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## Hypoglycemic effect of *Tournefortia hirsutissima* L., on *n*-streptozotocin diabetic rats

Adolfo Andrade-Cetto<sup>a,\*</sup>, Cristina Revilla-Monsalve<sup>b</sup>, Helmut Wiedenfeld<sup>c</sup>

<sup>a</sup> Laboratorio de Etnofarmacología, Fac. Ciencias, Universidad Nacional Autónoma de México, 04510 México DF, Mexico

<sup>b</sup> Unidad de Investigación Médica en Enfermedades Metabólicas Centro Médico Nacional SXXI, 06725 México DF, Mexico

<sup>c</sup> Pharmaceutical Institute, University of Bonn, An der Immenburg 4, D-53121 Bonn, Germany

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### Abstract

The hypoglycemic effect of aqueous and butanolic extracts from *Tournefortia hirsutissima* (Boraginaceae) was determined on neonatal induced streptozotocin diabetic rats (*n*-STZ). Oral administration of water extracts at doses of 20 and 80 mg/kg, and butanolic extracts (8 and 80 mg/kg) significantly lowered the plasma glucose levels in diabetic rats within 3 h. Glibenclamide was used as reference and showed similar hypoglycemic effect. Our results support the traditional use of the plant as a hypoglycemic agent; we observe a dose-dependent action of the extracts. HPLC analysis confirmed that the aqueous and butanolic extracts had the same chemical composition.

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**Keywords:** *Tournefortia hirsutissima*; Hypoglycemic effect; *n*-Streptozotocin diabetic rats; Type 2 diabetes

### 1. Introduction

Type 2 diabetes is a public health problem. According to the World Health Organization (WHO, 2006) more than 176 million patients are affected by this disease in the world. In México, the WHO estimates that the number of diabetic patients will increase from more than two million in 2002 to more than six million in 2030, which would imply that in a few decades, Mexico may have the highest rate of diabetes in the world. According to the Mexican health services, in 2001, diabetes was the first cause of mortality among the Mexican population (SSA, 2006). Due to the complications associated to diabetes like heart disease, retinopathy, nephropathy, and neuropathy, it is also a common cause of chronic morbidity and disability among the working population.

The use of medicinal plants is a tradition for the Mexican population; recently we documented the use of 306 species to

treat diabetes (Andrade-Cetto and Heinrich, 2005), however, only few species have been studied to proof their hypoglycaemic effect.

#### 1.1. Plant description

*Tournefortia hirsutissima* L. (Boraginaceae), traditional name: ‘Lagrima de San Pedro’, is a woody vinegar, with cylindrical pubescent stems, leaves are simple and pubescent, alternate and pinnately veined without stipules, compound inflorescences, terminal or axilar on helicoid cyme (bostrix), bisexual flowers: white or yellow to greenish, five lobulate, fruit, white berries (Campos-Villanueva et al., 2004). The plant is distributed from Florida, USA to Brazil.

#### 1.2. Plant background

*Tournefortia hirsutissima* was reported by Martínez (1954) for the treatment of rheumatism, and furthermore against diabetes (Andrade-Cetto and Heinrich, 2005). It is also used to treat kidney problems (Aguilar et al., 1994).

The traditional use against diabetes, to our group, was firstly reported by the people of the Mexican state Veracruz; nowadays its usage is found in the whole central part of Mexico.

**Abbreviations:** DER, ratio of the herbal drug to the native herbal drug preparation; BE, butanolic extract; WE, water extract; (*n*-STZ), neonatal induced type 2 diabetic rats.

\* Corresponding author at: Apartado Postal 70-359, 04511 México DF, Mexico. Tel.: +52 5556224834; fax: +52 5556224828.

E-mail address: [aac@hp.ciencias.unam.mx](mailto:aac@hp.ciencias.unam.mx) (A. Andrade-Cetto).

Alarcón Aguilar et al. (1998) administered a decoction of the stem wood at a dose of 45 mg/kg BW, to healthy rabbits on a glucose tolerance test (no diabetic model). They reported a significant decrease of the blood glucose levels only at 120 min after the first administration of 2 g/kg of glucose solution when compared with the control group. Recently, Ortiz-Andrade et al. (2005) reported the hypoglycemic effect of a related species, *Tournefortia hartwegiana*, on a type 1 diabetes model.

### 1.3. Animal model background

Here, we report for the first time the use of the neonatal induced streptozotocin (*n*-STZ) model for the study of the hypoglycemic effect of a Mexican plant. According to Verspohl (2002) and Portha et al. (2002), the *n*-STZ model is adequate for testing type 2 diabetes. 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 6–15 weeks of age, an impaired glucose disposal rate and significant beta-cell secretory dysfunction (type 2 diabetes) is observed (Verspohl, 2002).

## 2. Objective

The aim of the work was the study of the hypoglycemic effect of water and butanolic extracts of the plant on streptozotocin (*n*-STZ) induced diabetic rats and the analysis of the main constituents in those extracts.

## 3. Materials and methods

According to our previous studies (Andrade-Cetto et al., 2000, 2005; Andrade-Cetto and Wiedenfeld, 2001, 2004) and further definitions (Holmsted, 1991) and (Etkin, 2005), we performed an ethnopharmacological study with respect to ethnobotanical, phytochemical and pharmacological methodologies.

### 3.1. Ethnobotany

The traditional use of the plant was recorded at the market place of Sonora, México City and Veracruz, Veracruz; from there the use of the plant was traced to small stores at Palo Gacho, Veracruz and finally to a traditional healer in Rancho Viejo, Veracruz (200 km away from Mexico City) who sells the plant at those locations.

### 3.2. Plant materials

With the help of the main healer from Rancho Viejo, Veracruz, the plant was collected in its natural habitats in 2003, 2004 and 2005; botanically determined, voucher specimens were deposited at the IMSS Herbarium in Mexico City. (IMSS15006, IMSS15007 and IMSS 1008).

### 3.3. Preparation of the extracts and detection of compounds

Two different types of plant extracts were prepared from stem samples:

- (1) In order to obtain an aqueous extract, similar to the traditional recommendation, 15 g of coarsely fragmented stems were placed in a 2 L flask with 1 L of distilled boiling water for 10 min. The extract was filtered through a sieve and a fine nylon mesh, and lyophilized, resulting in 1.4 g of dry extract (WE), DER native 10.7:1. (Gaedcke and Steinhoff, 2003).
- (2) The butanolic extract (BE) was prepared as already described (Andrade-Cetto et al., 2000) from stem material (145 g) resulting in 2.83 g of extract DER native 51:1. The BE was used for the phytochemical detection of the main components by application on a 100 cm × 2 cm Polygoprep 60-30 C<sub>18</sub> (Macherey and Nagel, Düren, Germany) flash-column and eluted with H<sub>2</sub>O/MeOH/AcCN 70:15:15, 4 ml/min (10 ml fractions; monitored by UV-detection and controlled by Diode Array Detector HPLC).

### 3.4. Animals and induction of experimental 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. Non-diabetic control group received only buffer i.p.

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 (23 °C with 55% humidity) under a 12-h light:12-h dark cycle, at the Bioterium of the Science School, UNAM. The animal handling was in accordance with the Federal Government Legislation on Animal Care.

After 12 weeks, diabetes was identified by polydipsia, polyuria and by measuring fasting plasma glucose levels. Male and female rats with glucose levels >150 mg/dl were included in the study.

### 3.5. Experimental groups

The diabetic animals were classified into 7 groups (1–7) each of them with 11 rats. Group 1 non-diabetic control received 1.5 ml of physiological NaCl-solution (Vehicle), group 2 diabetic control received also 1.5 ml of physiological NaCl-solution (vehicle), rats of group 3 were treated with the standard oral hypoglycemic agent glibenclamide (3 mg/kg bodyweight (BW) in the same vehicle, groups 4 and 5 received WE (20 mg/kg bw) and WE (80 mg/kg bw), respectively, groups 6 and 7 received BE (8 mg/kg bw) and BE (80 mg/kg bw). The extracts were redissolved in 1.5 ml of physiological NaCl-solution and administered orally by a canule.

### 3.6. Collection of blood and determination of blood glucose

Blood samples were obtained from the tail vein (according to the Guideline 9 (3/10/99) IACUC, 1999) before the oral adminis-

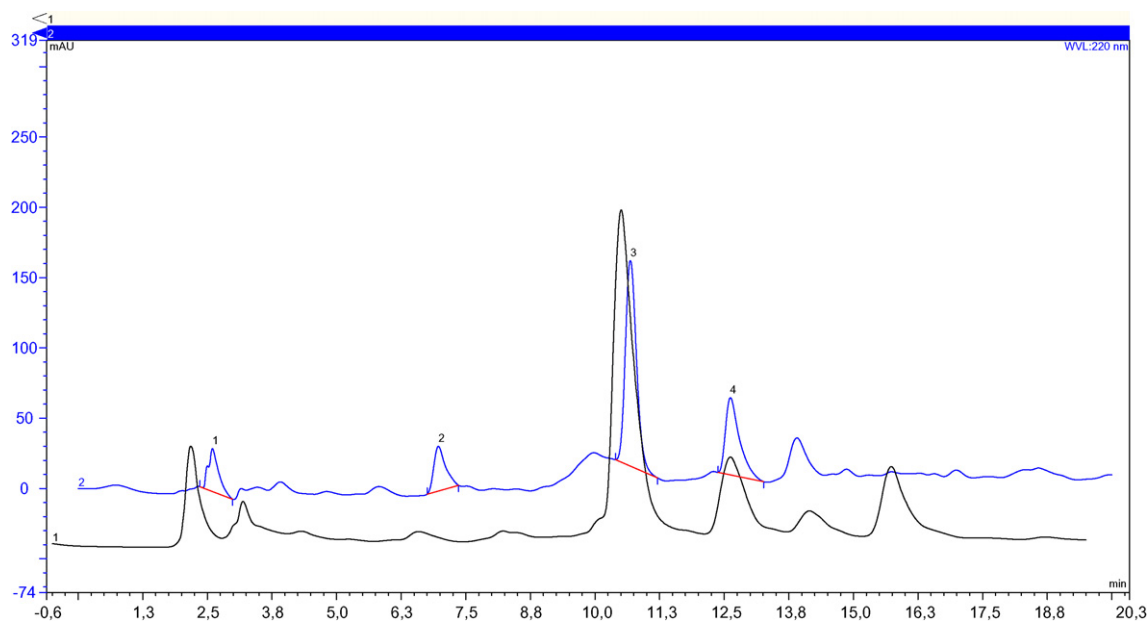


Fig. 1. DAD, HPLC of *Tournefortia hirsutissima*, lower line water extract, upper line BUOH extract; a main compound (number 3) can be observed in both extracts.

tration of the extracts or the vehicle (T0) and at times T60, T120 and T180 min thereafter. Thirty-two microliters of blood were used for each assay; the glucose concentration was measured in plasma serum with Reflotron equipment and confirmed by Accutrend GC and Accu-check compact equipments (Roche).

### 3.7. Statistical analysis

The data were statistically analyzed by one-way ANOVA followed by Tukey's test. The plasma glucose levels were expressed as the mean (S.E.M.).

## 4. Results

### 4.1. Ethnobotany

The results of our field study in Rancho Viejo Veracruz, the market places of Palo Gacho, Veracruz, and Veracruz City confirmed that *Tournefortia hirsutissima* is used as a hypoglycemic agent to treat type 2 diabetes. The plant is named by its Spanish name "Lagrima de San Pedro", because if the woody stem is transversally cut, one can see a "tear" inside.

The people drink the infusion of the dry stem after boiling 12–15 g in 1 l water, this infusion is named by the Spanish name; "agua de uso", which is drunk over the day. These results confirm the traditional use of *Tournefortia hirsutissima* for the treatment of type 2 diabetes.

### 4.2. Compounds

By HPLC analysis, we could detect one main (number 3 see Fig. 1) and further 3 compounds in minor concentration in the BE as well as in the WE (Fig. 1), by the HPLC–DAD spectra (Fig. 2), we could determinate that the compound number 3 is the same in both extracts.

### 4.3. Animal model

A comparison of the glucose levels of the *n*-STZ rats with those of the normal STZ-rats (STZ injection in adult state) (Andrade-Cetto et al., 2000, 2005; Andrade-Cetto and Wiedenfeld, 2001, 2004) shows that the here used model produces lower blood glucose values. Nevertheless, the STZ administration at a dosage of 90 mg/kg BW to neonatal rats (*n*-S2T) significantly elevated the blood glucose levels after 3 months compared with rats injected with acetate buffer alone (not diabetic control) (Table 1); these observations are according to Verspohl (2002) and Portha et al. (2002) for the development of a type 2 diabetic model.

### 4.4. Activity of the plant in diabetic rats

In our of type 2 diabetes animal model, the extracts showed significant hypoglycemic effect (Table 1). As demonstrated in this study, the effect of the administered extract is dose-dependant, the lower dose (20 mg/kg) of the WE only produced a statistical significant effect at 180 min after its administration, while with the higher dose (80 mg/kg), the effect started

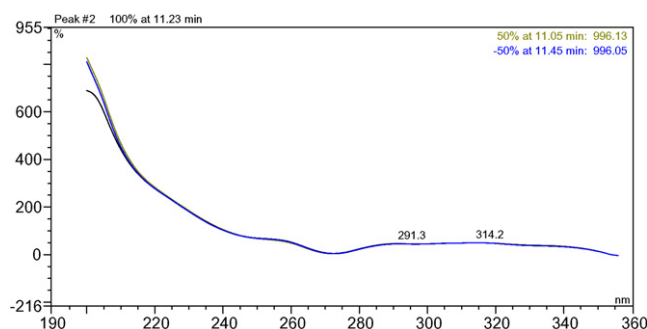


Fig. 2. DAD, HPLC spectra of compound number 3 present on both of extracts.

Table 1  
Effect of oral administration of extracts of *Tournefortia hirsutissima* stem on plasma glucose concentration in *n*-stz diabetic rats

Treatments	Plasma glucose levels (mg/dl) $\pm$ standard error			
	T0	T60	T120	T180
Control (+) no diabetic (vehicle)	113 $\pm$ 3	112 $\pm$ 6	114 $\pm$ 4	118 $\pm$ 4
Control (–) (vehicle)	195 $\pm$ 9 <sup>2</sup>	190 $\pm$ 9 <sup>2</sup>	190 $\pm$ 10 <sup>2</sup>	195 $\pm$ 9 <sup>2</sup>
Glibenclamide (3 mg/kg bw)	190 $\pm$ 16	158 $\pm$ 12 <sup>1</sup>	143 $\pm$ 4 <sup>a2</sup>	139 $\pm$ 4 <sup>b2</sup>
<i>Tournefortia hirsutissima</i> WE extract (20 mg/kg bw)	179 $\pm$ 5	170 $\pm$ 6	179 $\pm$ 4	172 $\pm$ 6 <sup>1</sup>
<i>Tournefortia hirsutissima</i> WE extract (80 mg/kg bw)	186 $\pm$ 2	154 $\pm$ 13 <sup>a</sup>	149 $\pm$ 8 <sup>b2</sup>	146 $\pm$ 9 <sup>b2</sup>
<i>Tournefortia hirsutissima</i> BE extract (8 mg/kg bw)	190 $\pm$ 7	187 $\pm$ 6	177 $\pm$ 7	169 $\pm$ 7 <sup>a1</sup>
<i>Tournefortia hirsutissima</i> BE extract (80 mg/kg bw)	187 $\pm$ 5	166 $\pm$ 5 <sup>a2</sup>	166 $\pm$ 4 <sup>a2</sup>	168 $\pm$ 5 <sup>b2</sup>

The values represent the mean  $\pm$  S.E.M. as compared with control time intervals, super index 1, 2 mean statistical significance against the diabetic control group (or de not diabetic group in the case of the diabetic control) with  $p < 0.05$  and  $p < 0.01$ , respectively, sub index a, b, mean statistical significance against time 0 of the same group with  $p < 0.05$  and  $p < 0.01$ , respectively.

after 120 min ongoing until 180 min, compared with the control group. If the groups are compared against themselves (time 0), the same lowering effect with the same statistical significance is observed, there is only one difference at time 60 where the WE (80 mg/kg) has statistical significance.

The lower dose of the BE (8 mg/kg BW) produced also a statistical reduction of glucose levels after 180 min, while the higher dose (80 mg/kg BW) produced the hypoglycemic effect from 60 up to 180 min; when the groups are compared against their own time 0 the same effect with statistical significance can be observed.

In this animal model, glibenclamide (3 mg/kg) produced a significant decrease in plasma glucose from 60 up to 180 min.

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

## 5. Discussion

Our ethnopharmacological studies confirmed the ethnobotanical data for the plant *Tournefortia hirsutissima* which is traditionally used as an infusion of the dried stem by the Mexican population against type 2 diabetes.

Our results indicate that the *n*-STZ diabetic animal group develop a moderate type 2 diabetes; however the animals are in better conditions on the ongoing experiments (with lower blood-sugar concentrations); it confirms that the (*n*-STZ) model is suitable for investigations on type 2 diabetes.

The extracts of *Tournefortia hirsutissima* stem showed a hypoglycemic effect in (*n*-STZ) rats and the dose-dependent effect was observed for the water and butanolic extracts. The administered doses are equivalent to the doses given daily traditionally to diabetic people (dosage of 20 mg/kg WE in rat = 9 g/l men and 80 mg/kg WE in rat = 36 g/l WE in men), the first is a single dose and second is a whole day dose (four times) for one person, furthermore, we demonstrated that a traditional dose can lower plasma glucose levels in the animal model.

In the study performed in 1998, the authors report only an "anti-hyperglycemic" effect at 120 min after a glucose charge was administrated to healthy rabbits, herewith, we demonstrate

the hypoglycemic effect in a dose-dependant way which is independent of the external administration of glucose, furthermore, it was observed since 60 until 180 min with a single administration of a traditional used dose.

As we detected that in both extracts (BE and WE), the same constituents are contained and that the pharmacological effect is similar and dose-dependant; it can be assumed that those compounds are related with the observed efficacy.

Summarizing, it could be proofed that the traditional use of *Tournefortia hirsutissima* as a hypoglycaemic agent is justified and that extracts from this plant show a dose-dependant activity which is comparable to the standard hypoglycaemic drug glibenclamide.

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