

Hypoglycemic effect of *Acosmium panamense* bark on streptozotocin diabetic rats

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Abstract

The hypoglycemic effects of water and butanolic extracts prepared from the bark of *Acosmium panamense* (Fabaceae) were studied in diabetic rats (streptozotocin (STZ)-induced). Oral application of water extracts at doses of 20 and 200 mg/kg and of butanol extracts at doses of 20 and 100 mg/kg significantly lowered the plasma glucose levels in diabetic rats within 3 h. Glibenclamide was used as reference and showed similar hypoglycemic effect like the extracts.

Three structurally new compounds were isolated from the plant and shown to be the main constituents in both extracts.

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1. Introduction

Acosmium panamense (Benth.) Yacolev, syn, *Sweetia panamensis* Benth., traditional names “Guayacán” and “Bálsamo amarillo” is a tree up to 40 m high, growing in form of codominant species in the tropical rain forest. The main characteristic of the tree is a tall, straight trunk pyramidal treetop with ascendant branches. The external cortex is plain, scamosus, and dark gray, the inner cortex is yellow and bitter (Pennington and Sarukhán, 1998).

In Oaxaca State the plant is traditionally used for the treatment of stomach pain, respiratory problems, diarrhea, malaria, and “marsh fever”. The plant medicine is prepared as an infusion of the bark and it is taken orally 1–2 times per day. In addition, *Acosmium panamense* is utilized to treat diabetes in the village of Sotapan, Veracruz (Leonti, 2002).

Phytochemical studies of the plant yielded to the isolation of several quinolizidine alkaloids like acosmine and acosminine, hydroxysparteine, as well as lupinane alkaloids (Balandrin and Kinghorn, 1982; Argueta, 1994; Veitch et al., 1997; Nuzillard et al., 1999).

As the described compounds were isolated from alcoholic plant extracts, the aim of our study was to investigate

the hypoglycemic effect of hydrophilic extracts from *Acosmium panamense* in streptozotocin (STZ)-induced diabetic rats and to identify the main chemical constituents in these extracts.

2. Materials and methods

According to our previous studies (Andrade-Cetto et al., 2000; Andrade-Cetto and Wiedenfeld, 2001) and related definitions (Holmsted, 1991), we performed an ethnopharmacological study focuses on ethnobotanical, phytochemical, and pharmacological methodologies.

2.1. Ethnobotany

Ethnobotanical studies were conducted during several visits to the community of San Felipe Usila, Oaxaca, in the period 1997–1999. We followed the method already described (Andrade-Cetto, 1999; Andrade-Cetto and Wiedenfeld, 2001). Diabetic people were identified by the local health services and local healers. All ethnobotanical data were collected through structured and unstructured interviews with the traditional healers and the diabetic people, respectively. The data were referred to plant samples (mini-herbarium) collected at its natural habitats, and then stored as herbarium vouchers for exact identification.

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2.2. Materials

With the help of traditional healers and diabetic people, samples of *Acosmium panamense* were collected in San Felipe Usila, Oaxaca, Mexico. Their identity was confirmed and voucher specimen (IMSS14649) was deposited at the IMSS herbarium in Mexico City.

2.3. Preparation of the extracts and isolation of compounds

Plant extracts were prepared from bark samples (300 g) as already described (Andrade-Cetto et al., 2000), resulting in 36 g of aqueous extract (WE) and 3.12 g of butanolic extract (BE). The latter was used for the phytochemical identification of the main components. The BE was applied on a 100 cm × 2 cm Polygoprep 60–30 C₁₈ (Macherey & Nagel, Düren, Germany) flash-column and eluted with H₂O/MeOH/AcCN 80:10:10, 4 ml/min (10 ml fractions; monitored by UV-detection and controlled by HPLC).

2.4. Animals

Male Wistar rats were used; 8 weeks old (weighting 200–250 g) obtained from the Bioterium of the Science School, UNAM, and acclimatized 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 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 seven groups (1–7) each of them with 11 rats. Group 1 as the control received 1.5 ml of physiological NaCl-solution (vehicle), the rats of group 2 were given the standard oral hypoglycemic agent glibenclamide (3 mg/kg bodyweight (bw)) in the same vehicle, while groups 3 and 4 received WE (20 mg/kg bw) and WE (200 mg/kg bw), groups 5 and 6 received BE (20 mg/kg bw) and BE (100 mg/kg bw), respectively. Group 7 received a mixture of the isolated compounds (M) 20 mg/kg. 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 and at times 60, 120, and 180 min thereafter. Thirty-two microliters of blood were used for each assay; the glucose concentration was measured in plasma serum with a Reflotron equipment and confirmed by a Accutrend GCEquipment (Roche).

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 our field study in San Felipe Usila confirmed that *Acosmium panamense* is mainly used as a hypoglycemic agent against diabetes type II. The tree is locally named by his Spanish name “Guayacán” or by his Chinantec name Amać nain. In general the people drink the infusion of the bark after boiling 20–30 g in 1 l water. This tea is taken orally during the day as “agua de uso”. Those results confirm the previously reported use of *Acosmium panamense* for the treatment of diabetes type II.

3.2. Compounds

Besides caffeic acid we found three pyrones. The structures of the isolated compounds **1**, **2**, and **3** (Fig. 1), were determined by GC-mass and by homo- and hetero-nuclear 2D-NMR correlated spectroscopy. Besides the already known **1**; desmethylangonine (= 6[(*E*)-2-(4-hydroxyphenyl)vinyl]-4-methoxy-2*H*-pyron-2-one) we isolated as new compounds its; β-D-O-glucoside **2**, and its; β-D-O-di(1-6)glucoside **3**. (Wiedenfeld and Andrade-Cetto, 2003).

3.3. Activity in diabetic rats

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

In our diabetic rats, the extracts as well as the isolated compounds both showed significant hypoglycemic effects (Table 1 and Fig. 2).

The water extract at doses of 20 mg/kg bw showed activity at 180 min, with a significant reduction ($P < 0.01$) of plasma glucose levels. The water extract at a dosage of 200 mg/kg bw showed the same activity at 180 min, but the significance was higher ($P < 0.001$). The maximum effect of both water extracts was observed after 180 min of treatment.

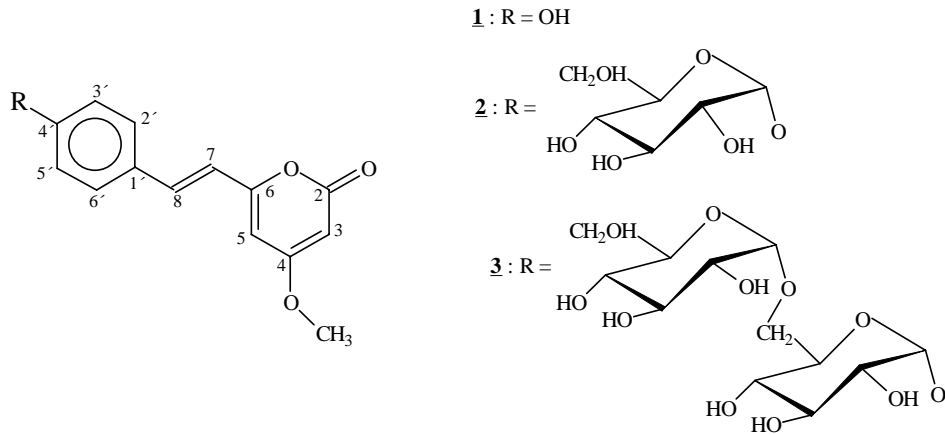


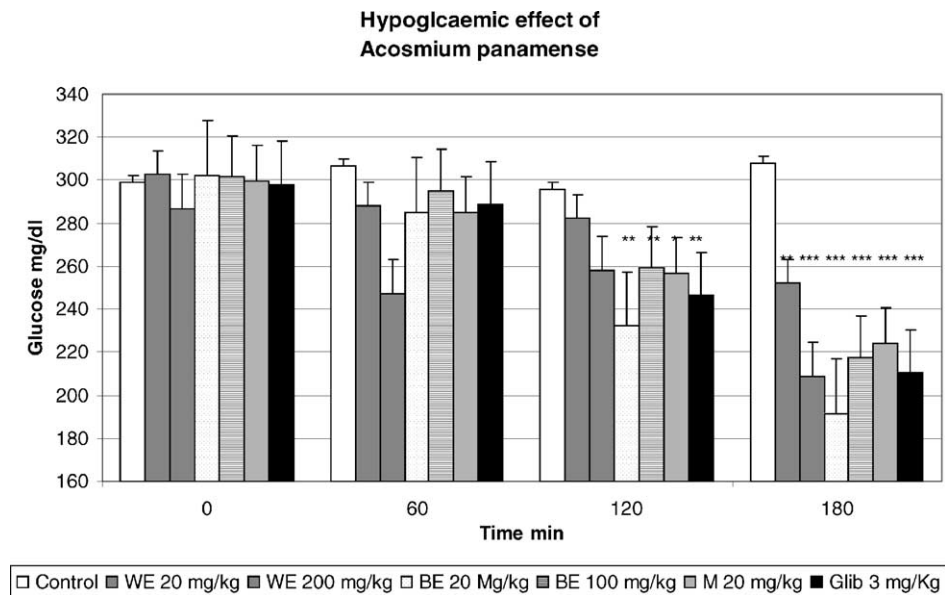
Fig. 1. Structures of isolated compounds 1 to 3.

Table 1
Effect of oral administration of aqueous and butanolic extracts of *Acosmium panamense* bark on plasma glucose concentration in diabetic rats

Dose (mg/kg)	Plasma glucose (mg/ml)			
	0 h	1 h	2 h	3 h
Control (Saline 2.5 ml)	299 ± 9	307 ± 9	296 ± 12	308 ± 11
Glibenclamide (mg/kg)	298 ± 8	289 ± 14	246 ± 13**	211 ± 14***
Water extract (20 mg/kg)	303 ± 11	288 ± 13	282 ± 15	252 ± 15**
Water extract (200 mg/kg)	287 ± 11	247 ± 12	258 ± 14	208 ± 12***
Butanol extract (20 mg/kg)	302 ± 6	285 ± 6	232 ± 16**	192 ± 20***
Butanol extract (100 mg/kg)	301 ± 13	395 ± 12	259 ± 10**	218 ± 15***
Mixture of 2, 3 (20 mg/kg)	300 ± 10	285 ± 19	257 ± 12*	224 ± 13***

The values represent the mean ± S.E.M.

The number of rats was 11 in all cases. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ as compared with control time intervals.



* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ as compared with control time intervals.

Fig. 2. Effect of oral administration of water and butanolic extract of the bark of *Acosmium panamense* in diabetic rats. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ as compared with control time intervals.

The butanolic extract led to a significant decrease in plasma glucose level compared with the control, at doses of 20 mg/kg bw. The effect was significant after 120 min with $P < 0.01$ ongoing with $P < 0.001$ at 180 min. At doses of 100 mg/kg bw the activity was significant from 120 to 180 min with $P < 0.01$ and $P < 0.001$, respectively. The maximum activity was observed after 180 min comparable to the water extract.

The mixture of **2** and **3** showed a similar activity with $P < 0.01$ at 120 min ongoing to 180 min. The glibenclamide group (3 mg/kg) produced a significant decrease with $P < 0.05$ at 120 min ongoing to $P < 0.01$ at 180 min.

Those results indicate that there is no significant difference between the tested plant preparations in comparison to glibenclamide (standard hypoglycemic drug) and a mixture of the isolated compounds **2** and **3**.

4. Discussion

Our ethnopharmacological studies as well as our experimental pharmacological data confirm that *Acosmium panamense* is traditionally used in San Felipe Usila as an infusion of the bark for the treatment of diabetes type II and there exist a clear hypoglycaemic activity in our animal studies.

The diabetes induction by STZ and the use of glibenclamide in this animal model were discussed previously (Andrade-Cetto et al., 2000; Andrade-Cetto and Wiedenfeld, 2001).

Both, water and butanolic extracts of *Acosmium panamense* bark produce a hypoglycemic effect in rats. The water extracts show a significant activity after 180 min; the higher dosage shows a higher activity ($P < 0.001$) compared with the lower one ($P < 0.01$). The butanolic extracts lead to a similar activity after 120 min up to 180 min at $P < 0.001$. A similar effect can be observed by giving a mixture of isolated compounds. Those data lead to the assumption that the mixture of compounds **2** and **3** witch are the main constituents in the water as well as in the butanolic extract could be responsible for the measured activity.

Further studies will be done to determine the kind of action of the whole extract as well as for the isolated compounds.

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