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Role of prostaglandin/cAMP pathway in the diuretic and hypotensive effects of purified fraction of *Maytenus ilicifolia* Mart ex Reissek (*Celastraceae*)

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ABSTRACT

Ethnopharmacological relevance: Although *Maytenus ilicifolia* is used in Brazilian folk medicine as a diuretic drug, no study has been conducted to this date in order to evaluate this ethnopharmacological statement. So, the aim of this study was to evaluate possible mechanisms involved in acute diuretic activity of the ethanolic supernatant of the infusion (SEI) obtained from *Maytenus ilicifolia* and to assess its relationship with a hypotensive activity by a bioassay-guided fractionation using normotensive Wistar rats.

Material and methods: The preparation obtained from the infusion (SEI) and their respective fractions ($Fr \cdot H_2O$ and $Fr \cdot EtOAc$) were orally administered in a single dose to rats. The urine excretion rate, pH, density, conductivity and content of Na^+, K^+, Cl^- and HCO_3^- were measured in the urine of saline-loaded animals. Samples of the concentration of electrolytes, urea, creatinine, aldosterone, vasopressin and angiotensin converting enzyme (ACE) activity were evaluated in collected serum. The hypotensive activity and the involvement of nitric oxide, bradykinin and prostaglandin/cAMP pathway in the hypotensive and diuretic effects were also determined.

Results: Water and Na^+ excretion rate were significantly increased by $Fr \cdot EtOAc$ and the arterial pressure was significantly reduced, while the urinary excretion of potassium and chloride were reduced. Pre-treatment with indomethacin or DDA (2′,5′-dideoxyadenosine) significantly reduced the hypotensive and diuretic activity observed. All other parameters evaluated were not affected by any treatment.

Conclusion: The present study reveals that $Fr \cdot EtOAc$ obtained from *Maytenus ilicifolia* may present compounds responsible for diuretic and hypotensive activities, and this effect, could involve the prostaglandin/cAMP pathway.

1. Introduction

Cardiovascular diseases constitute the leading cause of disability and death worldwide. Among cardiovascular diseases hypertension features prominent position because in most cases there are no symptoms and its control has been ineffective in several countries (Rapeport and Middlemost, 2012). Common clinical strategies to achieve the reduction of blood pressure include the use of inhibitors of renin-angiotensin-system, beta-blockers, calcium channel blockers and diuretics (McManus et al., 2012). In Brazil, diuretic drugs, especially thiazides, are used as first-line drugs in the treatment of hypertension (Sociedade Brasileira de Cardiologia et al., 2010). Despite their high efficiency, thiazide diuretics are associated with a high incidence of adverse effects such as electrolyte imbalance, metabolic disorders, development of glucose intolerance and sexual dysfunction (Ellison and Loffing, 2009).

Recent studies demonstrate that research on natural products used in folk medicine as diuretics, are rising gradually in recent decades, and may become a valuable tool in the treatment of various human pathologies (Wright et al., 2007). Despite the great effectiveness of some medicinal plants such as diuretic drugs, there are few reports describing the underlying mechanisms responsible for these activities (Gasparotto Junior et al., 2012; Kazama et al., 2012).

Maytenus ilicifolia Mart. ex Reissek (*Celastraceae*), popularly known in Brazil as “espinheira santa” (holy spines), is a native

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plant from southern Brazil, Paraguay, Uruguay, and northern Argentina (Lorenzi and Matos, 2002). Besides their ornamental attributes they have been widely used in folk medicine to treat digestive disorders (gastritis and ulcers), diabetes, and kidney diseases (Mariot and Barbieri, 2007). In Brazil, it is currently marketed under license from National Agency of Sanitary Surveillance (ANVISA) for the treatment of gastric diseases.

In their chemical composition the literature describes the presence of various secondary metabolites, especially terpenoids (Itokawa et al., 1991; Ohsaki et al., 2004; Shirota et al., 1994a) and flavonoids (Leite et al., 2001), such as quercetrin, kaempferol (Tiberti et al., 2007) and catechins (de Souza et al., 2008a). The pharmacological properties attributed to this plant in preclinical studies include antiulcer activity (Baggio et al., 2007; Souza-Formigoni et al., 1991), cytotoxic and antimutagenic (Horn and Vargas, 2003; Shirota et al., 1994b), vasorelaxant (Rattmann et al., 2006) and hypotensive effects (Crestani et al., 2009).

Although *Maytenus ilicifolia* is used in Brazilian folk medicine as a diuretic drug, no study has been conducted to this date in order to evaluate this ethnopharmacological statement. So, the aim of this study was to evaluate possible mechanisms involved in acute diuretic activity of the ethanolic supernatant of the infusion (SEI) obtained from *Maytenus ilicifolia* and to assess its relationship with a hypotensive activity by a bioassay-guided fractionation using normotensive Wistar rats.

2. Materials and methods

2.1. Drugs and chemicals

Hydrochlorothiazide (HCTZ), *N*-hippuryl-L-histidyl-L-leucine hydrate (HIP-HIS-LEU), *o*-phthalaldehyde, *N*-ω-Nitro-L-Arginine Methyl Ester (L-NAME), icatibant acetate (HOE-140), indomethacin, 2′,5′-dideoxyadenosine (DDA) and captopril, were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Methanol, ethyl acetate and formic acid were HPLC grade (Tedia). Water was Milli-Q (Millipore).

2.2. Phytochemical study

2.2.1. Plant material

Leaves from *Maytenus ilicifolia*, collected in the region of Curitiba (Southern Brazil), were donated by the Central de Produção e Comercialização de Plantas Medicinais, Aromáticas e Condimentares do Paraná Ltda (Curitiba, PR, Brazil). The plant was identified by Dr. Olavo Guimarães (Department of Botany, Universidade Federal do Paraná, Curitiba, PR, Brazil). Voucher specimens are deposited at the Herbarium of this University under numbers 30,842 and 43,795.

2.2.2. Sample preparation

Leaves from *Maytenus ilicifolia* were submitted to infusion. A volume of 1 L boiling water was poured over 100 g of dried, ground leaves, the container was closed, and the extraction was allowed to proceed until room temperature was reached (~6 h). The infusion extract was treated with 3 volumes of EtOH, which gave rise to a precipitate and an ethanolic supernatant (SEI; 20.61 g). A portion of SEI (10 g) was solubilized in H_2O (100 mL) and partitioned with EtOAc (100 mL; ×5), giving rise an aqueous fraction (Fr · H_2O; 64% yield), and an organic fraction (Fr · EtOAc; 36% yield). All preparations were freeze-dried and maintained at room temperature until the experiments were performed.

2.2.3. Sample analysis (liquid chromatography–mass spectrometry)

The samples from liquid/liquid partition were examined by ultra performance liquid chromatography (UPLC, Waters-Acquity) composed by a binary solvent delivery and a photo diode array (PDA) detector. The samples at 2 mg/mL were prepared in H_2O–MeOH (7:3 v/v) and the analysis was carried out on a reversed phase BEH-Phenyl column 1.7 μm (2.1 × 50 mm). The binary solvent was composed by (A) 0.1% aqueous formic acid (v/v) and (B) methanol. The linear solvent gradient was: initial (B) at 3 to 50% at 8 min, returning to 3% at 10 min, and additional 3 min at 3% (B) were held to system re-equilibration. The column was heated at 60 °C and the samples held at room temperature (22 °C). The injection volume was 2 μL and the compounds were detected at λ 200–400. The mass spectrometry was electrospray ionization (ESI), triple quadrupole (Quattro-LC, Waters), operating at atmospheric pressure ionization (API). The negative ionization mode was used for detecting compounds, at m/z 100–1100, with energies at 2.7 kV on capillary and 80 V on cone. Nitrogen was used as nebulizer and desolvation gas at 350 L/h in *offline* mode and 850 L/h in *online* (LC-MS) mode. In *offline* mode, samples were injected with a syringe pump (KD-Scientific), at 10 μL/min.

2.3. Pharmacological studies

2.3.1. Animals

We used male Wistar rats (3–4 months old) from the colony of the Universidade Paranaense, maintained under standard laboratory conditions, with a constant 12 h light/dark cycle and controlled temperature (22 ± 2 °C). Standard pellet food (Nuvital®, Curitiba/PR, Brazil) and water were available ad libitum. All experimental procedures adopted in this study were previously approved by the Institutional Ethics Committee of the Universidade Paranaense (authorization number 20763-2011).

2.3.2. Assessment of diuretic activity: Experimental design

The diuretic activity was determined accordingly to the method described previously (Kau et al., 1984), with minor modifications (Gasparotto Junior et al., 2009). The animals were separated in different groups (n=5) for the acute (single dose) study. Rats were fasted overnight with free access to water and subjected to the stated treatment as described below. Each animal was placed in an individual metabolic cage 24 h before the commencement of treatments for environmental adaptation.

2.3.2.1. Acute diuretic activity. Before the treatments, all animals received physiological saline (0.9% NaCl) in an oral dose of 5 mL/100 g to impose a uniform water and salt load (Benjumea et al., 2005). The first group received vehicle (deionized water) orally and it was used as control. Different groups of rats received, by oral route, SEI (30, 100 and 300 mg/kg), Fr · H_2O (15, 50 and 150 mg/kg), Fr · EtOAc (10, 30 and 100 mg/kg), or HCTZ (hydrochlorothiazide, 10 mg/kg). The 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. Electrolyte concentrations (Na^+, K^+, Cl^- and HCO_3^-), pH, density and conductivity were estimated from urine sample of each rat at the end of the experiment (8 h).

2.3.2.2. Analytical procedures. For serum analysis, blood samples were collected in conical tubes after decapitation. Plasma and serum were obtained by centrifugation (2000 rpm, 10 min, 4 °C), and stored at −20 °C until their analysis. The levels of urinary and plasmatic Na^+ and K^+ were quantified by flame photometry (Quimis model Q398112). The concentrations of Cl^- and HCO_3^- were quantified by titration. pH and conductivity were directly