EFFECTS OF THE AQUEOUS EXTRACT LEAVES OF *CELTIS DURANDII* ENGLER (ULMACEAE) ON CONSTRICTION OF RAT AORTA

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Summary

Celtis durandii is a medicinal plant highly used in Cameroon for the treatment of cardiovascular disorders. The vasorelaxant effects of the aqueous extract leaves 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. In the presence of Tetraethylammonium (10^{-6} M), the extract at concentrations of 1.75 mg/ml, 5.25 mg/ml and 8.75 mg/ml, did not produce significant modifications between the vasorelaxation induced by the extract in the normal physiological medium. Relaxation elicited by *C. durandii* were not significantly affected by glybenclamide (10^{-6} M), a non selective K^+ channel blocker. Indomethacine (10^{-6} M) significantly inhibited relaxation induced by the plant extract. At concentration of 1.75 mg/ml, 5.25 mg/ml, and 8.75 mg/ml, relaxation was 15.64 %, 36.53 %, and 72.12 % respectively. These findings indicate that the vasorelaxation effect of the aqueous extract leaves of *C. durandii* may be mediated at least in part by prostacyclin.

Keywords: *Celtis durandii* / Vasorelaxation / Aorta / Rat

Introduction

The high cost of manufactured drugs has led close to 80 % of the population of the developing world to turn to phytotherapy [1]. Each of the majority of these plants has several properties that phytotherapists exploit to treat illnesses such as hypertension, flu, kidney problems, ear and eye infections [2]. The importance of traditional medicine has mobilized many institutions into research or medicinal plants.

Celtis durandii (Ulmaceae) is a medicinal plant used empirically in Cameroon by traditional healers in the treatment of various illnesses such as: migraine, epilepsy, painfull menstruation, cardio-vascular disorders, especially arterial hypertension, and renal disorders [3-8]. Phytochemical studies have revealed the presence of proteins, alkaloids and tannins [9-10] in leaves. In order to popularize this plant and propose a low risk treatment for the sick, it is necessary to carry out pharmacology studies as well as studies of the vasodilatation properties of the plant material used by the traditional healers. In the present study, we have evaluated the effects of the aqueous extract leaves of *Celtis durandii* Engler (Ulmaceae) on constriction of rat aorta muscles

Materials and Methods

Animals

The experiments were carried out on Wistar rats aged from 3 to 4 months and weighing 150 to 250 g. the animals were raised in the Animal House of the University of Yaoundé I. They were fed a standard laboratory diet (S.P.C. Ltd, Bafoussam, Cameroon) and given water ad libitum.

Plant extract

Fresh leaves of *C. durandii* were collected around Yaoundé in October. A voucher specimen N° 6291/SRF CAM documenting the collection was identified at the National Herbarium, Yaoundé and is on deposit there. The traditional method of extraction consisted of grinding 1 kg of leaves in 1L distilled water for 24 hours, then filtering through a Whatman paper n°3. The filtrate was stored at 4°C, then subdivided in 20 ml fraction and dried at 30°C for 72 hours. 0.1 g drug weight of extract was obtained per ml of *C. durandii* extract. Phytochemical analysis of the extract revealed the presence of flavonoïds, phenols, sterols, alkaloids, cetones and triterpenes.

Tissue preparation and experimental procedure

Experiments were performed on 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. All adhering tissue from the thoracic aorta were cleaned and then cut into helica strips (1.0 mm x 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 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 [11]. After Ach. testing, the aorta strips were washed with PSS three time during the next hour, prior to the next sequence [12].

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 minutes 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 Indomethacine (10^{-6} M), a cyclooxygenase inhibitor and the relaxant effect of the plant extract was tested. The ability of glibenclamide (10^{-6} M), blocker of ATP-sensitive K^+ channels [12], 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 strip 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

Statistical analysis

Data are expressed as mean ± SEM (n = 5), n representing the number of rats used for each experiment. 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.

Results

KCl- induced contractions

The aqueous extract leaves of *C. durandii* in the incubation of the rat aorta. The minimum dose utilized, 1.75 mg/ml, provokes a non significant relaxation of the KCl-induced contraction of the aorta. The force of contraction decreases from 188.91 ± 22.77 mgf to 159.36±27.77mgf, i.e. 15.64 % of reduction. At a concentration of 3.5 mg/ml, the percentage relaxation was 16.74 %. *C. durandii*, at a concentration of 5.25 mg/ml provoked a significant relaxation of KCl-induced contraction of 225.31 ± 26.17 mgf to 142.98 ± 17.26 mgf, i.e. 36.54 % of reduction. The reduction was 53.18 % at a concentration of 7 mg/ml whereas the force of contraction decreases from 231.38 ± 21.90 mgf to 108.32 ± 20.55 mgf. At a concentration of 8.75 mg/ml, *C. durandii* provoked a 72.12 % reduction in the contraction of the rat aorta induced by KCl (60 mM). The force of contraction decreased from 394.31 ± 19.85 mgf to 113.31 ± 16.74 mgf.

C. durandii provoked, on the intact aorta, a concentration-dependent relaxation. The reduction in the force of contraction increased from 15.64 % to 72.12 %, respectively, for concentrations of 1.75 mg/ml and 8.75 mg/ml. The ED_{50} value established was 5.78 ± 3.36 mg/ml.

In the absence of the endothelium, the extract reduces the tension induced by KCl by 10.42 % and 27.37 % at a concentration of 1.75 mg/ml and 5.25 mg/ml respectively. The maximum concentration of the aqueous extract leaves of *C. durandii* (8.75 mg/ml) in a single dosage produced on endothelium-free aorta fragments a relaxation of 59.20 %. This result indicates that there is a significant difference between the action of the extract on intact aorta fragments (72.12 % of relaxation) and its action on endothelium-free aorta contracted in KCl (60 mM).

In the presence of Tetraethylammonium (10^{-6} M), the extract at concentrations of 1.75 mg/ml, 5.25 mg/ml and 8.75 mg/ml, did not produce significant modifications between the vasorelaxation induced by the extract in the normal physiological medium and in the presence of Tetraethylammonium. At the concentration of 1.75 mg/ml, the relaxant effect of the extract decreased from 248.98 ± 19.59 mgf to 216.65 ± 2.44 mgf, i.e. a reduction of 14.59 %. At a concentration of 5.25 mg/ml the relaxation was 37 %. At the maximum concentration (8.75 mg/ml), the relaxant effect of the extract decreased from 496.22 ± 17.69 mgf to 108.37 ± 13.68 mgf, a reduction of 78.22 % (fig. 1).
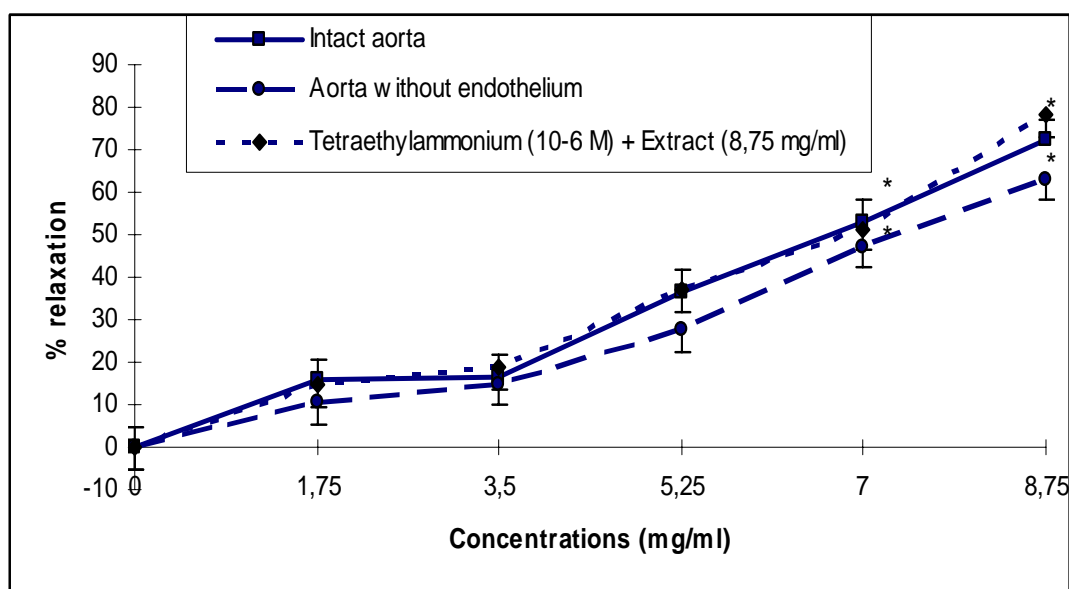

Figure 1: Vasorelaxant activity of the aqueous leaves extract of *C. durandii* on the isolated rat aorta precontracted with KCl (60 mM), in presence of Tetraethylammonium (10^{-6} M). Each value represent the percentage of relaxation as mean ± SEM (n = 5), P < 0.05, significant difference compared to the intact aorta precontracted with KCl (60 mM).

The introduction in the incubation medium of Glibenclamide (10^{-6} M) 5 minutes before the contractile response produced by KCl and 20 minutes before the addition of *C. durandii* extract did not provoke any significant modifications of the vasorelaxant effects of *C. durandii*. At a concentration of 1.75 mg/ml and 5.25 mg/ml, the relaxation induced by the extract was 13.48 % and 35.28 % respectively. At the maximum concentration of the extract, the tension decreases from 468.60 ± 5.30 mgf to 112.65 ± 8.10 mgf, i.e. 75.96 % reduction after the incubation of the organ in Glibenclamide (10^{-6} M) (fig.2).

Norepinephrine - induced contractions

In the absence of norepinephrine, the extract induced a relaxation of 22.74 % and 38.61 % respectively at concentrations of 1.75 mg/ml and 3.5 mg/ml. This dose-dependent relaxation decreased from 272.98 ± 12.99 mgf to 155.98 ± 12.63 mgf at a concentration of 5.25 mg/ml, giving a relaxation of 42.85 %. The relaxation (significant P< 0.05) was 60.36 % at a concentration of 7 mg/ml. At a concentration of 8.75 mg/ml, *C. durandii* provokes a significant (P< 0.05) relaxation of the norepinephrine-induced contraction. The force of contraction decreased from 477.31 ± 10.61 mgf to 107.85 ± 9.42 mgf, showing a reduction of 77.50 %. The ED_{50} value established was 5.91 ± 3.36 mgf.

On endothelium-free aorta fragment, the leaves extract of *C. durandii* at a concentration of 1.75 mg/ml reduces the force of contraction induced by norepinephrine from 216.65 ± 23.73 mgf to 190.64 ± 22.09 mgf, showing a reduction of 12 %. At concentrations of 5.25 mg/ml and 8.75 mg/ml, the relaxation was 46.85% and 60.75 % respectively. Thus, the aqueous extract of *C. durandii* provokes the relaxation of the norepinephrine-induced contraction of the aorta (intact as well as endothelium-free).
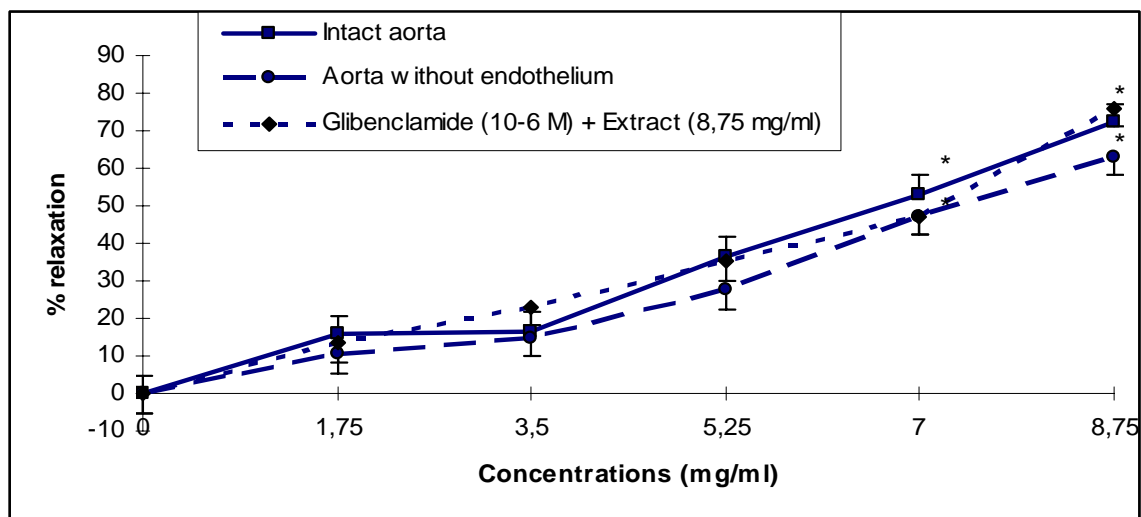

Figure 2: Effects of the aqueous leaves extract of *C. durandii* on the isolated rat aorta precontracted with KCl (60 mM and in presence of Glibenclamide (10^{-6} M). Each value represent the percentage of relaxation as mean $\pm$ SEM (n = 5), P < 0.05, significant difference compared to the intact aorta precontracted with KCl (60 mM).

Indomethacine (10^{-6} M) provoked a significant inhibition (P< 0.05) of the relaxant activity of rat aorta. At a concentration of 1.75 mg/ml, the tension decreased from 225.31 ± 8.66 mgf to 201.31 ± 9.32 mgf, with a reduction of 10.65 %. At the maximum concentration (8.75 mg/ml), the force of contraction decreased from 268.60 ± 13.00 mgf to 155.98 ± 8.10 mgf, i.e. a reduction of 41.9 %. At concentration of 1.75 mg/ml, 5.25 mg/ml, and 8.75 mg/ml, relaxation was 15.64 %, 36.53 %, and 57.12 % respectively. The contractions induced by the extract in normal physiological medium were reduced respectively by 10.65 %, 32 %, and 41.9 % after prior treatment of the intact aorta with Indomethacine (fig. 3).
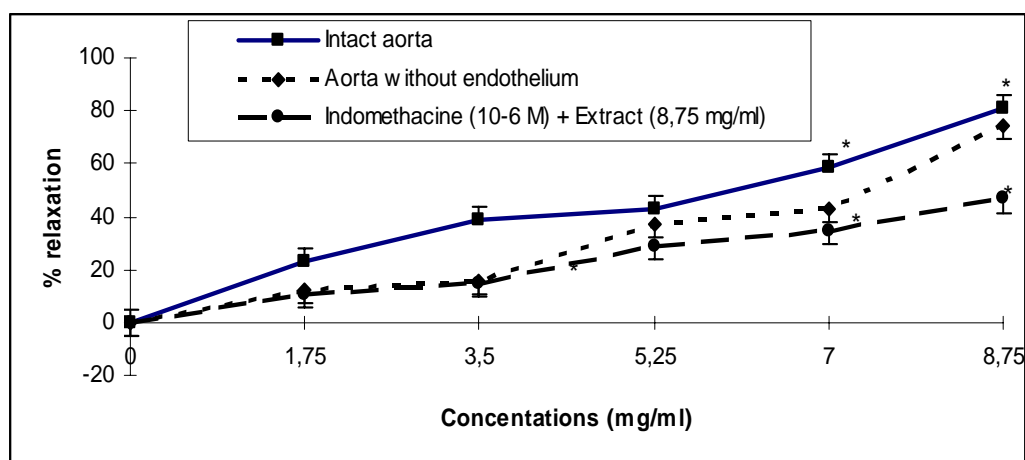

Figure 3: Effects of Indomethacine (10^{-6} M) on the vasorelaxant activity induced by the aqueous leaves extract of *C. durandii* on the isolated rat aorta precontracted with norepinephrine (10^{-4} M). Each value represent the percentage of relaxation as mean $\pm$ SEM (n = 5), P < 0.05, significant difference compared to the intact aorta precontracted with norepinephrine (10^{-4} M).

Effects of pharmacological substances

Nifedipine (40 µg/ml) provoked a decrease of the potassic contraction (60 mM) of intact fragments of rat aorta. The force of contraction decreased from 244.03 ± 8.03 mgf to 99.65 ± 14.69 mgf, a significant (P<0.05) 59 % reduction in the force of contraction.

Acetylcholine (10^{-5} M) reduced the force of contraction of intact aorta fragments induced by KCl (60 mM). The force of contraction decreased from 236.58 ± 14.14 mgf to 151.65 ± 18.12 mgf, a reduction of 36 %.

When the aorta is deprived of epithelium, acetylcholine does not provoke relaxation from KCl-induced contraction. Instead, a contraction was observed. Thus, from 233.65 ± 16.91 mgf, the force of contraction increases to 372.64 ± 18.64 mgf, an increase of 37.29 %.

Discussion

The effects of the aqueous leaves extract of *C. durandii* were examined on the contraction of the rat aorta induced by KCl and norepinephrine. The results obtained showed that the aqueous leaves extract of *C. durandii* is able to provoke a dose dependent vasorelaxation on intact aorta fragments or aorta fragments without endothelium contracting in KCl or in norepinephrine. The endothelium is known to play an important role in the control of vascular tonus [12-13]. Endothelial cells respond to physical and chemical stimuli by the production of vasorelaxing substances such as bradykinine, prostacycline and nitrogen monoxide [14]. *C. durandii* may contain vasoactive substance capable of relaxing contractions of the aorta induced by KCl (60 mM) or norepinephrine (10^{-4} M). at a concentration of 60 mM, the KCl provokes on the rat aorta a biphasic contraction with a rapid and transitory phasic component and a slow tonic component with a progressive modification of the tonus. According to Duarte et al. [12] and Shimodan and Sunano [15], this contraction may due to the depolarisation propagated along the plasmalemma, provoking the opening of potential sensitive Ca^{2+} channels thus causing an influx of Ca^{2+}. The increase in intracellular free Ca^{2+} concentration stimulates reactions which result in the contraction. According to Shimodan and Sunano [15], the phasic component is due to a rapid depolarisation with opening of fast potential-dependent Ca^{2+} channels where Ca^{2+} and Na^+ ions pass whereas the tonic component is the result slow depolarisation which admits Ca^{2+} and Mg^{2+} ions. Duarte et al [12] showed that the tonic component of the contraction is the result of an increase in entry of extracellular Ca^{2+}. After 30 to 45 minutes of action of 4.15 mg/ml of *C. durandii*, the percentage relaxation was 57.12 % with the intact aorta, and 49.20 % with endothelium-free aorta contracting in KCl. The ED_{50} established, was 3.12 ± 3.36 mg/ml with the intact aorta contracting in KCl and 2.91 ± 3.36 mg/ml with the intact aorta contracting in norepinephrine. The percentage relaxation depends also on the duration of the incubation. After 15 min. of action of 4.15 mg/ml of *C. durandii*, the percentage relaxation is 18 %. The increase in percentage of relaxation with time could be explained by the increase in the percentage of active principle-receptor complexes of *C. durandii*. This linkage receptor –active principle seems reversible since after inhibiting contraction, the act of washing readers the muscle capable of responding to a fresh stimulation. The relaxation is not the result of the opening of K+ channels as, according to Hamilton and Weston [16] and Gilani [17], any factor entailing the opening of K+ channels can only inhibit contraction if the concentration of KCl is inferior to 30 mM. the effects of *C. durandii* remind us of the action of acetylcholine. The results show that the contraction induced by KCl is not easily relaxed by the extract as well as by acetylcholine. This could be explained by its mechanism of action. In effect, acetylcholine provokes relaxation instead by stimulating muscarinic receptors.

This leads to the synthesis and liberation of a calcium-dependent factor named EDRF (Endothelium derived relaxation factor). According to Ignarro [18], this factor crosses the membrane of smooth muscle cells to activate the soluble guanylate cyclase, leading to the synthesis of cGMP. The increase in cGMP concentration leads to relaxation [19-20]. In effect, cycliques nucleotides play an important role in vascular smooth muscle relaxation. cAMP can dilate vascular smooth muscle either by causing a phosphorylation of the myosine light chain kinase [12], or by increasing the capture of Ca^{2+} by the sarcoplasmic reticulum, or finally by acting by other means, thus provoking the reduction of free cytosolic Ca^{2+} [21]. In the case of the acetylcholine induced relation, the cGMP may inhibit calcium influx and the liberation of Ca^{2+} from the reticulum [22]. This would explain the vasorelaxation provoked by the extract which would be due to a direct action on smooth muscle cells. *C. durandii* probably contains substances capable of inhibiting the component of the contraction due to a calcium influx through potential-dependent calcium channels. In the presence of Indomethacine (10^{-6} M), at concentrations of 1.75 mg/ml, 3.5 mg/ml, and 8.75 mg/ml, a significant difference was observed between the relaxation provoked by the extract on the aorta contracting in norepinephrine (22.74 %, 42.84 %, and 72.99 %, respectively), and the aorta incubated in Indomethacine (10.65 %, 32.07 %, and 41.90 %, respectively). Indomethacine is an inhibitor of cyclo-oxygenase, a producer of prostanoïd metabolites from arachidonic acid [19-20]. The pre-treatment of the intact aorta with a selective inhibiter of K-ATP-dependent channels, Glibenclamide (10^{-6} M) [23], did not provoke significant modifications of the vasorelaxant activity of the aqueous leaves extract of *C. durandii*. This seems to exclude any possible activation of K-ATP-dependent channels. In the presence of non specific K-ATP-dependent channel inhibitor, Tetraethylammonium (10^{-6} M) [12] the aqueous leaves extract of *C. durandii* did not provoke significant modifications of the vasorelaxant activity.

In conclusion, the results from this study show that the leaves aqueous 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 Indomethacine.

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