Vasodilating Properties of Extracts from the Leaves of Musanga cecropioides (R. Brown)

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The mechanisms of action involved in the hypotensive properties of the aqueous extract of the leaves of Musanga cecropioides were investigated. The effect of the aqueous leaf extract of M. cecropioides, found to contain mostly saponins, flavonoids and procyanidins, was investigated on vascular smooth muscle and also in an in vivo direct invasive blood pressure study in both normotensive and hypertensive rats. The hypotensive or antihypertensive properties of the extracts appear to be due partly to a direct or indirect vasodilator effect and also to some α1- and β2-adrenergic blocking effects. The extract also exhibited significant endothelium-dependent vascular smooth muscle relaxation, accounted for by the release of nitric oxide (NO), and induced significant angiotensin converting enzyme (ACE) inhibitory effects thereby supporting its vasodilator mechanism of action. Copyright © 2002 John Wiley & Sons, Ltd.

Keywords: vasodilator; hypotensive; saponins; flavonoids; procyanidins; nitric oxide.

INTRODUCTION

Musanga cecropioides (MC) R. Brown, commonly known as the ‘umbrella tree’ in tropical Africa is used in the treatment of sexually transmitted diseases, cough, bronchopulmonary infections, to calm epileptic seizures, in hypertension and to ease delivery in childbirth. The abortifacient and uterotonic properties of the aqueous leaf extract in rats have been reported (Kamanyi et al., 1996), and also the blood pressure lowering effect and endothelium dependent relaxation effect of the aqueous leaf extract (Kamanyi et al., 1991; Dongmo et al., 1996).

Several triterpenes and their derivatives have been identified and isolated from the stem bark (Lontsi et al., 1987) and procyanidins and the flavonoids isovitexin and isoqueritrin from the leaf (Franck et al., 1997).

In the present study, attempts were made to determine the possible mechanisms of action involved in the blood pressure lowering properties of the plant extract. Preliminary chemical analysis of the plant extract was carried out to determine the possible active principles responsible. We therefore investigated the blood pressure effect of the various chemical components extracted using the invasive technique, and the direct effect of the plant extract on vascular smooth muscle (aorta) and the effect of the plant extract on angiotensin converting enzyme (ACE) activity.

MATERIALS AND METHODS

Preparation of plant material. Fresh leaves of MC were collected along the Yaounde-Douala highway in Cameroon in May and December 1995. The plant material was identified at the national herbarium, Yaounde, where a voucher specimen was deposited under identification label NHMC-0030. These leaves were sun-dried and powdered. 1 kg of this was extracted in 3 L of boiling water for 48 h, filtered, and the filtrate (2.5 L) further divided into two fractions. One fraction (1 L), representing the aqueous extract (A Ext) was evaporated to dryness leaving a brown mass (yield 26%) 0.35 g of this extract was dissolved in distilled water to produce a stock solution of 30 mg/mL from which further dilutions to be used in the trials were made. The other portion, (1.5 L) was used for the extraction of saponins while fresh portions were prepared for extraction of flavonoids and procyanidins to be used in the different pharmacological tests.

Preparation of saponins. The 1.5 L portion of the aqueous extract was partitioned four times in n-butanol. The n-butanol was concentrated and the residue obtained was dissolved in methanol then precipitated with ethyl acetate. This resulted in saponins (Sap), confirmed by their foaming properties and haemolysis of red blood cells. Haemolytically active saponins are detected as white zones over a reddish background by thin layer chromatography.

Flavonoids and procyanidins. The powdered leaves (200 g) were extracted with hexane followed by methanol. The methanol extract was suspended in water, and heated under reflux for 5 min at 60 °C, filtered, and after the removal of chlorophyll extraction with EtOAc was carried out. The evaporated residue fractionated on a Sephadex LH 20 column by elution with EtOAc, followed by 80% aqueous methanol. Five fractions (A–E), were collected. These fractions were submitted to thin layer chromatography (TLC) using ethylacetate–formic acid–glacial acetic acid–water (100:11:11:26) as the mobile phase, and compared with the reference
The PSS was bubbled continuously with 95% O$_2$ and 5% CO$_2$ gas mixture. The pH of the medium was maintained to produce relaxation on aortic rings precontracted with NA.

After spraying with anisaldehyde–sulphuric acid reagent, the TLC of fraction C was heated at 100 °C. The appearance of a red colour revealed the presence of procyanidins.

In vitro studies

Blood pressure study. Wistar strain normotensive rats (NTR, 110–130 mmHg) and hypertensive rats (HPR, 165–175 mmHg) weighing 180–250 g were used in this study.

The rats were anaesthetized by intraperitoneal administration of 1 mL/100 g body weight of 15% urethane (ethyl carbamate) solution. The femoral vein was cannulated and connected to a pressure transducer, model Gould P23 ID, for blood pressure measurement. Arterial blood pressure was detected and recorded on a Beckmann polygraph.

A comparison of the fall in the blood pressure effect of the aqueous leaf (0.2–0.4 mg/kg body weight) extract in the presence and in the absence of phenolamine (0.2 µg/kg body weight) or salbutamol (0.2 mg/kg body weight) was performed. Rats were pretreated with reserpine (1 mg/kg/day, p.o), or with propranolol (3 mg/kg/day) for 2 days, and the effect of the extract of MC on the blood pressure was evaluated as described above.

Statistical analysis. Comparison of the mean values was done using the Student’s t-test. A p value less than 0.05 was considered as significant and p less than 0.01 as highly significant.

Drugs used and sources. Hydrochlorothiazide (Ciba, France), propranolol (I.C.Pharma), heparin, n-butanol, methanol, ethyl acetate and ethyl carbamate, phenolamine, acetylcholine, noradrenaline (Sigma). Salbutamol, angiotensin converting enzyme.

ACE-inhibitory test. An angiotensin converting enzyme (ACE)-inhibitory test was done using an in vitro method with dansylglycine as substrate, according to Elbl and Wagner (1991) modified by Hansen et al. (1995). This technique involves the use of ACE from rabbit lung with dansylglycine as a substrate. The method is based on the ACE-catalysed cleavage of the chromophore-fluorophore labelled substrate, dansyltriglycine, into dansylglycine, which is quantitatively measured by HPLC. Test solutions were prepared by dissolving 1 mg extract in 1 mL assay volume. All samples were analysed in duplicate by the HPLC procedure described by Hansen et al. (1995).

Figure 1. (A) Blood pressure lowering effect of the aqueous leaf extract of MC in normotensive rats (○) pretreated with propranolol (●) or reserpine (◇), n = 5. (B) Blood pressure lowering effect of the aqueous leaf extract of MC in hypertensive rats (●) pretreated with propranolol (○) or reserpine (◇), n = 6, * p < 0.05, ** p < 0.01.
RESULTS

The aqueous leaf extract and saponins (0.02–0.8 mg/kg) from this extract produced a dose-dependent fall in blood pressure between 3% and 55% in normotensive rats and 5%–53% in hypertensive rats (Figs 1, 2). Phentolamine (2mg/kg) administered intravenously 5 min before induced more than 62% potentiation of the hypotensive effect of the plant extract (Fig. 3A, B), while salbutamol (0.2mg/kg) produced an almost 30% reduction in the hypotensive effect induced by 0.4mg/kg of the aqueous extract of MC. The effect of the extract on arterial blood pressure of rats pretreated with propranolol or with reserpine showed a shift of the dose-response curves to the right (Figs 1, 2).

MC extract and ACh manifested endothelium-dependent relaxation of aortic ring segments precontracted by noradrenaline. Relaxation induced by MC extract was 21.8% ± 2.4% (n = 8), while the maximum relaxation produced by ACh was 85.3% ± 4.7% (Fig. 3A and C). Both ACh and MC failed to produce any relaxation in aortic rings without endothelium (−E) precontracted with NA aortic (Fig. 3B and D). Exposure of the aortic rings to methylene blue (MB) prevented ACh-induced (n = 4) and MC-induced (n = 4) relaxation.

In the angiotensin converting enzyme (ACE)-inhibitory test, a concentration of 0.33mg/mL of the methanol extract of MC exhibited a 100% ACE-inhibitory effect. Another fraction obtained from the liquid–liquid extraction with ethyl acetate exhibited an ACE inhibitory effect of 37.5% ± 3% at the same concentration of 0.33mg/mL, while a further fraction containing only monomeric procyanidins inhibited the enzyme activity by 52.2% ± 4.5% at the same concentration of 0.33mg/mL.

DISCUSSION

The aqueous extract of MC produced a fall in blood pressure in anaesthetized normotensive and hypertensive rats, suggesting that this extract may possess both hypotensive and antihypertensive principles.

The fall in arterial blood pressure occurred immediately after the administration of the aqueous extract and saponins from MC leaf extract, suggesting a possible action of this extract on the heart or a relaxant effect on the vascular smooth muscles (Ebeigbe and Ezimokhai,
direct smooth muscle depressant or a direct sympathomimetic action on the heart.

The methanol extract of the leaves of MC containing mostly flavonoids and procyanidins exhibited potent ACE inhibitory effect and also produced vascular smooth muscle relaxation. It could thus contain compounds that will provoke the release of endothelium-dependent relaxant factor (EDRF), identified as nitric oxide (Palmer et al., 1987). This is thought to be responsible for the regulation of vascular smooth muscle tone by stimulating the production of cyclic guanosine monophosphate (cGMP) (Vanhoucke et al., 1986) and also producing vascular smooth muscle relaxation resulting in a reduction of vascular tone and peripheral resistance (Kimura et al., 1986) and hence a fall in blood pressure.

The methanol extract as well as the EtOAc extract of MC showed a significant ACE inhibitory activity. Phytochemical studies of the leaves revealed the presence of some flavonoid and propanidin types that exhibited ACE inhibitory activity. It has been shown that among the flavonoids, some derivatives of 8-hydroxychromone and chromone and also polyhydroxyflavones with a phenolic hydroxyl group in a position 2' or 4' of the β-ring inhibited ACE (Elbl and Wagner, 1991). Active flavonoids are characterized by a phenolic hydroxyl group (Wagner, 1993). Some procyanidin small polymers (2- and 3-unit in particular) produced an ACE inhibitory activity. It is likely that the ACE inhibitory activity of the leaf extract of MC may be due to the presence of flavonoids and procyanidins, showing Musanga cecropioideis to be a rich and promising source of cardiovascular drugs which may be exploited for the production of new phytomedicines.

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REFERENCES


