Phytomedicine 23 (2016) 214–219

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.com/locate/phymed

Potent antihypertensive effect of *Hancornia speciosa* leaves extract

Grazielle C. Silva[a,b], Fernão C. Braga[c], Virgínia S. Lemos[b], Steyner F. Cortes[a,*]

[a] *Department of Pharmacology, Universidade Federal de Minas Gerais, Av. Antonio Carlos, 6627, 31270-901 Belo Horizonte, MG, Brazil*
[b] *Department of Physiology and Biophysics, Institute of Biological Sciences, Universidade Federal de Minas Gerais, Av. Antonio Carlos, 6627, 31270-901 Belo Horizonte, MG, Brazil*
[c] *Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universidade Federal de Minas Gerais, Av. Antonio Carlos, 6627, 31270-901 Belo Horizonte, MG, Brazil*

ARTICLE INFO

Article history:
Received 9 April 2015
Revised 11 December 2015
Accepted 16 December 2015

Keywords:
Antihypertensive effect
Nitric oxide
Hydrogen peroxide
Hancornia speciosa Gomes
Resistance arteries

ABSTRACT

Background: Hancornia speciosa Gomes is an herb traditionally used in Brazil for blood pressure control.
Purpose: The present work investigated the antihypertensive effect of an extract from *Hancornia speciosa* leaves (SFH) and analyzed its underlying mechanisms of action.
Methods: Hypertension was induced in mice by surgical removal of a kidney and by subcutaneous administration of a pellet with deoxycorticosterone. Vasodilatation was measured in mesenteric arteries with a wire myograph. Nitrites were measured by fluorescence with 2,3-diaminonaphthalene and H_2O_2 was measured with carbon microsensors.
Results: SFH (0.03, 0.1 or 1 mg/kg; po) induced a dose-dependent, long-lasting reduction in the systolic blood pressure in conscious DOCA-salt hypertensive mice (DOCA). Administration of SFH produced a significant increase in the plasmatic level of nitrites. The systemic inhibition of nitric oxide synthase by L-NAME (20 mg/kg) reduced its antihypertensive effect. SFH also induced a concentration-dependent vasodilatation of mesenteric resistance arteries contracted with phenylephrine, which was more potent in arteries from DOCA mice. Removal of the endothelium or pretreatment with L-NAME or catalase reduced the vasodilator response for SFH. The nitrite production induced by SFH was significantly bigger in mesenteric arteries from DOCA than in SHAM mice. However, the production of H_2O_2 induced by SFH was twice higher in DOCA mice.
Conclusion: Altogether, our results point to an antihypertensive effect of SFH due to a reduction in peripheral resistance through the production of NO and by a mechanism involving an increased production of H_2O_2 in the mesenteric arteries from hypertensive mice. These findings are further evidence to support the use of *Hancornia speciosa* by traditional medicine as an antihypertensive drug.

Introduction

Hypertension is a major risk factor that predisposes to cardiovascular disorders and is responsible for a large morbidity and mortality in patients (Lawes et al. 2008). Hypertension is closely related to the development of an impaired vascular function associated with an endothelial dysfunction and oxidative stress (Endemann and Schiffrin 2004). In this sense, several animal models of hypertension present a similar profile of impaired vascular function associated with an endothelium dysfunction (Ghiadoni et al. 2012).

One of the main problems related to the management of hypertension is the adherence to the treatment, which is estimated as 57% of the patients (Naderi et al. 2012). The interruption of treatment is highly associated with the failure in restoring the physiological blood pressure, mostly resulting from resistant hypertension that reaches approximately 20% of treated individuals (Cushman et al. 2002). The side effects related to the treatment are the primary reason for non-adherence to the treatment (Naderi et al. 2012). Therefore, there still are spaces for the development of new drugs with the ability to improve the vascular function by acting on targets that regulate the vascular smooth muscle contraction and revert or reduce the endothelial dysfunction (Fu et al. 2014). Phytochemicals consumed on a diet or as traditional medicines have been associated with a reduction in cardiovascular diseases (Hertog et al. 1993) and reduction of blood pressure

Abbreviations: (SFH), extract from *Hancornia speciosa* leaves; (NO), nitric oxide; (H_2O_2), hydrogen peroxide; (DOCA), deoxycorticosterone; (DAN), 2,3-diaminonaphthalene; (SBP), systolic blood pressure; (NOS), nitric oxide synthase.

* Corresponding author. Tel.: +55 31 3409 2726; fax: +55 31 3409 2695.
E-mail address: sfcortes@icb.ufmg.br, steyner1@msn.com (S.F. Cortes).

http://dx.doi.org/10.1016/j.phymed.2015.12.010
0944-7113/

(Faraji and Tarkhani 1999). Therefore, the investigation of plants used in traditional medicine for the treatment of hypertension may lead to the development of new drugs.

Hancornia speciosa Gomes (Apocynaceae), commonly known as "mangabeira", is a plant species found in savanna-like vegetation in Brazil. The chemistry of the ethanolic extract from *H. speciosa* leaves comprises flavonoids, chlorogenic acid and L-(+)-bornesitol (Endringer et al. 2009; Ferreira et al. 2007). This extract inhibited the angiotensin-converting enzyme (ACE) (Serra et al. 2005) and promoted vasorelaxant effects in rat aorta (Ferreira et al. 2007). More recently, our group demonstrated that a refined dry extract from *H. speciosa* leaves (SFH) has a hypotensive effect in mice by a mechanism dependent on inhibition of ACE and increased production of NO in mice (Silva et al. 2011).

The present study aimed at investigating the antihypertensive effect of the SFH and its respective mechanism of action in hypertensive mice.

Materials and methods

Hancornia speciosa *leaves extraction*

The leaves of *Hancornia speciosa* Gomes (checked with http://www.theplantlist.org) were collected in São Gonçalo do Rio Preto, Minas Gerais state, Brazil, in October 2003 (voucher specimen BHCB 49895, deposited at the Herbarium of the Universidade Federal de Minas Gerais – UFMG). After drying at 40 °C for 72 h in a ventilated oven, the leaves were grinded in a knife mill. The extract of *Hancornia speciosa* leaves (SFH) was prepared by a two-step process: 250 g of the ground leaves were exhaustively percolated with 96% EtOH (total volume: 17.5 l) at room temperature. The ethanol extract was evaporated under reduced pressure to give 69 g of a dark residue. It was then submitted to column chromatography over silica gel (0.2–0.5 mm; 37.0 × 6.8 mm i.d.) sequentially eluted with (500 ml each) *n*-hexane, dichloromethane, dichloromethane / ethyl acetate (1:1 v/v), ethyl acetate and ethyl acetate / methanol (1:1 v/v). Following, the dichloromethane / ethyl acetate (1:1) eluate (500 ml) was evaporated under vacuum to furnish 31.4 g of the dry extract SFH. According to EMA guideline (European Medicines Agency (HMPC) 2010), the extract is "other herbal preparation" declared as: refined dry extract from *Hancornia speciosa* Gomes, leaves (DER = 8:1). Extraction solvent: ethanol 96% v/v.

Since bornesitol and flavonoids are related to the biological activity of the extract (Endringer et al. 2014; Pereira 2012; Ferreira et al. 2007), they were chosen as analytical markers. The content of bornesitol (7.75 ± 0.78% w/w) was determined using a HPLC method developed by us (Pereira et al. 2012), whereas total flavonoids (14.52 ± 0.44% w/w) were quantified by a spectrophotometric method (Pereira 2012). Both methods were validated according to ICH guideline (International Conference on Harmonization (ICH) 1996).

Animals

All experimental protocols were performed in accordance with guidelines for the humane use of laboratory animals at our Institute and were approved by local ethics committee (protocol # 227/08, UFMG). Male Swiss mice (12–15 week-old) were used. All animals were obtained from CEBIO (Centro de Bioterismo – Instituto de Ciências Biológicas, UFMG). Free access was allowed to the standard diet (Labina, São Paulo, Brazil), and tap water was supplied ad libitum. All mice were maintained at eight per cage at a constant temperature (24 °C), with 12-h dark/light cycle.

Induction of hypertension

Mice (25–30 g) were unilaterally nephrectomized under anesthesia using a solution containing ketamine (500 mg/ml) and xylazine (20 mg/ml), administered once by intraperitoneal route. The skin over the left flank was shaved, and a 1.5 cm incision was made through the skin and underlying muscle caudal to the rib cage. The left kidney was externalized and removed after ligation of the renal artery and vein with 4-0 silk sutures (Ethicon, Inc, Somerville, NJ, USA). The muscle and skin layers were then closed separately with 4-0 silk sutures. A small area between the shoulder blades was shaved, and a 1 cm incision was made for implanting DOCA pellets to provide a dose of 1 mg/kg. The DOCA-salt mice were given water containing 0.9% NaCl and 0.2% KCl. The sham mice were also unilaterally nephrectomized, but they did not receive a DOCA pellet and were given tap water. All mice were placed on standard pellet rodent chow. After recovery, the mice were housed under standard conditions for 4 weeks, after which their systolic BP was determined by the tail-cuff method (Silva et al. 2013).

Blood pressure measurements

SBP and HR were measured by the tail-cuff method (Gross and Luft 2003) using the XBP1000 series rat tail blood pressure system (Kent Scientific, Torrington, USA). Conscious rats were conditioned in restraints in a warming chamber controlled at 37 °C for no more than 5 min. Thereafter, an integrated sensor cuff was placed at the tail and used to take at least 7 different pressure measurements from SBP. Measurements were taken every 15 or 30 min for 3 h and recorded using a DI-194RS data acquisition system (Dataq, Akron, USA) connected to a personal computer. After the measurement of the basal SBP, each animal randomly received 0.03, 0.1, 1 mg/kg of SFH or 100 mg/kg captopril by oral route.

Nitrite dosage in the serum

Nitrite (NO_2^-) was measured by using the Griess reaction with modifications (Grisham et al. 1996; Silva et al. 2011). The animals were treated by intragastric gavage with 1 mg/kg SFH or 20 mg/kg L-NAME or saline. One hour after, the animals were sacrificed by decapitation. Briefly, an aliquot (100 μl) of mice serum was added to a microtiter plate and the enzymatic treatment was started by adding 10 UL/ml *Aspergillus* nitrate reductase (Sigma, São Paulo, Brazil), 1 M HEPES buffer (pH 7.4), 0.1 mM FAD, 1 mM NADPH. After homogenization, the mixture was incubated for 30 min, at 37 °C. 1500 U/ml lactate dehydrogenase and 100 mM pyruvic acid were then added and mixed for 10 min at 37 °C. Following the above enzymatic treatment steps, 500 μl of the sample was added to each freshly prepared 500 μl Griess. The absorbance of each sample was then determined at 540 nm, and total nitrite concentrations were calculated from the slope of the standard curves established using known concentrations of nitrite. The water was used as blank solution.

Myograph studies

Mice were euthanized by decapitation. The abdomen was immediately opened and the mesenteric arcade removed. The branch II of the mesenteric resistance arteries in the mice were cleaned of fat and connective tissue, and a segment of approximately 1.6–2.0 mm in length was removed as previously described (Silva et al. 2013). The segments were then mounted in physiological salt solution (PSS) of the following composition (mmol/l): 119 NaCl; 4.7 KCl; 0.4 KH_2PO_4; 14.9 $NaHCO_3$; 1.17 $MgSO_4$; 2.5 $CaCl_2$, and 5.5 glucose. Mechanical activity was recorded isometrically