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Hydroalcoholic extract and pure compounds from *Senecio nutans* Sch. Bip (Compositae) induce vasodilation in rat aorta through endothelium-dependent and independent mechanisms

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ABSTRACT

Ethnopharmacological relevance: Senecio nutans Sch. Bip. (Compositae) is an endemic plant of South America, and is used in herbal medicine in Andean communities for treating acute mountain sickness. Currently, the direct effects of hydroalcoholic extract of *S. nutans* (HAE *S. nutans*) or its isolated compounds on the vascular system are not well described. The aim of this study was to determine the effects and mechanism of action of *S. nutans* on vascular function in healthy rats.

Material and methods: Seven compounds were isolated from the HAE *S. nutans*, and their structures were characterized using spectroscopic techniques as 1D and 2D NMR, and mass spectrometry. Vascular reactivity experiments were carried out in rat aorta. *S. nutans*-dependent vasodilation and phenylephrine-dependent contraction were measured in endothelium-intact and endothelium-denuded aortic rings of male rats.

Results: Seven pure compounds were isolate from HAE *S. nutans*, but two pure compounds showed significant vasodilation in rat aorta: 4-hydroxy-3-(3-methyl-2-butenyl)acetophenone (compound **E**) and 5-acetyl-6-hydroxy-2-isopropenyl-2,3-dihydrobenzofurane (compound **G**). Although HAE *S. nutans* induced vasodilation in absence of endothelium, the vasodilation in intact aorta, via NO, was higher. HAE *S. nutans* reduced calcium-dependent contraction in endothelium-intact, but not in endothelium-denuded aortic rings.

Conclusion: HAE *S. nutans* and its isolated compounds caused vasodilation in rat aorta in absence of endothelium, suggesting its vasodilator properties is endothelium-dependent (NO) and or independent, and may involve a modulation of the calcium channels. This result is of clinical interest as potential therapy control of blood pressure.

1. Introduction

Senecio nutans Sch. Bip. belongs to the family Compositae and it is a perennial shrub about 20–60 cm high, that grows in habitat with heights fluctuating between 3500 and 5000 m above sea level in the Andes of Chile, Argentina, Peru and Bolivia (Villagrán et al., 2003). The branches and leaves from *S. nutans* are taken in the Andes of northern Chile as an infusion in water to lower blood pressure, in a bid to counteract the effects of acute mountain sickness (Giberti, 1983; Pérez et al., 1999; Serra, 2001). In Peru, this plant is known by the vernacular names 'piruaya', 'chachacoma', 'chacharume' and 'huishcash'. Its leaves are used as a gastric antispasmodic in the treatment of gastric ulcers, while an infusion from its leaves is useful for the treatment of altitude sickness. The leaves are also employed as a veterinary antiparasitic and the aerial parts are used as flavouring agents and to dye wool (Belaunde et al., 2007).

Sesquiterpenes, *p*-hydroxyacetophenones, benzofurans, benzopyrans, monoterpenoids, eremophilanes and germacranos derivatives of shikimic acid have been reported as isolated compounds from *S. nutans* (Dupre et al., 1991; Loyola et al., 1985). Dihydroeuparin from *S. nutans* has hypotensive effect in rats (Gallardo and Araya, 1982b), p-hydroxyacetophenone, a

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hypotensive effect in frog and iguana (Gallardo and Araya, 1982a), sesquiterpenes vasodilate the corpus cavernosum of Guinea pig (Hnatyszyn et al., 2003).

To the best of our knowledge, no vascular reactivity and endothelial mechanistic study has been reported on pure compounds (**A**-**G**) isolated from hydroalcoholic extract of *S. nutans* (HAE *S. nutans*). This prompted us to carry out these studies to evaluate the biological activity of plant extract from the aerial parts of *S. nutans*, using accepted pharmacological and organ bath methodologies used in vascular reactivity experiments in rat aorta (Ajay et al., 2007; Rameshrad et al., 2016). This study is designed to research the vasodilation induced by HAE *S. nutans* and pure compounds, and the possible mechanism(s) of action in rat aorta.

2. Material and methods

2.1. Plant material

The branches, leaves and inflorescences from *S. nutans* were collected in Toconce (22°15′11.16″ S 68°5′44.68″ W; at 3.788 m.s.n.m), North of Chile, II Region of Antofagasta. The specimen was identified by Dr. Roberto Rodríguez, Departamento de Botánica, Facultad de Ciencias Naturales y Oceanográficas. Universidad de Concepción, Concepción, Chile. One specimen was deposited for collection of herbarium (registration number CONC 139.929).

2.2. Extract preparation

Aerial parts: the plant material was spread and dried in the shade at room temperature, and with the help of a mechanical mill was finely grounded. A mass of 2.0 kg of dry and powdered plant was deposited into a cotton bag with 4 L of a mixture EtOH:H_2O (1:1) for 72 h inside a glass beaker at room temperature. Then, the resulting solution was filtered (Whatman No. 4 filter paper) and concentrated on a rotary evaporator (50 °C) to a quarter of the initial volume. This procedure was repeated several times, until the final solution was colorless. The concentrate obtained was freeze-dried using a 4.5 FreeZone, Labconco lyophilizer. The lyophilized hydroalcoholic extract was stored at 4 °C until use. The yield of extraction was 19%.

2.3. Extraction, fractionation and isolation of pure compounds

The hydroalcoholic extract was re-suspended in distilled water and extracted successively with *n*-hexane, chloroform, ethyl acetate and *n*-butanol. The yield of *n*-hexane extract was negligible, and *n*-butanol extract did not cause vasodilation (data not shown). The other organic solutions were concentrated on a rotary evaporator and lyophilized. The chloroform and ethyl acetate extracts were subjected to successive chromatography gravity column packed with silica gel, using solvents with increasingly polar mixtures of toluene: EtOAc (6:4 to 2:8), PE: EtOAc (9:1 to 7:3), $CHCl_3$-MeOH (1:9 to 3:7) and monitored by thin layer chromatography (TLC). Complex fractions were subjected to chromatography on flash purification using a Biotage SP-4 system with ultraviolet detector. The mixtures were deposited in cartridges KP-Sil (120 × 150 mm) in flash column 12 + M, each sample was eluted with isocratically mixtures of PE: EtOAc (9:1 to 4:6) using a flow of 12 mL/min and monitored at 240 nm. Metabolites with minor impurities were purified by gravity column chromatography and successive crystallizations using different solvent mixtures at low temperature.

The melting points of the isolated metabolites were determined using an Electrothermal 9100 (ThermoFisher Scientific) system. The structural elucidation was performed using the appropriate spectroscopic information. 1D and 2D NMR spectra of ^1H and ^{13}C were recorded using a Bruker Avance 400 MHz. Molecular masses were determined by mass spectrometry of high-resolution electron impact, EI-HRMS, on a Micromass AutoSpec - Last NT. Infrared spectra were recorded in KBr pellets using Nicolet Avatar FT-IR 330 equipment.

2.4. HPLC-DAD analysis

The hydroalcoholic extract was analyzed by HPLC-DAD. The system used for analysis was a Agilent Technologies 1260 Infinity equipment (Agilent Technologies, Santa Clara, United States) consisting of a 1260 Quat Pump, a 1260 DAD VL UV diode array detector, 1260 TCC column oven, 1260 ALS autosampler and a OpenLab software. A MultoHigh 100 RP 18–5 μ (250 × 4.6 mm) column (CS-Chromatographie Service GmbH- Germany) maintained at 25 °C was used. Approximately 8 mg of each methanol extract were dissolved in 1.5 mL MeOH, filtered through a 0.45 μm PTFE (polytetrafluoroethylene) filter (Waters) and submitted to HPLC-DAD analysis. The compounds were monitored at 250 nm and 330 nm. UV spectra from 200 to 500 nm were recorded for peak characterization. The HPLC analysis was performed using a linear gradient consisting of 1% formic acid (A) and methanol (B) as follows: 0–5 min by 25% (B); 5–30 min, from 25 to 60% (B); 30–35 min, from 60 to 75% (B); 35–40 min, from 75 to 60% (B); 40–50 min, back to 25% (B). The flow rate was 1 mL/min. The volume injected was 20 μL.

2.5. Animals

For vascular reactivity experiments, Male Sprague–Dawley rats (6–8 weeks of age, 130–180 g) from the breeding colony at the Antofagasta University were used. All animals were housed in a temperature-controlled, light-cycled (08:00–20:00 h) room with ad libitum access to drinking water and standard rat chow (Champion, Santiago). In this study 25 rats were randomly allocated into two groups. These rats were used for experiments on vasodilation (n = 10), and contractile response (n = 15). The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the U. S. National Institutes of Health (NIH Publication revised 2013), and the local animal research committee approved the experimental procedure used in the present study (number CEIC REV/2013).

2.6. Isolation of aortic rings

Rats were sacrificed through cervical dislocation. The thoracic aorta was quickly excised and placed in cold (4 °C) physiological Krebs-Ringer bicarbonate buffer (KRB) containing (x10^{-3} M): 4.2 KCl, 1.19 KH_2PO_4, 120 NaCl, 25 Na_2HCO_3, 1.2 $MgSO_4$, 1.3 $CaCl_2$, and 5 D-glucose (pH 7.4). Rings (3–5 mm and 2–4 mg) were prepared after connective tissue was cleaned out from the aorta, taking special care to avoid endothelial damage. Aortic rings were equilibrated for 40 min in KRB at 37 °C by constant bubbling with 95% O_2 and 5% CO_2.

2.7. Vascular reactivity experiments

Aortic rings from the same animal were studied in duplicate, using different vasoactive substances (phenylephrine [PE], KCl). To study the effect of hydroalcoholic extract or pure compounds on vascular reactivity in rat aorta, we performed different protocols. A stock solution in DMSO (10^{-3} M) was prepared with pure compounds and then, dilutions in KRB were added in the bath. In the first protocol, the aortic rings were pre-contracted with 10^{-6} M PE, and then increasing concentrations of *S. nutans* or pure