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Original Article

Sesquiterpene lactones from *Hedyosmum brasiliense* induce *in vitro* relaxation of rat aorta and *corpus cavernosum*

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ABSTRACT

Hedyosmum brasiliense Miq., Chloranthaceae, has been used in Southern Brazil as a sedative, anti-inflammatory, and aphrodisiac. In this study, endothelium-intact and endothelium-denuded rat aortic rings and strips of *corpus cavernosum* were used to investigate the relaxant effects of an hexane fraction of leaves of *H. brasiliense* and its sesquiterpene lactones 13-hydroxy-8,9-dehydroshizukanolide, podoandin, and elemanolide 15-acetoxy-isogermafurenolide. The incubation of hexane fraction of leaves of *H. brasiliense* resulted in significant relaxation of endothelium-intact aortic rings previously contracted by phenylephrine. In addition, 13-hydroxy-8,9-dehydroshizukanolide and podoandin displayed a clear concentration-dependent ability to relax endothelium-intact (~85 to 90%) and endothelium-denuded (~45 to 55%) rat aortic rings. A less pronounced vascular relaxation was recorded when 15-hydroxy-isogermafurenolide was tested. Interestingly, in tissues previously incubated with the nitric oxide synthase inhibitor L-NAME (100 μM), both 13-hydroxy-8,9-dehydroshizukanolide and podoandin had their effects in endothelium-intact vessels reduced to the same degree of relaxation observed in endothelium-denuded aortic rings. Podoandin, 13-hydroxy-8,9-dehydroshizukanolide, and 15-acetoxy-isogermafurenolide (100 μM) were also able to relax precontracted *corpus cavernosum* strips by 49.5 ± 3.9%, 65.9 ± 7.3% and 57.9 ± 5.5%, respectively. Our results demonstrated that 13-hydroxy-8,9-dehydroshizukanolide, podoandin and 15-acetoxy-isogermafurenolide, isolated from *H. brasiliense*, generate both endothelium-dependent and -independent relaxation of rat aortic rings, as well as being able to induce *in vitro* relaxation of rat *corpus cavernosum*. Importantly, the endothelium-dependent effect is fully dependent on nitric oxide production. Considering that penile erection depends on both relaxation of cavernosal smooth muscle and inflow of blood for the cavernous bodies, this is the first study reporting experimental evidence supporting the aphrodisiac properties of *H. brasiliense*.

Introduction

Hedyosmum brasiliense Miq. is a member of the Chloranthaceae family widely distributed in Southern Brazil. It is popularly known as "cidrão", "cidreira" and "erva-de-bugre", among others. In folk medicine, it is indicated for the treatment of migraine, depression and diseases of ovary, and has been used as sedative, anti-inflammatory, and aphrodisiac (Reitz, 1965). Experimental evidence demonstrated that solutions obtained from *H. brasiliense* present analgesic (Trentin et al., 1999a), hypnotic-, anxiolytic- and antidepressant-like effects in rodents (Tolardo et al., 2010; Goncalves et al., 2012). Nevertheless, at least to our knowledge, the aphrodisiac effect has never been investigated.

Sesquiterpene lactones are a group of secondary metabolites found in several species, such as Acanthaceae, Amaranthaceae, Apiaceae, Aristolochiaceae, Burseraceae, Coriariaceae, Illiciaceae, Magnoliaceae, Menispermaceae, Lamiaceae, Lauraceae, Polygonaceae, Winteraceae, and Chloranthaceae (Picman, 1986; Kawabata and Mizutani, 1988; Cao et al., 2008; Hu et al., 2013). These compounds are well known for their antifeedant and protective roles in plants, mainly against infections by viruses, bacteria, and fungi (Nawrot and Harmatha, 2012; Chadwick et al., 2013). The potential therapeutic effects of sesquiterpene lactones have been widely investigated in animal models, revealing anti-inflammatory (Merfort, 2011; Ferrari et al., 2013), antimicrobial (Ordonez et al.,

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2011; Ciric et al., 2012), antitumor (Zhang et al., 2005), and antiviral effects (Ozcelik et al., 2009; Rossini et al., 2012; Mohammed et al., 2014), among others. In addition, their biological effects include vascular relaxation as demonstrated by several studies (Campos et al., 2003; Hong et al., 2005). The structure of sesquiterpene lactones consists of three isoprene units and a lactone group, which may accordingly be classified with their carbon skeletons. There are about 100 types of carbonskeletons, and although the majority of the sesquiterpene lactones are classified into seven main groups known as germacranolides, guaianolides, elemanolides, eremofilanolides, eudesmanolides, pseudoguaianolides and xanthanolides (Schmidt, 2006), phytochemical studies have shown lindenanolides as the main chemical markers of the Chloranthaceae family (Kawabata and Mizutani, 1988). Importantly, some of the biological effects found after the administration of solutions obtained from *H. brasiliense* in rodents were, at least in part, dependent on the activity of sesquiterpene lactones, such as the lindenanolide onoseriolide or 13-hydroxy-8,9-dehydroshizukanolide (HDS) (Trentin et al., 1999a), and the guaianolide podoandin (Tolardo et al., 2010; Goncalves et al., 2012).

Given the popular usage of *H. brasiliense*, its high amounts of sesquiterpene lactones and the putative involvement of these compounds to explain some of the popular uses of this plant, we designed this study to investigate the ability of HDS, podoandin, and the recently described elemanolide 15-acetoxy-isogermafurenolide (IGM-A; Amoah et al., 2013), to induce *in vitro* relaxation of rat *corpus cavernosum* and aorta.

Materials and methods

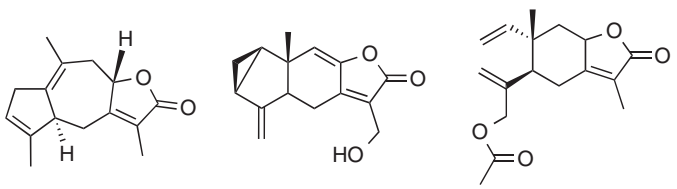
Plant material

Hedyosmum brasiliense Miq., Chloranthaceae, was collected from the municipal area of Antonio Carlos in the Santa Catarina state (Brazil). It was identified by the botanist Professor Dr. Ademir Reis and a voucher specimen (number 2031) was deposited at the Lymann Bradford Smith Herbarium (Univali, Itajaí, SC, Brazil).

Preparation of the hexane fraction and isolation of compounds

Fresh leaves (5 kg) of *H. brasiliense* were subjected to extraction with bi-distilled ethanol for 15 days. The solvent was subsequently removed under reduced pressure using a rotary evaporator. The recuperated solvent was used to re-extract the plant material twice, thereby resulting into a total of 210 g of crude extract. The crude extract (190 g) was subjected to liquid:liquid partition to yield hexane (16 g), CH_2Cl_2 (4 g), EtOAc (13 g), and the residual aqueous (74 g) fraction. All fractions were stored at −18 °C. About 2 g of the hexane fraction (HFHB) was kept for biological and pharmacological tests and the rest was subjected to flash silica gel column chromatography (240–400 mesh). It was eluted with a gradient of 0–70% CH_2Cl_2 in hexane (200 ml) followed by 0–70% EtOAc in CH_2Cl_2 (200 ml), yielding eight sub-fractions (A–H).

The sub-fraction B was recrystallized to afford the guaianolide podoandin (**1**; 300 mg). It was identified comparing its spectroscopic data with already published data (Blay et al., 2000; Kubo et al., 1992). There was a spontaneous crystallization of the previously described (Trentin et al., 1999b) lindenanolide 13-hydroxy-8,9-dehydroshizukanolide (**2**, HDS; 40 mg) from sub-fraction C. The elemanolide 15-acetoxy-isogermafurenolide (**3**, IGM-A; 35 mg) was isolated from sub-fraction F, as previously detailed (Amoah et al., 2013).



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Drugs and reagents

Acetylcholine (ACh) chloride, phenylephrine hydrochloride (PE), and Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME), as well as the salts used for the preparation of the physiological saline solution (PSS) were obtained from Sigma–Aldrich Co. (St Louis, MI, USA). All reagents used for preparation of the hexane fraction and the isolation of sesquiterpenes were of analytical grade. The stock solution of HDS, podoandin, and IGM-A were dissolved in pure DMSO. A concentrated solution of hexane fraction was prepared using DMSO and deionized water (1:10). All working solutions of HDS, podoandin, and IGM-A, which were applied in the *in vitro* experiments were, however, prepared in physiological saline solution.

Animals

Male Wistar rats (3–4 months) were provided by the Central Vivarium facilities of the Universidade Federal de Santa Catarina. The animals were maintained under controlled light–dark cycle (12/12 h) and temperature (22 ± 2 °C), with free access to water and chow. This study was performed in accordance with the NIH guidelines for animal experimentation. In addition, the Institutional Ethics Committee for Animal Use (CEUA) of the Universidade Federal de Santa Catarina (UFSC, Brazil) approved all procedures adopted in this study (authorization number 5371190815).

Isolation of corpus cavernosum *and aorta*

Immediately before the experiments, the animals were euthanized by anesthesia overdose (ketamine and xylazine, *i.p.*) and had their thoracic aorta and penis carefully excised and kept in physiological saline solution (PSS, composition in mM: 130.3 NaCl, 4.7 KCl, 1.6 $CaCl_2{\cdot}2H_2O$, 1.18 KH_2PO_4, 1.17 $MgSO_4$, 14.9 $NaHCO_3$, 5.5 D-glucose) warmed at 37 °C. After removing adherent tissues, the aorta was cut into rings of approximately 3–4 mm. The dorsal penile vein and spongy tissue were carefully removed from the penis and the *corpus cavernosum* was divided into two longitudinal strips. Both aortic rings and strips of *corpus cavernosum* were mounted in organ baths containing PSS (37 °C, continuously aerated with 95% O_2/5% CO_2) and connected to force–displacement transducers for recording of isometric force. The experiments using the aortic rings were conducted in endothelium-intact and endothelium denuded preparations. To remove the endothelium, the lumen of the vessels was gently rubbed with a small wire before the setup in the organ baths. A stabilization period of 60 min was allowed for all preparations, which were maintained at a resting tension of 3 g (aortic rings) or 250 mg (strips of *corpus cavernosum*). The isometric tension was recorded using a digital data acquisition system (PowerLab®) coupled to a computer running the software LabChart v. 7.1 (both from ADInstruments, Castle Hill, Australia).