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Hypoglycemic effect of *Cecropia obtusifolia* on streptozotocin diabetic rats

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Abstract

The hypoglycemic effects of water and butanolic extracts prepared from leaves of *Cecropia obtusifolia* (Cecropiaceae) were examined in streptozotocin induced diabetic rats. A single oral administration of a water extract at doses of 90 and 150 mg/kg and of a butanol extract at doses of 9 and 15 mg/kg significantly ($P < 0.05$) lowered the plasma glucose levels in diabetic rats after 3 h administration. Glibenclamide was used as reference and showed similar hypoglycemic effect to the tested extracts at a dose of 3 mg/kg. The flavone, isoorientin and 3-caffeoylquinic acid (chlorogenic acid), were isolated as the important constituents of the plant and were identified as the main constituents in both extracts, too.

Keywords: *Cecropia obtusifolia*; Hypoglycemic effect; Streptozotocin; Diabetes; Isoorientin; Chlorogenic acid

1. Introduction

Cecropia obtusifolia Bertol. (Cecropiaceae), traditional names; 'Guarumbo' and 'Chancarro', is a monopodic tree 20 m tall, growing in form of secondary vegetation in the tropical rain forest. The main characteristics of the tree are a tall, straight, hollow trunk, stratified treetop with few large branches growing horizontally from the trunk. The leaves are in a spiral disposition located at the top of the branches and are simple, peltate or deeply palmate, with a deep green color in the upper face and gray at the under surface, the outer bark is flat. It is a fast-growing pioneer tree from tropical America, the hollow septate twigs are inhabited by ants (Pennington and Sarukhán, 1998).

The plant was first mentioned for the treatment of diabetes by Martínez (1936), since which time several ethnopharmacological reports can be found which describe its use. Traditionally the leaves, bark and root of the plant are boiled in water and the resulting infusion is drunk throughout the day (Argueta, 1994).

The hypoglycemic effect of the water extract was demonstrated on alloxan diabetic mice (Pérez et al., 1984) and on hyperglycemic rabbits (Roman-Ramos et al., 1991).

Water decoctions of 'Guarumbo' are used traditionally for the treatment of diabetes type II, particularly in the Mexican states of Oaxaca and Hidalgo. The plant is also sold in several traditional markets, either alone or mixed with other plants as an anti-diabetes complex remedy (Andrade-Cetto, 1999).

The aim of this study was to investigate the hypoglycemic effect of several extracts from *C. obtusifolia* in streptozotocin-induced diabetic rats and to identify the main chemical constituents in the plant and the tested extracts.

2. Materials and methods

2.1. Ethnobotany

Ethnobotanical studies were performed during several short visits to the communities of Tlanchinol, Hidalgo, and San Felipe Usila, Oaxaca, in Mexico

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during 1994–1999 to confirm the previously reported use of *C. obtusifolia* as a treatment for diabetic illness. In both cases we followed the same method: diabetic people were identified by the local health services and local healers; information was collected about the plant and its special usage based on structured and unstructured interviews with both the traditional healers and the diabetic people. All data were referred to plant samples (mini-herbarium) collected at its natural habitats and stored as herbarium vouchers for exact identification.

2.2. Materials

With the guidance of traditional healers, samples of *C. obtusifolia* were collected in Tlanchinol (Hidalgo) and San Felipe Usila (Oaxaca), Mexico. Their identity was confirmed by Andrade-Cetto and voucher specimens were deposited at the IMSS Herbarium in Mexico City.

2.3. Preparation of the extracts and isolation of compounds

Plant extracts were prepared from leaf samples (300 g) as already described (Andrade-Cetto et al., 2000), resulting in a yield of 45 g of aqueous extract (WE) and 4.5 g of butanolic extract (BE). The latter was used for the phytochemical identification of the main components. The BE was applied on a 100 × 2 cm Polygoprep 60–30 C_{18} (Macherey & Nagel, Düren, Germany) flash-column and eluted with H_2O/MeOH/AcCN 80:10:10, 4 ml/min (10 ml fractions; **1**: fr. 16–18, **2**: 23–26). The resulting fractions were monitored by HPLC (ET 250/8/4 Nucleosil 120–5 C_{18}, Macherey & Nagel; 0.04 m H_3PO_4/AcCN/MeOH, 0–9 min.: 85/8/7 to 70/15/15, to 20 min.: 70/15/15; 1.5 ml/min.; 220 and 255 nm UV-det. R_t of **1**: 5.1 min., **2**: 9.5 min.). Prep. HPLC (SP 250/10 Nucleosil 120–7 C_{18}, Macherey & Nagel) was used for final purification yielding compounds **1** (12 mg), **2** (8mg).

The structures (Fig. 1) were established by spectroscopy.

Here, for **1** the NMR data give evidence for a caffeoylquinic acid. The structure of **1** is especially determined by the ^{1}H coupling of H-3, H-4 and H-5. Thus, the values of 5.12 ppm, 8.5 and 9.0 Hz (C-3H), 3.53 ppm, 9.0 and 2.8 Hz (C-4H) as well as 3.94 ppm, 2.8, 2.4 and 1.5 Hz (C-5H) proof for the quinic acid part a $^1C_4$-form leading to the conclusion that **1** shows the structure of chlorogenic acid (3-caffeoylquinic acid). These date are similar to those described earlier within a range of 0.4 ppm (^{1}H) and 3 ppm (^{13}C), respectively (Corse et al., 1966; Kelley et al., 1976). The ^{1}HNMR data for **2** established the possible structure of a flavone by the aromatic protons at 7.37, 7.35, 6.82 and 6.35 ppm (C-6′H, C-2′H, C-5′H, C-8H) and the olefinic proton at C-3 at 6.55 ppm. The high-field shift of C-1″H (4.55 ppm) indicated the C-glucosidation at C-6. The ^{1}H as well as the ^{13}CNMR-data (within a range of 2 ppm) are in agreement with those already reported earlier for isoorientin (Markham et al., 1982).

2.4. Animals

Male Wistar rats, 8 weeks old (weighting 280–300 g) obtained from the Bioterium of the Science School, UNAM, and acclimatised with free access to food and water for at least 1 week in an air conditioned room (25 °C with 55% humidity) under a 12-h light:12-h dark cycle prior to the experiments.

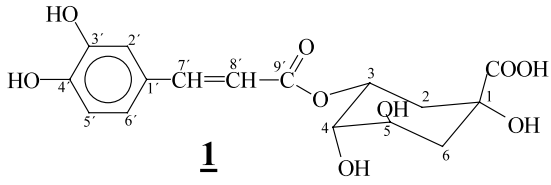
2.5. Induction of experimental diabetes

Diabetes was induced by a single intraperitoneal injection of a freshly prepared streptozotocin (STZ) solution (Sigma, No. 242-646-8) (50 mg/kg in acetate buffer 0.1 M, pH 4.5) to overnight-fasted rats. Control rats received only the buffer.

Diabetes was identified by polydipsia, polyuria and by measuring non-fasting plasma glucose levels 48 h after injection of STZ. Animals, which did not develop more than 250 mg/dl glucose levels, were rejected.

2.6. Experimental groups

The diabetic animals were classified into six groups (1–6) each of them with 11 rats and two groups with six rats (7–8). Group 1 as a control received 1.5 ml of physiological NaCl-solution (vehicle), group 2 was given a standard oral hypoglycemic agent,



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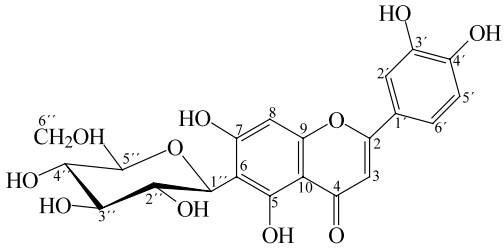

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Fig. 1. Structure of **1** and **2**.

Table 1
Traditional Uses of *Cecropia obtusifolia*

Zone	Local names	Part used	Preparation and administration
San Felipe Usila, Oaxaca, Voucher IMSS11496	Chancarro, Amac'ma	Leaves	Dry leaves are boiled in 1 l water, and the infusion is drunk during the day[a]
Tlanchinol, Hidalgo, Voucher IMSS14140	Chiflador, Hormiguillo	Leaves	Dry leaves are Boiled in 2l water, the infusion is drunk during the day[a]

[a] The amount of boiled plant material goes from three leaves approximately 36 g to five leaves approximately 60 g.

glibenclamide (3 mg/kg bodyweight (bw)), in the same vehicle, while groups 3 and 4 received WE (90 mg/kg bw) and WE (150 mg/kg bw), groups 5 and 6 received BE (9 mg/kg bw) and BE (15 mg/kg bw), respectively. Group 7 received compound **1**, Chlorogenic acid (10 mg/kg bw), and Group 8 received compound **2**, Isoorientin (10 mg/kg bw). The extracts were redissolved in 1.5 ml of physiological NaCl-solution and administered orally by a canule.

2.7. Collection of blood and determination of blood glucose

Blood samples were taken from the tail vein before oral administration of the extracts or the vehicle (time 0) and 60, 120 and 180 min thereafter. Thirty-two μl of blood were used for each assay; the glucose concentration was measured in plasma serum with a Reflotron equipment (Boehringer-Mannheim).

2.8. Statistical analysis

The data were statistically analyzed by unpaired *t*-test. The plasma glucose levels were expressed as the mean (S.E.M.).

3. Results

3.1. Ethnobotany

The results of the field study are summarized in Table 1, where the local name is listed, the voucher herbarium number the used parts of the plant and the preparation and administration. We confirmed that the main use of *C. obtusifolia* is as a hypoglycemic agent. Normally the people drink the infusion of the leaves after boiling between three (approximately 36 g) and five (approximately 60 g) leaves in 1 l water.

3.2. Identification of compounds

3.2.1. Chlorogenic acid (*1*)

LC-MS *m/z* (rel. int): 355.25 $[M]^+ + 1$ $C_{16}H_{19}O_9$ (100) (calc. 355.39), 320.21 (11.5) $C_{16}H_{16}O_7$, 248.15 (73.5) $C_{13}H_{12}O_5$, 220.08 (35) $C_8H_{12}O_7$.

3.2.2. ^1H NMR (δ = ppm) data

7.44 (1H, d, $J_{7',8'} = 15.8$ Hz, C-7′H), 7.04 (1H, d, $J_{2',6'} = 1.5$ Hz, C-2′H), 6.95 (1 H, dd, $J_{6',5'} = 8.2$ Hz, $J_{6',2'} = 1.5$ Hz, C-6′H), 6.76 (1H, d, $J_{5',6'} = 8.2$ Hz, C-5′H), 6.26 (1H, d, $J_{8',7'} = 15.8$ Hz, C-8′H), 5.12 (1 H, dd, $J_{3,2} = 8.5$ Hz (calc. 11 Hz), $J_{3,4} = 9.0$ Hz (calc. 9.2 Hz), C-3H), 3.94 (1H, ddd, $J_{5,4} = 2.8$ Hz (calc. 3 Hz), $J_{5,6\alpha} =$ 2.4 Hz (calc. 3.6 Hz), $J_{5,6\beta} = 1.5$ Hz (calc. 2.8 Hz), C-5H), 3.53 (1 H, dd, $J_{4,3} = 9.0$ Hz, $J_{4,5} = 2.8$ Hz, C-4H), 1.95 (1 H, dd, $J_{6\alpha,6\beta} = 11.9$ Hz, $J_{6\alpha,5} = 2.4$ Hz, C-6H_α), 1.81 (2H, d, $J_{2,3} = 8.5$ Hz, C-2H_2), 1.67 (1 H, dd, $J_{6\beta,6\alpha} = 11.9$ Hz, $J_{6\beta,5} = 1.5$ Hz, C-6H_β). ^{13}C NMR (δ = ppm) data: 168.0 (s, C-9′), 148.9 (s, C-4′), 146.1 (s, C-3′), 146.0 (d, C-7′), 126.7 (s, C-1′), 122.8 (d, C-6′). 116.8 (d, C-5′), 115.5 (d, C-8′), 115.1 (d, C-2′), 76.7 (s, C-1), 73.6 (d, C-4), 72.2 (d, C-3), 72.0 (d, C-5), 40.0 (t, C-2), 38.4 (t, C-6).

3.2.3. Isoorientin: (*2*)

^1H NMR: (400 MHz, DMSO-d_6, δ in *ppm*) data: 7.37 (C-6′H, dd, $J = 8.1$ Hz, 1.9 Hz, 1H), 7.35 (C-8H, s, 1H), 6.82 (C-5′H, d, $J = 8.1$ Hz, 1H), 6.55 (C-3H, s, 1H), 6.35 (C-2′H, d, $J = 1.9$ Hz, 1H), 4.55 (C-1″H, d, $J = 9.9$ Hz, 1H), 3.63 (C-5″H, d, $J = 10.7$ Hz, 1H), 3.40 (C-2″H, dd, $J = 10.5$ Hz, $J = 6.3$ Hz, 1H), 3.20 (C-3″H, d, $J = 8.5$ Hz, 1H), 3.17 (C-4″H, t, $J = 8.2$ Hz, 1H), 3.15 (C-6″H_2, t, $J = 8.0$ Hz, 2H). ^{13}C NMR: (100 MHz, DMSO-d_6, δ = *ppm*) data: 182.1 (C-4, s), 164.1 (C-2, s), 160.9 (C-7, s), 157.4 (C-9, s, C-5, s), 150.6 (C-3′, s), 146.3 (C-4′, s), 121.9 (C-1′, s), 119.8 (C-6′, d), 116.8 (C-5′, d), 113.4 (C-2′, d), 109.5 (C-6, s), 103.1 (C-3, d), 102.9 (C-10, s), 95.1 (C-8, d), 81.8 (C-5″, d), 79.4 (C-3″, d), 73.9 (C-1″, d), 70.9 (C-2″, d), 70.7 (C-4″, d) 61.9 (C-6″, t).

3.3. Activity in diabetic rats

STZ administration at a dosage of 50 mg/kg bw to normal rats significantly ($P < 0.001$) elevated the blood glucose levels compared with rats injected citrate buffer alone as reported (El-Fiky et al., 1996) for albino rats.

In our diabetic rats the extracts as well as the two isolated compounds both showed significant hypoglycemic effects. Table 2 and Fig. 2.

The water extract at doses of 90 mg/kg bw showed activity at 60 min, with a significant reduction ($P <$ 0.001). After 60 min the significance was reduced to

Table 2
Effect of oral administration of aqueous and butanolic extracts of *Cecropia obtusifolia* aerial parts on plasma glucose concentration in diabetic rats

Dose (mg/kg)	Plasma glucose (mg/ml) at			
	0 h	1 h	2 h	3 h
Control (Saline 2.5 ml)	316 ± 4	324 ± 5	314 ± 5a	312 ± 4
Glibenclamide (mg/kg)	311 ± 6	298 ± 6***	262 ± 6***	245 ± 8***
Water extract (90 mg/kg)	305 ± 8	282 ± 7***	278 ± 8**	259 ± 11***
Water extract (150 mg/kg)	308 ± 7	281 ± 7***	256 ± 7***	257 ± 8***
Butanol Extract (9 mg/kg)	310 ± 6	292 ± 8**	265 ± 8***	260 ± 11***
Butanol Extract (15 mg/kg)	303 ± 8	276 ± 7***	262 ± 7***	257 ± 6***
1 Chlorogenic acid (10 mg/kg)	304 ± 6	270 ± 10***	247 ± 12***	226 ± 11***
2 Isoorientin (10 mg/kg)	303 ± 5	265 ± 8***	229 ± 11***	221 ± 12***

The values represent the mean ± S.E.M. The number of rats for **1** and **2** was eight for the rest of the groups was = 11; $^{**}P<0.005$, $^{***}P<0.001$ as compared with control time intervals.

$P < 0.005$, and went down again at 120 min to $P < 0.001$. The water extract at doses of 150 mg/kg bw showed activity from 60 to 180 min with $P < 0.001$.

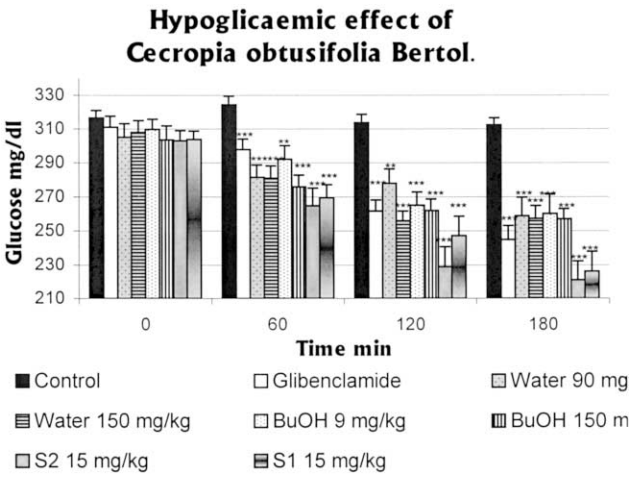

Fig. 2. Effect of oral administration of water and butanolic extract of aerial parts of *Cecropia obtusifolia* in diabetic rats. The number of rats was 11 in all cases. $^{**}P < 0.005$ and $^{***}P < 0.001$ as compared with control time intervals.

The maximum effect of the water extracts was observed after 180 min.

The butanolic extract led to a significant decrease in plasma glucose level compared with the control, at doses of 9 mg/kg bw the effect was significant after 60 min with $P < 0.005$ ongoing with $P < 0.001$ at 120 and 180 min. At doses of 15 mg/kg bw the activity was significant since 60 min until 180 min with $P < 0.001$. The maximum activity was observed after 180 min comparable to the water extract.

Chlorogenic acid **1** and the Isoorientin **2** showed a similar activity with $P < 0.001$ at 60 min ongoing to 120 and 180 min, with the same significance and the glibenclamide group (3 mg/kg) produced a significant decrease compared with the controls, with $P < 0.001$ at 60 min until 180 min.

Those results support acceptance of the null hypothesis that there is no significant difference between the tested plant mediums in comparison to glibenclamide (= standard hypoglycemic drug).

4. Discussion

Our own ethnopharmacological studies confirm the earlier reported data for the plant *C. obtusifolia* which is traditionally used as an infusion of (mainly) dried leaves by the Mexican population against type II diabetes.

There is no previous report about a hypoglycemic activity of isoorientin or chlorogenic acid. However, isorientine is reported to show antimicrobiological and antispasmodic activities (Afifi et al., 1999). Besides this, a hypoglycemic activity of some flavonoids is described and reported in literature (Lamba et al., 2000).

The STZ diabetes induction and the use of glibenclamide in this animal model were previously discussed (Andrade-Cetto et al., 2000).

Both, water and butanolic extracts of *C. obtusifolia* produce hypoglycemic effects in rats. This is in accordance with a previous report where water infusions of the plant at unspecified concentration and administered doses were used in alloxan diabetic mice (Pérez et al., 1984). Furthermore our results are similar to those, which described a hypoglycemic effect of a water extract of the same plant in hyperglycemic rabbits at doses of 528 mg/kg (Roman-Ramos et al., 1991). In contrast to the last study which described the maximum effect at 240 and 300 min. we found that the effect is significant after 60 min $P < 0.005$ and had an increased effect ($P < 0.001$) at 120 and 180 min.

Summarizing this present study we found activities from 60 min until 180 min for both extracts. These data are comparable to those that we found after glibenclamide administration.

Thus, one can speculate that the water as well as the butanolic extract of *C. obtusifolia* may possess a glibenclamide-like effect.

Besides this, we demonstrated that the main components in the plants are isoorientin and chlorogenic acid and that both substances are mainly present in both extracts, too. As these compounds showed also significant hypoglycemic activity it may be assumed that these compounds are involved in the hypoglycemic effect of *C. obtusifolia*.

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