RESEARCH ARTICLE

Roles of Ca2+ sensitive K+ channel, phosphodiesterase inhibitor, and β adrenergic activity in *Tridax procumbens* leaf extract relaxation of trachea smooth muscle in male Wistar rats

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ABSTRACT

**Background:** Although traditional use of *Tridax procumbens* aqueous leaf extract (TPALE) in the management of respiratory disorders is documented, validated scientific evidence is scarce. **Aim and Objectives:** Trachea smooth muscle (TSM) relaxant activity of TPALE ingestion was investigated in the presence or absence of key TSM relaxant agents. This was with the aim at elucidating relaxant activity of TPALE on TSM. **Materials and Methods:** Contractile activity of TSM excised from TPALE treated (100 mg/kg) and non-treated rats was assessed pre - and post-incubation in salbutamol (10^{−4} M), theophylline (10^{−4} M), caffeine (10^{−4} M), naringin (10^{−4} M), and naringenin (10^{−4} M) using organ chamber connected to a force isometric transducer (Model 7004; Ugo-Basile V Arese, Italy). **Results:** TPALE treatment significantly inhibited contractile activity in TSM. TPALE treated rats showed significantly inhibited contractile activity of the TSM pre (45.6%) and post-incubation (35%) in theophylline when compared to control pre (90.6%) and post-incubation (60%). Incubation of TSM from control and TPALE treated rats in salbutamol, significantly inhibited contractile activity (33.2%) and (37.2%), respectively. After incubation in caffeine, TSM from TPALE treated rats showed significant inhibition in the contractile activity (30.7%) as TSM from control post-incubation (38.4%). TSM of TPALE-treated group pre-incubation showed significant inhibition in contractile activity (33.2%) and (37.2%), respectively. After incubation in caffeine, TSM from TPALE treated rats showed significant inhibition in the contractile activity (30.7%) as TSM from control post-incubation (38.4%). TSM of TPALE-treated group pre-incubation showed significant inhibition in contractile activity (40.4%) when compared to pre-incubation (45%) and control pre - and post-incubation, respectively (52% and 90%). **Conclusion:** Calcium-activated K+ channels, β2 adrenergic stimulation, and antioxidant activity contribute to the mediation of relaxant activity by TPALE in TSM.

**KEY WORDS:** Trachea smooth muscle; Relaxant activity; *Tridax procumbens*; Theophylline; Salbutamol; Naringenin

INTRODUCTION

Asthma, bronchitis, and chronic obstructive pulmonary disease are common respiratory diseases characterized by reversible airway obstruction and airway hypersensitivity.[1,2] Obstructions and hypersensitivity may arise due to thickening (As a result of infiltration of inflammatory cells), mucus secretion, epithelial shedding, hypertrophy/hyperplasia of airway smooth muscle, and inflammation of the airways smooth muscle.[3]

Treatments for asthma, bronchitis, obstructive pulmonary disease, and other airway diseases are still challenging considering the side effects of drugs used in management.[4,5] Consequently, the search for drugs with minimal side
effects will always be a worthwhile endeavor. Plants parts and derivatives of plants have always been resourceful sources in drug discovery and development. According to Chang et al.,[4] 50% of prescription drugs in the past 25 years were developed from natural products and their derivatives.

*Tridax procumbens* (Linn) (Asteraceae) is widely distributed in many parts of the world. Conventionally, its leaves have long been used for the treatment of asthma, cough, and other respiratory diseases.[7] Enhanced contractility coupled with impaired relaxation of the airway smooth muscles is implicated in airway hyper-responsiveness and asthma.[8] Although there exists no experimental evidence supporting trachea smooth muscle (TSM) relaxant activity of *T. procumbens* aqueous leaf extract (TPALE), other studies have reported on the antihypertensive and portent smooth muscle relaxant activity of the aqueous extract of the leaf in corpus cavernosum,[9] aortic rings,[10] and mesenteric artery.[11,12]

This study, therefore, investigated the mechanistic activity of TPALE in TSM relaxation. Roles of salbutamol (a β2 adrenergic stimulant), naringin and naringenin (antioxidant/anti-inflammatory flavones that stimulate the opening of Ca²⁺ sensitive K⁺ channel), caffeine (phosphodiesterase IV inhibitors and actin depolymerizer), and theophylline (Non-selective phosphodiesterase inhibitor and activator of large-conductance Ca²⁺ - activated K⁺ channel) in TPALE-mediated air-way smooth muscle relaxation were studied.

**MATERIALS AND METHODS**

**Plant Material Collection and Preparation of Leaf Aqueous Extract**

Leaves of the *T. procumbens* plant were collected from the Lagos State University College of Medicine Complex. After collection, a sample of the leaf was properly identified by a certified taxonomist in the Department of Botany of the University. Aqueous leaf extract was then prepared as we have previously described.[9]

**Animals and Experimental Design**

Ten male adult Wistar strain rats (200–250 g) fed standard rats pelleted feed with water were used. They were appropriately housed singly in cages and allowed to acclimatize for 2 weeks in the animal house before treatment was commenced. Standard procedures on animal use and care were adhered to. The study was granted ethical approval by the Animal Ethics Committee of the Lagos State University College of Medicine (AREC/2019/013). Two Groups of five animals each were then treated through oral gavage daily with distilled water (control) and 100 mg/kg of aqueous leaf extract of *T. procumbens* for 6 weeks.[9]

**TSM Preparation and In Vitro Experimental Contractile Studies**

Trachea tissues were surgically removed after sacrifice by CO₂ asphyxiation. Dissected trachea tissues were put inside a petri-dish containing physiological salt solution (PSS). Then, the TSM were separated and six rings were tied together using a thread with the cartilage and smooth muscles facing opposite directions. Each tracheal strip was suspended in a 50 ml chamber of the organ bath. These chambers contain PSS with the following composition (m/mol): NaCl (119.0); KCl (4.7); KHPO₄ (1.2); MgSO₄ (1.2); NaHCO₃ (15.0); CaCl₂ (1.6); And glucose (11.5). The temperature of the organ/tissue bath was maintained at 37°C and the solution was bubbled with 95% O₂ and 5% CO₂ gas mixture (pH 7.35–7.40). The TSM were anchored using a thread at both ends, with one end anchored to the hook in the organ chamber and the other end connected to a force isometric transducer (Model 7004; Ugo-basile Varese, Italy) which was in turn connected to a data capsule acquisition system model 17400 for recording isometric contractions.

**Drugs and Chemical**

Salbutamol and caffeine were purchased from AK Scientific, 30023 Ahern Ave, Union City, CA 94587 United States. Acetylcholine (Ach), theophylline, naringenin, and naringin were sourced from Tocris online store UK.

**Experimental Studies**

**Cumulative dose responses of TSM to Ach**

The tissue was allowed to stabilize in the physiological solution for 90 min. During this period, it was stimulated 3 times at 30 min intervals with Ach (10⁻⁶ M). Dose-response of the tracheal strip to Ach (10⁻⁶–10⁻⁴ M) was thereafter determined cumulatively. Responses were ensured to be at a steady level before the addition of another dose. Tissues were washed 3 times between each drug administration.

**Contractile activity after incubation**

1. Effect of β 2 adrenergic stimulation on the contractile activity of the TSM in TPALE treated and non-treated groups was investigated by incubating TSM from the two Groups in salbutamol (10⁻⁴ M) for 15 min. Contractile responses to cumulative doses of Ach (10⁻⁶–10⁻⁵ M) were then determined and recorded

2. Effect of phosphodiesterase IV inhibition and actin depolymerization on the contractile activity in the TSM of TPALE treated and non-treated groups was investigated by incubating the TSM from the two Groups in caffeine (10⁻³ M) for 15 min. Subsequently, contractile responses to cumulative doses of Ach (10⁻⁶–10⁻⁵ M) were determined and recorded

3. Influence of non-selective phosphodiesterase inhibitor, and large-conductance calcium-activated potassium channel
activity was investigated by incubating TSM from TPALE treated and non-treated groups in theophylline (10^{-4} M) for 15 min. Contractile responses to cumulative doses of Ach (10^{-9}–10^{-5} M) were then determined and recorded.

4. Effect of anti-inflammatory and antioxidant flavones activity on TPALE-mediated contractile activity of the TSM was investigated by incubating the TSM from the two Groups in naringenin and naringin (10^{-4} M) for 15 min. Contractile responses to cumulative doses of Ach (10^{-9}–10^{-5} M) were determined and recorded.

Statistical Analysis and Presentation of Data
Results were presented as a mean ± SEM. Data were analyzed using GraphPad Prism (Version 5) statistical software. One-way analysis of variance with Dunnett’s post hoc was determined where applicable. $P < 0.05$ were considered statistically significant.

RESULTS

TPALE-mediated Contractile Activity of TSM in the Presence of Theophylline
TPALE treatment significantly inhibited contractile activity in TSM. TPALE treated rats showed significantly inhibited contractile activity of the tracheal smooth muscle pre (19%, 28.7%, 31.2%, 39.8%, and 45.6%) and post-incubation (16.6%, 22.9%, 24.3%, 32.9%, and 35%) in theophylline when compared to control pre (20.6%, 45%, 60%, 82.5%, and 90.6%) and post-incubation (20.5%, 32%, 41.7%, 51.5%, and 60%) [Figure 1].

TPALE-mediated Contractile Activity of TSM in the Presence of Salbutamol
Incubation of TSM from control and TPALE treated rats in salbutamol, significantly inhibited contractile activity (10.3%, 14.4%, 22.7%, 31.2%, and 33.2%) and (16.3%, 23.7%, 27.8%, 35.6%, and 37.2%), respectively. Contractile activity was also significantly inhibited in TSM of TPALE treated group pre-incubation in salbutamol (45%) compared to TPALE non-treated group pre-incubation (90.6%) [Figure 2].

TPALE-mediated Contractile Activity of TSM in the Presence of Caffeine
TSM from TPALE treated rats exhibited significantly inhibited contractile activity (45%) when compared to control (90%). After incubation in caffeine, TSM from TPALE treated rats showed comparably significant inhibition in contractile activity (11.9%, 17.5%, 23.1%, 28.5%, and 30.7%) as TSM from control (13.1%, 20.1%, 26.9%, 31.5%, and 38.4%) [Figure 3].
TPALE-mediated Contractile Activity of TSM in the Presence of Naringin and Naringenin

TSM of TPALE-treated rats preincubation showed a significant inhibition in contractile activity (20%, 28.7%, 31.2%, 41.8%, and 45%) when compared to the TSM of TPALE-treated Group (19.5%, 32%, 42.3%, 59.3%, and 60.3%) and control (18%, 35%, 47.8%, 64.5%, and 72.5%) post-incubation in naringin [Figure 4]. However, incubation of TSM of TPALE-treated rats in naringenin significantly inhibited contractile activity (40.4%) when compared to pre-incubation (45%) and control (52% and 90%) [Figure 5].

**DISCUSSION**

This study attempted to elucidate the mechanism involved in TPALE induced relaxation of TSM. Specifically, this study investigated the influence of theophylline, salbutamol, caffeine, naringin, and naringenin in TPALE-induced relaxation of TSM. In general, this study found that TPALE treatment significantly mediated inhibition of contractile activity in TSM. This study also observed that contractile activity in the TSM of TPALE treated rats was significantly inhibited pre- and post-incubation in theophylline (35%) when compared to control (90%, 60%) pre- and post-incubation, respectively. This is indicative of the fact that TPALE treatment enhanced mechanisms involved in theophylline-mediated relaxation of the TSM. It also suggests that TPALE treatment may not impair mechanisms involved in the theophylline-induced relaxant activity of the TSM. Theophylline is a non-selective phosphodiesterase inhibitor and is regarded as a major bronchodilator in the treatment of human asthma. Early studies have suggested that the relaxant activity of TPALE is also partly related to reduced Ca²⁺ sensitivity and activation of Ca²⁺-sensitive potassium channels. *Tamarix dioica* which shares similar phytochemical constituents such as saponins, flavonoids, and tannins with *Tridax procumbens* was reported to have spasmylytic, a bronchodilator, and vasorelaxant activity that was facilitated through the opening of the K⁺ channel. These put together indicate a very important role for Ca²⁺ sensitive K⁺ channel in TPALE-mediated TSM relaxation.

In this study, incubation of TSM from control and TPALE treated rats in salbutamol, significantly inhibited contractile activity (33.2%) and (37.2%), respectively. This we suggest signifies the contribution of β₂ adrenoceptors in the relaxant activity mediated by TPALE treatment. Although cAMP levels were not estimated in this study, stimulation of β₂ adrenergic receptors is known to activate adenylyl cyclase, through receptor-associated G proteins, to increase cAMP levels to mediate smooth muscle relaxation. β₂ adrenoceptor agonists have long been widely used as agents for the treatment of asthma. The use of β₂ adrenoceptor agonists is based on the fact that bronchial muscles are mainly controlled by β adrenoceptors, whose stimulation causes bronchodilation. β2 adrenoceptor agonists, such as isoprenaline and salbutamol, usually exhibited both a potent trachea relaxing activity and high β₂ selectivity. Incubation of TSM in caffeine resulted in a significant reduction of contractile activity in the control (38.4%) when compared to TSM from TPALE treated group. Caffeine action on relaxant activity of TPALE indicates that mechanisms involved in caffeine relaxation of TSM are important for TPALE relaxant effects on TSM. Caffeine (1, 3, 7-trimethylxanthine), is reportedly a weak trachea relaxant. Apart from the phosphodiesterase IV inhibition reported for its relaxant activity on trachea muscle, Tazzeo et al. also suggest...
that relaxation of TSM by caffeine can occur through actin depolymerization. Pertaining to naringin and naringenin, TPALE treated rats showed significant inhibition in contractile activity post-incubation in naringenin (40.4%) than post-incubation in naringin (60.3). This shows that TPALE action is better enhanced by naringin than naringenin. Furthermore, in this study, TSM from TPALE treated group pre-incubation reduced contractile activity (45%) than control post-incubation in naringin and naringenin, respectively, (73% and 52%). This suggests that TPALE anti-inflammatory/antioxidant properties could be more potent than that of naringin and naringenin. Chemical compounds/constituents of medicinal plants are known to contribute greatly to their activity. Phenolic properties of *Lamium album* were reportedly responsible for the relaxant activity of the plant extract in rat TSM.\(^{[18]}\) *T. procumbens* is also reported to possess strong antioxidants and flavonoids.\(^{[19,20]}\) One of the key properties of TPALE identified and suggested for being responsible for its vasorelaxant activities was the presence of antioxidants and flavonoids. Flavonoids are known to possess hemodynamic and smooth muscle relaxant activity.\(^{[21,22]}\) Studies have reported that naringin and its major metabolite naringenin (isoflavones isolated from citrus fruit) possess antioxidant, anti-inflammatory, and antitussive effects *in vivo and in vitro*.\(^{[23,24]}\) Both are also reported to remarkably reduce hyper-responsiveness in various animal models of respiratory diseases.\(^{[25]}\) Naringin and naringenin have been shown to relax TSM by opening of the big-conductance calcium-activated K\(^+\) channel which subsequently causes membrane hyperpolarization and reduces calcium influx.\(^{[26]}\) It is imperative to state that hyperpolarization and involvement of Ca\(^{2+}\) sensitive K\(^+\) channel in TPALE relaxant activity of vessels have been earlier reported,\(^{[11]}\) and it is rational to suggest its involvement in the relaxation of TSM judging from results in this study.

It is apposite to state here that the TSM used in this study is from a normotensive animal. Induction of experimental asthma with the use of appropriate blockers and receptor typing will further elucidate the particular receptors/mechanisms and pathways involved in TPALE relaxant activity in TSM. This is a limitation we hope to investigate in future studies.

**CONCLUSION**

TPALE treatment induced TSM relaxation that was enhanced with theophylline, salbutamol, caffeine, and naringenin. Incubation in naringin, however, did not enhance the relaxation induced by TPALE-treatment on the TSM. Ca\(^{2+}\) sensitive K\(^+\) channel, β2 adrenoceptor stimulation, and the potent antioxidant/anti-inflammatory activity are suggested to play key roles in TPALE TSM relaxant activity. Findings from this study provide a scientific basis for the reported traditional use of TPALE in respiratory tract disorder.

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