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Endothelium Mediated Aortic Relaxation and Blood Pressure Lowering Effect of a Procyanidin Rich Fraction of the Stembark Extracts of *Erythrophleum suaveolens*

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Abstract

A procyanidin-rich fraction of the stembark extracts of *Erythrophleum suaveolens* induced significant dose-dependent (0.1–100 µg/ml) relaxation of the guinea pig and rat aortic ring segments precontracted with noradrenaline (10 µM) and potassium chloride (KC1, 30 mM). However, the extract produced more significant relaxation in aortic rings with endothelium intact (+E) than in those without endothelium (−E). The plant extract produced a relaxation effect of 54.35 ± 3.53% in rat aortic rings in the presence of endothelium. The relaxation was 10.18 ± 1.64% in the rat endothelium-denudated aortic rings. The procyanidin fraction (30 µg/ml) also prevented NA-induced contraction of aortic rings while 10 mg/kg provoked a fall in blood pressure of over 30 mm Hg a few seconds after extract administration with gradual return to the initial level. A 0.33 mg/ml procyanidin fraction also produced over 86.6% inhibitory effect on angiotensin converting enzyme (ACE) activity, indicating a possible blood pressure lowering effect through vasodilation. These results indicate vasodilating properties on aortic rings which are endothelium mediated due to the liberation of nitric oxide leading to the lowering of blood pressure.

Keywords: Procyanidin, endothelium mediated relaxation, blood pressure, precontracted, noradrenaline.

Introduction

The search for cheap and more readily available sources of new pharmaceutical products from plants is the new challenge facing phytochemists, pharmacists, pharmaceutical and medicinal chemists, and pharmacologists today.

Many scientific investigations have been carried out with medicinal plants leading to the isolation and characterisation of new substances which have also been tested pharmacologically in laboratory animal models with some satisfactory and encouraging clinical trials. The urge today is to look for new pharmaceutical products from medicinal plants. The genus *Erythrophleum* is one of these medicinal plants which have been extensively studied. This genus is known to contain 12 species with four well identified in Africa namely: *Erythrophleum suaveolens* (Guill. et Perr.), *Erythrophleum africanum* (Bentham), *Erythrophleum lasianthum* (A. Chev.) and *Erythrophleum ivorense* (A. Chev).

Erythrophleum suaveolens is a species represented only in tropical Africa. It is commonly used as a poison or an ordeal brew for persons suspected of witchcraft or serious crime (Dalziel, 1937). The cold infusion of the stembark is sometimes used as a purgative and in Cameroon, the crushed bark is applied to the fugative swellings of filaria. The Mbamois of the Centre province of Cameroon associate the aqueous stembark extract of *E. suaveolens* with *Belina acuminata* for the treatment of various inflammatory diseases such as asthma (Bouquet, l969). The decoction is also used in Zaire for the treatment of tuberculosis, angina and bronchitis (Bouquet, 1969). Bioassays of some extracts from *E. suaveolens* have shown anti-inflammatory and analgesic activities (Dongmo et al., 2001).

With the search for new pharmaceuticals possessing anti-inflammatory, cardiovascular (vasodilating and blood

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pressure lowering), and angiotensin-converting enzyme inhibitory properties, a good number of scientific investigations have been carried out on a number of African medicinal plants. We here report the results of our findings on the cardiovascular effects of some extracts of the stembark *of E. suaveolens* in the rat and guinea pig.

Materials and methods

Collection and preparation of the plant materiel

E. suaveolens was collected in March 1999 and the whole plant material was identified by Mr. Nole Tsabang of the Institute for Medical Research and Medicinal Plant Studies (IMPM). A voucher specimen was deposited at the National Herbarium at Obili, Yaoundé, for future reference.

Animal material

Guinea pigs weighing 250–300 g and rats weighing 170–200 g were used in all experiments. The aortic ring segments were isolated from these animals for the *in vitro* studies of the vasodilating properties of the plant extracts while anaesthetised rats were used for *in vivo* or direct, non-invasive blood pressure studies.

The solutions used

The bath solution for *in vitro* trials was a modified Krebs-Henseleit solution of the following composition (mM): NaCl, 115; KCl, 4.7; $CaCl_2$, 2; $NaHCO_3$, 25; KH_2PO_4, 1.2; $MgCl_2$, 1.2; glucose, 11.5. Other solutions used were acetylcholine: 0.5 mM, Sigma Chemical, USA; ascorbic acid: 150 mM, Sigma Chemical, USA; KCl: 3.75, Merck, Germany; noradrenaline: 10 mM, Sigma Chemical, Germany.

Preparation of animal tissues

Guinea pigs weighing 250–300 g and Wistar rats weighing 170–200 g, were killed by cervical dislocation and the thoracic aorta quickly removed, freed of adhering connective tissues, cut into 2–4 mm ring segments and preserved in warm aerated physiological solution maintained in the organ bath.

For the *in vivo* direct blood pressure studies, Wistar rats, 170–200 g, were anaesthetised by the intraperitoneal administration of 1 ml/100 g body weight of 15% urethane (ethyl carbamate). The femoral vein was canulated for the administration of plant extract and the carotid artery was canulated for the blood pressure measurement through a blood pressure transducer model S5021, Panlab Laboratories, Barcelona, Spain.

Blood pressure variation was detected and recorded on a polygraph, type Havard Universal oscillograph.

Preparation of the extract

160 g of the dried bark of *E. suaveolens* was reduced to very fine powder and extracted respectively over a Soxhlet with hexane for 48 h to eliminate lipophilic compounds followed by MeOH. The MeOH extract was evaporated in vacuum over a rotavapor to obtain a brown powder (10 g). This brown powder was further dissolved in water and treated with ethyl acetate (liquid-liquid extraction). The EtOAc phase was evaporated and the residue obtained as a brown powder was fractionated and purified by polyamide column chromatography with MeOH as eluant to give four principal fractions: alkaloids (A), a mixture of alkaloids and procyanidins (B), monomeric procyanidins (C), and a mixture of monomeric and oligomeric procyanidins (D). A comparative HPLC fingerprint analysis of these fractions revealed the presence of procyanidins monomers [(+)-catechin, and (−)-epicatechin] for fraction C, while fraction D contained monomeric and oligomeric procyanidins, according to the techniques used by Bartolome et al. (1996), Nonaka and Nishiota (1982), and Prieur et al. (1994). Figure 1 summarizes the procedure in the extraction and fractionation of *E. suaveolens.*

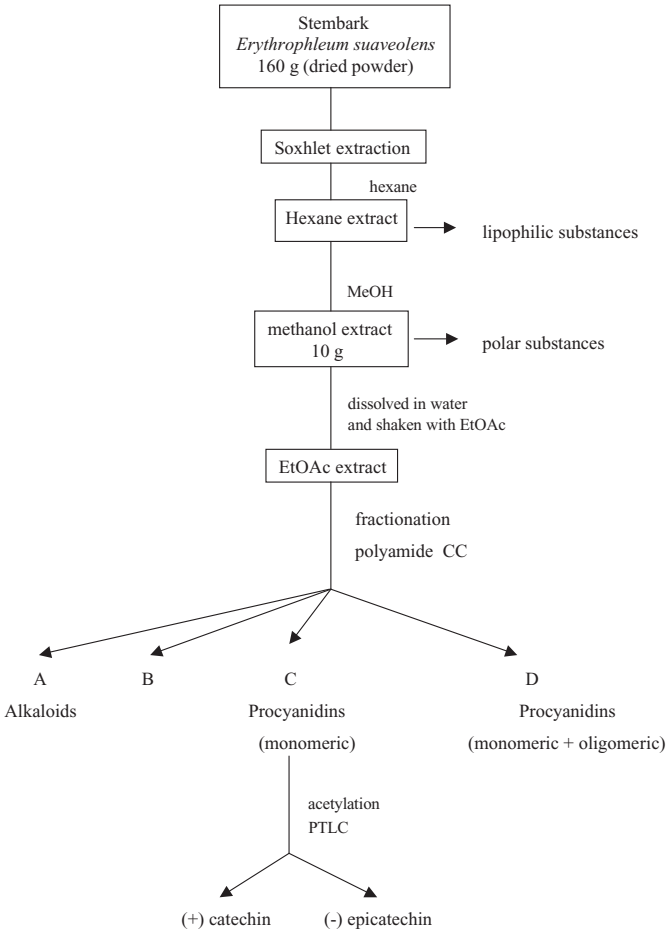

Figure 1. Diagram of extraction and fractionation of *Erythrophleum suaveolens*.

Experimental procedure

Investigating the response of aortic rings in the presence of the various plant extracts

For the study of the effect of endothelium on these responses, rat aortic rings with endothelium (+E) and without endothelium (–E) were used. The effective removal of endothelium was done by gently rubbing the inner lining of the lumen of the aortic rings a number of times. The absence of endothelium was tested experimentally by the failure of acetylcholine (1 µM) to elicit relaxation of aortic rings precontracted with NA (1 µM) (Fig. 2). For the test of the presence and integrity of endothelium, the aortic ring fragments were precontracted with noradrenaline, while relaxation was supposed to be produced in aortic rings with endothelium intact 2–5 min by at least 45% after the addition of ACh (1 µM). However, the functional integrity of aortic ring segments was tested by eliciting them with 30 mM KCl in the organ bath. Aortic rings which showed a contraction force of at least 4 mN were retained for further trials (Müller, 1997).

The effect of the procyanidin rich fraction of *E. suaveolens* extract was investigated by introducing it into the bath solution containing aortic ring segment with (+E) and without (–E) endothelium while the effect of the various fractions of *E. suaveolens* (MeOH extract, alkaloid rich fraction, EtOAc fraction, procyanidin rich fraction) was investigated on guinea pig aortic rings precontracted with 30 µM KCl.

The effect of the procyanidin rich fraction on guinea pig aortic rings precontracted with 10 µM NA (noradrenaline) was also evaluated in guinea pig aortic rings with endothelium intact. The same experiment was carried out with rat aortic ring segments.

ACE inhibitory properties of the plant extract

The effect of the procyanidin fraction on ACE inhibition was performed using the test method proposed by Elbl and Wagner (1991) and modified by Hansen et al. (1995).

In vivo direct blood pressure study

In the anaesthetised rat, administration of procyanidin fractions (10 mg/kg) was done through the canulated femoral vein and blood pressure variation recorded through the canulated carotid artery, using a Panlab, Barcelona, pressure transducer model S5021 on a polygraph, type Havard Universal oscillograph.

Results

Effect on aortic segments

The procyanidin rich fractions induced dose-dependent relaxation with a final response of about 60% and 86% of the contractile response induced by 30 µM KCl or NA (10 µM), respectively (Fig. 3A), on guinea pig aortic ring segments with endothelium intact. The methanol extract and alkaloids, identified in the plant extract, instead potentiated the contractile effect induced by 30 µM KCl (Fig. 3B) and NA (10 mM).

Figure 3C shows the effect of fraction D rich in procyanidins (0.1–100 mg/ml) on rat aortic rings in the presence (+E) and absence (–E) of endothelium. This fraction induced a concentration-dependent relaxation which was more significant in the aortic rings with endothelium than in those without endothelium. With a bath concentration of 100 µg/ml of fraction D, the relaxation reached 54.35 ± 3.53 and 10.18 ± 1.64% with and without endothelium, respectively (Table 1).

ACE inhibitory effect

Table 2 shows the inhibitory effect of angiotensin converting enzyme. A dose of 0.33 mg/ml of each of these extracts produced an inhibitory effect of 16.1 to 86.6%.

Effect on blood pressure

Intravenous administration of fraction D (procyanidin rich fraction) (10 mg/kg) provoked a fall in blood pressure of 30 mm Hg reaching a maximum level a few seconds after plant extract administration, with a gradual return to the initial level in a few minutes while MeOH (30 mg/kg) extract induced an increase in blood pressure of 24 mm Hg after an equally sharp fall (Fig. 4).

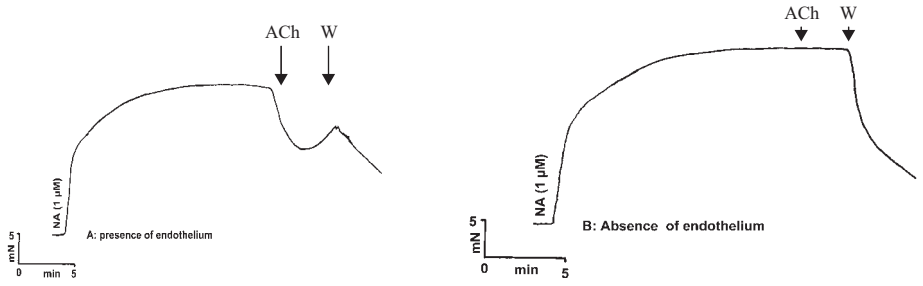

Figure 2. Verifying the integrity of aortic segments by inducing contractions with noradrenaline (NA) and relaxation with acetylcholine (Ach) in the presence or in the absence of endothelium.

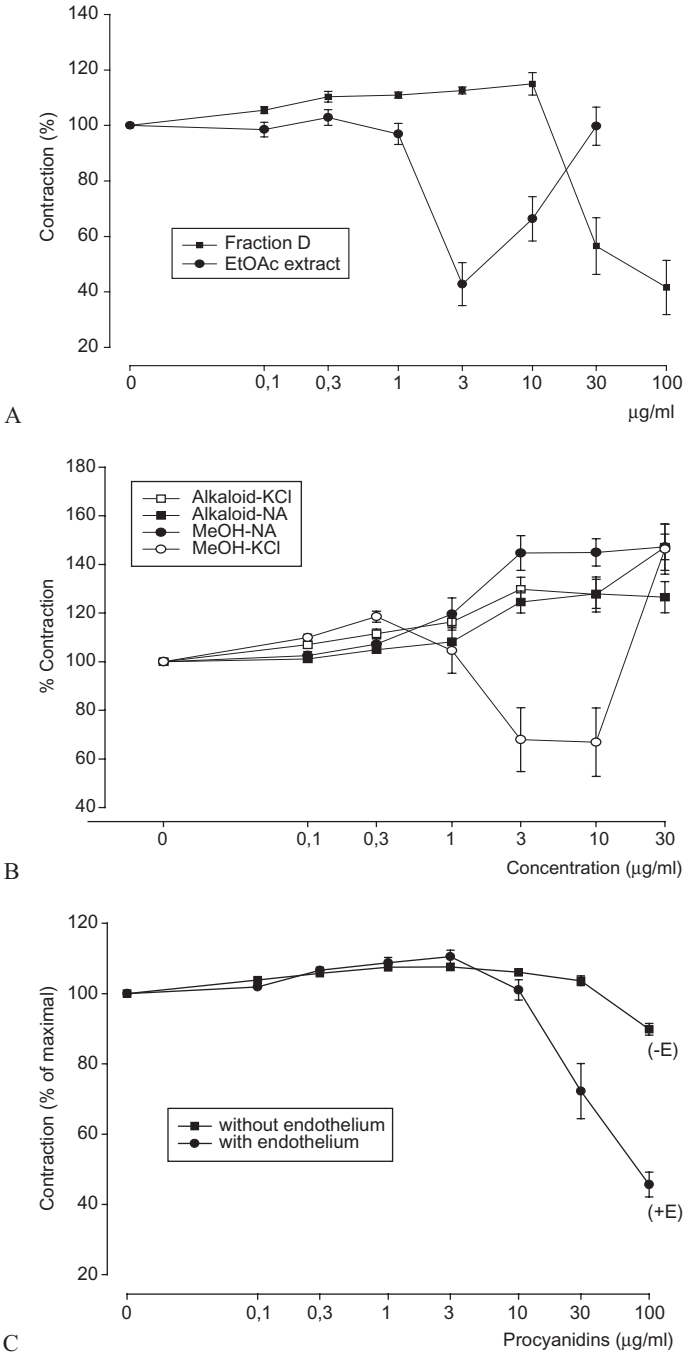

Figure 3. (A) Effect of fraction D and EtOAc extract of *E. suaveolens* on the contractile response of rat aortic rings induced by NA (10 µM). (B) Effect of MeOH extract and alkaloids from E. suaveolens on the contractile response of guinea pig aortic ring segments induced by NA (10 µM) or by KCl (30 mM). (C) Relaxation effect of procyanidin rich fraction of *E. suaveolens* (30 µg/ml) on contractile response of rat aortic rings in the presence (+E) and in the absence (–E) of endothelium contraction is expressed as the percentage of maximum contration (100%) induced by NA (1 µM).

Discussion

The methanol extract of *E. suaveolens* potentiated the contractile effect induced by NA and KCI on the aortic rings. Alkaloids of *E. suaveolens* also produced similar effects. The chemical study of the bark of *E. suaveolens* revealed a high presence of alkaloids. It was demonstrated that some of these alkaloids have a positive inotropic effect and may be acting in the same way as digitalis while others, like cassaine and cassaidine, provoked hypertension in dog and cat, due probably to vasoconstriction. Bamgbose (1974) also demonstrated some hypertensive effect with the aqueous extract of *E. suaveolens* collected in Nigeria. There is the possibility, therefore, that the observed vasocontriction effect of the MeOH extract of *E. suaveolens* could be due to its content in alkaloids which manifested similar effects in the present studies. Already Khan et al. (1963) demonstrated that the increase in arterial systolic and diastolic pressure could not be due to a direct effect of alkaloids (cassaine) on adrenergic receptors found on vascular smooth muscle wall but that this hypertension, apparently, was mediated by an increase release of catecholamines.

In contrast, the rich procyanidin content of the fractions D and EtOAC of *E. suaveolens* provoked a vasorelaxation of aortic rings precontrated with NA and KCl. Procyanidins extracted from *Crataegi folium* produced vasorelaxant effects like those induced by IBMX (3-isobutyl-l-methylxanthin) (Muller, 1997). This similarity presupposes that the effect induced by the procyanidins from *E. suaveolens* depends on the intracellular increase of cGMP. This hypothesis can only be confirmed by measuring the intracellular concentration of cAMP and cGMP. Another probable mechanism of action of the procyanidin rich extract could be a stimulation of adenylate cyclase or guanylate cyclase or also by activation of PKA or PKG (Furchgott & Zawadski, l980). From the results of the present investigations, we can conveniently postulate that the relaxant effect of the extract of *E. suaveolens* could be due to its procyanidins contents while the vasoconstrictor effect would be due to alkaloid content of plant extract. The relaxant effect induced by the procyanidins fraction was more significant in aortic fragments with endothelium intact than in fragments devoid of endothelium. This is due to the release of nitric oxide, an endothelium derived relaxing factor (EDRF).

This procyanidin rich fraction could thus contain precursors responsible for the release of EDRF (nitric oxide) which is responsible for the regulation of vascular muscle tone (Furchgott & Zawadski, 1980; Palmer et al. 1987). The resulting vasodilatory effect consequently produces blood pressure fall.

The inhibition of angiotensin converting enzyme (ACE) activity by some extracts of *E. suaveolens* would also be due to the procyanidin content as was shown by Inokuchi et al. (1985) and Wagner et al. (1991). According to Meunier et al. (1987), small polymers of procyanidins of two or three units are more active. It should be noted, however,

Table 1. Effect of fraction D of *E. suaveolens* on rat aorta rings precontracted with noradrenaline (NA, 1 µM), in the presence (+E) and in the absence (–E) of endothelium. Values were expressed as mean maximum relaxation force, and concentration of extract in bath inducing maximal relaxation in µg/ml. Mean ± s.e.m.

Organ	Reference force with NA (1 µM)	Relaxation E_{max}	Concentration at E_{max} (µg/ml)	n
With endothelium (+E)	24.08 ± 2.03	54.35 ± 3.53	100	6
Without endothelium (–E)	28.24 ± 3.50	10.18 ± 1.64	100	6

Table 2. Inhibitory activity of fractions of the *E. suaveolens* extracts on ACE.

Extract	Concentration (mg/ml)	Inhibition (%)
EtOAc extract	0.33	86.6
Fraction C	0.33	16.6
Fraction D	0.33	26.1

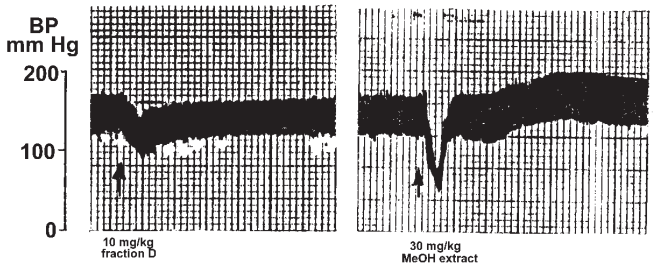

Figure 4. Blood pressure (BP) lowering effect of the procyanidin rich fraction (fraction D) of the extract of *E. suaveolens* in the rat. The effect of the MeOH extract (alkaloid rich fraction) is also presented.

that an angiotensin converting enzyme (ACE) inhibitory activity does not necessarily imply potent anti-hypertensive properties. Additional study is continuing at our laboratory.

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References

Bamgbose (1974): Preliminary studies on the pharmacology of the water soluble extract of *Erythrophleum (guineense) suaveolens*. *J Pharmacy Drug Res 1*: 32–41.

Bartolomé B, Hernandez T, Bengoechea ML, Quesada C, Gomez Cordoves C, Estrella I (1996): Determination of some structural features of procyanidins and related compounds by photodiode-array detection. *J Chromatog 723*: 19–23.

Bouquet A (1969): Féticheurs et médécines traditionnelles du Congo (Brazzaville) ORSTOM Paris.

Dalziel JM (1937): The useful plants of West tropical Africa. The Crown agents for the colonies, London. 192 pp.

Dongmo AB, Kamanyi A, Anchang MS, Chungag-Anye Nkeh B, Njamen D, Nguelefack TB, Nole T, Wagner H (2001): Anti-inflammatory and analgesic properties of the stem bark extracts of *Erythrophleum suaveolens* (Caesalpiniaceae), Guillemin & Perrottet. *J Ethnopharmacol 77*: 137–141.

Furchgott RF, Zawadzki JV (1980): The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature 288*: 373–376.

Elbl G, Wagner H (1991): A new method for the in vitro screening of angiotensin converting enzyme (ACE), using the chromophore and fluorophore labelled substrate dansyl-triglycine. *Planta Med 57*: 137–141.

Hansen K, Nyman U, Wagner SU, Adersen A, Gudiksen L, Rajasekharan S, Pushpangada P (1995): In vitro screening of traditional medicines for antihypertensive effect based on inhibition of angiotensin converting enzyme (ACE). *J Ethnopharmacol 48*: 43–51.

Inokuchi JI, Okabe H, Yamauchi T, Nagamatsu A, Nowaka GI, Nishioka I (1985): Inhibitors and angiotensin-converting enzyme in crude drugs: II. *Chem Pharm Bull 33*: 264–269.

Khan JB, Van Atta RA, Johnson GL (1963): Some effects of cassain on cardiac vascular dynamic in dog. *J Pharm Exp Therap 142*: 215–222.

Meunier MT, Villie F, Jonadet M, Bastide J, Bastide P (1987): Inhibition of angiotensin I converting enzyme by flavanolic compounds: In vitro and in vivo studies. *Planta Med 53*: 12–15.

Müller B (1997): Untersuchungen zur Wirkung von Pflanzenextrakten und Pflanzeninhaltsstoffen auf das kardiovaskuläre System durch Kontraktionskraftmessung an isolierten Aortenringen und Papillarmuskeln., Thesis, Institut für Pharmazeutische Biologie, LMU München.

Nonaka GI, Nishioka I (1982): Tannins and related compounds. VII. Phenylpropanoid-substituted epicatechins,

cinchonains from *Cinchona succiruba*. *Chem Pharm Bull 30*: 4268–4276.

Palmer R, Ferridge AG, Moncada S (1987): Nitric oxide release accounts for the biological activity of endothelium derived relaxing factor. *Nature 27*: 524–530.

Prieur C, Rigaud J, Cheynier V, Moutounet M (1994): Oligomeric and polymeric procyanidins from grape seeds. *Phytochemistry 36*: 781–784.

Wagner H, Elbl G, Lotter H, Guinea M (1991): Evaluation of natural products as inhibitors of angiotensine (ACE). *Pharm Pharmacol Lett 1*: 15–18.