



Ethnopharmacological communication

Hypoglycemic and antihyperglycemic effect of *Witheringia solanacea* in normal and alloxan-induced hyperglycemic ratsCristina Herrera^{a,*}, Pedro M. García-Barrantes^a, Franklin Binns^a, Marianela Vargas^b, Luis Poveda^c, Sandra Badilla^a^a Instituto de Investigaciones Farmacéuticas, Facultad de Farmacia, Universidad de Costa Rica, San José, Costa Rica^b Laboratorio de Análisis Clínicos, Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica^c Herbario Juvenal Valerio Rodríguez, Escuela de Ciencias Ambientales, Universidad Nacional, Heredia, Costa Rica

ARTICLE INFO

Article history:

Received 13 June 2010

Received in revised form

10 September 2010

Accepted 2 October 2010

Available online 16 October 2010

Keywords:

Witheringia solanacea

Solanaceae

Antidiabetic activity

Hypoglycemic activity

Antihyperglycemic activity

Alloxan

ABSTRACT

Aim of the study: *Witheringia solanacea* is a small shrub that belongs to the Solanaceae family. The plant is used as an antidiabetic in Costa Rican herbal medicine. The aim of this study was to evaluate the hypoglycemic and antihyperglycemic activity of the aqueous extract of *W. solanacea* leaves in rodent models.

Materials and methods: A crude extract of *W. solanacea* leaves was prepared in boiling water and the aqueous filtrate was lyophilized. A single oral dose of 250, 500 and 1000 mg/kg of the extract was evaluated for hypoglycemic activity in a glucose tolerance test in normal rats and for antihyperglycemic activity in alloxan-induced (140 mg/kg) diabetic rats. The blood glucose level was determined at different times by the glucose oxidase method.

Results: Dosage of 500 and 1000 mg/kg of the extract significantly decreased ($p < 0.05$) blood glucose levels in the glucose tolerance test in normal rats after 1 h, there was no significant difference observed at 250 mg/kg. Dose of 500 mg/kg of the extract significantly reduced ($p < 0.05$) blood glucose levels in alloxan induced hyperglycemic rats at 4 and 5 h.

Conclusions: In the present study, the hypoglycemic and antihyperglycemic potential of the *W. solanacea* was demonstrated in rats. These results give support to the traditional use of *W. solanacea* as antidiabetic herbal medicine.

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1. Introduction

Diabetes Mellitus is the most common endocrine disease and affects nearly 10% of the world population (Burke et al., 2003). For a long time, diabetics have been treated with medicinal plants based on traditional medicine information. Several plant species have proven hypoglycemic effects (Alarcon-Aguilara et al., 1998; Barbosa-Filho et al., 2005; Gurib-Fakim, 2006; Lans, 2006). Despite the presence of antidiabetic medicines in the market, the search for more effective and safer hypoglycemic agents has continued to be an important area of research.

Witheringia solanacea L'Hér (Fig. 1) is a small shrub which belongs to Solanaceae family. The plant is distributed in southern Mexico, Central and South America; growing typically 2000 m above the sea level (Stone and Pierce, 2005). In Costa Rica, *W. solanacea* is also known as "sulfatillo", in Panama, this species

is known by the Kuna Indians as "Tinanguakiguid" (Caballero-George et al., 2001) and in Mexico is called "merengena" or "hierba cimarrona" (Jacobo-Herrera et al., 2006). *Witheringia asterotricha* is considered a synonym of *W. solanacea* (Bohs, 2000).

Phytochemistry of this species is unknown, but its chemical composition is probably related with *Witheringia coccoloboides* from which physalins were isolated (Jacobo-Herrera et al., 2006). Physalins are a group of substances with a secosteroidal chemical structure with antimycobacteria, antitumoral and anti-inflammatory activities reported (Vieira et al., 2005; Jacobo-Herrera et al., 2006).

W. solanacea is used in Latin American countries as anti-inflammatory, antimicrobial agent, anti-hypertensive and for management of general pain and gastrointestinal disorders (Caballero-George et al., 2001; García et al., 2006; Jacobo-Herrera et al., 2006; De la Torre et al., 2008). In Costa Rican traditional medicine, a water decoction of the aerial parts and roots of *W. solanacea* is employed as an antidiabetic medicine; however there are no reports of its hypoglycemic effect in the literature. This fact emphasizes the importance of carrying out studies in order to evaluate the effects of the treatment with this plant. In this study, we

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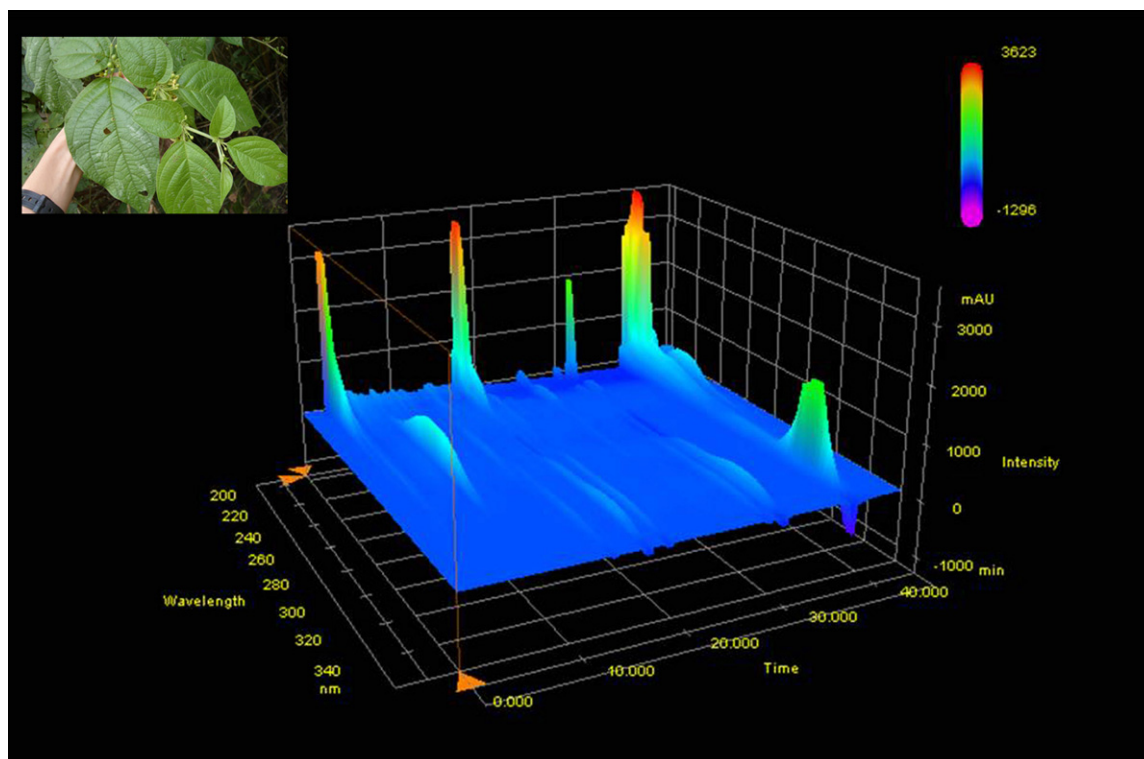


Fig. 1. Chromatography fingerprint of the *Witheringia solanacea* aqueous extract obtained by HPLC-DAD. Inset, left, picture of *Witheringia solanacea* L'Hér. Photo: C. Herrera.

evaluate the hypoglycemic and antihyperglycemic activity in normal and alloxan induced hyperglycemic rats of an aqueous extract of *W. solanacea* leaves.

2. Materials and methods

2.1. Drugs

Alloxan was obtained from Sigma Co. and glibenclamide from Raven Laboratories (San José, Costa Rica). Alloxan was dissolved in saline solution for intraperitoneal administration and glibenclamide was dissolved in distilled water for oral administration.

2.2. Animal

Male Sprague–Dawley rats with a weight between 230 and 260 g obtained from the Biological Assays Laboratory Bioterium, of the Universidad de Costa Rica, were used after approval of the protocol by the University Bioethics Committee (CICUA). Rats were maintained under standard conditions of temperature $22 \pm 2^\circ\text{C}$, light/dark cycles of 12 h, and food and water *ad libitum*.

2.3. Plant collection

Aerial parts of *W. solanacea* were collected during June 2009 near Guapiles, Limón, Costa Rica. The botanical identity of the plant material was authenticated by Dr. Luis Poveda (Universidad Nacional, Costa Rica) and the voucher specimen was deposited in the “Juvenal Valerio Rodríguez” herbarium (Universidad Nacional, Costa Rica) under the acquisition number JVR12469, for future references. Leaves were allowed to air dry at room temperature, and then ground using a cutter mill to obtain a coarse powder.

2.4. Preparation of the extracts

Extract of the dried leaves powder was prepared in boiling water, at a ratio 1:10 (w/v). The extract was filtered, and lyophilized to produce a brown powder; the percentage yield obtained was 2.05%.

2.5. Preliminary phytochemical screening

Chromatography fingerprint analysis was performed in Shimadzu LC20A equipment using a C_{18} column Phenomenex (250–4.6 mm) with a 5-mm particle size, and Photodiode Array Detector (DAD, SPD-M20A, Shimadzu). The mobile phases consisted of eluent A (water acidified with trifluoroacetic acid 0.01%) and eluent B (methanol). The gradient utilized was the following: 0–15 min 0–40% B; 15–35 min 40% B; 35–40 min 40–100% B; 40–45 min 100%. Total runtime was 45 min with a solvent flow rate of 1.0 ml/min, and the injection volume of 20 μl at concentration of 1 mg of the lyophilized extract in 1 ml of water. LC Solution Software was used for data collection.

Also, the crude aqueous extract was subjected to preliminary phytochemical analysis to determine the presence of secondary metabolites groups such as alkaloids, saponins, tannins, flavonoids and anthraquinones following standard published protocols (Lock de Ugaz, 1994; Evans, 2002).

2.6. Experimental procedure

The extract was dissolved in distilled water and administered orally. Saline solution and glibenclamide at 5 mg/kg were administered orally as negative and positive controls, respectively. The blood samples were drawn from the saphenous vein and the blood glucose level was determined by the glucose oxidase method with the GlucoSure Plus[®] glucose analyzer, which was validated. The change ratios of blood glucose levels were calculated for each animal according to the following formula: $100 + 100 \times (\text{postdrug}$

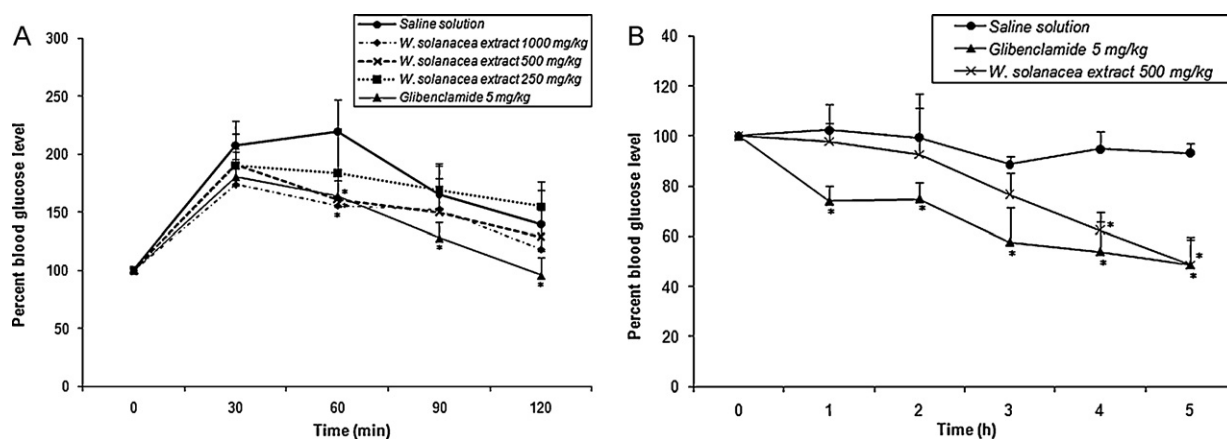


Fig. 2. Hypoglycemic and antihyperglycemic activity of aqueous extract of *W. solanacea* leaves in rats. (A) Percentage reduction of blood glucose produced by *W. solanacea* leaves at different doses after oral administration in glucose load rats ($n=6$). (B) Percentage reduction of blood glucose produced by *W. solanacea* leaves at 500 mg/kg after oral administration in alloxan induced hyperglycemic rats ($n=4$). *Significant difference from control ($p < 0.05$).

blood glucose level – predrug blood glucose level)/(predrug blood glucose level).

2.7. Hypoglycemic activity: oral glucose tolerance model

The rats were fasted for 14 h prior to oral glucose tolerance testing. The aqueous extract of *W. solanacea* leaves was administered at different doses (250, 500 and 1000 mg/kg). At 10 min after the administration of the treatments, 2 g/kg of glucose was administered to each rat. The blood glucose level was measured before the treatment and at 30, 60, 90 and 120 min after the treatment.

2.8. Antihyperglycemic activity in alloxan-induced hyperglycemia model

Hyperglycemic condition was induced by a single intraperitoneal injection of alloxan at 140 mg/kg. After 48 h of administration, hyperglycemia was confirmed and rats showing blood glucose levels above 250 mg/dl were selected for the following studies. Approximately 50% of the rats were hyperglycemic. Blood glucose concentration was measured at 0, 1, 2, 3, 4 and 5 h after the treatment (500 mg/kg).

2.9. Statistical analysis

The results were expressed by the means \pm standard deviation. The statistical difference was determined by Student's *T* test. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Phytochemical screening

The Chromatography fingerprint of the *W. solanacea* aqueous extract obtained by HPLC-DAD is showed in Fig. 2. The qualitative phytochemical screening of the extract showed the presence of tannins, alkaloids and saponins. The extract gave a negative result to anthraquinones, and flavonoids, when determined by ferric chloride, Shinoda's and Wilson's tests.

3.2. Hypoglycemic activity

There was no significant difference observed among the *W. solanacea* extract at 250 mg/kg and saline solution groups in the oral glucose tolerance model. However, the administration of the

W. solanacea extract at 500 and 1000 mg/kg significantly decreased ($p < 0.05$) blood glucose levels in rats at 60 min compared to saline control group (Fig. 2A). At these doses all animals were conscious and the activity and the behavior were normal compared to the control. Preliminary assays showed that doses of 2000 mg/kg are not toxic for the animals, while a dose of 3000 mg/kg presents toxic effects (unpublished data). Glibenclamide (5 mg/kg) produced a significant reduction ($p < 0.05$) in blood glucose compared to the group treated with saline solution at 60, 90 and 120 min.

3.3. Antihyperglycemic activity

There was a significant decrease ($p < 0.05$) in blood glucose levels in alloxan-induced hyperglycemic rats treated with 500 mg/kg of *W. solanacea* extract at 4 and 5 h with a percentage reduction in blood glucose levels of 39% and 54%, respectively (Fig. 2B). At this time all animals were conscious and behavior was normal. Glibenclamide (5 mg/kg) produced a significant reduction ($p < 0.05$) in blood glucose compared to the group treated with saline solution at 1 h (34%) and was still significant at 5 h (61%).

4. Discussion and conclusions

In the present study, the hypoglycemic and antihyperglycemic potential of *W. solanacea* was demonstrated in normal and alloxan induced hyperglycemic rats. At a dose of 500 mg/kg of the extract, there was an inhibition of the increase in blood sugar levels in the oral glucose tolerance model, at 60 min in a dose-dependent manner and a reduction of nearly 50% of the blood glucose in alloxan treated animals after 5 h. Alloxan is known for its selective pancreatic islet β cell cytotoxicity and has been extensively used to induce a hyperglycemic state in animals (Lenzen, 2008). Our results showed that *W. solanacea* can compensate the metabolic alterations occurring in alloxan induced hyperglycemic rats.

These results indicate the presence of antidiabetic active principles in the aqueous extract of *W. solanacea* leaves. The phytochemical screening shows the presence of tannins, alkaloids and saponins; however, further work is necessary to fractionate, purify and identify the active water soluble principles present in the leaves of *W. solanacea*.

Finally, our results give support to the traditional use of *W. solanacea* as an antidiabetic herbal medicine and provide a warning for its hypoglycemic toxicity potential because people that consume this plant for other indications may be affected by the hypoglycemic activity. However, further studies should be carried

out to confirm its antidiabetic activity, determine its toxicity, mechanism of action and phytochemistry.

Acknowledgements

The authors are thankful to Professor Guy Lamoureux from the Universidad de Costa Rica for his help in the reviewing of this article and to Andrea García and Lucía Granados for their help in the experimental procedures.

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