HYPOGLYCEMIC EFFECT OF CECROPIA PELTATA L. ON N5-STZ TYPE 2 DIABETIC RATS

Adolfo Andrade-Cetto [a]* René Cárdenas [b] and Brenda Ramírez-Reyes [a]

[a] Lab. Etnofarmacología, Fac. de Ciencias, Universidad Nacional Autónoma de México, México DF.
[a] Lab.Biologìa Animal Exp., Fac. de Ciencias, Universidad Nacional Autónoma de México, México DF.

***Corresponding author: aac@fciencias.unam.mx**

Summary

Cecropia peltata is a plant highly appreciated in the Mayan communities of the south west part of México for the treatment of type 2 diabetes. In this work the hypoglycemic effect of the aqueous (**Ae-P)** and the butanolic (**Be-P)** extracts of the plant were tested in n5-stz diabetic rats. F The **Ae-P** showed a hypoglycemic effect only at 200 mg/kg bw after 180 min of administration, whereas 20 mg/kg bw did not show any effect. The **Be-P** at 27 mg/kg bw showed effect only at 180 min., whereas at 60 mg/kg bw showed an hypoglycemic effect since 60 min up to 180 min. Since *C. peltata* extracts exerted a lower hypoglycemic effect than that previously observed with *Cecropia obtusifolia*, after we compared the phytochemical profile of both species we observe that *C. peltata* presents lower concentrations of Cholorogenic acid, with this results one can assume that a good hypoglycemic effect of both Cecropia species is related with the amount of Cholorogenic acid and Isoorientin present in the extracts.

Key Words: Neonatal induced diabetic rats, medicinal plants, type 2 diabetes, *Cecropia*.

Introduction

Type 2 diabetes is one of the primary threats to human health, due to its increasing prevalence, chronic course and disabling complications. The natural history of type 2 diabetes begins with a period of insulin resistance, with augmented pancreatic insulin secretion. As the disease progresses, pancreatic function falters and is no longer able to meet peripheral demands. As a result, insulin levels fail to keep up with the body requirements. The disease is characterized by increased circulating glucose concentrations, associated with abnormalities in carbohydrate, fat and protein metabolism and a variety of microvascular, macrovascular, neurologic and infectious complications (1).

In Mexico about 10.6% of the population between 20 and 69 years old is affected by the disease, ranging around the ninth place worldwide (FMD, 2007). The use of medicinal

plants among the Mexican population is a tradition. The use of 306 species for the treatment of type 2 diabetes has been already reported (2). Among them *Cecropia obtusifolia* and *Cecropia peltata* have been reported as hypoglycemic plants.
Among these plants *Cecropia peltata* L. was selected under the basis of our field work in Yucatan, Mexico (3).

In our previous studies, the hypoglycemic effect of *Cecropia obtusifolia* was demonstrated in stz-diabetic rats. The two main compounds (Cholorogenic acid, **CA** and Isoorientin, **IO**) occurring on the active extracts were isolated (4). Also we have demonstrated the effect of the aqueous extract in type 2 diabetic patients and correlated the hypoglycemic effect with the presence of the previously isolated compounds (5). In a previous work (6) the hypoglycemic effect of *Cecropia obtusifolia* and *Cecropia pelatata* were compared in a non-diabetic animal model, which led to the conclusion that *C. pelatata* exerts as strong hypoglycemic effect as *C. obtusifolia*. The authors related the effect to the Cholorogenic acid content.

A possible mechanism of action of both Cecropias could be by reducing hepatic glucose output, due to the inhibition of glucose 6 phosphatase by chlorogenic acid, which can simultaneously target gluconeogenesis and glycogenolysis (2).

The n5-stz diabetic model is well recognized for the study of type 2 diabetes (7). 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 to 15 weeks of age, an impaired glucose disposal rate and significant beta-cell secretory dysfunction (type 2 diabetes) is observed (7). We have already proved the suitability of this model for plant testing (8).

Cecropia peltata L. (Cecropiaceae), traditional Maya name “X cooch”, is a monopodic tree 15 m tall, growing as secondary vegetation in the tropical forest. It is highly appreciated in the Mayan communities for the treatment of type 2 diabetes, this use was already demonstrated by our group (9).

The aim of the present study was to investigate the hypoglycemic effect of the water and butanol extracts of *C. peltata* in streptozotocin (n5-stz)-induced diabetic rats and compare the phytochemical profile of the plant with the previously studied *Cecropia obtusifolia* **(4).**

Methods

Extracts Preparation.
For the phytochemical comparison of *C. peltata* with the previously studied compounds from *C. obtusifolia*, the butanol (Be-P) extract was prepared from dry leaves as already described (10). The leaves were collected near Chikinzonot Yucatan Mexico. A voucher specimen was deposited at the Herbarium IMSS, 14696. After the first phytochemical results more leaves were collected at two different locations near Playa del Carmen, Quintana Roo México and Chetumal, Quinta Roo, Mexico. For *C. obtusifolia* a new butanol extract (Be-O) was prepared from the already described plants (4, 5)

The Be-P was used for the phytochemical analysis of the plant by HPLC as follows. An extract sample was applied to a Nucleosil 60-30 C_{18} (Macherey & Nagel, Düren, Germany) column and eluted with H_2O/MeOH/AcCN 70:15:15, 4 ml/min monitored by DAD-HPLC, Beckman System Gold with 32 Karat software. The Be-P was compared with the original isolated samples from *C. obtusifolia* Be-O described in (4).
In addition, for the pharmacological testing, an aqueous extract (Ae-P) equivalent to the traditional preparation was prepared from dry leaves as previously described (5). In our previous studies (9, 4, 11) we had demonstrated that the butanol extract has the same phytochemical composition as the water extract, but the active compounds are more concentrated in the former.

Experimental animals and induction of type 2 diabetes.
Five-day old Wistar rats (weighing 10–12 g) received 90 mg/kg i. p. of STZ (Sigma, No. 242-646-8) in acetate buffer 0.1 M, pH 4.5. Control rats received only the buffer. After 4 weeks of age, rats were separated from their mothers and acclimatized with free access to food and water in an air conditioned room (25° C with 55% humidity) under a 12:12 h light: dark cycle, at the Bioterium of the Faculty of Sciences, UNAM. The animal handling was in accordance with the Federal Government legislation of animal care. After 12 weeks, diabetes was identified by measuring fasting plasma glucose levels. Animals with glucose levels <155 mg/dl were not included in the study.

Experimental groups and Blood Collection.
The diabetic animals were classified into seven groups (2-8), each of them with eleven rats. One non diabetic group (1) was included as control, which received 1.5 ml of physiological NaCl-solution (Vehicle). Group 2 diabetic control received also 1.5 ml of physiological NaCl-solution (vehicle), Group 3 were treated with the standard oral hypoglycemic agent glibenclamide (3 mg/kg bodyweight (BW) in the same vehicle, Group 4 received a higher dose of glibenclamide (5 mg/Kg), Groups 5 and 6 received **Ae-P** at 20 and 200 mg/kg bw, respectively, Groups 7 and 8 received **Be-P** at 27 and 60 mg/kg bw, respectively. The extracts were re-dissolved in 1.5 ml of physiological NaCl-solution and administered orally with the use of a canule.

Blood samples were obtained from the tail vein (according to the Guideline 9 from IACUC, 3/10/99) before the oral administration of the extracts or the vehicle (T0), and at times 60, 120 and 180 min thereafter. Thirty-two microliters of blood were used for each assay. Glucose concentration was measured in plasma with Reflotron equipment and confirmed by Accutrend GC and Accu-check compact equipments (Roche).

Statistical analysis.
The data were statistically analyzed by one-way ANOVA followed by Tukey's test. Plasma glucose concentrations are expressed as the mean ± standard error.

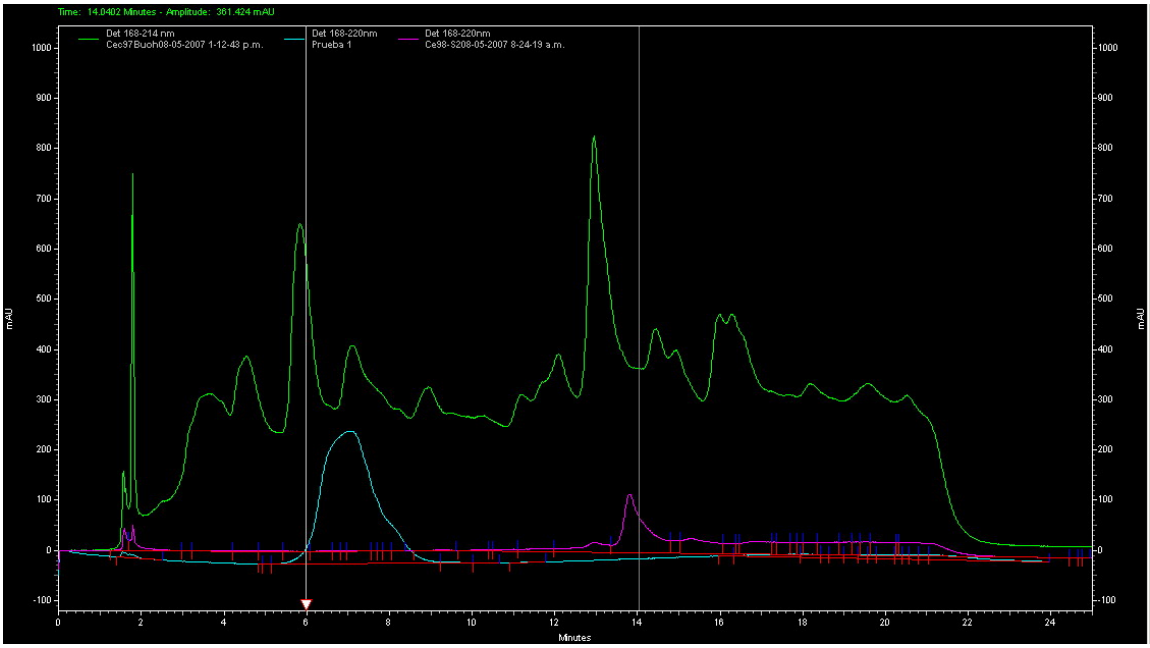
Results

Phytochemistry.

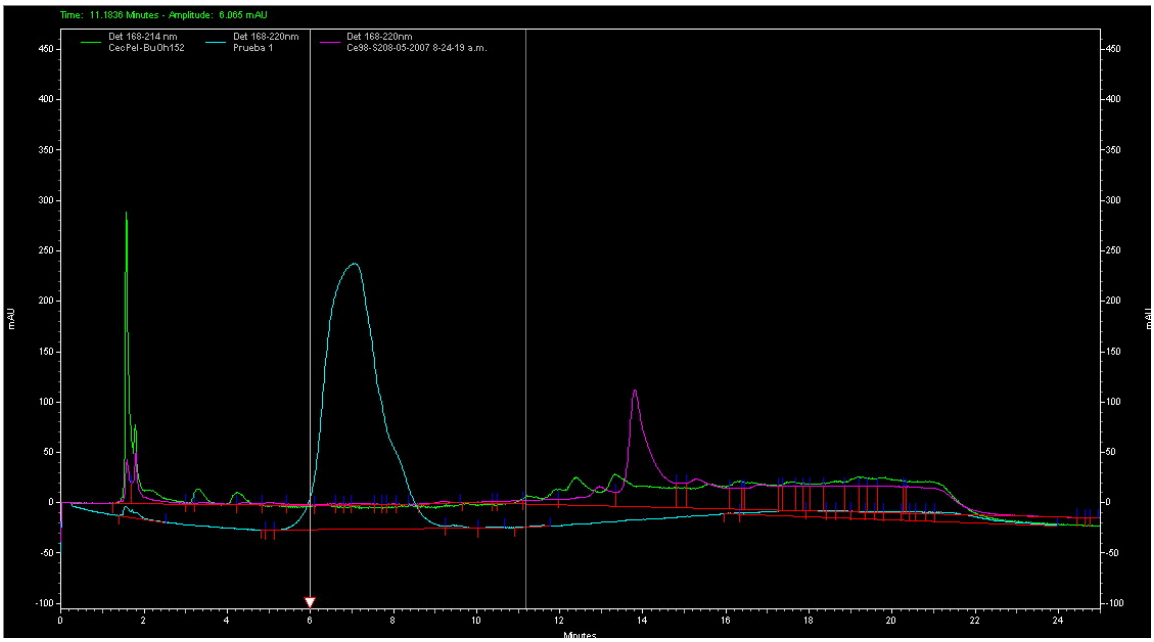
The analysis of *C. obtusifolia* **Be-O** used in the present study showed that it contained both **CA** and **IO**, in the same concentration as in the extract previously reported (ref; graphic 1). However, after doing the same analysis with *C. peltata* **Be-P** from Chikinzonot, Yucatan, we noticed that **CA** was not present in the extract (graphic 2). The analysis of leaf extracts from the other two locations (Playa del Carmen and Chetumal, Quintana Roo, Mexico) showed the same results (data not shown). The tree with the highest concentration of metabolites in the phytochemical profile (graphic 3) was selected for the pharmacological testing.

Hypoglycemic effect of C. pelatata.

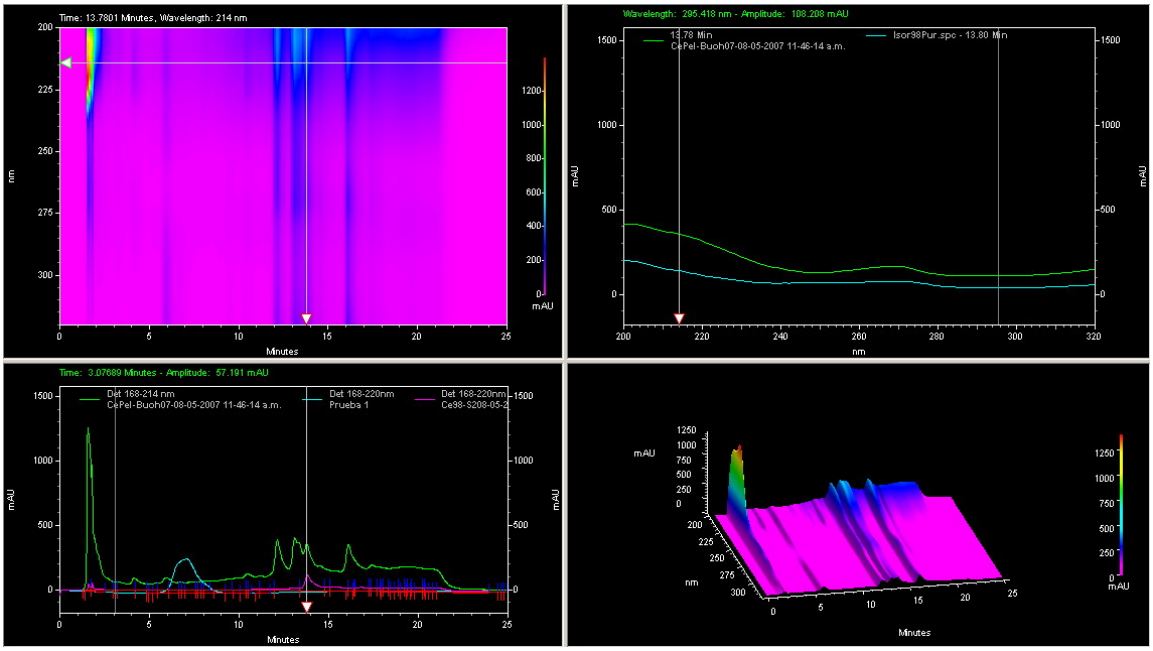
The diabetic group (2) showed a significant hyperglycemia when compared against the control group (1) since time 0. The two different doses of glibenclamide administered to the diabetic animals from groups 3 and 4, exerted a hypoglycemic effect since time 60 up to the end of the experiment (180 min). However group 4 (5 mg/kg) showed a higher hypoglycemic effect at times 120 and 180 min than group 3 (3 mg/kg). The **Ae-P** showed a hypoglycemic effect only at the higher dose at time 180 min (group 6), while group 5 did not show effect. The **Be-P** at lower dose (group 7) showed effect only at 180 min., whereas the higher dose showed an hypoglycemic effect since 60 min up to 180 min (Figure 1).



Graphic 1. UV-HPLC at 220 nm of *Cecropia obtusifolia* (green line) compared with Cholorogenic acid (blue line) and Isoorientin (purple line).



Graphic 2. UV-HPLC at 220 nm of *Cecropia peltata* (green line) compared with Cholorogenic acid (blue line) and Isoorientin (purple line).



Graphic 3, *Cecropia peltata* DAD-HPLC spectra Upper left. Down right, 3d spectra .Upper Right UV-Chromatogram Spectra at 220 nm (green line) compared with Cholorogenic acid (blue line) and Isoorientin (purple line). Upper right Isoorientin from the present extract compared with the extract previously published.

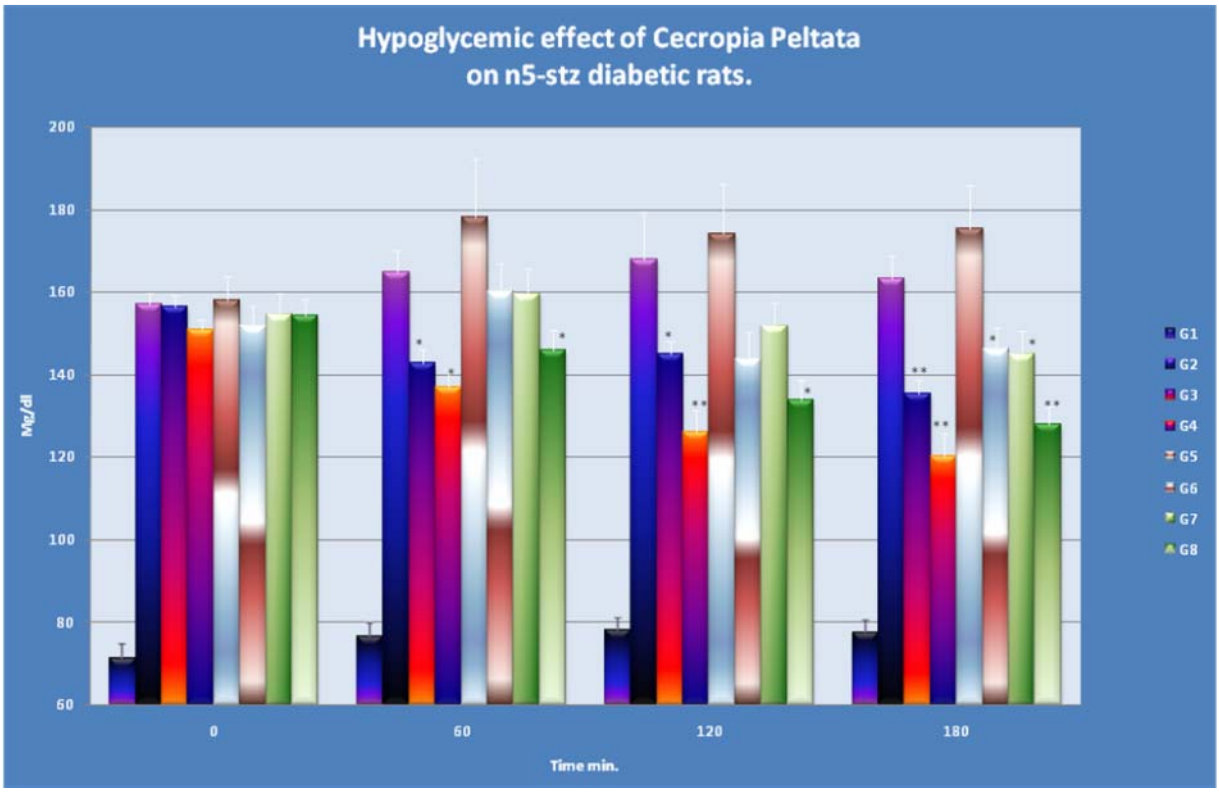


Figure 1, Hypoglycemic effect of C. Peltata on n5-stz diabetic rats. G1, non diabetic control; G2, diabetic control; G3, glibenclamide 3mg/kg; G4, glibenclamide 5 mg/kg; G5, Ae-P 20 mg/kg; G6, Ae-P 200 mg/kg; G7, Be-P 27 mg/kg; G8, Be-P 60 mg/kg. * $p<0.05$, ** $p<=.005$ vs G2

Discussion

According to the present results we confirmed that the n5-stz rat is a suitable model for the study of type 2 diabetes. Furthermore, we proved on this model that the hypoglycemic effect produced by the oral administration of glibenclamide is dose dependent.

The hypoglycemic effect of *Cecropia peltata*, is not as clear as the previously observed with *C. obtusifolia* (4), even when the here tested plant extracts were used at higher doses.

The water extract (**Ae-P**) exhibited a hypoglycemic effect only at the higher dose after 180 min. With the **Be-P** extract, the lower dose was as effective as the higher dose of the **Ae-P**, and the higher dose of **Be-P** was the most effective, with the glycemia been significantly lower earlier and decreasing with time. This seems to be due to the higher concentration of compounds of *Cecropia peltata* reached in the **Be-P**.

In the here presented phytochemical results it can be observe that *C. peltata* contains lower concentrations of cholorogenic acid (**CA**) and this can be the reason why at lower doses the

extracts did not produce any hypoglycemic effect. Our results disagree with those of Nicasio (6), who reported higher concentrations of **CA**. This difference could be due to a variation on the **CA** content of *C. peltata* by phenotypic or seasonal variation. Since we report the phytochemical analysis of several trees, we can assume that the content of cholorogenic acid in *C. peltata* is in general lower than in *C. obtusifolia*.

With the here presented results, we can propose that the chlorogenic acid content in Cecropia species plays an important role in their hypoglycemic effect. Similarly, we also suggest that the isoorientin content can also be responsible of the strength of the hypoglycemic effect.

The here presented results suggest that Cecropia species could be further developed as phytomedicines and for observe a hypoglycemic effect it is necessary the presence of the two main compounds occurring in the extracts.

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