Available online at www.sciencedirect.com
ScienceDirect
Phytomedicine 16 (2009) 1151–1155
Phytomedicine
www.elsevier.de/phymed

SHORT COMMUNICATION

Endothelium-dependent vasorelaxant effects of the essential oil from aerial parts of *Alpinia zerumbet* and its main constituent 1,8-cineole in rats

Nilson V. Pinto[a], Ana Maria S. Assreuy[a], Andrelina N. Coelho-de-Souza[a], Vania M. Ceccatto[a], Pedro Jorge C. Magalhães[b], Saad Lahlou[a], José Henrique Leal-Cardoso[a,*]

[a]*Instituto Superior de Ciências Biomédicas, Universidade Estadual do Ceará, Fortaleza, CE, Brazil*
[b]*Departamento de Fisiologia e Farmacologia, Universidade Federal do Ceará, Fortaleza, CE, Brazil*

Abstract

Vasorelaxant effects of essential oil of *Alpinia zerumbet* (EOAZ) and its main constituent, 1,8-cineole (CIN) were studied. In rat isolated aorta preparations with intact endothelium, EOAZ (0.01–3000 μg/ml) induced significant but incomplete relaxation of the phenylephrine-induced contraction, an effect that was abolished by removal of vascular endothelium. However, at the same concentrations (0.01–3000 μg/ml corresponding to 0.0000647–19.5 mM), CIN induced a complete vasorelaxant effects ($IC_{50} = 663.2 \pm 63.8$ μg/ml) that were significantly reduced in endothelium-denuded rings ($IC_{50} = 1620.6 \pm 35.7$ μg/ml). Neither EOAZ nor CIN affected the basal tonus of isolated aorta. Vasorelaxant effects of both EOAZ and CIN remained unaffected by the addition of tetraethylamonium chloride (500 μM) or indomethacin (10 μM) into the bath, but were significantly reduced by N^G-nitro-L-arginine methyl ester (100 μM). It is concluded that EOAZ induces a potent vasorelaxant effect that could not be fully attributed to the actions of the main constituent CIN, and appears totally dependent on the integrity of a functional vascular endothelium. The data is novel and corroborate the popular use of *A. zerumbet* for the treatment of hypertension.

Keywords: *Alpinia zerumbet*; 1,8-Cineole; Essential oil; Isolated thoracic aorta; Nitric oxide

Introduction

Alpinia zerumbet or *speciosa* [K. Schum (Zingiberaceae)] is an aromatic plant abundant in north-eastern Brazil, where it is commonly known as ''colônia''. Infusions or decoctions of leaves from *A. zerumbet* are commonly used for their diuretic and antihypertensive properties (Matos 2001). Leaves of *A. zerumbet* possess an essential oil content of 0.2–1% of the plant dry weight, composed principally of mono and sesquiterpenes (Oliveira 1994). Decoctions of leaves from *A. zerumbet* were shown to induce a slight diuresis and decrease in blood pressure in healthy volunteers without affecting the renal function parameters (Laranja et al. 1991). Previously, we showed that intravenous (i.v.) treatment of normotensive rats with essential oil of *A. zerumbet* (EOAZ) and one its main constituent, 1.8-cineole (CIN) dose-dependently decreased mean arterial pressure (Lahlou et al. 2002a, b). Such an effect remained unaffected by bilateral vagotomy or i.v. hexamethonium pretreatment, suggesting that it may result from vasodilatory action of EOAZ directly upon vascular smooth muscle rather than withdrawal of sympathetic tone (Lahlou et al. 2003). As no information is available in the literature regarding the vascular effects of EOAZ, the present investigation was undertaken to assess the potential vascular effects of the

*Corresponding author. Tel.: 55 85 3101 9815; fax: 55 85 3101 9810.
E-mail address: lealcard@gmail.com (J.H. Leal-Cardoso).

0944-7113/$ - see front matter © 2009 Elsevier GmbH. All rights reserved.
doi:10.1016/j.phymed.2009.04.007

EOAZ and CIN in rat isolated thoracic aorta, and to elucidate the mechanisms underlying this vascular activity.

Material and methods

EOAZ leaves was obtained as previously described (Lahlou et al. 2002a; Lahlou et al. 2003) and had the following composition (in % of oil weight): 1,8-cineole (33.29), terpinen-4-ol (19.40), p-cimene (11.04), sabinene (6.92), caryophyllene oxide (5.56), β-pinene (3.54), γ-terpinene (3.16) and other minor constituents. A voucher specimen (no. 27138) is deposited in the Herbarium Prisco Viana of the Federal University of Ceará. The composition of Tyrode solution (pH 7.4) was (mM): NaCl 136, KCl 5, $MgCl_2$ 0.98, $CaCl_2$ 2, NaH_2PO_4 0.36, $NaHCO_3$ 11.9 and glucose 5.5. CIN (Sigma, purity ⩾99.0%) and EOAZ were first dissolved in dimethyl sulfoxide (DMSO) up to 10% of the total volume, made up with distilled water brought to the chosen concentration with Tyrode's solution and sonicated just before use. A stock of indomethacin chloride (Sigma) was prepared in 70% ethanol. All other drugs (Sigma) were dissolved in distilled water.

Male Wistar rats (250–350 g) were cared for in compliance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institute of Health (NIH Publication 85–23, revised 1996). All procedures described here had prior approval from the local animal ethics committee. Rats were stunned and then sacrificed by cervical dislocation, thoracic aorta was removed and cut transversally into cylindrical ring-like segments (2–3 mm) attached to steel wire triangular pieces (0.3 mm diameter), which were mounted in 5-ml organ baths containing perfusion medium continuously bubbled with 95% O_2 and 5% CO_2 at 37 °C. Endothelium-containing strips were stretched with a passive tension of 0.5 g and tension was recorded using an isometric force transducer (Grass Model FTO3, Quincy, MA, USA) connected to a PC-based Dataq acquisition system (PM-1000, CWE Inc., Akron, OH, USA). The vasorelaxant effects of EOAZ and CIN were studied according to a previously described protocol (Interaminense et al. 2007). Some experiments have been performed in endothelium-denuded rings precontracted by phenylephrine (PHE; 0.1 μM). The endothelium was removed immediately after dissection by gentle rubbing of the aortic lumen with a stainless steel wire. The absence of ACh-induced vasorelaxant effects was taken as evidence that the preparation was effectively stripped of endothelium.

Data are expressed as mean ± s.e.m. Significance ($P<0.05$) of the results was assessed by means of paired and unpaired Student's t-tests, and one- or two-way analysis of variance (ANOVA), followed by Benferroni's multiple comparison tests, when appropriate. IC_{50} values were calculated by interpolation from semi-logarithmic plots.

Results

In aortic rings with ($n=8$) or without ($n=7$) vascular endothelium, EOAZ (0.01–1000 μg/ml) and CIN (0.01–1000 μg/ml) had no significant effect on the tissue basal tone (data not shown).

In endothelium-containing preparations pre-contracted with PHE (0.1 μM), cumulative addition of the EOAZ (0.01–3000 μg/ml, $n=8$) (Fig. 1A) or CIN (0.01–3000 μg/ml corresponding to

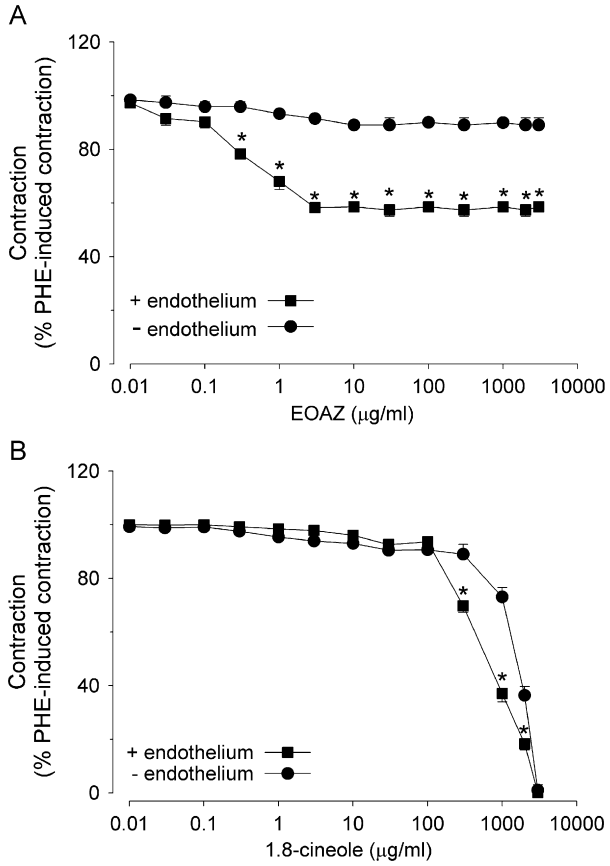

Fig. 1. Effects of increasing concentrations of the essential oil of *Alpinia zerumbet* (EOAZ; 0.01–3000 μg/ml) (A) and its main constituent, 1,8-cineole (0.01–3000 μg/ml corresponding to 0.0000647–19.5 μmM) (B) on the contraction induced by phenylephrine (0.1 μM) in rat aortic rings with ($n=8$ and 12, respectively) or without ($n=6$ and 11, respectively) functional endothelium. Vertical bars indicate s.e.m.. Responses are expressed as % of PHE-induced contraction before EOAZ or 1,8-cineole addition. *$P<0.05$ by Bonferroni's test with respect to control responses in absence of EOAZ or 1,8-cineole.