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Hypotensive and vasorelaxant effects of the procyanidin fraction from *Guazuma ulmifolia* bark in normotensive and hypertensive rats

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Abstract

The aim of the study: was to investigate the *in vivo* and *in vitro* cardiovascular activity of a procyanidin fraction (PCF) obtained from acetone extract of *Guazuma ulmifolia* bark which has traditionally been used as an antihypertensive agent.
Results: 10 mg/kg PCF doses orally administered to sugar-fed hypertensive rats decreased both the systolic arterial pressure and the heart rate, whereas the same doses intravenously administered induced arterial hypotension which was attenuated by N^G-nitro-L-arginine methylester (L-NAME 31 mg/kg) pretreatment. In these experiments we employed carbachol as a positive control test. The PCF reduced the contraction induced by norepinephrine (1×10^{-7} M) in isolated aortic rings of normotensive ($IC_{50} = 35.3 \pm 12.4$ ng/mL) and sugar-fed hypertensive ($IC_{50} = 101.3 \pm 57.2$ ng/mL) rats. This relaxant activity was inhibited by either vascular endothelium removal or L-NAME (30 μM) pretreatment, while indomethacin (10 μM) or atropine (10 μM) had no effect. Preliminary analysis of the PCF by HPLC–DAD–MS and FAB^+ mass spectrometry allowed the detection of the main components such as the complex of procyanidin oligomers consisting mainly of tetramers and trimers.
Conclusions: *Guazuma ulmifolia* bark possesses long-lasting antihypertensive and vasorelaxing properties linked to the endothelium related factors, where nitric oxide is involved.

Keywords: *Guazuma ulmifolia*; Sterculiaceae; Antihypertensive; Vasorelaxant; Rat aorta; Procyanidins

1. Introduction

Hypertension is a major risk factor for stroke, myocardial infarction, heart and kidney failure. Worldwide hypertension is estimated to cause 7.1 million premature deaths and 4.5 % of the disease burden (World Health Organization, 2002). Treating hypertension has been associated with about a 40% reduction in the risk of stroke and about a 15% reduction in the risk of myocardial infarction (Collins et al., 1990). In consequence, current clinical practice guidelines identify lowering blood pressure as a priority in the treatment of people with hypertension (Lonn, 2004). However, the hypertension remains inadequately managed everywhere (Mancia et al., 2002), and in spite of the large number of antihypertensive drugs and combinations, most people in developing countries have poor access to modern health care. Therefore, pharmacological validation of medicinal plants or ethnomedical treatment methods could greatly benefit populations with poor economic resources. The bark from *Guazuma ulmifolia* Lam (Sterculiaceae) commonly known as "guacimo" or "mutamba", is used as antihypertensive in the

Abbreviations: AcOEt, ethyl acetate; Cl_3Fe, ferric chloride; DAD, diode array detector; ESI, electrospray ionisation; FAB, fast atom bombardment; HOAc, acetic acid; HPLC, high performance liquid chromatography; HR, heart rate; HV, high voltage; LC, liquid chromatography; L-NAME, N^G-nitro-L-arginine methylester; MAP, mean arterial pressure; Me_2CO, acetone; MeOH, methanol; MS, mass spectrum; NO, nitric oxide; PCF, procyanidin fraction; PDA, photodiode array detection; RDA, retro Diels–Alder; SAP, systolic arterial pressure; TLC, thin layer cromatography.

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Panama folk medicine (Caballero-George et al., 2001), while in Perú the entire plant is used by traditional healers for diabetes and hypertension treatments (Agapito and Sung, 2003). However, its popular preparation as antihypertensive agent is not described in detail; it is only mentioned that the entire plant infusion is orally ingested. In contrast, the doses recommended for respiratory and gastrointestinal affections (Cáceres, 1999) are fully detailed (2–3 g three times per day in infusion).

Guazuma ulmifolia is a native tree widely used in Latin America for the treatment of a variety of diseases, particularly for gastrointestinal disorder treatments (Heinrich et al., 1992; Cáceres et al., 1993; Hör et al., 1995); stomach aches (Comerford, 1996); diabetes Mellitus (Alarcón-Aguilara et al., 1998); malaria and syphilis (Morton, 1977) and as uterine contraction stimulants (García-Barriga, 1975). The phytochemical studies carried out with *Guazuma ulmifolia* bark have led to the isolation of procyanidins consisting mainly of epicatechin and catechin units (Hör et al., 1996). These compounds have also been related to antihypertensive activities (Cheng et al., 1993). However, this plant has neither been specifically studied for its cardiovascular effects nor for its antihypertensive action mechanism. Therefore, the aim of the present study was to analyze, the effects of a procyanidin fraction (PCF) obtained from acetone extract of *Guazuma ulmifolia* bark on the rat's cardiovascular system in order to determine its contributions as an antihypertensive agent.

2. Materials and methods

2.1. Plant material and extraction

The bark of *Guazuma ulmifolia* was collected at Amatlán, Tepoztlán in the Mexican state of Morelos, September 2002. The corresponding voucher specimen of the plant (09B561-2830) remains at the medicinal herbarium at the Instituto Mexicano del Seguro Social (IMSS) for future reference. The dry bark (1 kg) was previously defatted with hexane and macerated at room temperature with Me_2CO (5× 1000 mL, 24 h each). The filtered extracts were combined and evaporated to reduced pressure obtaining a deep red solid amorphous extract. Extract yield was 70.2 g.

2.2. Fractionation and isolation

Crude extract (70 g) was partitioned with AcOEt. Two parts were obtained from this process: the AcOEt soluble part (14.3 g), and the insoluble part (55.4 g). This latter part was put within 500 mL MeOH–Me_2CO 1:1 (v/v). From this mixture, a part was settled down. The rest was subjected to evaporation. 33 g of a dark red glassy residue was obtained. This material was named PCF and it gave a positive procyanidin green colour with Cl_3Fe and red colour when it was examined by TLC on silica gel (Merck) with vanillin–H_2SO_4 reagent. TLC was eluted using Me_2CO–toluene–AcOEt–MeOH–HOAc 5:3:3:1:1 (v/v/v/v/v). The PCF was subjected to solid phase extraction on a Bond Elute C18 cartridge (1 cm × 3 cm, Varian) to FAB^+-MS and HPLC–DAD–ESI/MS analysis. The spectrometric analysis was performed using a Waters HPLC system (Milford, MA, USA) consisting of a 1525 binary pump and a 2996 PDA detector. All experiments were carried out in positive ion mode with a mass spectrometer Esquire 6000 Ion trap (Bruker Daltonics, Billerica, MA, USA). FAB-MS (positive ion mode) was obtained by a JEOL JMS-SX102A instrument using 3-nitrobenzylic alcohol as a matrix. 20 mg PCF was dissolved into 1 mL of H_2O–MeOH 80:20 and injected into the C18 cartridge. Then 2 mL of H_2O–MeOH 80:20 (v/v) was also applied into the cartridge for rinsing. The achieved retained sample was eluted with a mixture of 2 mL H_2O–MeOH 25:75 (v/v). This mixture displaced the PCF and it shows an intense narrow ring going down, which was monitored visually.

Analysis of PCF by analytical HPLC was performed using a Zorbax 300 Extend C18 column (150 mm × 4.6 mm i.d.; 5 μm) maintained at 25 °C. Solvent system: A 0.1% formic acid, B acetonitrile. A gradient was performed during 0–45 min from 90 to 0% A. After each run, the chromatographic system was equilibrated with 10% B for 30 min. Flow rate of 1 mL/min; injection volume: 20 μL; sample concentration: 10 mg/mL in H_2O–MeOH 25:75 (v/v). DAD conditions: 280 nm.

ESI-MS conditions: positive ion mode; scan range: 50–1300 *m/z*; Nebulizer gas (N_2) 30 psi; dry gas (N_2) 10 mL/min; dry temperature 365 °C; HV capillary 4000 V; HV end plate offset −500 V; capillary exit 134.4 V and trap drive 70.8.

2.3. Anesthetized rats' preparation

Experimental protocols were supported by CONACyT (MO292 and Q42096) and followed the national (NOM-062-ZOO-1999 y NOM-087-SEMARNAT-SSA1-2002) and international official guidelines (*Guide for the care and Use of Laboratory Animals*. INH Publication 85-23, revised in 1985). The animals were anesthetized with a mixture of chloralose–urethane (50:800 mg/kg) i.p. administered. Three cannulas were inserted into a femoral artery, a femoral vein, and trachea in order to get blood pressure measurements, administrate drug and allow spontaneous breathing. Mean arterial pressure (MAP) was recorded with a pressure transducer Gould–Statham P231D (Grass Instrument Division, Astro Med, West Warwick, R1) connected to the arterial cannula. The signal from the transducer was electronically damped and registered by a Model 79 Grass polygraph. Heart rate (HR) was simultaneously recorded through another channel from the polygraph with a Grass 7P4 tachograph triggered by the pulse waves from the unfiltered transducer signal. After a 15 min stabilization period, only one administration, the whole acetone extract (50 mg/kg), PCF (10 mg/kg) or the saline solution (0.2 mL/100 g) was injected i.v. as a bolus in the first series of experiments. An additional positive control group received carbachol (1 μg/kg-min during 2 h). In view of the short-lasting cardiovascular effects of carbachol, this drug was administered by infusion in order to elicit more prolonged responses. In these series MAP and HR recordings were immediately taken during 120 min and their values were registered every10 min. In a second series, the rats were pretreated with L-NAME administered