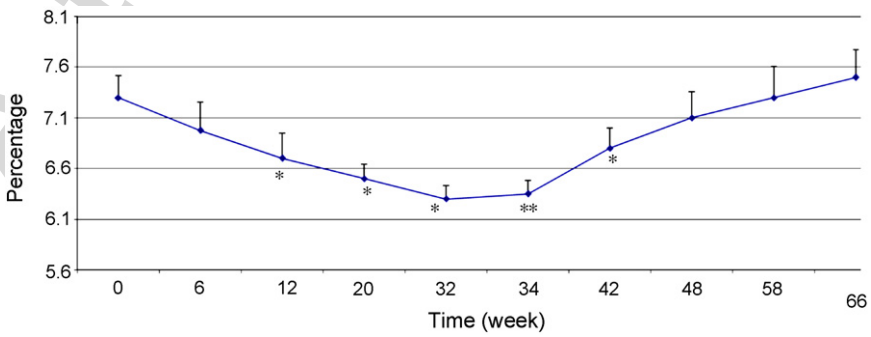

Fig. 2. Reduction of the glucose values after administration of aqueous leaves extract of *Cecropia obtusifolia* to type 2 diabetic patients. The symbol (*) shows statistical significance at $p \leq 0.05$ and the symbol (**) shows statistical significance at $p \leq 0.01$ when compared to T0.

Table 2
Mean values and standard error of the concentrations of glucose, cholesterol, triglycerides, insulin, $HbA_{1c,}$ ALT, AST and ALKP

	Basal (T0)	32 Weeks with treatment (T1)	Suspension of treatment (T2)	34 Weeks without treatment (T3)
Glucose	145 ± 7	120 ± 3a	125 ± 3	154 ± 7a
Cholesterol	217 ± 10	226 ± 5	234 ± 5	241 ± 3
Triglycerides	164 ± 21	159 ± 21	165 ± 17	177 ± 9
Insulin	7.34 ± 1.23	7.16 ± 0.98	5.81 ± 0.84	5.82 ± 0.79
HbA_{1c}	7.3 ± 0.22	6.35 + 0.13a	6.5 ± 0.13	7.5 ± 0.27a
ALT	35 ± 9	37 ± 8	36 ± 10	36 ± 11
AST	29 ± 10	32 ± 9	32 ± 11	31 ± 11
ALKP	50 ± 4	48 ± 5	50 ± 6	51 ± 6

Letters on column (T1) indicate significance respect to the beginning of the treatment (T0) $p \leq 0.01$. Letters on column (T3) indicate significance respect to the end of treatment (T1) $p \leq 0.01$.

After suspending the *Cecropia obtusifolia* treatment, the reduced levels of blood glucose were maintained for 4 weeks but they began to increase thereafter. At the end of the 34 weeks of follow-up of the study, the patients' glucose levels were significantly higher than those detected at the end of the treatment and also higher than the ones detected at the beginning of the treatment (basal).

The HbA_{1c} values increased after the suspension of the *Cecropia obtusifolia* treatment in the same way as those of the glucose levels. The reduction persisted for 2 weeks, subsequently increasing during the final 34 weeks of the study. At the end of the study, HbA_{1c} levels were also significantly higher than those detected at the end of the treatment and higher than at the start of the study.

4. Discussion

Ethnobotanical studies have demonstrated that the infusions of *Cecropia obtusifolia* have been used for a long time by the Mexican traditional healers to treat diabetes, but there are not enough scientific controlled studies that can demonstrate its hypoglycemic effect.

Since diabetes is a chronic disease, a long-term therapy is required. So, we administered the *Cecropia obtusifolia* extracts for 32 weeks in order to demonstrate the hypoglycemic effect.

According to the method the traditional healers recommend, a daily dose of the aqueous extract of *Cecropia obtusifolia* was administered. Our results proved a significant and sustained hypoglycemic effect of the *Cecropia obtusifolia* aqueous extract after 18 weeks of the daily administration of 13.5 g leaves extract that contained 2.91 mg of chlorogenic acid and 2.4 mg of isoorientin. The patients' reduced concentrations of glucose were maintained during the entire treatment and for an additional 4 weeks after suspending treatment. Subsequently, the concentrations of glucose increased to significantly higher levels than those detected at the end of the treatment and to levels that were higher than the basal ones, even while the patients continued with the same diet and exercise regimes. This provides evidence that the plant extract, not diet and exercise, was responsible for the reduction of the glucose levels.

The administration of the *Cecropia* extract clearly reduced the HbA_{1c} levels, a gold standard that confirms a long-range level of the blood glucose, confirming the hypoglycemic effect of the extract. Suspending treatment significantly increased the HbA_{1c} levels.

The hypoglycemic effect of the extracts of *Cecropia obtusifolia* administered to type 2 diabetic patients has been reported only in one previous study. But the hypoglycemic effect found in the Herrera-Arellano et al. (2004) study cannot be attributed only to the plant extract since the patients were additionally receiving different doses of glibenclamide. No hypoglycemic drug was administrated throughout our study. Therefore, the hypoglycemic effect detected is due exclusively to the *Cecropia obtusifolia* extracts.

In 32 weeks of treatment using 13.5 g plant/day/patient, no antihyperlipidemic effect was observed. We could not detect any significant reduction of cholesterol or triglyceride levels as reported by Herrera-Arellano et al. (2004), it is important to establish that the type 2 diabetic patients included in our study were not hyperlipidemic as were those of the Herrera-Arellano et al. (2004) study.

Throughout the entire study, we did not find significant changes of the ALT, AST and ALKP levels. This indicates that the treatment with *Cecropia obtusifolia* is not hepatotoxic and that it guarantees the biosecurity of using these extracts as an antihyperglycemic treatment.

No significant changes in the patients' insulin levels could be detected, leading to the conclusion that the *Cecropia's* hypoglycemic effect cannot be attributed to the induction of insulin secretion, as was also reported by Sezik and Aslan (2005) who proposed that other mechanisms may be responsible for the hypoglycemic effect of isoorientin (Sezik and Aslan, 2005).

No changes in the blood pressure were detected during the study. Body weight showed no changes, since the glucose levels were not as high as to provoke the excessive breakdown of tissue protein that is a characteristic condition in patients with non-controlled diabetes.

The treatment adherence was of 100%, far better than the adherence reported for hypoglycemic drugs and no adverse effects were reported.

5. Conclusions

The results of this study clearly demonstrated that the aqueous extracts of *Cecropia obtusifolia*, containing isoorientin and chlorogenic acid as active compounds, have a significant hypo-

glycemic effect, with no hepatotoxicity or adverse effects. The results clearly demonstrate that the *Cecropia obtusifolia* aqueous extracts have no direct effect on insulin secretion, so other mechanisms must be responsible for its hypoglycemic effect. According to these results, *Cecropia obtusifolia* has a great potential to be further developed into a phytomedicine. Further studies related to the development of a phytomedical preparation from *Cecropia obtusifolia* are being carried out by our group.

Acknowledgments

This work was supported by IMSS project IMSS 2004/160 and partially by DGAPA, PAPIIT project IN 204703.

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