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Effects of methylene chloride/methanol leaf extract of *Celtis durandii* Engler (Ulmaceae) on constriction of rat aorta

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Celtis durandii is a medicinal plant widely used in some part of Cameroon for the treatment of cardiovascular disorders. The vasorelaxant effects of the methylene chloride/methanol leaf extract of *C. durandii* were examined on isolated rat thoracic aorta. The relaxant effects of *C. durandii* on vascular preparation from rat aorta precontracted with KCl or norepinephrine was concentration dependent. This relaxing effect was significantly reduced with KCl-induced contraction following mechanical damage to the aortic endothelium. Relaxation elicited by *C. durandii* was not significantly affected by glibenclamide (10^{-6} M), a selective inhibitor of K-ATP-dependent channels or tetraethylammonium (10^{-6} M), a non selective K^+ channel blocker. Indomethacin (10^{-6} M) significantly inhibited relaxation induced by the plant extract. These findings indicate that the vasorelaxation effect of the methylene chloride/methanol leaf extract of *C. durandii* may be mediated at least in part by prostacyclin.

1. Introduction

Celtis durandii (Ulmaceae) is a medicinal plant used empirically in Cameroon by traditional healers in the treatment of various illnesses such as migraine, epilepsy, painful menstruation, cardio-vascular disorders, especially arterial hypertension, and renal disorders (Hauman 1948; Watt and Brandwigt 1962; Keay et al. 1964; Letouzey 1968; Troupin 1978; Eschborn 1984). In the central region of Cameroon, it is used as an antihypertensive agent. Phytochemical studies have revealed the presence of proteins, alkaloids and tannins (Oates et al. 1977; Baranga 1983) in the leaves.

Research on cameroonian medicinal plants reputed to have cardiovascular effects will help us to scientifically validate their traditional use and to rationalize local use of the plant extracts for the management of these diseases. In the present study, we have evaluated the effect of the methylene chloride/methanol leaf extract of *C. durandii* on the contraction of rats aorta muscles.

2. Investigations and results

The vasorelaxant effects of the methylene chloride/methanol leaf extract of *C. durandii* was assessed in rat vascular smooth muscle following a method previously described (Duarte et al. 1995; Dimo et al. 1998).

2.1. KCl-induced contractions

At final concentrations between 0.83 mg/ml and 4.98 mg/ml, *C. durandii* extract had no significant effect on baseline tension in aortic strips. The relaxant effect of *C. durandii* on vascular preparations from rat aorta precontracted with KCl (60 mM) are shown in Fig. 1. *C. durandii* extract induced relaxation of the precontracting strips in a concentration-dependent manner. The maximum relaxation at the highest dose of the plant extract when aortic muscle was precontracted with KCl were 83% and 63%, respectively, on endothelium-intact arteries and endothelium denuded arteries. There was a significant difference between effects on intact and denuded aortic strips in all concentration tested. Acetylcholine (10^{-5} M), a drug with known

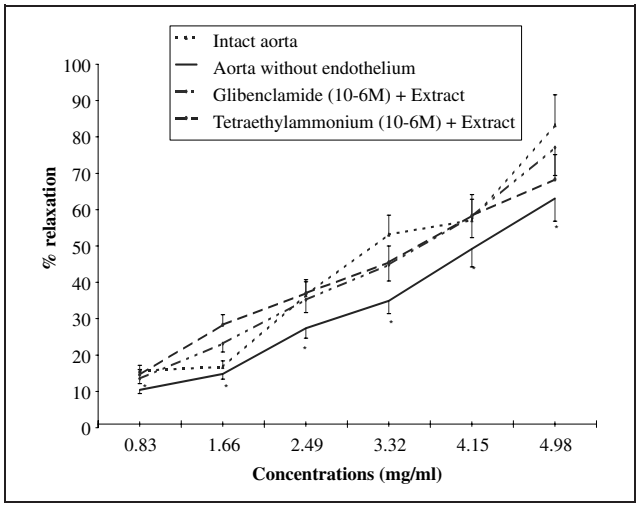

Fig. 1: Vasorelaxant activity of the leaf methylene chloride/methanol extract of *C. durandii* on the rat aortic strips precontracted with KCl (60 mM). Each value represent the percentage of relaxation ± SEM (n = 5). *P < 0,05, significant difference compared to the intact aorta precontracted with KCl (60 mM)

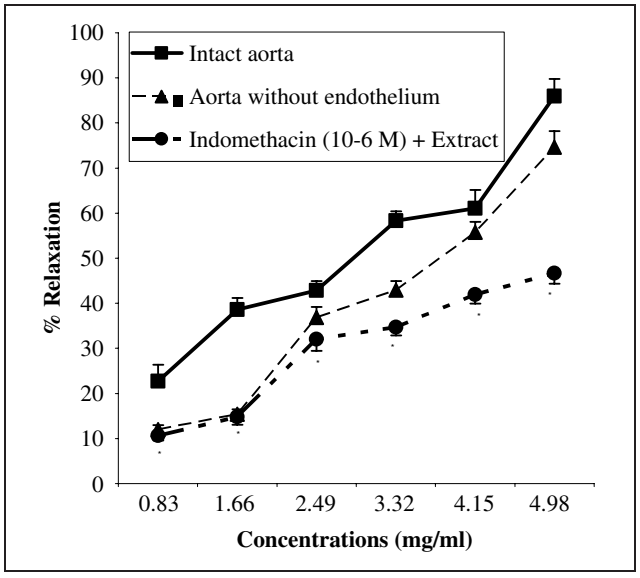

Fig. 2: Effects of the leaf methylene chloride/methanol extract of *C. durandii* on the rat aortic strips precontracted with norepinephrine (10^{-4} M). Each value represent the percentage of relaxation ± SEM (n = 5). *P < 0,05, significant difference compared to the intact aortic precontracted with norepinephrine (10^{-4} M)

vascular smooth muscle relaxant properties, used as the standard drug produced a relaxation of intact aorta (36%). Pretreatment of intact aortic strips with the specific inhibitor of ATP-dependent K^+ channels, glibenclamide (10^{-6} M) or a non-specific K^+ channel blocker, tetraethylammonium (10^{-6} M), had no significant effects on *C. durandii*-induced relaxation of aortic strips. In addition, it was observed (data not shown) that incubation of the non precontracted rat aortic strips with the plant extract (4.98 mg/ml) inhibited the contractile response induced by KCl (60 mM) by 83%.

2.2. Norepinephrine-induced contractions

Rat aortic strips precontracted with norepinephrine (10^{-4} M) were relaxed dose-dependently by the methylene chloride/methanol leaf extract of *C. durandii* (Fig. 2). The relaxation of aorta muscle precontracted with NE increased with increasing doses of *C. durandii* extract, from 23% at the dose of 0.83 mg/ml to 86% at the dose of 4.98 mg/ml on intact aorta and from 12% to 75% at respective doses on denuded aortic strips. The effect of the plant extract on norepinephrine-induced contractions was not altered in the absence of the functional endothelium when compared to endothelium-intact rings. Incubation of intact aortic strips with indomethacin (10^{-6} M) significantly shifted the concentration effect curves of *C. durandii* to the right (Fig. 2). The relaxation provoked by the plant extract (4.98 mg/ml) on intact aorta muscle was higher (86%) than that (46%) obtained in the presence of indomethacin.

3. Discussion

The presented results indicate that the methylene chloride/methanol leaf extract of *C. durandii* induces concentration-dependent relaxation in rat aortic strips precontracted with KCl or norepinephrine. The vasorelaxation effect of the plant extract on KCl-precontracted aorta was significantly reduced by endothelium removal which indicated that its relaxant effect is related to the release of endothelium-derived factors. The vascular endothelium played an important role in the controlling of vascular tone (Corvol 1993; Fitzpatrick et al. 1995). Endothelial cells respond to physical and chemical stimuli by production of vasorelaxing substances such as bradykinine, prostacyclin and nitrogen monoxide (Corvol 1993). The possible mechanism of action of *C. durandii* extract may involve the production of NO in the endothelium, which results in the formation of cGMP, causing the relaxation of the preparation contracted by KCl. However, the present results using norepinephrine contraction indicate that, the relaxation induced by *C. durandii* in aorta precontracted with norepinephrine was not significantly affected by endothelial removal. The cause of this contradiction is unclear. The relaxation action of the plant extract was sgnificantly reduced in endothelium intact aorta contracted with norepinephrine in the presence of indomethacin, a cyclooxygenase inhibitor (Duate et al. 1995). Thus, the vasorelaxation caused by *C. durandii* was mediated at least in part by prostacyclin. Glibenclamide (a selective inhibitor of K-ATP-dependent channels, Calixto and Cabrini 1997) and tetraethylammonium (a non specific K-ATP-dependent inhibitor, (Duarte et al. 1995) had no effect on *C. durandii*-induced relaxation, thereby excluding opening of K-ATP-dependent channels to the vasorelaxation of rat aortic rings of the plant extract.

The contractile responses induced by high KCl (60 mM) are due to the influx of extracellular Ca^{2+} through L-type voltage-sensitive channels (Godfraind et al. 1986; Carmeliet 1986; Gilani et al. 1994) and have been used to provide a simple means of studying drug with possible Ca^{2+} entry blocking properties (Duarte et al. 1995). The plant extract (4.98 mg/ml) inhibited these contractile responses (83%), which suggested that it inhibited Ca^{2+} through voltage-sensitive channels. In the present experiment, there are no data which suggest the release of intracellular calcium. It appears that the relaxation response to *C. durandii* extract may be due to the blockade of the influx of extracellular Ca^{2+}.

In conclusion, the results from this study show that the methylene chloride/methanol leaf extract of *C. durandii* is able to provoke a concentration-dependent vasorelaxation of the rat aortic strips. The vascular relaxation induced by *C. durandii* may be partially mediated by the activation of endothelial cyclo-oxygenase which is sensitive to inhibition by indomethacin.

4. Experimental

4.1. Animals

Male Wistar rats (150–250 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*.

4.2. Plant material

Fresh leaves of *C. durandii* were collected around Yaounde in October. A voucher specimen N° 6291/SRF CAM documenting the collection was identified at the National Herbarium, Yaounde and is on deposit there. The leaves were sun-dried and ground into powder. Then, air dried powdered leaves of *C. durandii* (1 kg) were macerated in 6 L of a mixture of methylene chloride/methanol (CH_2Cl_2/CH_3OH 1 : 1) at room temperature for a week with occasional shaking, filtered, and concentrated to dryness on a rotary evaporator under reduced pressure to afford a deep green CH_2Cl_2/CH_3OH extract (340 g), with an extraction yield of 34%. This extract (100,0 g) were dissolved in two drops of Tween 80 (1%) and the solution adjusted to 10 ml with distilled water to obtain a stock solution of 10 mg/ml. Further dilution was made in physiological salt solution. The final tween concentration did not produce significant effect on contractile response. Phytochemical analysis of the CH_2Cl_2/CH_3OH extract of *C. durandii* revealed the presence of flavonoïds, phenols, sterols, alkaloïds, cetones and triterpenes.

4.3. Tissue preparation and experimental procedure

Experiments were performed on the isolated rat thoracic aorta. Male Wistar rats were killed by cervical dislocation and their thoracic aortas were removed and placed in oxygenated physiological salt solution containing in mM: NaCl 147, KCl 5.6, $CaCl_2$ 2.6, NaH_2PO_4 0.66, CO_3NaH 11.9, $MgCl_2$ 0.24, Glucose 11. The thoracic aorta was cleaned of all adhering tissue and then cut into helical strips (1.0 mm × 10 mm). The endothelium was kept intact in some aorta strips, but in another group of experiments, the endothelium was removed from the aorta by rubbing the luminal surface with cotton thread. One end of the strip was attached to a hook at the bottom of an organ chamber and the other end connected to the sensitive element of the isometric transducer couple UGO BASILE two channel "GEMINI" 7070 recorder. Preparation were submitted to a basal tension of 1 g and were allowed to equilibrate for 60 min, during which the bath solution was renewed every 15 min. The organ bath was maintained at 37 °C, pH 7.4, and bubbled continuously with air. CO_2 was removed from air by passing through a 30% NaOH solution. Then, endothelium integrity was functionally assessed by evaluating the ability of acetylcholine (ACh, 10^{-5} M) to produce relaxation of preparations precontracted with norepinephrine (NE 10^{-4} M). Preparations were considered to contain a viable endothelium when Ach. evoked relaxations exceeding 64% of precontraction, and were considered to be endothelium denuded when ACh failed to cause relaxation (Furchgott and Zawadski 1980). After Ach testing, the aorta strips were washed with PSS three time during the next hour, prior to the next sequence (Dimo et al. 2003).

Following the equilibration period, concentration response of strips with or without endothelium were studied by precontracting each aortic strip with KCl (60 mM) or 10^{-4} M norepinephrine for 30 min and then allowing them to relax in the presence *of C. durandii* extract. Only one agonist was used in each experiment. When the contractile response to each agonist was stable, aortic strip was challenged with respective doses of *C. durandii*. All concentrations are expressed as final bath concentrations.

In the second group of experiments, tissues containing an intact endothelium were incubated for 30 min with indomethacin (10^{-6} M), a cyclooxygenase inhibitor and the relaxant effect of the plant extract was tested. The ability of glibenclamide (10^{-6} M), a blocker of ATP-sensitive K^+ channels (Duarte et al. 1995), to antagonize the relaxant effect of *C. durandii* was tested on the contraction induced by 60 mM KCl. Additionally, the relaxant effect of *C. durandii* extract on KCl induced contraction was studied with aortic strips pretreated with tetraethylammonium (10^{-6} M). Glibenclamide and tetraethylammonium were added to the bath after the contractile responses induced by KCl reached steady-state values and 30 min before the addition of the plant extract.

4.4. Statistical analysis

Data are expressed as mean ± SEM, n representing the number of rats used in each experiment. Changes in aortic tension were expressed in percentage of either norepinephrine- or KCl-induced tension. The one way analysis of variance (ANOVA) of the "Mintab" program was used to determine statistical significance of differences between treatments. $P < 0.05$ was considered to be statistically significant.

References

Baranga D (1983) Changes in chemical composition of food parts in the diet of colobus monkeys. J Biochem Ecology 64: 668.

Calixto BJ, Cabrini AD (1997) Herbal medicine catuama induices endothelium-dependent and independent vasorelaxant action on isolated vessels from rats, guinea-pigs and rabbits. Phytother Res 11: 32–38.

Carmeliet E (1986) Calcium channel-antagonist and the cardiovascular system. Acta cardiol XLI: 133–146.

Corvol P (1993) L'endothélium, site de production et du métabolisme des peptides vasoactifs. Méd/Sci 9: 1050–1060.

Dimo T, Rakotonirina VS, Kamgang R, Tan VP, Kamanyi A, Bopelet M (1998) Effects of leaf aqueous extract of *Bidens pilosa* (Asteraceae) on KCl- and norepinephrine-induced contraction of rat aorta. J Ethnopharmacol 60: 179–182.

Dimo T, Azebaze AGB, Kamgang R, Rakotoririna S, Nkengfack AE (2003) Vasodilator effect of the methylene chloride/methanol extract of *Erythrina indica* Linn. J Cam Aca Sci 3 (3), 207–212.

Duarte J, Perez-Vizcaino F, Torres AI, Zarzuelo A, Jimenez J, Tarmago J (1995) Vasodilator effects of visnagin in isolated vascular smooth muscle. Eur J Pharmacol 286: 115–122.

Eschborn FD (1984) Flore analytique du Togo: Phanérogames. Rapport Agence de Coopération Culturelle et Technique, Paris, p. 482–483.

Fitzpatrick DF, Hirschfield SL, Ricci T, Jantzen P, Coffey RG (1995) Endothelium-dependent vasorelaxation caused by variuos plant extracts. J Cardiovas Pharmacol 26: 90–95.

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

Gilani HA, Jambaz KH, Zaman M, Lateef A, Tariq SR, Ahmad HR (1994) Hypotensive and spasmolytic activities of crude extract of *Cyperus scariosus*. Arch Pharm Res 17: 145–149.

Godfraind T, Miller R, Wibo M (1986) Calcium antagonists and calcium entry blockade. Pharmacol Rev 38: 324.

Hauman L (1948) Flore du Congo belge et du Rwanda-urundi, préparée par le comité exécutif de la flore du Congo belge et le jardin botanique de l'état. Spermaphytes vol I. Institut National pour l'Etude Agronomique du Congo belge, Bruxelles, p. 39–43.

Keay RWJ, Onochie CFA, Stanfield DP (1964) Nigerian trees Federal Department of Forest Research Ibadan; Nigeria vol II. Printed at the Nigerian National press LTD; Apapa, and published by the Department of Forest Research, Ibadan, p. 160.

Letouzey R (1968) Ulmacées et Urticacées. Muséum National d'Histoire Naturelle. Laboratoire de phanérogamie. Direction de A. Aubrevilles, Paris, p. 3–44.

Oates JF, Swain T, Zantorska J (1977) Secondary compounds and food selection by colubus monkeys. J Biochem Eco 5: 317–321.

Troupin G (1978) Flore du Rwanda, spermatophytes. Musé Royal de l'Afrique centrale – Tervuren, Belgique, p. 127.

Watt JM, Brandwigk B (1962) Medicinal and poisonous plants of southern and eastern Africa. Second Edition E. & S. Livingstone LTD, Edinburgh and London, p. 1032.