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Original Article

Evaluation of the diuretic activity of the ethanolic extract of *Geranium seemannii* Peyr. in Wistar rats

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ABSTRACT

Background: Although *Geranium seemannii* Peyr. is widely used in Mexican traditional medicine, there is as yet no scientific study to explore the validity of this practice. Therefore, the aim of the present study was to evaluate the diuretic activity of the ethanolic extract of *G. seemannii* Peyr. to provide evidence about its effect.

Methods: *G. seemannii* Peyr. was orally administered to adult male Wistar rats at 25 and 50 mg/kg, and its diuretic activity was evaluated and compared to the reference drug furosemide (20 mg/kg, administered intraperitoneally). Acute toxicity and lethality (LD_{50}) of the extract were assessed.

Results: Compared to control rats, there was significantly higher urinary output, as well as sodium, potassium and chloride ion excretion, in animals administered the ethanolic extract of *G. seemannii* Peyr. This effect was dose dependent, and there was no evidence of either acute toxicity or lethality with twice the maximum dose employed.

Discussion: The diuretic activity of some plants has been attributed to the presence of flavonoids, and *G. seemannii* Peyr. has a relatively large concentration of ellagitannins. This could be responsible for the effect demonstrated herein, which was similar to that produced by the reference drug furosemide. The mechanism of action of furosemide is by inducing a loss of water through the inhibition of NaCl reabsorption. The results suggest that this receptor-mediated mechanism may account for the diuretic effect of *G. seemannii* Peyr. as well.

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Conclusion: The findings in the present work support the possible use of *G. seemannii* Peyr as a diuretic agent and, representing the first report of the diuretic activity of this specie.

1. Introduction

Diuretics drugs increase the rate of urine flow and adjust the volume and composition of body fluids. Drug-induced diuresis is beneficial for the treatment of many maladies such as congestive heart failure (CHF), chronic renal failure, nephritis, cirrhosis, hypertension and pregnancy-induced toxemia.[1,2] However, many of the diuretics currently used in clinical practice have been associated with a number of adverse effects, including electrolyte imbalance, metabolic alterations, the onset of diabetes, activation of the renin-angiotensin and neuroendocrine systems, and impairment of sexual function.[2,3] Therefore, it is important to consider alternatives that have greater effectiveness and fewer side effects. Many of the herbs used in folk medicine have yet to be scientifically evaluated for their effectiveness and safety.[4] Geraniums are widely used in Mexican traditional medicine as antidiarrhoeal,[5] among other uses. Some pharmacological studies report hypotensive and astringent activity,[6] hepatoprotective and antiviral activity,[7] as well as anti-oxidant[8] and anti-inflammatory activity.[9]

Aerial parts of *Geranium seemannii* Peyr. is used in infusions as a kidney analgesic, mild astringent, and anti-inflammatory agent.[10] The chemical characterization of some *Geraniaceae* family plant species, such as *bellum, potentillaefolium* DC, *robertianum,* and *thunbergii*, has identified sugars, fatty acids, flavonoids, and tannins.[11] *G. seemannii* Peyr. has been employed as a diuretic in some indigenous areas of Mexico for centuries, but this use still lacks a scientific basis. The aim of the present study was to evaluate the diuretic activity of ethanolic extract of *G. seemannii* Peyr.

2. Materials and method

2.1. Plant collection

Specimens of *G. seemannii* Peyr. were collected when the plant was in blossom in June and July of 2010, in the municipality of Epazoyucan, Hidalgo State, Mexico. A voucher specimen (J. M. Torres Valencia 61) is preserved in the Herbarium of the Biological Research Center at the Universidad Autónoma in Hidalgo, and was identified by Professor Manuel González Ledesma of that institute.

2.2. Ethanolic extraction of G. seemannii Peyr.

The air-dried aerial part of the plant (1.5 kg) was extracted successively with a hexane, ethyl acetate, methanol and aqueous solution. Extractions in these organic solvents were all conducted by heating the solid plant residue in the appropriate solvent at reflux for 6 h, while the water extract was obtained by maceration at room temperature for 7 days. Filtration and evaporation of the extracts afforded green viscous oils (hexane, 7 g; EtOAc, 21 g; MeOH, 417 g and water, 123 g). Hexane and EtOAc extracts were dissolved in MeOH at 50 °C, then left at 0 °C for 12 h. Afterward, insoluble fatty materials were removed by filtration. The filtrate was evaporated under vacuum to give defatted extracts.[12] Ethanolic extract was tested on the basis that was the evidence showed increased activity in acute diuresis. The dose of 25 mg/kg of the extract was obtained from the average consumption of an infusion of 8 g of plant per 70 kg of body weight, and the dose of 50 mg/kg was tested to evaluate a possible dose dependent effect.

2.3. Experimental animals

Adult male Wistar rats (250–300 g) were housed in transparent polycarbonate cages of 50 × 28 cm, two per cage. Animals were maintained in a room that had little noise, a controlled temperature (22–25 °C), 8 to 10 air changes per minute, and natural lighting. They were given food (a standard rodent diet of Purina lab chow) and water *ad libitum*, and underwent an adaptation period of three days. The experimental protocol was approved by the Institutional Animal Ethics Committee and is in accordance with Mexican federal regulations for animal experimentation and care (NOM-062-ZOO-1999, Ministry of Agriculture, Mexico City, México).

2.4. Acute oral toxicity study

To determine acute oral toxicity, the method of acute oral toxicity at fixed doses was used.[13] The extract was administered at doses of 5 mg/kg to 100 mg/kg, with animals showing no notable signs of toxicity. The 50% lethal dose was found to be greater than 100 mg/kg, which is twice the highest dose (50 mg/kg) used for evaluation of a possible diuretic effect.

2.5. Evaluation of diuretic activity

Animals were maintained under standard condition of temperature and humidity and underwent for an adaptation period of three days. The animals were divided into four groups ($n = 6$). Group 1, as the negative control, received normal saline solution (25 ml/kg oral administration); group 2 received the reference diuretic, furosemide (Lasix, SANOFI-AVENTIS) at 20 mg/kg administered intraperitoneally[14,15]; groups 3 and 4 received the ethanolic extract of *G. seemannii* Peyr. at 25 mg/kg p.o. and 50 mg/kg p.o. respectively, in normal saline solution (25 ml/kg p.o.) and the diuretic activity was carried out based on the method of Lipschitz et al.[16]