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Endothelium-dependent and independent vasorelaxant effect of *Terminalia superba* (Combretaceae) on rat aorta

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Abstract

Terminalia superba (Combretaceae) is a plant which is used in Cameroon for the treatment of many diseases including arterial hypertension. The vasorelaxant effect of the aqueous stem bark extract of *T. superba* was evaluated on the isolated aorta rings of the rat constricted with KCl (60 mM) or norepinephrine (10-5 M). Cumulative concentrations (20-100 µg/mL) of *T. superba* provoked a dose-dependent relaxation of the thoracic aorta precontracted by norepinephrine or KCl. The maximum vasorelaxant activity of *T. superba* was 107.24 ± 7.01% on the intact aorta and 102.48 ± 19.09% on the denuded aorta contracted by norepinephrine. The evaluation of the effects of the aqueous extract of *T. superba* on the intact aorta precontracted by KCl showed a maximum relaxation of 68.43 ± 2.51% at a final concentration of 100µg/mL. The vasorelaxation induced by *T. superba* (100 µg/mL) on the intact aorta precontracted by norepinephrine was significantly reduced in the presence of Nw-nitro-L-arginine methyl ester (54.98 ± 6.0%, $p<0.01$), tetraethylammonium (58.93 ± 5.30%, $p<0.05$) or propranolol (69.39 ± 4.03%, $p<0.05$). Indometacin (10-4 M), or glibenclamide (5 µM), did not modify significantly the vasorelaxant effect of the plant extract. These results suggest that the vasorelaxation elicited by *T. superba* was not mediated via endothelium-derived prostacyclin or ATP-sensitive K^+ channels. The direct effects of *T. superba* seem to be mediated by beta-adrenergic receptors and potassium channels other than potassium ATP-dependent channels. The results of this study could explain, at least partly, the use of this plant in empirical treatment of arterial hypertension.

Keywords: *T. superba*, Aorta, Endothelium, Contraction, Vasorelaxation.

Introduction

A number of medicinal plants have been widely used for the treatment of hypertension and several other ailments.[1] Plants like *Bidens pilosa*, *Celtis durandii*, and *Brillantesia nitens* have shown great potential as antihypertensives and they have been included in various herbal preparations.[2-4] *Terminalia superba* is a big tree with deciduous leaves, attaining 30 to 50 m in height and 120 cm stem diameter. It is widely distributed in the dense humid forests, semi-deciduous forests and also in easily flooded and secondary forests. It has a broad distribution in West and Central Africa. Its stem bark is used in folk medicine for the treatment of gastroenteritis, diabetes, female infertility, hypertension and abdominal pain.[5] The trypanocidal properties[6] anti-diabetic properties[7] and analgesic activities[8] of *T. superba* have been reported. Chemical analysis carried out on the methanol-water extract of the stem bark of *T. superba* has revealed the presence of flavonoids, terpenoids and tannins.[8]

On the basis of animal experiments, it has been suggested that systemic blood pressure elevation is associated with increased vascular tone which is the degree of constriction of blood vessels relative to their maximal diameters in the dilated state. Vascular tone is influenced by both the endothelium and vascular smooth muscle.[9] We recently reported that *T. superba* methanol extract caused endothelium-independent relaxations of rat aorta. These relaxations were not affected by L-NAME, an inhibitor of nitric oxide synthesis and were not fully abolished by K^+ channel inhibitors, suggesting other mechanisms of action.[10]

The mains objectives of this study were to investigate the vasorelaxant activity of the aqueous extract of *T. superba* stem bark, the fraction used in folk medicine as a remedy against hypertension, and to characterize its possible mechanism of action.

Materials and method

Plant material

The stem bark of *T. superba* was harvested in DJOUM locality, South province of Cameroon and authenticated at the National Herbarium, Yaounde by comparison with herbarium voucher specimen no. 19652/HNC earlier collected by Leeuwenberg. The sample was dried in the shade and ground into powdered form for extraction.

Animals

The experimental animals were albino Wistar rats weighing 180-220 g, raised in the Animal House of the Faculty of Science, University of Yaounde I. They were fed with a standard laboratory diet (Lanavet, Garoua, Cameroon) and given tap water ad libitum. They were kept at an ambient temperature under a 12 hour dark-light cycle. The study was approved by the institutional animal ethics committee (Reg. No. FWA-IRB00001954).

Preparation of crude extract

Two hundred grams of dried powdered material was boiled in 2 L distilled water for 30 minutes and then left to macerate during the cooling time. The resulting liquid extract was filtered and then concentrated under reduced pressure (45°C). The extraction yield was 8.18%.

Reagents

Norepinephrine, acetylcholine, indomethacin, Nw-nitro L-arginine methyl ester, propranolol, tetraethylammonium, potassium chloride, nifedipine and glibenclamide were purchased from Sigma Chemical Company (St. Louis, MO, USA). All chemicals used were of the highest purity commercially available. All solutions were made fresh in sufficiently high concentrations so that only very small aliquots were used for the experimentation. All the drugs and *T. superba* extract were freshly dissolved in distilled water before use.

Aortic preparation and mounting

Wistar rats (180-220 g) were sacrificed by cervical dislocation. The thoracic aorta was carefully removed and placed in krebs buffer solution (containing in mM: NaCl 118, KCl 4.8, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 11.1, pH 7.4) where adhering fat and connective tissue were cleaned. The aortas were cut into rings approximately 3-4 mm length. In some experiments, the vascular endothelium was removed intentionally by gently rubbing the internal surface using cotton thread. The aortic rings were mounted suitably between two stainless steel wire hooks in organ chamber filled with 20 mL of physiological salt solution maintained at 37°C and bubbled continuously with a 95% O2/ 5% CO2 mixture. One wire was anchored to the plastic holder of the organ chamber and the other was connected to an isometric force transducer. The isometric transducer was connected to a hemodynamic recorder Biopac Student Lab (MP 35) and computer. A resting tension of 1 g was applied to each aortic ring and they were allowed to equilibrate for 60 min, during which the bath solution was renewed every 15 min. Functional integrity of endothelium was confirmed by evaluating the ability of acetylcholine (10-5 M) to produce relaxation of preparations precontracted with norepinephrine. Preparations were considered to contain a viable endothelium when ACh evoked relaxations exceeding 80% of precontraction, and were considered to be endothelium free when ACh failed to cause a relaxation.[11, 12] After ascertaining the presence or absence of endothelium, rings of aorta washed with physiological salt solution three times during the next hour, were contracted by norepinephrine (10-4 M) or KCl (60 mM). At steady tension level, *T. superba* extract (20-40-60-80-100 µg/mL) was added cumulatively into the bath at 7 min. intervals which allowed each dose to reach a steady level before the addition of the subsequent dose.

To investigate the possible mechanism of action of extract, experiments were carried out in the presence of a non selective cyclooxygenase inhibitor, indomethacin (10-4 M); an inhibitor of NO-synthase, Nw-nitro-L arginine methyl ester (L-NAME, 10-4 M); a selective inhibitor of potassium ATP-dependent channels, glibenclamide (5 µM); a non selective potassium channel blocker, tetraethylammonium (TEA, 5 µM) or a non selective β-adrenoceptor blocker, propranolol (10-6 M).[13] Indomethacin or L-NAME was added in krebs solution 15 min. before contractions with norepinephrine, and then relaxed by cumulative dose of *T. superba* aqueous extract (20 - 100 µg/mL). Glibenclamide, TEA or propranolol was added at steady tension level of norepinephrine contractions but 15 min before the addition of *T. superba* aqueous extract (20-100 µg/mL). To investigate β-adrenoceptor mediating vascular relaxation, the effect of extract was examined in the presence of L-NAME and propranolol on intact aortas rings contracted by norepinephrine.

Statistical analysis

Group comparisons were performed by ANOVA (one way analysis of variance) with posthoc analysis using Newman-keuls test. Values are presented as means ± SEM; $p<0.05$ was considered to indicate statistical significance.

Results

Cumulative concentrations of *T. superba* (20-100 µg/mL) caused dose-dependent vasorelaxation of the intact aorta on norepinephrine- or KCl-induced contractions, reaching a maximum of 107.24 ± 7.01% or 68.43 ± 2.51% at 100 µg/mL, respectively. *T. superba* aqueous extract also induced vasorelaxation of denuded aorta in KCl or norepinephrine-induced contractions which reach a maximum of 15.64 ± 4.3 and 102.48 ± 19.09% at 100 µg/mL, respectively (Figure 1A and B).

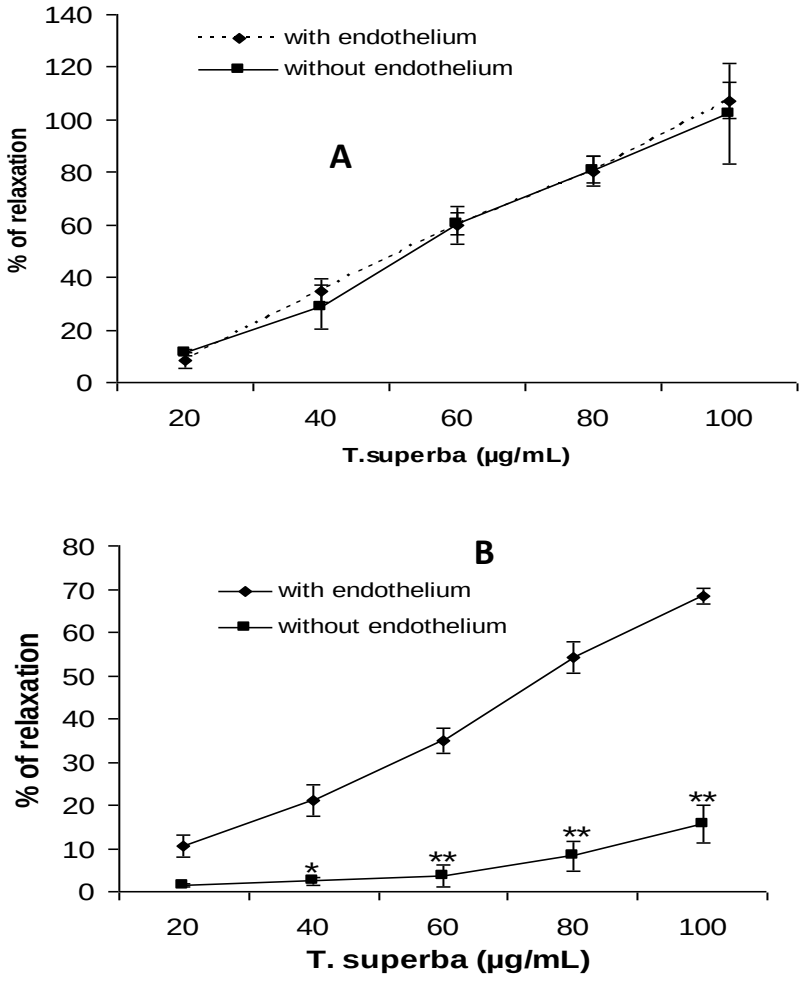

Figure 1: Relaxant effects of *T. superba* aqueous extract on endothelium-intact and -denuded rat aortas precontracted by norepinephrine (A) or KCl (B)

Data are expressed as mean ± SEM, n=5. *p <0.05, **p<0.01 as compared with relaxation of aorta ring with endothelium

Nifedipine cumulatively (10-20-30-40 µg/mL) used as reference drug caused neither maximal vasorelaxation of nor epinephrine-induced contraction of the aorta with endothelium (66.93 ± 6.32%) and without endothelium (30.23 ± 4.31%) at 40 µg/mL. In KCl-induced contractions, there was no significant difference between relaxation provoked by nifedipine (40 µg/mL) in intact (97.75±2.18) and denuded (95.83±1.34) aortas. Precontracted endothelium-intact aortic rings were relaxed dose-dependently by cumulative concentrations (10-8-10-5 M) of acetylcholine reaching a maximum of 83.46 ± 3.85% at 10-5M. In contrast, endothelium-intact aortic rings pre-treated with L-NAME (10-4 M) or endothelium-denuded aortic rings showed no relaxation after acetylcholine administration (data not shown).

The vasorelaxant effect of the aqueous extract of *T. superba* was examined with various compounds reported as inhibitors of vasodilation. Vasorelaxant effect of the aqueous extract of *T. superba* on intact aorta rings precontracted by nor epinephrine was not significantly affected by indomethacin or glibenclamide, while propranolol, TEA or L-NAME significantly reduced the effect of the plant extract as compared to the control group (Figure 2 and 3).

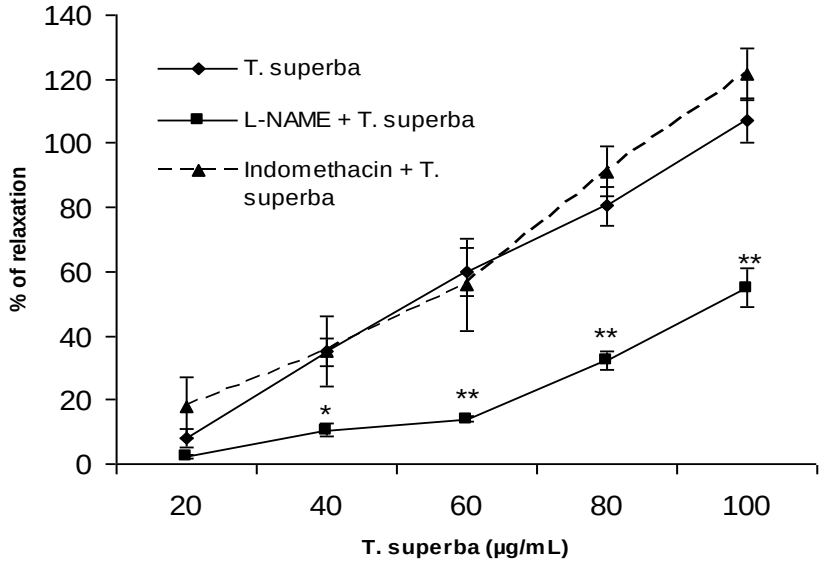

Figure 2: Effects of L-NAME (10-4 M) or Indometacin (10-4 M) on vasorelaxation induced by the aqueous extract of *T. superba* on the neither intact aorta rings precontracted with nor epinephrine

The values are given as mean ± SEM, n = 5. *p<0.05, **p<0.01, as compared with the control (*T. superba*)

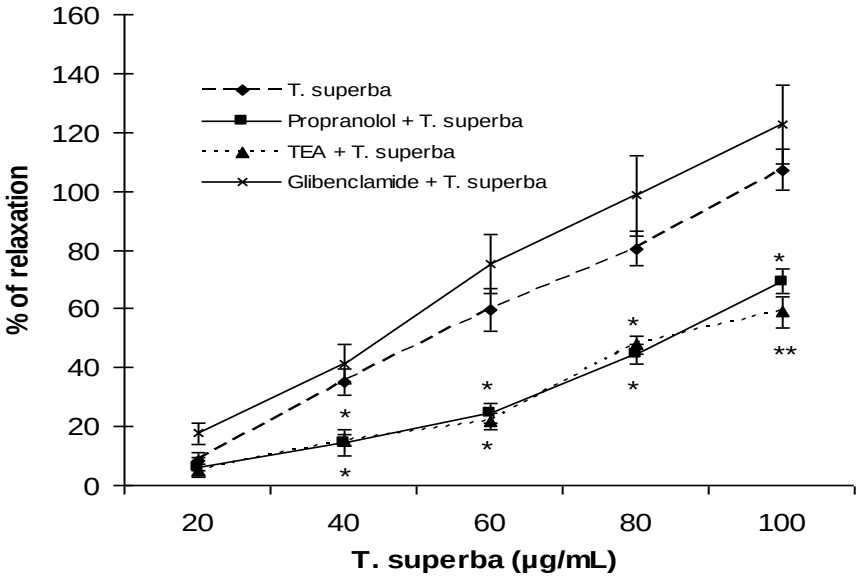

Figure 3: Effects of TEA (5 µM), Glibenclamide (5 µM) or Propranolol (10-6 M) on vasorelaxation induced by aqueous extract of *T. superba* on intact aorta ring precontracted with nor epinephrine.

The percentage of relaxation induced by the plant extract (100 µg/mL) in the presence of these antagonisms was 121.49 ± 8.07%, 122.72 ± 13.25%, 54.98 ± 6.01%, 58.93 ± 5.30% and 69.39 ± 4.03%, respectively, with indomethacin, glibenclamide, L-NAME, TEA and propranolol as compared to the effect on intact aorta rings precontracted by nor epinephrine (107.24 ± 7.01%). In the presence of L-NAME and Propranolol, the vasorelaxant effect of the extract was significantly reduced, reaching a maximum of 36.05 ± 2.18% at 100 µg/mL (Figure 4). This inhibitory effect of L-NAME and propranolol on *T. superb* induced relaxation was greater than the effect of L-NAME or propranolol alone.

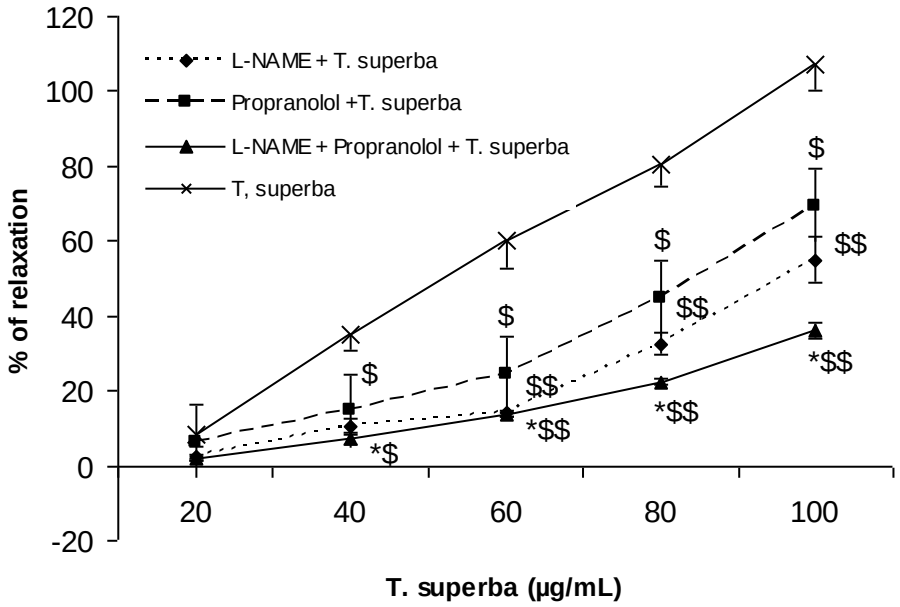

Figure 4: Effects of L-NAME and/or Propranolol on vasorelaxation induced by the aqueous extract of *T. superba* on aorta precontracted by nor epinephrine.

Each point represents mean ±SEM, n=5. *p<0.05, as compared with the vasorelaxation in the presence of L-NAME. $ p<0.05, $$p<0.01, as compared to vasorelaxation induced by *T. superba* alone.

Discussion

The stem bark aqueous extract of *T. superba* is a crude drug empirically used by traditional healers in Cameroon to manage hypertension. It is known that vasorelaxant substances can be beneficial for the treatment of hypertension.[14-15]

In this study, we demonstrated that the aqueous extract of *T. superba* exerted both endothelium-dependent and –independent relaxant effects in rat thoracic aorta. The ability of the extract to relax intact or denuded aortic rings suggests that the extract has a direct relaxant effect on vascular smooth muscle.[14]

A Ca^{2+}-channel blocker, nifedipine caused the dilatation of aorta with/without endothelium precontracted by norepinephrine, but its vasorelaxant effect was weaker than that induced by the plant extract. Indeed, the vasorelaxant effect of the extract on intact aorta precontracted by KCl (60 mM) was weaker than that obtained with norepinephrine (10-5M). The vasoconstrictor action of norepinephrine is due, at least in part, to an increase in calcium sensitivity of the contractile apparatus.[17] The relaxant effects of some therapeutically used drugs like nitroglycerine, β-adrenoceptor agonists such as betaxol and propranolol are primarily due to a decrease in calcium sensitivity of the contractile apparatus. Thus, our results suggest that the aqueous extract of *T. superba* may not have a Ca^{2+}-channel blocker activity because it is known that Ca^{2+}-channel blockers relax completely the contraction induced by KCl.[18, 19] as shown in our study. The plant extract may have interfered with calcium sensitivity of the contractile apparatus. The weakness of *T. superba* in relaxing KCl-constricted rings may also be due to the elimination of the chemical gradient of K^+ efflux. These results may also suggest that *T. superba* aqueous extract primarily inhibits the α-receptor-operated Ca^{2+} channels.

The vasorelaxant effect of *T. superba* aqueous extract was significantly reduced by L-NAME, an inhibitor of nitric oxide-synthase. However, in the presence of indomethacin,

a non selective cyclooxygenase inhibitor, the effect of the extract was not significantly modified. On the other hand, our results showed no significant difference between vasodilationsproduced by *T. superba* extract on aortic rings with or without the endothelium. This implies that T. superba causes vascular relaxation by two mechanisms: a direct effect on vascular smooth muscle that is independent of the endothelium and a mechanism that is dependent on the presence of a functional endothelium.Our results are similar to those of Ayano et *al*.[20] who reported anendothelium-dependent and –independent vasorelaxant action of Cinnamaldehyde, a crude drug of Cinnamomi Cortex on isolated rat aorta

To study this mechanism, we evaluated the effect of various inhibitors such as TEA, glibenclamide or propranolol on extract induced relaxation. We found that TEA and propranolol decreased significantly the vasorelaxant effect of the extract. It is well established that TEA, a non specific inhibitor of K^+-channels inhibit K^+ efflux and the resulting hyperpolarisation leads to vasodilation.[18, 21] The inhibitory effect of TEA on vasorelaxation of the *T. superba* aqueous extract suggests that extract may act at least partly on K^+-channels. This hypothesis is supported by the weak vasorelaxant effect of the extract on intact aorta precontracted by KCl since an increase of extracellular K^+ inverted the chemical gradient across the plasmic membrane leading to potassium influx[18, 19] and so, may attenuate the effects of the extract through potassium channels. The fact that Glibenclamide failed to inhibit the vasorelaxant effect of the aqueous extract of *T. superba* clearly demonstrates that its action on K^+-channels is not mediated through K^+-ATP channels. This result is different from those previously obtained with the methanol extract of T. superba.[10] In fact; we have shown that methanol extract of *T. superba* acts on K^+-ATP channels. This difference can be explained by the solvent used for extraction. Water is more polar than methanol and may thus extract some compounds which cannot be obtained with methanol. The inhibitory effect of propranolol on vasodilation effect of the plant extract suggests a β-adrenoceptor activity, since it is known that the β-adrenoceptor agonists may induce vasorelaxation on rat aorta.[13, 22] It has also been reported that β-adrenoceptors agonists (e.g., isoprenaline) act through a beta-adrenoceptor on the endothelium to raise cAMP and that this may, directly or indirectly, release nitric oxide to evoke vascular relaxation via increase in cGMP.[23] It has been suggested that action of β-adrenoceptor agonists can be linked to the L-arginine/NO pathway and given that such an endothelium-dependent mechanism has been proposed in rat thoracic aorta,[22-24] we investigated β-adrenoceptor mediating vascular relaxation of the extract. In the presence of L-NAME and propranolol, the percentage of relaxation induced by the plant extract was significantly lower than that recorded in the presence of L-NAME or propranolol alone. This result suggests a synergic action of β-adrenoceptor and L-arginine/NO pathway, indicating that β-adrenoceptor activity of *T. superba* aqueous extract could be independent of the L-arginine/NO pathway. It appears, therefore, that the aqueous extract of *T. superba* stem bark possesses in part NO-related dependent vasorelaxant activity and an endothelium-independent vasorelaxant activity by its directs effects.

The direct effects of the plant extract could be mediated by β-adrenoceptor and some potassium channels other than potassium ATP-dependent channels. Since it is unclear which specific potassium channel is involved, the precise mechanism by which aqueous extract of *T. superba* induces vasorelaxation awaits further investigation.

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