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Diuretic activity of *Withania aristata*: An endemic Canary Island species

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Abstract

This study reports on the pharmacological evaluation of the diuretic activity of an infusion and a methanol extract of *Withania aristata* Ait. in laboratory rats. Water excretion rate, pH, density, conductivity, and content of Na^+, K^+ and Cl^- were measured in the urine of rats subjected to hypersaline conditions. Both the infusion and the methanol extract showed a significant diuretic effect compared with non-treated controls, with notable increases in the rates of water and sodium excretion. There was also a potassium retention effect. The diuretic effect did not appear to be related to the potassium content in the material tested, but did have some relation to its content of active polar compounds. The results justify the use of *Withania aristata* as a diuretic agent in folk medicine of the Canary Islands.

Keywords: *Withania aristata*; Diuretic activity; Medicinal plant; Urinary excretion; Rats; Folk medicine

1. Introduction

Withania aristata Ait. (Solanaceae) is an endemic species of the Canary Islands. It is popularly known as "orobal" or "sáquido" (Darias et al., 1986). The plant grows wild on many of the central islands, and is common in ravines and at the bases of mountains, in soils which are somewhat nitrified and humid. It is common in the thickets of thermophilic forests (Pérez-Paz and Hernández, 1999).

This species forms a bush with variegated foliage which can be tree-like in size, although not reaching over 4 m in height. Its bark is coarse and grey in color, and its fragile branches form a dense mass. The plant bears greenish flowers which arise from the axillae of the leaves on peduncles. The fruit is globose and orange-colored (initially green), fleshy, and enveloped in a thin, resistant calyx (Kunkel, 1992).

This species has wide use in folk medicine practice on the islands due to the wide variety of medicinal properties attributed to it; including its use as a scarring agent, antispasmodic, for rheumatic problems, eye problems and otitis, as well as for insomnia, constipation, and urinary pathologies. Ingestion of the fruit strongly stimulates urine production, making it useful against hydropesia (Jaén, 1984, 1989; Darias et al., 1986, 1989, 2001; Pérez-Paz and Hernández, 1999).

Partial studies on the chemical composition of *Withania aristata* have isolated withanolides – types of steroid lactones – including withaferine A and withanolide D, among other constituents (González et al., 1972, 1974). Other compounds found were phytosterols, oleoresins and withaminol (Valera and Santos, 2002). Other *Withania* species from other parts of the world such as *Withania somnifera* and *Withania coagulans* have been submitted to numerous chemical studies, also encountering withanolides which are compounds characteristic of the Solanaceae and in particular the genus *Withania* (Ganzera et al., 2003). These products have demonstrated interesting properties such as anti-inflammatory, immunosuppressive, anti-stress, anxiolytic, cicatrizant, fungicidal and trypanosomacidal effects (Al-Hindawi et al., 1992; Choudhary et al., 1995; Habtemariam, 1997; Manickam et al., 1997; Bhattacharya et al., 2000; Jayaprakasam and Nair, 2003; Abe et al., 2006; Machiah et al., 2006). Until the present, however, no formal studies had been made on the biological activities and medicinal properties of *Withania aristata*.

The present study, using laboratory mice and rats, is thus the first formal attempt to demonstrate the diuretic efficacy of hot water infusion and methanol extract of the plant.

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2. Materials and methods

2.1. Plant material

Withania aristata was harvested from the Santa Cruz Coast in a place called Taganana in Tenerife, Canary Islands (Spain) at 75 m altitude above sea level, in March 2003, and labeled Exp. NE. UTM E381093-N3160004. Voucher specimens were deposited in the La Laguna University Herbarium (TFC 44199).

2.2. Extracts preparation

The leaves of flowering and immature fruiting *Withania aristata* were air-dried in an oven at 40 °C for 4 days and then the dry plant was cut and ground to a powder mechanical milling.

Then three aqueous extracts at 5, 10 and 15% from the dried powdered plant material were prepared by mean traditional method applied in Canaries. Amounts of 5, 10 and 15 g, respectively of pulverized plant material were each placed in 100 ml distilled boiling water and left at room temperature 15 min to infuse, and then were filtered. Five millilitres per kilogram body weight (bw) of each infusion was then given orally to individual rats (equivalent to doses of 0.25, 0.50 and 0.75 g/kg). The infusions were freshly prepared just prior to administration. In a second test procedure, the dried powdered plant material was submitted to a continuous extraction in a soxhlet extractor for 5 days using 100% methanol as a solvent. The solvent was then eliminated by vacuum distillation in a rotary vacuum evaporator (Buchler Corp.), representing a yield of 10.39% of the dry material extracted. The methanol residue obtained was dissolved in distilled water just before administration, and administered at doses of 100 and 200 mg/kg bw in a volume of 5 ml/kg bw.

2.3. Animals

Male albino Sprague-Dawley rats (180–210 g) and male and female albino Swiss mice (20–24 g) obtained from the Central Animal House, University of La Laguna, were used for the experiments, according with the guidelines of the European Community Council Directive 86/609.

2.4. Drugs

Hydrochlorothiazide (HCTZ; Sigma Chemical Co.) was used as a reference diuretic drug.

2.5. Acute toxicity test

Groups of 10 mice, 5 male and 5 female weighing 20–24 were used for administration of the infusion and MeOH extract of *Withania aristata*. The animals had free access to standard commercial diet and water *ad libitum* in a 12/12 h light–dark cycle at 22 °C. The test infusion at 2.5 g/kg bw (0.4 ml/20 g bw) and MeOH extract at 1 g/kg bw, respectively, were administered orally by means of a gastric catheter. Food was withdrawn 16 h before the start of the experiment. The mice were observed for symptoms of toxicity for 15 days in terms of weight loss, and autonomic and neurobehavioral alterations. On the 15th day, the animals were sacrificed and their vital organs were individually observed for overt pathology.

2.6. Diuretic activity

Diuretic activity was determined following the methods of Kau et al. (1984), with minor modifications. Male rats were divided into seven groups of eight animals each, in laboratory cages. They were fed laboratory diet *ad libitum* and allowed free access to drinking water. They were exposed to a 12/12 h light–dark cycle at 22 °C. Eighteen hours before testing, the animals were fasted overnight, with free access to tap water only. Then all animals were given an oral loading of normal saline (5% bw). Subsequently, three groups of rats were orally administered 5 ml/kg bw of the 5, 10 and 15% infusions of *Withania aristata,* two groups of rats were orally administered 5 ml/kg bw of the methanol extract at doses 100 and 200 mg/kg of weight, respectively, and other two groups of rats were orally administered 5 ml/kg bw p.o. of HCTZ at doses 10 and 25 mg/kg, respectively. Control rats received the same amount of deionised water (5 ml/kg bw). Immediately after administration, the rats were paired and placed in metabolism cages. Urine was collected in a graduated cylinder and its volume was recorded at 2 h intervals for 8 h. Cumulative urine excretion was calculated in relation to body weight and expressed as ml/100 g bw. Electrolyte (Na^+, K^+, Cl^-) concentrations, pH, density and conductivity were estimated from a pooled urine sample of each pair of rats at the end of the experiment (8 h) and expressed as mequiv./100 g bw.

2.7. Analytical procedures

Na^+ and K^+ concentrations were measured using a Jenway Corp. model PFP7 flame photometer. The instrument was calibrated with standard solutions containing different concentrations of Na^+ and K^+. Cl^- concentrations were determined by direct potentiometry, using an ion-selective chloride electrode (Orion 9417B) and an Ag/AgCl reference electrode with a double junction (Orion 90-02). The potentials were measured with an Orion Ionalyzer 901. KNO_3 2 M was used as a standard in all the determinations; pH and conductivity were directly determined on fresh urine samples using a HI-8424 Hanna Instruments pH-meter and a LF-320 WTF conductivity meter, respectively. Density estimation was made by weighing with a Mettler AE163 (±0.1 mg) analytical balance on urine volume measured with a Nichiryo micropipette.

2.8. Statistical analyses

Results are expressed as the mean values ± S.E. (standard error of mean) of four pairs of rats. The statistical evaluation was carried out by analysis of variance. The difference between the means of treated groups and the non-treated control groups was evaluated by the Student's unpaired *t*-test.