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Original Research Article

Effects of aqueous leaf extract of *Tridax procumbens* on contractile activity of corpus cavernosum in *N*-nitro-L-arginine methyl ester-induced hypertensive male rats

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ABSTRACT

Objective: This study investigated the effects of aqueous leaf extract of *Tridax procumbens* (ALETP) on contractile activity of corpus cavernosum in *N*-nitro-L-arginine methyl ester (L-NAME)-induced hypertensive male rats.

Methods: Twenty normal, adult male rats (130–150 g) were divided into four groups of five rats each. Group I (control) was given normal saline (0.6 mL/kg) and group II was given L-NAME (40 mg/kg) for 6 weeks. Groups III and IV also received L-NAME (40 mg/kg) for 6 weeks but were further co-treated with 100 and 200 mg/kg of ALETP, respectively, from week 4 to week 6. All treatments were given orally. Strips of corpus cavernosum from each of the four groups were exposed to increasing concentrations of acetylcholine (ACh) and sodium nitroprusside (SNP) (10^{-9}–10^{-5} mol/L) after contraction with phenylephrine (10^{-7} mol/L) to test for a dose–response effect. Response to potassium and calcium was also measured after cumulatively adding potassium and calcium (10–50 mmol/L) to potassium- and calcium-free organ chamber. Isometric contractions were recorded through an Ugo Basile data capsule acquisition system.

Results: Mean arterial blood pressure was significantly reduced in the ALETP co-treated group compared to the control and L-NAME-only groups ($P < 0.05$). Cavernosa strips from ALETP co-treated rats exhibited significant inhibition of contraction in response to phenylephrine, potassium chloride, and calcium chloride ($P < 0.05$). Relaxation in response to Ach and SNP was also significantly impaired in cavernosa strips from the L-NAME-only treated group ($P < 0.05$), while ALETP co-treated groups showed enhanced percentage relaxation.

Conclusion: ALETP treatment of L-NAME-induced hypertensive rats promotes a relaxant effect on isolated cavernosa strips. ALETP shows potential in correcting erectile dysfunction in hypertension.

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1. Introduction

Hypertension and antihypertensive drugs have been associated with incidences of sexual dysfunction and detrimental effects on erectile contractility [1,2]. Reports have associated elevated blood pressure with an increased occurrence of erectile dysfunction (ED), while successful control of blood pressure is associated with improvement of this ailment [1]. ED is a chronic condition that exerts a negative impact on male self-esteem, including interpersonal and family relationships. The pathogenesis of ED is usually complex, involving organic, physiologic, endocrine and psychogenic factors [3]. In addition to hypertension and associated cardiovascular risk factors, major health concerns such as metabolic syndrome, hyperlipidemia and diabetes have become relevant to the analysis of ED [4–6]. While studies [1,2] have shown that ED is more frequent in patients with hypertension when compared with normotensive subjects, it is unknown whether the higher prevalence of ED is a result of hypertension, antihypertensive treatment, or a combination of both. From previous studies, one out of five cases of ED is

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due to adverse effects of drug use [7]. Older antihypertensive drugs such as central-acting, β-blockers and diuretics have already been confirmed to cause ED, while newer antihypertensive drugs like calcium antagonists, angiotensin-converting enzyme inhibitors and angiotensin receptor blockers have either neutral effects or may even have a positive effect on sexual function [8,9]. Literature abounds with studies on the vaso-relaxant and antihypertensive potential of *Tridax procumbens* leaf (family Asteraceae) [10–13]. On hypertension, Salahdeen et al. [10] reported that intravenous injection of crude extract of *T. procumbens* caused bradycardia, which was associated with a fall in arterial blood pressure in normotensive rats. *T. procumbens* leaf extract was also shown to exhibit a non-specific relaxant activity in isolated aortic smooth muscle preparation [11], suggesting that the vaso-relaxant effect may be due to vasodilator properties of *T. procumbens* leaf extract. Ethanolic extracts of *T. procumbens* were also reported to induce relaxation in corpus cavernosum excised from normotensive rats [14]. Nitric oxide (NO) plays an important role in blood pressure regulation, thrombosis and atherosclerosis [15]. Vascular relaxation of smooth muscles is usually reduced in hypertensive patients as a result of NO deficiency, and this potentially leads to development of endothelial dysfunction in hypertensive models [16,17]. It has been proven that over 80% of ED cases are caused by vascular alterations, suggesting that the severity of ED is directly related to the presence of vascular risk factors (hypertension, hyperlipidaemia, atherosclerosis and diabetes) that cause severe endothelial dysfunction [18,19]. The mechanisms involved in vascular endothelium dysfunction are characterized by the alteration of vascular tone, which may be due to deficiency in the relaxation process of smooth muscle cells located in the corpus cavernosum and the wall of the small arteries as well as by occlusion of the cavernosal arteries, due to atherosclerosis. This indicates that an impaired release of NO, caused by endothelial dysfunction, could affect the smooth muscle cell activity during penile erection and also contributes to the loss of its anti-atherogenic protection [19,20].

It is noteworthy that earlier cited studies on the vaso-relaxant activities of *T. procumbens* are in normotensive animals and a literature search offers nothing on the mechanistic effect of *T. procumbens* treatments on contractile activity in the corpus cavernosum of hypertensive rats. This study therefore investigated the effects of treatment with aqueous leaf extract of *T. procumbens* (ALETP) on contractile activity of the corpus cavernosum excised from *N*-nitro-L-arginine methyl ester (L-NAME)-induced hypertensive rats.

2. Materials and methods

2.1. Plant material and preparation of ALETP

T. procumbens leaves were collected from open grassland on the Ojo and Ikeja campuses of Lagos State University. The plant leaf was identified by a certified taxonomist of the Forestry Research Institute, Ibadan, where a specimen with voucher number 1008876 was deposited. Preparation of ALETP was done by aqueous extraction as previously reported in our studies [13,21]. Air-dried leaves were pulverized and 500 g was placed in a conical flask containing 500 mL of distilled water. The mixture was shaken thoroughly before being allowed to stand for 24 h. Filtrate was then obtained using Whatman filter paper, and concentrated by evaporation in a water bath (35–40 °C) [13,21]. The yield for the extraction was 24% of a light brown powdery ALETP.

2.2. Experimental animals, design and treatments

Twenty healthy, adult male (10 weeks) Wistar albino rats (130–150 g) were obtained from animal house, Lagos State University College of Medicine. The animals were acclimatized for 2 weeks under conventional laboratory conditions (12-hour light–dark cycle at 18–26 °C and relative humidity of 30%–70%) and allowed free access to standard pellet diet (Ladokun Feeds Nig Ltd) and water. After acclimatization, the animals were randomly divided into four groups of five animals each. Group I received normal saline (0.6 mL/kg) as normal control and group II received L-NAME (40 mg/kg) via single daily oral gavage for six weeks to induce hypertensive rats. Groups III and IV also received L-NAME (40 mg/kg) for six weeks but were further treated with 100 and 200 mg/kg of ALETP, respectively, from the 4th to 6th week. Treatments for groups III and IV were delivered via single daily oral gavage. During the period of study, body weights and blood pressure of the animals were monitored using a HCB 1002 scale (Adams Equipment, UK) and a Kent non-invasive blood pressure apparatus (CODA 4.0). Ethical guidelines (animal handling and care) approved by the Lagos State University College of Medicine Research and Ethics Committee were strictly adhered to.

2.3. Drugs

Chemicals used for the preparation of physiological salt solution (PSS) are: calcium chloride ($CaCl_2$), glucose, magnesium sulphate, potassium dihydrogen phosphate, potassium carbonate and sodium chloride. All chemicals and drugs used were of the highest analytical grade. Drugs used for the dose–response of the tissues include potassium chloride (KCl), acetylcholine (ACh), sodium nitroprusside (SNP), phenylephrine and $CaCl_2$ (purchased from Tocris, UK). L-NAME was bought from AK Scientific, Inc. (CA, USA).

2.4. Tissue preparation of the corpus cavernosum

The male rats were anesthetized with sodium pentobarbital (60 mg/kg) before they were sacrificed by cervical dislocation. The penis was carefully surgically removed *en bloc*, to keep the tunica albuginea intact, and placed in a petri-dish containing PSS. Then the corpus cavernosal tissues were isolated and carefully dissected from the surrounding tunica albuginea. Each corpus cavernosum was suspended in a 50 mL organ bath chamber. These chambers contained PSS with the following composition (mmol/L): NaCl (119.0), KCl (4.7), $KHPO_4$ (1.2), $MgSO_4$ (1.2), $NaHCO_3$ (15.0), $CaCl_2$ (1.6) and glucose (11.5). The temperature of the organ bath was maintained at 37 °C and bubbled with a 95% O_2 and 5% CO_2 gas mixture (pH 7.35–7.40). Each corpus cavernosum was anchored with a hook to a force transducer (Model 7004; Ugo Basile Varese, Italy) connected to a data capsule acquisition system (Model 17400) for recording isometric contractions. Initial tension of 2 g was applied to the anchored cavernosa strips and an equilibration period of 90 min was observed before the start of dose-response determination.

2.5. Dose–response of corpus cavernosum to phenylephrine, potassium and calcium

The corpus cavernosum tissue was contracted with phenylephrine (10^{-9}–10^{-5} mol/L) after the tissue equilibration period. Dose–response to potassium was determined by adding potassium (10–50 mmol/L) progressively to the potassium-free solution in the organ chamber. Dose–response to calcium was determined by adding $CaCl_2$ (10–50 mmol/L) progressively to the calcium-free solution in the organ chamber. Calcium and potassium chloride additions to calcium- and potassium-free solution were conducted to assess the effect of calcium influx through voltage-dependent calcium channels and the inhibitory effect on receptor-operated calcium channels, respectively. SNP and ACh were used to determine if the effect of ALETP treatment on corpus

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