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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]

Immediately after administration by gavage using an 18 G intragastric cannula, the animals were placed in metabolic cages (1 per cage), especially designed to separate urine and feces, and kept at a controlled temperature of 22–25 °C. At the end of 12 h, the volume of urine collected was measured. During this period, no food and water was available to the animals. During the two-week experimental period, the parameters measured were body weight (before and after the test period), total urine volume, and concentration of Na^+, K^+ and Cl^- in the urine. Na^+, K^+, Cl^- concentrations were determined by an ion sensitive electrode (Roche Hitachi 917) automatic analyzer. After the experiment, animals were sacrificed by ether anesthesia.[17]

2.6. Statistical analysis

Results are expressed as the mean ± SEM. Data was analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. A value of $p < 0.001$ was considered statistically significant.

3. Results

3.1. Acute toxicity test

The LD_{50} was estimated to be greater than 100 mg/kg. The experimental extracts of *G. seemannii* Peyr. were used in concentrations of 25 mg/kg and 50 mg/kg, with animals showing no signs of acute toxicity. No macroscopic alterations were noted in the viscera of the treated rats.

3.2. Effect on urine volume

The animals were observed with no signs of dehydration at 12 h intervals. The reference diuretic (furosemide) significantly increased urine output compared to the control ($p > 0.001$), with a diuretic index of 2.86. Administration of the test drug at 25 and 50 mg/kg also resulted in a significant increase in urine volume, although less than that found with the reference drug. The diuretic index for these two doses was 1.49 and 1.75, respectively, compared to 2.86 found for furosemide (Table 1).

Table 1 – Effect of oral administration of the ethanolic extract of *Geranium seemannii* Peyr. on urinary volume.

Group	Urine volume (ml/100 g/12hr)	Diuretic index (12 h interval)
Control	6.28 ± 0.12	–
Furosemide	18.00 ± 0.40**	2.86
EEG 25 mg/kg	9.41 ± 0.18*	1.49
EEG 50 mg/kg	11.00 ± 0.42*	1.75

EEG (Ethanolic Extract of *Geranium seemannii* Peyr.).
Values are expressed as the mean ± SEM; *$p < 0.001$ compared to the control group, **$p < 0.001$ compared to Furosemide group (ANOVA followed by Dunnett's test).
Diuretic index = volume of test group/volume of control group.

3.3. Effect on urinary electrolyte excretion

Ethanolic extract of *G. seemannii* Peyr. showed a significant increase in the excretion of sodium, potassium and chloride in a dose dependent manner. Moreover, a dose dependent increase in the Na^+/K^+ ratio was also found. The increase in electrolyte excretions with the ethanolic extract (at both doses) was less than that found with furosemide (Table 2).

4. Discussion

There are few reports on the diuretic activity of the *Geraniaceae* species. One study reported use of the aqueous extract of *Geranium robertianum* L in conditions requiring increased diuresis, such as cystitis, oliguria, urethritis, pyelonephritis, hypertension and gout.[10]

The diuretic effect of the orally administered ethanolic extract of *Geranium seemannii* Peyr. was evaluated in normal adult male Wistar rats and compared with that produced by furosemide, a loop diuretic widely used in clinical practice. Diuresis has two components: an increase in urine volume (water secretion) and a net loss of solutes (i.e., electrolytes) in the urine. These processes may result from suppression of renal tubular reabsorption of water and electrolytes into the blood stream. Administration of the *Geranium seemannii* Peyr. extract showed a significant increase in urine output and electrolyte excretion ($p < 0.001$) in a dose dependent manner (Tables 1 and 2), indicating the possibility of intrinsic and causal action, possibly receptor-mediated.

Some herbs induce diuresis by stimulating the thirst center in the hypothalamus and thereby enhancing fluid intake.[18,19] Some plants elicit diuresis due to their high salt content.[20] Such nonspecific mechanisms are unlikely to be involved in the effect of the test compound, in spite of the high Na^+ level in urine, because the extract of *G. seemannii* Peyr. did not alter the osmolarity or specific gravity of urine. Thus, the diuretic effect is not related to an osmotic mechanism. Furthermore, osmotic diuretics are inactive when administered orally, and for this reason are usually administrated intravenously.[20] The diuretic effect of *G. seemannii* Peyr. is also unlikely to be due to an impairment of the action of an antidiuretic hormone, because such impairment causes polyuria with low osmolarity.

The reference drug furosemide showed a marked increase in urine volume and in urinary excretion of Na^+ and Cl^-, with a similar pattern as that found with the ethanolic extract of *Geranium seemannii* Peyr. (Tables 1 and 2), suggesting a similar mechanism of action in both cases. Furosemide, like other loop diuretics, acts by inhibiting NKCC2, the luminal Na^+-K^+-$2Cl^-$ symporter in the thick ascending limb of the Henle loop. It also abolishes the corticomedullary osmotic gradient and blocks negative as well as positive free water clearance.[21,22] By inhibiting the transporter, the loop diuretics reduce the reabsorption of NaCl in the kidney and also diminish the lumen-positive potential that derives from K^+ recycling. This electrical potential normally drives divalent cation reabsorption in the loop. Thus, by reducing this loop potential, diuretics induce an increase in Mg^{2+} and Ca^{2+}.[23]

Table 2 – Effect of oral administration of the ethanolic extract of *Geranium seemannii* Peyr. on urinary electrolyte excretion.

Treatment	Dose	Total Na^+ (μmoles/kg)	Total K^+ (μmoles/kg)	Total Cl^- (μmoles/kg)	Na^+/K^+ ratio
Normal saline	25 ml/kg	1555 ± 14.02	704 ± 7.34	579 ± 15.01	2.20
Furosemide	20 mg/kg	3345 ± 4.80**	1345 ± 11.06**	2034 ± 15.78**	2.48
EEG	25 mg/kg	2043 ± 4.42*	1000 ± 10.11*	2003 ± 10.30*	2.04
EEG	50 mg/kg	2427 ± 0.73*	1104 ± 6.23*	2095 ± 13.20*	2.19

EEG (Ethanolic Extract of *Geranium seemannii* Peyr.).
Values are expressed as the mean ± SEM.
*$p < 0.001$ compared to the control group, **$p < 0.001$ compared to Furosemide group (ANOVA followed by Dunnett's test).
†Na^+/K^+ ratio = Total Na^+/Total K^+.

Some flavonoids have shown a diuretic effect. Several isoflavonoids, including genistein and daidzein, have been reported to cause inhibition of the Na^+-K^+-$2Cl^-$ cotransporter, as well as an increase in natriuresis and kaluresis.[24] Moreover, the flavonoid crisine has been shown to induce a significant increase in urine flow, glomerular filtration and Na^+ and K^+ excretion. Recently, it was reported that seven methoxyflavonoids actively bound to adenosine receptor A_1, provoking antagonism and therefore dieresis and sodium excretion.[25]

In the present study, in reference to the elimination of Na^+, K^+ and Cl^-, the extract of *G. seemannii* Peyr. showed a greater natriuretic than kaluretic effect. The Na^+/K^+ ratio can define the nature of the diuretic mechanism. The Na^+/K^+ ratio for furosemide is approximately 1, meaning that it eliminates the two electrolytes equally. On the other hand, with tiacids this ratio is less than one (with a greater excretion of K^+ than Na^+), and with spironolactone it is greater than one (with a lower excretion of K^+ than Na^+).[26]

There is an association between urine volume and Na^+ concentration in the urine. This is logical, considering that the action mechanism of a great number of diuretics on the market is by decreasing the reabsorption of this ion, which induces osmosis of water out of the organism.[26]

The isolation and chemical characterization of the compounds present in different endemic species of the geranium gender found in the State of Hidalgo, México, showed the presence of tannins and flavonoids, mainly a high percentage of ellagitannins (5–16%),[12] The most abundant ellagitannin is geraniin, described as a crystalizable tannin that was isolated from *Geranium thunbergii* Sieg et Zucc by Okuda.[27] Hence, tannins are probably responsible for the diuretic effect of *G. seemannii* Peyr.

5. Conclusion

The present study demonstrates the diuretic activity of the ethanolic extract of *G. seemannii* Peyr., which increased urinary volume and electrolyte (sodium, potassium and chloride) excretion. The diuretic pattern of the ethanolic extract was similar to that of the reference drug (furosemide), suggesting a similar mechanism of action. Further study of *G. seemannii* Peyr. is necessary in order to isolate the compounds present in this species, as well as identify which compounds are responsible for the diuretic effect shown by the ethanolic extract. Additionally, it is necessary to determine the mechanism or mechanisms of action involved in the diuretic effect.

Conflicts of interest

All authors have none to declare.

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