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In vitro vascular smooth muscle contractile activity of *Aspilia africana* extract on rat aortic preparations

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Aspilia africana is widely used in ethnomedical practice in Africa for its ability to stop bleeding, even from a severed artery, as well as promote rapid healing of wounds and sores, and for the management of problems related to cardiovascular diseases. In the present paper, the methylene chloride/methanol extract of *A. africana* leaves was tested for its contractile activity *in vitro*. Rings of rat aorta, with or without an intact endothelium, were mounted in tissue baths, contracted with norepinephrine, and then exposed to the plant extract. The effect of the extract was also assessed on the baseline tension of aortic rings in normal and calcium-free PSS. At the lower doses, *A. africana* slowly re-inforced contractions induced by norepinephrine and relaxed precontracted tension at the highest concentration. The relaxant activity of the extract was endothelium-independent and was not modified by pre-treatment with N^w-nitro-L-arginine methyl ester or indomethacin, suggesting that its effect was not mediated by either nitric oxide or prostacyclin. *A. africana* extract induced slow and progressive increase in the basal vascular tone which was partially endothelium-dependent. In calcium-free PSS, a high proportion of the contractile activity was inhibited (77%), suggesting that *A. africana* contractile activity in vascular tissue depends, in part, on extracellular calcium.

1. Introduction

Several plant species have been used empirically in African ethnomedicine [1–4]. Sofowora [5] has indicated that tropical plant species are a natural resource for bioactive principles, some of which play an important role in therapeutic and/or curative activities against pathogenic organisms.

Aspilia africana C. D. Adams (Asteraceae) is highly reputed in folk medical practice for its ability to stop bleeding, even from a severed artery, as well as promote rapid healing of wounds and sores [6, 7]. In Cameroon, the plant is commonly known as "haemorhage plant". It is a hairy, scabrous herb, lignous at the base, with opposite, ovate-lanceolate leaves and deep yellow flowers. It is a secondary formation species occuring from Senegal to Cameroon [8]. The species is very variable and separable into at least four varieties (*africana, minor, ambigua, guinneensis*). The variety *africana*, described by Hutchinson and Dalziel [9] as a spreading herb or scrambling shrub growing up to 6 feet high, was used in this study.

In some parts of Africa, especially in the western region of Cameroon, the plant is used in the treatment of protracted menstruation, malaria, gastritis and lower abdominal pain [8]. A decoction of the fresh plant has also been recommended for pulmonary hemorrhage [10] and particularly for the management of problems related to high blood pressure, probably due to its reported diuretic effect. It has been reported that substances which lower blood pressure through a diuretic effect could act elsewhere on the vascular system [11–14]. In the present study, the contractile effect of the methylene chloride/methanol extract of *A. africana* on rat thoracic aorta rings was investigated.

2. Investigations and results

2.1. Effects of A. africana on norepinephrine-induced contractions in rat aorta rings

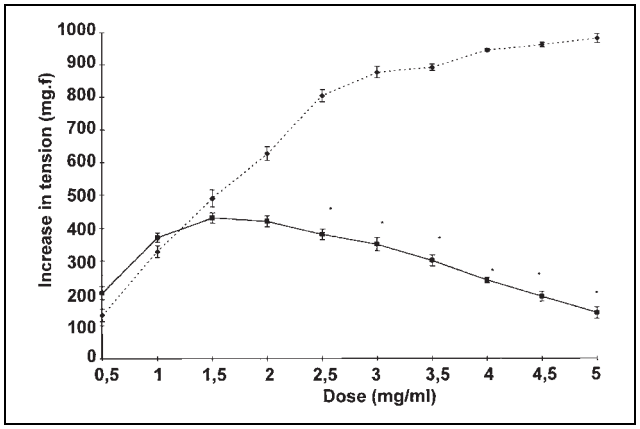
A. africana extract, at lower concentrations (0.5–1 mg/ml) slowly re-inforced the vasoconstriction induced by 10^{-6} M norepinephrine (NE). The maximal contractile effect ($21.62 \pm 3.54\%$) was recorded in aortic rings with intact endothelium 25 min after addition of 1 mg/ml of extract. A similar re-inforcement of tone by the extract was observed in NE-contracted rings without endothelium but the increase in tone was not significant. The Table shows that further cumulative increases in extract concentration (1.5–5 mg/ml) caused a reduction of the submaximal tension in a concentration-dependent manner. Maximum relaxations (93.87 ± 0.54 and $87.40 \pm 3.03\%$) were observed at the concentration of 3 mg/ml and 5 mg/ml on intact and denuded aortic rings, respectively. There were no significant differences in IC_{50} values (1.58 ± 0.08 and 1.69 ± 0.05) between aortic rings with or without endothelium, respectively. In addition, pre-treatement (30 min) with N^w-nitro-L-arginine methyl ester (L-NAME) (10^{-4} M) did not influence significantly the *A. africana* (3.5 mg/ml)-induced relaxation ($79.45 \pm 4.85\%$, $n = 8$), but this concentration of L-NAME completely abolished the maximal relaxation ($70.45 \pm 5.09\%$, $n = 7$) induced by 10^{-5} M ACh in arterial strips with intact endothelium. In these strips, relaxation induced by *A. africana* (3.5 mg/ml) following pretreatment with indomethacin (10^{-4} M) was $80.22 \pm 4.85\%$ ($n = 8$).

Table: Vascular relaxing activity of methanol/methylene chloride extract of *A. africana*

Dose (mg/ml)	Relaxation (%)	
	With endothelium	Without endothelium
0.5	-13.52 ± 2.02[a]	-4.57 ± 1.06[a]
1	-1.62 ± 3.54[a]	-0.27 ± 1.39[a]
1.5	44.69 ± 10.52	41.55 ± 6.66
2	83.25 ± 2.65	65.96 ± 3.45
2.5	90.46 ± 1.21	80.34 ± 0.35
3	93.87 ± 0.54	82.20 ± 2.93
3.5	89.97 ± 1.21	84.11 ± 2.97
4	89.64 ± 1.19	86.23 ± 3.03
4.5	90.11 ± 0.31	85.57 ± 3.53
5	91.97 ± 0.68	87.40 ± 3.03

Results are expressed as percent relaxation ± S.E.M, caused by cumulative doses of *A. africana* ($n = 8$).

[a] Extract-induced contraction of tissue is expressed as % increase in tension above norepinephrine (10^{-6} M)-induced contraction.



* Indicates significant difference ($p < 0.01$) from intact aorta rings.

Fig.: Effects of *A. africana* added cumulatively on the vascular tone of aortic rings with (+E) and without (−E) endothelium (n = 8) in the normal physiological salt solution. Each point represents the mean ± S.E.M.
···◆··· (+)Endethelium; —■— (−)Endethelium

2.2. Effects of A. africana on the basal vascular tone

A. africana extract caused a slow and progressive increase in the basal vascular tone. Concentration-response curves showing the effect of the extract on the basal vascular tone (Fig.) reveal that *A. africana*-induced contraction was partially endothelium dependent. Thus, at the maximal extract concentration (5 mg/ml), the developing tension was 957 ± 148 mgf in intact aortic rings (n = 8) compared with 140 ± 70 mgf in de-endothelialized rings (n = 8). The maximal tension developed in denuded aortic rings was 430 ± 50 mgf at the extract concentration of 1.5 mg/ml. Following incubation in Ca^{2+}-free PSS, the cummulative addition of extract to the bathing media did not cause a significant contraction of the aortic rings. The maximal tension developed in intact aortic rings was 218 ± 32 mgf (n = 8) at the highest extract concentration (5 mg/ml).

3. Discussion

The results of the present study show that low concentrations of the methanol/methylene chloride extract of *A. africana* cause further increases in tone in norepinephrine-contracted aortic rings. But at higher concentrations, the extract induced a dose-dependent relaxation of aortic rings. This effect was not suppressed by removal of the aortic endothelium. In contrast, the extract produced an endothelium/concentration-dependent vasoconstriction.

The vascular endothelium has been shown to play an important role in controlling vascular tone via the secretion of both relaxant and contractile factors [15, 16]. Endothelial cells respond to a variety of neurochemical and physical stimuli by releasing endothelium-dependent vasodilators such as nitric oxide (NO), which accounts for the biological activity of the endothelium-derived relaxing factor (EDRF) and prostacyclin [17–21]. The relaxing action of *A. africana* persisted in aortae with intact endothelium, in the presence of N^w-nitro-L-arginine methyl ester, a NO synthase inhibitor, and in the presence of indomethacin, a cyclooxygenase inhibitor. The concentration of L-NAME or indomethacin (10^{-4} M) used in this study is more than sufficient to fully inhibit NO synthase or cycloxygenase activity, respectively. This was verified in the study by testing the response to acetylcholine (ACh) (10^{-5} M), which was completely inhibited in the presence of L-NAME or indomethacin. Thus, the vasorelaxation caused by *A. africana* extract was not mediated by either endothelium-derived relaxing factor or prostacyclin.

A. africana extract produced vascular relaxation of the isolated rat aorta with an IC_{50} of approximately 1.6 mg/ml either with or without endothelium. These observations support the hypothesis that *A. africana* exerts a direct vasodilatory effect on the vasculature, which explains, at least in part, the use of this plant by the traditional healers in the management of cardiovascular diseases.

Our results show that *A. africana* at the lower concentration caused further increases in tone in norepinephrine-contracted intact aortic rings. It is well known that the contractions induced by NE are due partly to calcium release from intracellular stores and partly to the influx of extracellular calcium into the cell via receptor channels following the stimulation of α_1-receptors [22]. Carmeliet [23] and Gilani et al. [24] have also reported that high K^+ concentrations cause marked contractions in blood vessels by depolarization of smooth muscle fibres, leading to increased influx of calcium through L-type voltage-operated channels. Therefore, the increase in tone in norepinephrine-contracted aortic rings induced by the extract might probably be explained by the presence of potassium ions in the crude extract which increase the influx of extracellular calcium into the cell and/or release of calcium ions from intracellular stores of vascular smooth muscle. It is also possible that the extract contains coumpounds which directly activate the contractile machinery or sensitize it to respond to stimulating vascular endothelium contractile factors. Indeed, the extract in the presence of intact aortic rings, induced significant increses in vascular tone and the effect was reduced after the removal of endothelium. Akabue et al. [1] have shown that the topically applied alcoholic extract of *A. africana* was potently vasoconstrictive. Our results indicate that, in calcium-free buffer, maximal tension developed by the extract (5 mg/ml) was reduced by 77% compared with the tension provoked in normal PSS. Thus, *A. africana* contractile activity in vascular smooth muscle may partially by dependent on extracellular calcium, which suggests that calcium influx is the initiating event of contraction.

In conclusion, this study shows that the methanol/methylene chloride extract of *A. africana* can induce endothelium-independent relaxation of the rat aortic rings *in vitro*. The relaxation is not mediated by either NO or prostacyclin. The extract developed a slow and significant constriction of the intact aortic rings. Should the plant extract retain its activity *in vivo* after absorption, it could possibly prevent hypertension. Studies are currently under way to isolate and identify the active coumpounds in the plant extract and to determine *in vivo* and *in vitro* mechanisms of action.

4. Experimental

4.1. Materials

Norepinephrine (NE), acetylcholine (ACh), and N^w-nitro-L-arginine methyl ester (L-NAME) were from Sigma Chemical Co., St Louis, USA, while indomethacin was from Merk Sharp & Dohme, Great Britain. Ascorbic acid (0.57 mM) was added to each solution of NE, made up freshly every day.

4.2. Animals

Male Sprague-Dawley rats (300–400 g) raised in the animal house of the Faculty of Science, University of Yaounde I, were used. They were fed a standard laboratory diet (S.P.C. Ltd, Bafoussam, Cameroon) and given fresh water *ad libitum*.

4.3. Plant material

Plant material was collected from Nsimeyong neighbourhood in Yaounde-Cameroon, in August. The wole plant of *A. africana* was sun-dried and ground into a fine powder. 500 g were extracted exhaustively with 2 l of a 1 : 1 methanol/methylene chloride mixture for 48 h. The resulting solution was filtered and evaporated to dryness at 80 °C in a vacuum dessicator to obtain 32 g of dry material. 5 g of this extract were dissolved in 1 ml of dimethyl sulfoxide (DMSO) and the solution adjusted to 100 ml with distilled water to obtain a final extract concentration of 50 mg/ml.

4.4. Animal blood vessel preparations

Male Sprague-Dawley rats were killed by cervical dislocation followed by exsanguination. The thoracic aorta was carefully excised and adhering fat and connective tissue removed. The vessels were cut into rings 3–4 mm in length and some rings were denuded of endothelium by gentle rubbing of the luminal surface with cotton thread. The aortic ring was held in place by two opposite stainless steel wire hooks passed through the lumen. One hook was attached by a connecting thread to an isometric transducer connected to a Narco Bio-System Physiograph, and the other anchored to a plastic holder at the bottom of the organ chamber. The 10 ml organ bath, which was maintained at 37 °C, contained oxygenated (95% O_2 and 5% CO_2) Krebs buffer solution of the following composition (mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 11.1. The initial resting tension of 1 g was applied to the aortic ring followed by equilibration for 90 min, during which Krebs buffer was changed at 15 min intervals [25]. At the start of each experiment, tissues were contracted submaximally with norepinephrine (NE, 10^{-8} M) and then relaxed with ACh (10^{-5} M) to test for the intactness of the endothelium. ACh-induced relaxation less than 64% was taken as an indicator that endothelium was partially destroyed.

After ACh testing, the aortic rings were washed with physiological salt solution (PSS) three times during the hour that followed. To measure the relaxant effect of the extract, the rings were initially contracted with a single submaximal concentration of NE (10^{-6} M). Maximal tension was reached about 20 min later. This was followed by exposure of the aortic preparation to cumulative concentrations of the extract at 25 min intervals. The relaxant effect was measured for each cummulative dose as a percentage reduction of the maximal contraction induced by NE. Relaxation of aortic rings with or without endothelium were compared following application of the extract.

In the second group of experiments, tissues containing intact endothelium were incubated for 30 min with N^w-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor, or with indomethacin (10^{-4} M) to prevent production and release of prostacyclin. A single concentration of the extract was then tested. To determine the effect of *A. africana* extract on baseline tension in aortic rings, the extract was cumulatively added to the organ bath and the contractions of aortic rings with or without endothelium were compared.

To investigate the role of calcium-free buffer, contractions were measured using the following experimental protocol described by Cadene et al. [26]: the aortic rings were incubated in Ca^{2+}-free PSS for 45 min and then for 15 min in the presence of 1 mM ethylene glycol-bis (amino ethyl ether) N,N,N′,N′-tetraacetic acid (EGTA). The arterial rings were then washed 3 times in Ca^{2+}-free PSS for 10 min. The concentration of the extract in the bathing medium was then increased stepwise and the contractile effects recorded and the results expressed as grams of maximal tension developed after 10 min.

4.5. Statistical analysis

Statistical analysis was performed and means compared using the Student's *t*-test. Data are presented as mean ± S.E.M. Concentration-response curves were analysed to obtain the concentration of extract producing a 50% relaxation of the maximal contractile response (IC_{50}) using non linear curve fitting by means of a logistic equation.

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