

Hypoglycemic effect of *Equisetum myriochaetum* aerial parts on streptozotocin diabetic rats

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Abstract

The hypoglycemic effect of water as well as butanolic extracts prepared from aerial parts of *Equisetum myriochaetum* (Equisetaceae) was examined in streptozotocin induced diabetic rats. A single oral administration of the water extract (WE) at doses of 7 and 13 mg/kg and of the butanol extract (BE) at doses of 8 and 16 mg/kg significantly ($P < 0.001$) lowered the plasma glucose levels in diabetic rats after three hours of the administration. As a reference drug glibenclamide was used and showed, at a dose of 3 mg/kg, similar hypoglycemic effect like the tested extracts. Three kaempferol glucosides and one caffeoyl glucoside were isolated from the drug and were shown to be the main constituents in both extracts. © 2000 Published by Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Equisetum myriochaetum Schlecht and Cham (Equisetaceae, traditional name: ‘cola de caballo’) is a plant showing aerial stems 2–5-m high, branched with regular verticillies 2–23 mm in diameter with 16–48 channels, terminal strobile in the branches and in the main stem 10-mm long and 4 mm in diameter (Palacios-Rios, 1999). Ethnopharmacologically, the plant is described to

show activities against kidney diseases (Argueta, 1994).

Furthermore, water decoctions of ‘cola de caballo’ have been, traditionally, used to treat diabetes, especially in the southern Mexican state of Guerrero. It is reported that the administration of the aqueous extract of *E. myriochaetum* should reduce the blood glucose level in type 2 diabetic patients. The traditional healers use the aerial part of the dried plant; it is boiled in water and drunken daily as the so-called ‘agua de uso’.

Besides, this the plant is also sold in several medicinal markets mixed together with other

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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 *E. myriochaetum* in streptozotocin-induced diabetic rats.

Phytochemically, we could identify flavonol glycosides present as the main constituents in water as well as in butanol extracts (Wiedenfeld et al., 2000).

2. Materials and methods

2.1. Materials

Authentic samples of *E. myriochaetum* were collected in Xochipala Guerrerro, México. They were identified by Mónica Palacios-Rios, Ecological Institute at Jalapa Veracruz. A voucher specimen IMSSM 11266 was deposited at the IMSS Herbarium in México City.

2.2. Preparation of the extracts and isolation of compounds

The plant (aerial parts, 700 g) extracts were prepared as follows — the first extract was produced using the dried powdered material and extracted with water by refluxing during 4 h, the extract was lyophilized and stored in screw cap vials at 4°C (WE).

The second extract was prepared with the same quantity of plant material by Soxhlet extraction. Defatting with n-hexane (24 h) was followed by MeOH (48 h) extraction. The methanolic extract was evaporated under reduced pressure to dryness. The residue was partitioned in a mixture of CCl₄/80% MeOH 1:1. The MeOH/H₂O phase was evaporated to dryness. The residue was dissolved in n-BuOH/H₂O 1:1 the BuOH layer was dried by lyophilization and stored (BE).

For phytochemical identification of the main components, the BE was applied on a 100 × 2 cm Polyoprep 60–30 C₁₈ (Macherey and Nagel, Düren, Germany) flash-column and eluted with H₂O/MeOH/AcCN 80:10:10, 4 ml/min (10-ml fractions; **1**, fr. 29–35; **2**, 20–25; **3**, 15–19; **4**, 26–28). The resulting fractions were monitored by high

performance liquid chromatography (HPLC) (ET 250/8/4 Nucleosil 120–5 C₁₈, Macherey and Nagel; 0.04 M H₃PO₄/AcCN/MeOH, 0–9 min, 85/8/7–70/15/15–20 min, 70/15/15; 1.5 ml/min; 220 and 255 nm UV-det. *R*_t of **1**, 13.2; **2**, 11.0; **3**, 6.6; **4**, 9.5 min). Prep. HPLC (SP 250/10 Nucleosil 120–7 C₁₈, Macherey and Nagel) was used for final purification yielding compounds **1**, 25; **2**, 20; **3**, 12; **4**, 7 mg. The structures were identified by spectroscopical methods.

2.3. Animals

Male Wistar rats, 6-weeks-old (weighing 180–200 g) obtained from the Bioterium for the Science School were housed 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:dark cycle prior to the experiments.

2.4. 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.

The diabetic animals were classified into six groups, each of them with eleven rats. Group 1 as a control received 2.5 ml of physiological NaCl-solution (vehicle), group 2 was given a standard oral hypoglycemic agent, glibenclamide (3 mg/kg bodyweight (bw)) in the same vehicle, while groups 3 and 4 received WE (7 and 13 mg/kg bw, respectively) and groups 5 and 6 received BE (8 and 16 mg/kg bw, respectively). The extracts were redissolved in 2.5 ml of physiological NaCl-solution.

2.5. Collection of blood and determination of blood glucose

Blood samples were taken from tail vein before oral administration of the extracts or the vehicle

(time 0) and 60, 120 and 180 min thereafter. Blood (32 μ l) were used for each assay; the glucose concentration was measured in plasma serum with a Reflotron equipment (Boehringer-Mannheim).

2.6. Statistical analysis

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

3. Results

3.1. Identification of compounds

The preparative HPLC analysis yielding compounds **1**, kaempferol-3-*O*-sophoroside; **2**, kaempferol-3, 7-di-*O*- β -glucoside; **3**, kaempferol-3-*O*-sophoroside-4'-*O*- β -glucoside; **4**, caffeoyl-methylate-4- β -glucopuranoside. Structural data of **1** and **2** correspond to those found in literature (Markham et al., 1982; Budzianowski, 1990). Kaempferol-3-*O*-sophoroside-4'-*O*- β -glucopyranoside (**3**) was first isolated from *Asplenium septentrionale* and confirmed by FAB-MS and ¹HNMR as described (Wiedenfeld et al., 2000).

3.2. 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 diabetic rats, both extracts (WE and BE) showed significant hypoglycemic effects. The WE at a doses of 7 and 13 mg/kg bw lead to a significant decrease in the plasma glucose level compared with the controls; the effect was significant from 60 min with $P < 0.005$ ongoing with $P < 0.001$ at 120 and 180 min. The maximum effect was observed after 3 h.

The BE at doses of 16 mg/kg bw showed activity at 60 min, too, with a significant reduction of $P < 0.001$. After 60 min, the significance is reduced to $P < 0.01$, and went down again at 120 min to $P < 0.001$. At doses of 8 mg/kg bw the BE showed activity at 120 min with a significant reduction of $P < 0.001$. After 180 min, the significance is also reduced to $P < 0.001$, the maximum effect for both doses of the BE was observed after 3 h comparable to the WE.

The glibenclamide group (3 mg/kg) produced a significant decrease compared to the controls, with $P < 0.01$ at 60 min, going down to $P < 0.001$ at 120 and 180 min. These results provide evidence that there exists no significant difference between the plant extracts themselves as well as between the glibenclamide group compared with each of both extracts.

The non parametric Mann–Whitney rank test gave us the same results as the unpaired *t*-test. Table 1, shows the experiment's data. Fig. 1 shows the results of the treatment of diabetic rats after oral application of the plant extracts, glibenclamide and control rats.

Finally, preliminary tests with the isolated compounds from *E. myriochaetum* show that the kaempferol-3-*O*-sophoroside-4'-*O*- β -D-glucoside (**s3**) produces a blood-glucose lowering activity in a similar range like the complete BE. On account of less amounts of **3** this could only be tested with a small number of animals Table 1. Further experiments have to be carried out to probe the hypoglycemic effect.

4. Discussion

The STZ-induction in adult animals produces a type 2 diabetes mellitus model. STZ selectively destroys the pancreatic insulin secreting β -cells, what leaves less active pancreatic cells and results in a diabetes mellitus (Gilman et al., 1990).

The acute hypoglycemic action of glibenclamide is the stimulation of the insulin release and the inhibition of glucagon secretion. Findings indicate the effectiveness of glibenclamide in moderate diabetic rats, and ineffectiveness in severe diabetic animals (Ivorra et al., 1988, Sharma et al., 1997).

Table 1

Effect of oral administration of aqueous and butanolic extracts of *E. myriochaetum* aerial parts on plasma concentration in diabetic rats^a

Dose (mg/kg)	Plasma glucose (mg/ml) at			
	0 h	1 h	2 h	3 h
Control(saline 2.5 ml)	323 ± 5	326 ± 5	312 ± 4	317 ± 4
Glibenclamide (3 mg/Kg)	316 ± 5	298 ± 9**	273 ± 10***	247 ± 14***
Water extract (7 mg/Kg)	314 ± 5	294 ± 7**	261 ± 11***	251 ± 11***
Water extract (13 mg/Kg)	312 ± 6	292 ± 9**	258 ± 13***	249 ± 13***
Butanol extract (8 mg/Kg)	312 ± 12	304 ± 16	279 ± 12*	252 ± 17***
Butanol extract (16 mg/Kg)	316 ± 7	292 ± 5***	283 ± 9**	255 ± 11***
s3	314 ± 3	263 ± 12***	229 ± 6***	192 ± 8***

^a The values represent the mean ± S.E.M.; the number of rats for s3 was three, for the rest of the groups was 11; * $P < 0.01$; ** $P < 0.005$; *** $P < 0.001$ as compared with control time intervals.

Not totally destroyed β -cells result in a moderate diabetes and then hypoglycemic drugs like glibenclamide can stimulate those cells. In severe diabetes the β -cells are totally destroyed; with no glibenclamide effect.

The WE at a dosage of 7 and 13 mg/kg bw showed an activity from 60 min until 180 min whereas the activity of the BE at doses of 8 mg/kg bw start after 120 min while the dosage of 16 mg/kg bw also started at 60 min. The results of the present study with moderate diabetic animals demonstrate that the WE and BE of *E. myriochaetum* show a hypoglycemic activity in diabetic rats corresponding to the reported traditional use of the plant.

We found an activity comparable to that of glibenclamide, therefore, one can speculate that the WE and BE of *E. myriochaetum* may possess a glibenclamide-like effect stimulating the insulin secretion.

It could also be demonstrated that there are four main compounds (flavonol glycosides and one caffeoylglycoside) which are present as the main constituents in both extracts.

There exist already reports about hypoglycemic activities of Kaempferol derivatives containing plant extracts, Kaempferol 3-*O*-galactoside and Kaempferol 3-rhamno glucoside from *Bahuvia variegata* (Andrade-Cetto, 1999), Kaempferol 3-*O*-rhamnoside from *Zizhyphus rugosa* (Khosa et al., 1983), Kaempferol 3-*O*- β -glucopyranoside from *Morus insignis* (Basnet et al., 1993), and

Kaempferol-3-*O*-(2gal-rhamnosilobonoside) from *Sterculia rupestris* (Desoky and Youssef, 1997).

On account of our results and the reports about the activities of the compounds mentioned previously, it can be assumed that the kaempferol-3-*O*-sophoroside-4'-*O*- β -D-glucoside is one of the responsible factors for the hypoglycemic effect of *E. myriochaetum*.

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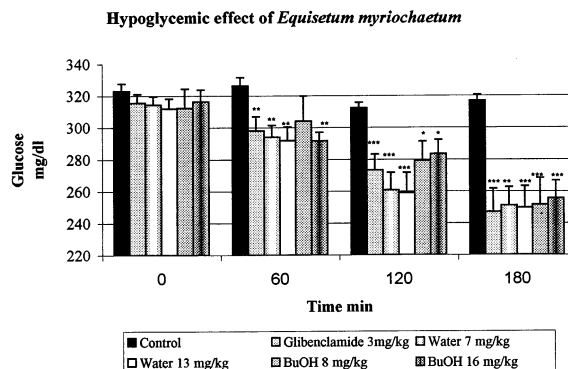


Fig. 1. Effect of oral administration of water and butanolic extracts of aerial parts of *E. myriochaetum* in diabetic rats. The number of rats was 11 in all cases. * $P < 0.01$, ** $P < 0.005$ and *** $P < 0.001$ as compared with control time intervals.

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