Vasorelaxant effects of aqueous leaf extract of *Tridax procumbens* on aortic smooth muscle isolated from the rat

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Abstract

*Tridax procumbens* is commonly used in traditional medicine in southern part of Nigeria for the treatment of hypertension. However, the mechanism of its antihypertensive properties remains unclear. Attempts were made to investigate the properties of direct actions of aqueous extract of the leaves of *T. procumbens* on mechanical responses of smooth muscles in aortic ring preparations isolated from the rat. Endothelium-intact aortic rings, isolated from the normotensive rats, had been pre-contracted with noradrenaline, and cumulative addition of the aqueous extract (0.15–1.05 mg/mL) to the bathing fluid induced a concentration-dependent relaxation. Aqueous extract of *T. procumbens* also attenuated the contractile responses to KCl and shifted the concentration-response curve to the right. The contractile responses to serotonin were also attenuated and the concentration-response curve was shifted to the right in the presence of the extract. The results of this study indicated that aqueous leaf extract of *T. procumbens* possesses vasodilatory effects on the aortic smooth muscles isolated from the rat. Based on these results, a possible mechanism involved in the relaxing actions of the extract on vascular smooth muscle was discussed. The results of this study may provide a scientific basis for the use of this extract to the treatment of hypertension in Nigerian traditional medicine.

Key words: *Tridax procumbens*, leaf aqueous extract, aortic ring, hypertension, vascular smooth muscle

Introduction

*Tridax procumbens* Linn. is a family of Asteraceae, and its common name includes coat buttons and tridax daisy in English, cadillo chisaca in Spanish, herbe caille in French, gharama in Hindi (Saxena and Albert, 2005) and kotobukigiku in Japanese. This plant is native of tropical America and naturalized in tropical Africa, Asia, Australia and India. It is a wild herb distributed throughout India.

Previously, we reported the possible antihypertensive effect of the aqueous extract of the leaves...
of this plant on rat: an intravenous injection of the crude extract of the leaves of *Tridax procumbens* caused reduction in both the blood pressure and the heart rate in anaesthetized healthy normotensive rats (Salahdeen et al., 2004). These effects were attributed to be produced by substances which activate muscarinic cholinergic receptors, since the hypotensive effect of the extract was significantly prevented by the pretreatment with atropine (Salahdeen et al., 2004).

However, the precise mechanism of the hypotensive effect of *Tridax procumbens* is not clear, although it is speculated that the action may involve a reflex which depends on the vagus being intact, in addition to the direct vasodilatory actions to vascular smooth muscle cells. The latter effect could not be confirmed with the *in vivo* experimental designs, and attempts were made to investigate the possible direct effects of the aqueous extract of leaves of *T. procumbens* on vascular smooth muscles, using isolated rat aorta.

**Materials and Methods**

*Ethical considerations*

Experimental protocols and procedures used in this study were approved by the Animal Ethics Committee of the Lagos State University College of Medicine and conform to the 1985 guidelines for laboratory animal care of the National Institute of Health (NIH).

*Plant material*

Fresh leaves of *T. procumbens* were collected from open grassland on the Ikeja Campus of the College of Medicine, Lagos State University, Lagos, Nigeria in July, 2011. Identification of the plant was carried out by the Taxonomist of the Lagos State University, Department of Botany. Following identification, a voucher specimen of the plant was deposited in the herbarium of the Botany Department of Lagos State University, Lagos State, Nigeria.

*Preparation of T. procumbens leaf aqueous extract*

Five hundred grams (500 g) of fresh *T. procumbens* leaves were air dried under shade at room temperature (26 ± 1°C) for a period of 2 weeks. The dried leaves were thereafter milled into fine powder in a warring commercial blender. The powdered leaves were macerated in distilled water and extracted twice, on each occasion with 100 mL of distilled water at room temperature (26 ± 1°C) for 48 hours (with occasional shaking). The resultant mixture was filtered and concentrated under reduced pressure in a rotary evaporator at 60 ± 1°C. Freeze-drying and solvent elimination of the resulting aqueous extract finally yield: 12.3% of a light brown, powdery crude *T. procumbens* leaf aqueous extract. Without any further purification, aliquot portions of *T. procumbens* were weighed and dissolved in distilled water (at room temperature) for use on each day of our experiments.

*Animals*

Healthy, young adult, male and female Wistar albino rats, weighing 250–300 g, were used. The animals were kept and maintained under conventional laboratory conditions of temperature, humidity and light, and allowed free access to food standard pellet diet (Live Stock feeds Nig. Ikeja Nigeria) and
drinking tap water *ad libitum*. All the animals used were fasted for 16 hours, but still allowed free access to drinking tap water before the commencement of our experiments.

**Drugs**

Chemicals used for preparing Krebs-Henseleit physiological solution (KHS) are: calcium chloride, glucose, magnesium sulphate, potassium dihydrogen phosphate, sodium bicarbonate and sodium chloride (E. Merck, Darmstadt, Germany). All other chemicals and materials were used at the highest analytical grade commercially available.

Noradrenaline hydrochloride and serotonin (5-hydroxytryptamine) were purchased from Sigma-Aldrich Chem. (St. Louise, MI, USA). The stock solutions of these chemicals were prepared by dissolving with distilled water, at concentrations of $10^{-2}$ M. The solutions were prepared fresh on the day of experiments.

**Tissue preparation**

Effects of *T. procumbens* on rat vascular smooth muscles were investigated in isolated, spontaneously-contracting descending thoracic aortic ring preparations of normotensive rats. Each rat was euthanised by halothane inhalation, thorax was opened, and the descending thoracic aortas (4 cm long) were quickly removed from the animal. It was freed of fat and connective tissues, and placed in a Petri dish containing physiological salt solution suspended in 50 mL Ugo Basile organ-baths chamber containing Krebs-Henseleit physiological salt solution (KHS) of composition, in mM: NaCl, 119.0; KCl, 4.7; NaHCO₃, 15.0; MgSO₄, 1.2; CaCl₂·2H₂O, 1.6; KH₂PO₄ 1.2 and glucose, 11.5; pH adjusted to 7.4. The bathing KHS was maintained at 36 ± 1°C and continuously aerated with 95% O₂ + 5% CO₂ gas mixture. The mounted aortic ring preparations (endothelium intact) were subsequently left to equilibrate for 60–90 min, under a passive tension of 1 g during which time the bathing physiological solution was changed every 30 min, after the tissues had been challenged with $10^{-7}$ M noradrenaline (NE). Extract and/or reference drug solutions used were added to the bath fluid cumulatively. Bath-application of *T. procumbens* extract and/or reference drug concentrations were repeated (where necessary) after washing out the previous extract or reference drug concentration 4–5 times, and allowing each tissue preparation to rest for 5–10 min, or until its tone returned to the baseline level. In order to make allowance for changes in tissue sensitivity, two isolated aortic rings were always set up at a time, one used as ‘control’ and the other one used as “test” (*i.e.*, *T. procumbens* extract- or reference drug-treated) preparations. ‘Control’ aortic rings were only treated with distilled water equivalent to the volume/s (0.1–0.8 mL) of bath applied *T. procumbens* extract or reference drug solution. *T. procumbens* extract - and/or reference drug-induced responses of the isolated venous smooth muscle preparations were recorded isometrically by means of Ugo Basile force-displacement transducers model 7004 which were coupled to Data capsule acquisition system Model 17400, for displaying and recording mechanical responses of isometric tension.

**Concentration-response relationship of the extract**

At the end of the equilibration period, the aortic rings were pre-contracted with either NE ($10^{-7}$ M) or KCl (60 mM) and, after the contraction had been peaked, a cumulative concentration-response test to the seven increasing concentrations (0.15–1.05 mg/mL) of extract was started from the lowest dose.
The effect of each concentration was allowed to stabilize before the addition of the next concentration of the extract. The response to each concentration was expressed as a percentage of the initial force produced by either NE or KCl. The concentration of the extract of *T. procumbens* which reduced the amplitude of contraction to half (EC\textsubscript{50}) the initial value was 0.45 ± 0.01 mg/mL in NE-precontracted aortic rings and 0.75 ± 0.01 mg/mL in KCl pre-contracted rings. These EC\textsubscript{50} concentrations of *T. procumbens* extract were used in the subsequent experiments.

**Concentration-response study to noradrenaline (NE) and KCl**

The aortic rings were exposed to cumulative concentrations of NE (10^{-11}–10^{-5} M) after incubating with the vehicle (0.2 mL distilled water) for 15 min, and the amplitude of contractions produced by each concentration were measured. The tissues were then washed by flushing with Krebs-Henseleit physiological solution for several times at 15 min interval, until the tension level came back to the initial resting level. After adequate rest the tissues were incubated with the extract (0.45 mg/mL) for 15 min, and the concentration-response study to NE was repeated in the presence of the extract. The same procedure was repeated for the concentration-response study to KCl (10–100 mM) with and without the extract (0.75 mg/mL), by using different sets of freshly stabilized aortic rings.

**Concentration-response test to serotonin**

In order to ascertain whether the observed responses of aortic rings to the extract involve endogenous serotonin (5-hydroxytryptamine), effects of exogenously applied serotonin on concentration-response relationship were examined, in the presence and absence of the extract (0.45 mg/mL). The amplitude of contractions produced by cumulative application of serotonin (10^{-9}–10^{-3} M) was measured, and they were expressed as percentage of the maximum force developed by the highest concentration of serotonin (equal to 10^{-3} M), in both conditions.

**Statistics**

Results are presented as means ± S.E.M. Data were analyzed using Student’s *t* test, and *P* values less than 0.05 were considered statistically significant.

**Results**

**Relaxation responses elicited by *T. procumbens* extract**

Figure 1 shows typical tracings of relaxation responses to *T. procumbens* extract recorded in aortic rings which had been pre-contracted with NE or KCl. The tension developed by NE (Fig. 1A) or by KCl (Fig. 1B) was successively inhibited by cumulative application of increasing concentrations of *T. procumbens* extracts. Removal of *T. procumbens* extracts from the bathing solution resulted in a recovery of tension produced by either NE or KCl (data not shown).

**Effects of *T. procumbens* extracts on concentration-response relationships to NE and KCl**

The concentration-response relationship of aortic rings to NE, with and without *T. procumbens*, is shown in Fig. 2. The curve obtained in the presence of *T. procumbens* was significantly shifted to the
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Right and the peak tension was attenuated, compared to the control curve \(P<0.05\). The concentration-response curve to KCl was shifted to the right significantly and the peak amplitude of contraction was attenuated compared to the control curve \(P<0.05\), in the presence *T. procumbens*.

**Effects of *T. procumbens* extracts to serotonin-induced contractions**

The concentration-responses curve to serotonin measured in the absence and presence of *T. procumbens* are shown in Fig. 4. Again, the curve was shifted to the right and the peak tension was attenuated significantly, after application of the extract of *T. procumbens* to the bath \(P<0.05\), Fig. 4. 

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**Fig. 1.** Typical tracings showing the vasorelaxant effects of graded concentrations of *T. procumbens* aqueous leaf extract on (A) noradrenaline (NE)-induced and (B) KCl-induced contractions in the endothelium-containing isolated aortic ring preparations obtained from normotensive rat. Arrows 1–7 represent cumulatively administered *T. procumbens* extracts (0.15, 0.3, 0.45, 0.6, 0.75, 0.9 and 1.05 mg/mL respectively). NE, KCl and *T. procumbens* were washed out at the open downward-arrow.
Discussion

This study indicated that the aqueous leaf extract of *T. Procumbens* had a vasorelaxant effect on the aortic rings isolated from the rat, possibly by modifying the Ca$^{2+}$-dependent mechanisms. The results showed that the contractile responses to NE and KCl were attenuated by the extract, as evidenced by the shifting of concentration-response curve to each contractile agent to the right and the maximal response to each agonist was depressed in the presence of *T. Procumbens*. Similar effects were also ob-
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served on the concentration-response curves for serotonin. The evidence that the aqueous extract of *T. procumbens* relaxed contractions induced by either NE or KCl, suggests that the actions of his extract are multiple, since these two contractile agents are known to induce contraction of vascular smooth muscle by two separate mechanisms: NE-induced contraction is produced by activating adrenergic receptors on the vascular smooth muscle membrane which leads to the mobilization of both extracellular and intracellular pools of Ca$^{2+}$, while the KCl-induced contraction is produced as a result of membrane depolarization which induces an increase in influx of Ca$^{2+}$ through voltage-dependent calcium channels (VDCC) (Bolton, 1979; Ebeigbe, 1982; Ebeigbe and Aloamaka, 1987). From our observations that aqueous leaf extract of *T. procumbens* induces comparable relaxation responses in contractions produced by either of the agonists, it was suggested that *T. procumbens* extract blocks Ca$^{2+}$ influx through interference with both voltage- and receptor-operated channels.

Similarly, the extract also produced relaxation responses in the serotonin-contracted aortic ring preparations. The main mechanism involved in the contraction of vascular smooth muscle by this agonist is considered to be produced through an increase in cytoplasmic Ca$^{2+}$ and phosphorylation of the regulatory light chains of myosin (Karaki *et al.*, 1997). It is well known that vasoconstrictive agonists activate multiple pathways that modulate the contractile response of smooth muscle, and protein kinase C (PKC) (Horowitz *et al.*, 1996), Rho family G proteins (Somlyo and Somlyo, 2000), nonreceptor tyrosine kinases (Hughes and Wijetunge, 1998) and extracellular signal-regulated kinases (ERK1/2) (Ishihata *et al.*, 2002) have been shown to play roles in smooth muscle contraction. Since the leaf aqueous extract of *T. procumbens* induces comparable relaxation responses produced by NE, KCl and serotonin, it is suggested a possible involvement of non-specific but ultimate interference with the availability of Ca$^{2+}$ for the contractile process.

These results are consistent with the earlier studies that the aqueous leaf extract of *T. procumbens* lowers the systolic, diastolic and heart rate (Salahdeen *et al.*, 2004), and it also prevents the development of hypertension in salt-loaded rats (Ikewuchi *et al.*, 2011). The hypotensive effect was
suggested to involve vasodilatations produced by both neuronal reflex responses and direct relaxing actions to smooth muscle cells, since the depressor and bradycardiac effects of the leaf extract had no effect on the increased blood pressure and heart rate elevation produced by adrenaline (Salahdeen et al., 2004). The present results confirmed that the extract of *T. procumbens* possessed a direct dilatory effect on vascular smooth muscle.

The phytochemical screening of *T. procumbens* revealed the presence of alkaloids, carotenoids, flavonoids (catechins and flavones) and tannins in the aqueous extract. Contents of luteolin, glucoluteolin, quercetin and isoquercetin have also been reported in *T. procumbens* leaf extract (Saxena and Albert 2005). Reports from several laboratory studies have shown that luteolin, glucoluteolin, flavonoids, quercetin and isoquercetin possess vasodilator effects in the isolated rat aorta and porcine coronary artery (Herrera et al., 1996), through the hyperpolarization of smooth muscle membrane via the activation of potassium channels (Xu et al., 2007), not through modification of Ca\(^{2+}\) efflux induced by NE, but through the inhibition of Ca\(^{2+}\) influx induced by KCl in rat aortae (Duarte et al., 1994).

Protein kinase C (PKC) has been proposed to play a key role in the maintenance of tonic contractions of vascular smooth muscles (Rasmussen et al., 1987). PKC extracted from the rat brain was inhibited by the plant flavonoids in a concentration-dependent manner, depending on the structure of flavonoid (Ferriola et al., 1989). Duarte et al. (1993) observed that the vasoconstriction induced by phorbol 12-myristate-13-acetate (PMA), a PKC activator, was inhibited by quercetin and related flavonoids in the rat aorta. From the result of our study, it is likely that the primary action of *T. Procumbens* extract in the flavonoid-induced vasodilation may be due to the inhibition of protein kinases, such as myosin light chain kinase, and possible involvement of some types of Ca\(^{2+}\)-sensitive kinases, including protein kinase C, is considered. Therefore it is not unreasonable to speculate that the vasodilatory effects of the extracts of *T. Procumbens* demonstrated in this study might therefore be in part due to the presence of flavonoids, including quercetin, in the extract.

In conclusion, the present study provided evidence for a direct dilatory effect of *T. Procumbens* extract on vascular smooth muscle. The present results support the ethno-medical application of *T. Procumbens* in the treatment of hypertensive disease. Further experimentation is needed in order to understand the precise mechanism of action in vascular smooth muscles by the extract.

References


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