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Original Article

Preliminary Mechanistic Study on the Trachea Smooth Muscle Relaxant Activity of Aqueous Leaf Extract of *Tridax Procumbens* in Male Wistar Rats

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Objectives: Aqueous leaf extract of *Tridax procumbens* (ALETP) has potent relaxant activity. However, this relaxant activity in respiratory smooth muscle remains uninvestigated. This study investigates the effect of ALETP on the contractile activity of tracheal smooth muscle (TSM) in adult male Wistar rats.

Methods: Twelve male Wistar rats divided into 2 groups and were treated with either 100 mg/kg of ALETP (ALETP treatment group) or vehicle (distilled water; control group) through oral gavage for 4 weeks. Dose responses of TSM from the 2 groups to acetylcholine (10^{-9} to 10^{-5} M), phenylephrine (10^{-9} to 10^{-5} M), and potassium chloride (KCl; 10^{-9} to 10^{-4} M) were determined cumulatively. Furthermore, cumulative dose responses to acetylcholine (10^{-9} to 10^{-5} M) after pre-incubation of TSM with atropine (10^{-5} M), L-NAME (10^{-4} M), indomethacin (10^{-4} M), and nifedipine (10^{-4} M), were determined.

Results: Treatment with ALETP substantially inhibited TSM contraction stimulated by cumulative doses of acetylcholine, phenylephrine, and KCl. Furthermore, preincubation of TSM from the 2 groups in atropine significantly inhibited contractility in TSM. Incubation in L-NAME and indomethacin also significantly inhibited contractility in TSM of ALETP-treated rats compared to that of controls. Contractile activity of the TSM was also inhibited significantly with incubation in nifedipine in ALETP-treated rats.

Conclusion: ALETP enhanced relaxant activity in rat TSM primarily by blocking the L-type calcium channel and promoting endothelial nitric oxide release. ALETP contains agents that may be useful in disorders of the respiratory tract.

Keywords: atropine contractile activity, calcium channel, l-name respiratory tract, therapeutic potential

INTRODUCTION

Plant/herbal preparations have long been used to treat several disorders [1], and a vast number of people trust their therapeutic potential [2]. Plants and plant by-products have yielded a significant number of medicines [1].

Several studies have reported the smooth muscle/vaso-relaxant and antihypertensive potential of *Tridax procumbens* leaf extract (TPE) [3-5]. *T. procumbens* has been used to produce emollients, beverages, and skincare products, and the extensive use of the plant is associated with its protective properties and phytochemical constituents [6].

A significant number of fatalities because of respiratory disorders and associated complications have been reported [7, 8]. The most commonly used drugs for asthmatic conditions are fast-acting β2-agonists [9] and anticholinergics [10]. However, for long-term regulation, antileukotrienes [11], cromolyn sodium [12], inhaled [13], oral [14] corticosteroids, and methylxanthines [15] are used.

Efforts for finding alternative or complementary medicines

with minimal adverse effects are intensifying. Although ALETP has remarkable smooth muscle relaxant activity, this activity in respiratory smooth muscle has not been studied. This study investigated the contractile activity of the tracheal smooth muscle (TSM) in male Wistar rats treated with ALETP. Contractile responses of TSM to acetylcholine (Ach), phenylephrine, and potassium chloride (KCl) were studied. Additional mechanistic investigation was performed by incubating TSM from the 2 groups in atropine (an anticholinergic), L-NAME (a nitric oxide synthase inhibitor), nifedipine (an L-type calcium channel inhibitor), and indomethacin (a non-specific cyclooxygenase inhibitor).

MATERIALS AND METHODS

1. Plant material

Between September and October 2019, fresh leaves of *T. procumbens* were sourced from campuses of Lagos State University. A sample specimen of the leaf was appropriately identified and certified by a taxonomist in the University. Voucher number for the leaf specimen is 1008876.

2. *T. procumbens* aqueous leaf extract preparation

Leaves were air-dried for 2 weeks, pulverized, and then soaked in distilled water for 72 hours. Whatman filter paper was then used to filter the mixture. The filtrate was evaporated in an incubator at 50℃. The yield of this preparation was 23.6% of a light brown powdery crude *T. procumbens* leaf aqueous extract. It was stored at –4℃, and fresh preparation was made for animal treatment.

3. Animals

Twelve male Wistar rats weighing 150-200 g were kept individually in cages for 2 weeks for acclimatization. They were fed standard rat feed and allowed access to water. This study obtained approval from Lagos State University College of Medicine Ethics Committee on Animal guideline (Ref. No: AREC/2019/013).

4. Experimental design

Twelve rats were separated into 2 equal groups. The control group was treated with distilled water (vehicle), whereas the other group was treated with 100 mg/kg of ALETP [16]. ALETP was administered by oral gavage, once daily, for 6 weeks.

5. Preparation of TSM

At the end of the 6-week treatment period, rats were sedated (30 mg/kg sodium pentobarbital) and then sacrificed by asphyxiation. The trachea were surgically removed. The TSM was separated and 6 rings were tied together using a thread with the cartilage and smooth muscles facing opposite directions. Each TSM was held in an organ chamber containing physiological salt solution (PSS) using a thread with one end coupled to a hook and the other to a force isometric transducer. The isometric transducer was sourced from Ugo Basile Italy (model 7004), and the isometric contractions were recorded by the data acquisition system (model 17400). Of note, the PSS was kept at a temperature of 37℃ with a continuous supply of gas mixture (95% oxygen and 5% carbon dioxide). The composition of the PSS was as described earlier [5].

6. Drugs and chemicals

AK Scientific (USA) supplied L-NAME, and Ach and KCl were procured through Tocris UK online store. Nifedipine was from Unicure Pharmaceutical Co. Ltd. Lagos, Nigeria, and indomethacin was from Jiangxi Pharmaceutical Co., Ltd. Jiangxi, China.

7. Experimental contractile studies on excised TSM

8. Cumulative dose responses of TSMs to phenylephrine, potassium chloride, and acetylcholine

The stabilization period for the tissue was 90 min during which the tissue was stimulated every 30 min. Cumulative dose responses of TSM toward Ach (10^{-9} to 10^{-5} M), phenylephrine (10^{-9} to 10^{-5} M), and KCl (10^{-9} to 10^{-5} M) were determined cumulatively. Steady responses were ensured before the addition of another dose or in-between drug applications; tissues were washed thrice.

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9. Contractile responses in TSM after pre-incubation

1) The involvement of calcium channel blockers in the contractile activity of the TSM in ALETP-treated and control groups was investigated by incubating TSM from the 2 groups in nifedipine (10^{-4} M; an L-type calcium channel inhibitor) for 15 minutes. Contractile responses to the cumulative dose of Ach (10^{-9} to 10^{-5} M) were then determined and recorded.

2) The activity of competitive muscarinic receptor antagonists in the TSM of ALETP-treated and control groups was investigated by incubating the TSM from the 2 groups in atropine (10^{-5} M) for 15 minutes. Subsequently, contractile responses to the cumulative doses of Ach (10^{-9} to 10^{-5} M) were determined and recorded.

3) The contributions of nitric oxide in ALETP-mediated contractile activity of the TSM were investigated by incubating TSM from ALETP-treated and control groups in a nitric oxide synthase inhibitor (L-NAME; 10^{-4} M) for 15 minutes. Contractile responses to the cumulative dose of Ach (10^{-9} to 10^{-5} M) were then determined and recorded.

4) Finally, non-specific COX inhibitor activity in ALETP-mediated contractile activity of the TSM was investigated by incubating the TSM from the 2 groups in indomethacin (10^{-4} M) for 15 minutes before the contractile response to the cumulative dose of Ach (10^{-9} to 10^{-5} M) were determined and recorded.

10. Statistics

Graphpad Prism statistical package (version 5) was used for analysis in this study. Where appropriate, 1-way analysis of variance (Dunnett post hoc test) was used, with $p < 0.05$ considered statistically significant.

RESULTS

Figs. 1, 2, and 3 present the maximum contraction (%) of the TSM from control and ALETP-treated rats to cumulative doses of PHE (10^{-9} to 10^{-5} M), KCl (10^{-9} to 10^{-5} M), and Ach (10^{-9} to 10^{-5} M). ALETP-treated rats showed significantly inhibited percentage TSM contraction compared to control rats. The contraction (%) with PHE (Fig. 1) in control rats was 14.4%, 39.2%, 53.3%, 77.7%, and 86.4% compared to 11.1%, 17.2%, 27.5%, 37.2%, and 45% in ALETP-treated rats. With KCl (Fig. 2), the percentage contraction in control rats was 17.5%, 39.6%, 56%, 70.8%, 78.3%, and 89.1% compared to 14.5%, 15.8%, 17.1%, 18.3%, 23.5%, and 25.5% in the ALETP-treated rats. With Ach (Fig. 3), the percentage contraction in control rats was 30.6%, 45%, 60%, 82.5%, and 90.6% compared to 25%, 28.7%, 31.2%, 41.8%, and 45% in ALETP-treated rats. TSM incubated in atropine (15 mins) resulted in significant inhibition of contraction in both control and ALETP-treated rats (13.5% [control] and

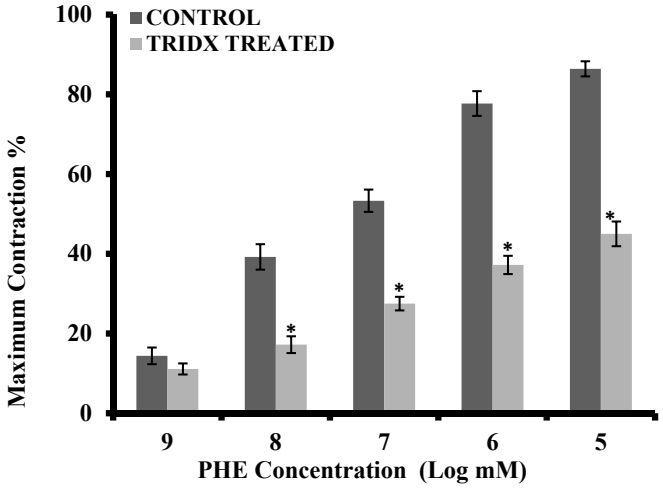

Figure 1. The percentage maximum contraction to Phenylephrine (10^{-9}-10^{-5} M) in control and *Tridax procumbens* (100 mg/kg) treated rat. N = 6, *p < 0.05.

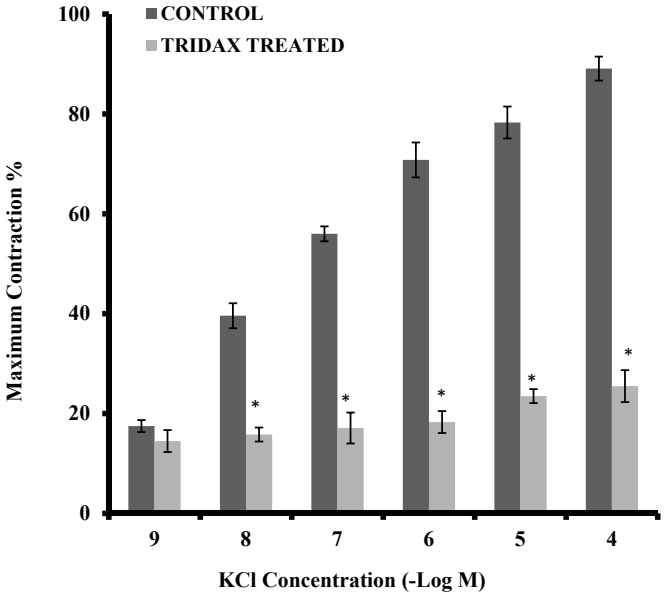

Figure 2. The percentage maximum contraction to cumulative doses of Potassium Chloride (10^{-9}-10^{-4} M) in control and ALETP (100 mg/kg) treated rat. N = 6, *p < 0.05.

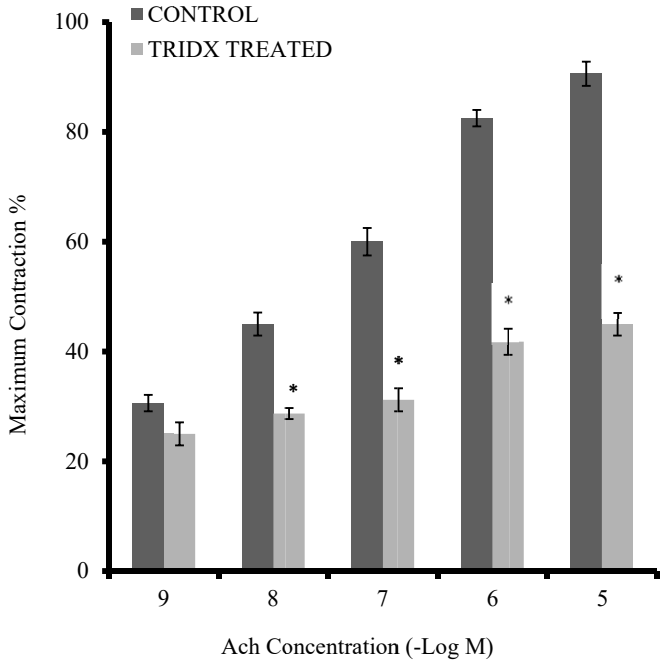

Figure 3. The percentage maximum contraction to cumulative doses of acetylcholine (10^{-9}-10^{-5} M) in control and ALETP (100 mg/kg) treated rat. N = 6, *p < 0.05.

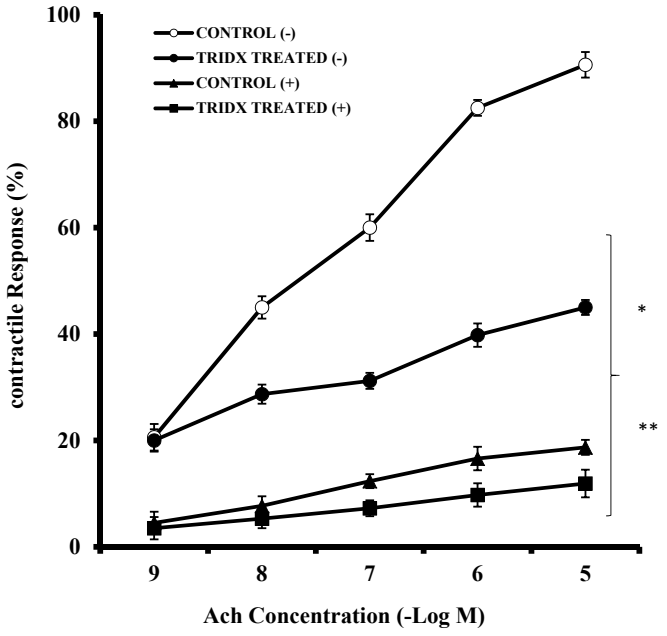

Figure 5. The contractile response (%) to cumulative doses of acetylcholine (10^{-9}-10^{-5} M) in TSM preincubated in L-NAME (10^{-4} M) from control and ALETP (100 mg/kg) treated rat. N = 6, *p < 0.05, **p < 0.01, + = with incubation, - = without incubation.

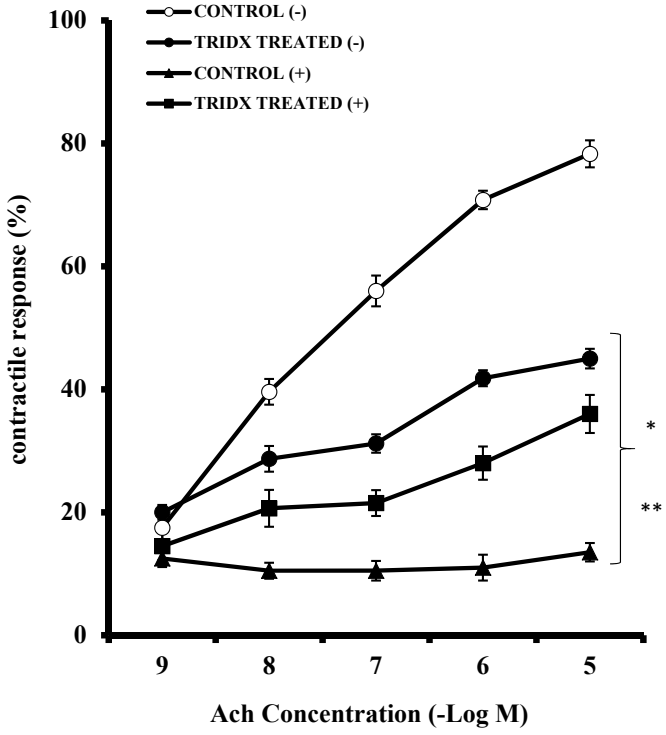

Figure 4. The percentage of contractile response to cumulative doses of acetylcholine (10^{-9}-10^{-5} M) in TSM preincubated in atropine (10^{-5} M) from control and ALETP (100 mg/kg) treated rat. N = 6, *p < 0.05, **p < 0.01, + = with incubation, - = without incubation.

30% [ALETP treated]) than in non-atropine-incubated TSMs (78.3% [control] and 45% [ALETP treated]; Fig. 4). Incubation of the TSM in L-NAME resulted in significant inhibition in contractile activity of TSMs obtained from control rats (18.7%) and ALETP-treated rats (11.9%) compared to pre-incubation control TSMs (90.6%; Fig. 5). In addition, incubation of TSM in indomethacin significantly inhibited contractile activity of the TSM from control rats (21.5%) and ALETP-treated rats (19.1%) compared to pre-incubation control TSMs (90.5%; Fig. 6). The contractile activity of TSM was also significantly inhibited with incubation in nifedipine in ALETP-treated rats (50.6%) when compared to pre-incubation control TSMs (90%; Fig. 7).

DISCUSSION

To our knowledge, this is the first study to report significant contraction inhibition by ALETP treatment in TSM in response to cumulative doses of respiratory smooth muscle constrictors like phenylephrine, Ach, and KCl. This observation provides empirical evidence for the relaxant property of ALETP in the smooth muscle of the respiratory tract for the first time. KCl induces contractility in TSM by stimulating the Rho A and Rho-kinase inhibitors. However, inhibitors of Rho-associated kinase

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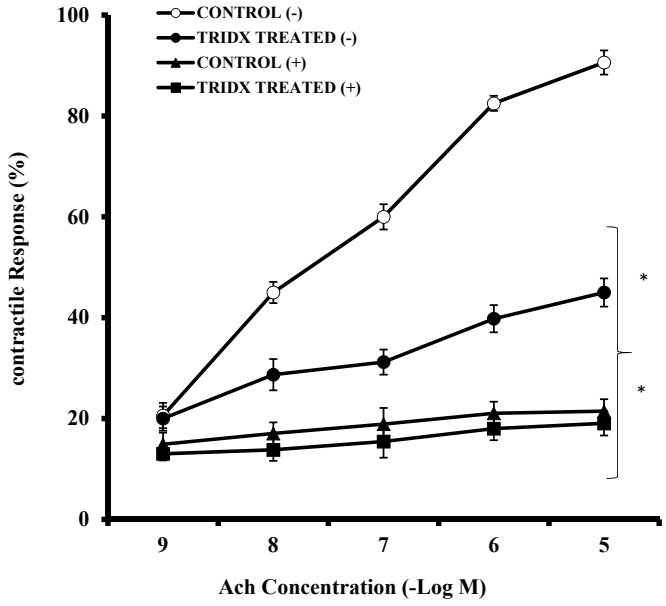

Figure 6. Contractile response (%) to cumulative doses of acetylcholine (10^{-9}-10^{-5} M) in TSM preincubated in indomethacin (10^{-4} M) from control and ALETP (100 mg/kg) treated rat. N = 6, *p < 0.05, + = with incubation, - = without incubation.

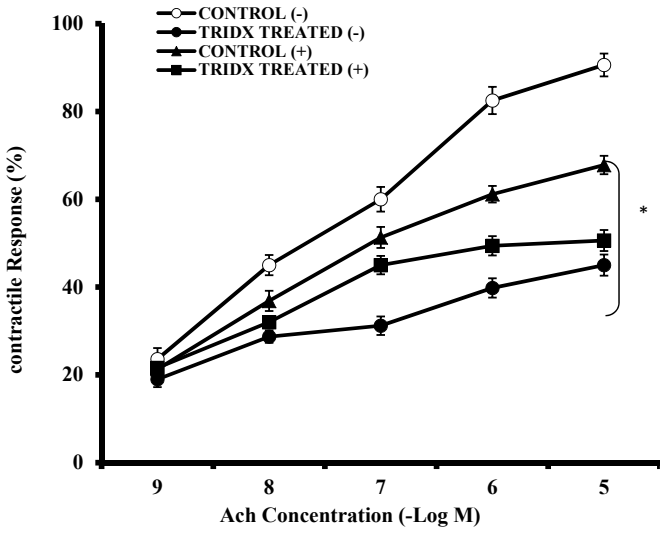

Figure 7. The contractile response (%) to cumulative doses of acetylcholine (10^{-9}-10^{-5} M) in TSM preincubated in nifedipine (10^{-4} M) from control and ALETP (100 mg/kg) treated rat. N = 6, *p < 0.05, + = with incubation, - = without incubation.

are found to inhibit KCl-induced contraction in TSM [17]. Ach is known to cause airway smooth muscle contraction by activating phospholipase to release inositol 1, 4, 5, triphosphate. This mobilizes calcium from the sarcoplasmic reticulum, resulting in increased intracellular calcium levels [18]. Prophylactic treatment with ALETP in this study significantly inhibited the contractile activity of excised TSM to cumulative doses of these airway smooth muscle contraction agonists (Ach, KCl). This observation suggests the underlying involvement of Rho A or Rho-kinase activation and calcium channel blocking in the relaxation of the TSM by ALETP.

We also investigated the involvement of calcium channel blockers in the relaxation induced by ALETP in TSM. We found that the contractile activity of the TSM was also significantly inhibited with incubation in nifedipine (an L-type calcium channel inhibitor) in ALETP-treated rats. The ability of ALETP to enhance nifedipine activity observed in this study indicates that it can help enhance the relaxation of respiratory tract muscle which could benefit an asthmatic patient with hyperactivity in tracheal/bronchial airways. Calcium-dependent mechanisms have previously been reported for ALETP-induced relaxant activity in normotensive rat aorta [3]. Specifically, non-specific and non-competitive inhibition of calcium influx and mobilization from stores were reported. ALETP treatment in this study exhibited its potential at preventing exaggerated contractile responses to key stimulants of the airway smooth muscles.

To further investigate the relaxant activity of ALETP, we observed TSM excised from the 2 groups preincubated in atropine (muscarinic receptor antagonist) and found significant inhibition of contractility in the TSM of ALETP-treated rats. This inhibition was however not as pronounced as that observed in control rats. This suggests that inhibitory muscarinic activity may not be key in ALETP-mediated relaxation of TSM. Atropine is a competitive antagonist that causes relaxation in the airway by preventing Ach from binding to type 3 muscarinic receptors [19].

The roles of nitric oxide synthase inhibitors and non-specific COX inhibitors (L-NAME and indomethacin, respectively) were also investigated. Incubation with L-NAME and indomethacin resulted in a significant inhibition in the contractile activity of TSM among ALETP-treated rats compared to control rats. The ability of ALETP to act as a nitric oxide donor in the endothelium is highlighted by significant relaxation of ALETP-treated TSM despite incubation with a nitric oxide synthase inhibitor (L-NAME). This hypothesis for the relaxant activity of ALETP has been reported in erectile tissues [5] and blood vessels [4]. Furthermore, the significant inhibition in contractile activity after incubation with indomethacin also indicates the inhibitory activity on prostaglandin synthesis/release by ALETP in TSM. However, the underlying mechanism remains unclear.

Literature review indicated that plants with therapeutic relaxant activity in airway smooth muscle exert their therapeutic effect by activating β2-adrenoceptors [20]. Other reported mechanisms include histamine receptor antagonism [21], inhibition of calcium channels [22], activation of K^+ channel [23], muscarinic receptor antagonism, and augmenting methylxanthine activity [24]. The relaxant activity of ALETP on TSM in this study can be attributed to the phytochemical constituents present in the plant. *T. procumbens* leaf contains saponins, flavonoids, and tannins [5]. Reports have shown that medicinal plants such as *Galago senegalensis* [25], *Tribulus terrestris*, and *Turnera diffusa* containing phytochemicals like saponins and flavonoids enhance relaxant activities [26]. In addition, naringin, a dihydroxyflavone from citrus fruits, has also been reported to stimulate TSM relaxation and attenuation of hyperresponsiveness in various models of animal respiratory diseases [27].

The antioxidant properties of ALETP were likely involved in mediating the airway smooth muscle relaxation in TSM observed in this study. Substantial antioxidants like quercetin, vitamin E, flavonoids, linoleic acid are reported to be present in ALETP [28]. These antioxidants may have offered protection against oxidative damage to trachea smooth muscle. Local mechanical and chemical irritation, hypoxia, and excess carbon dioxide can lead to airway smooth muscle contraction [29]. We did not determine the level of antioxidant enzymes in TSM in this study. The role of the antioxidant properties of ALETP in mediating its relaxant activities must be investigated.

CONCLUSION

This study indicates that ALETP treatment enhances relaxant activity in rat TSM partly by inhibitory muscarinic receptor activity and primarily by L-type calcium channel blocking, inhibition of prostaglandin release or synthesis, and promoting endothelial nitric oxide release. Phytochemical constituents and their pronounced antioxidant activities are likely involved in mediating muscle relaxant activity. ALETP contains agents that may be useful in disorders of hyperactivity or chronic obstructive diseases of the respiratory tract.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. Molecules. 2016;21(5):559.
2. Oyebode O, Kandala NB, Chilton PJ, Lilford RJ. Use of traditional medicine in middle-income countries: a WHO-SAGE study. Health Policy Plan. 2016;31(8):984-91.
3. Salahdeen HM, Idowu GO, Yemitan OK, Murtala BA, Alada AR. Calcium-dependent mechanisms mediate the vasorelaxant effects of Tridax procumbens (Lin) aqueous leaf extract in rat aortic ring. J Basic Clin Physiol Pharmacol. 2014;25(2):161-6.
4. Salahdeen HM, Adebari AO, Murtala BA, Alada AR. Potassium channels and prostacyclin contribute to vasorelaxant activities of Tridax procumbens crude aqueous leaf extract in rat superior mesenteric arteries. Afr J Med Med Sci. 2015;44(1):5-19.
5. Salami SA, Salahdeen HM, Ugbebor EC, Murtala BA, Raji Y. 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. J Integr Med. 2018;16(1):51-6.
6. Beck S, Mathison H, Todorov T, Calderón-Juárez EA, Kopp OR. A review of medicinal uses and pharmacological activities of *Tridax Procumbens* (*L.*). J Plant Stud. 2018;7(1):19-35.
7. Cukic V, Lovre V, Dragisic D, Ustamujic A. Asthma and chronic obstructive pulmonary disease (COPD) - differences and similarities. Mater Sociomed. 2012;24(2):100-5.
8. Forum of International Respiratory Societies. The global impact of respiratory disease. 2nd ed. Sheffield: European Respiratory Society; 2017. 10-24 p.
9. Yates DH, Kharitonov SA, Barnes PJ. Effect of short- and long-acting inhaled beta2-agonists on exhaled nitric oxide in asthmatic patients. Eur Respir J. 1997;10(7):1483-8.
10. Gross NJ. Anticholinergic agents in asthma and COPD. Eur J Pharmacol. 2006;533(1-3):36-9.
11. Chauhan BF, Ducharme FM. Anti-leukotriene agents compared

to inhaled corticosteroids in the management of recurrent and/or chronic asthma in adults and children. Cochrane Database Syst Rev. 2012;2012(5):CD002314.
12. Breslin FJ, McFadden ER Jr, Ingram RH Jr. The effects of cromolyn sodium on the airway response to hyperpnea and cold air in asthma. Am Rev Respir Dis. 1980;122(1):11-6.
13. Guilbert TW, Morgan WJ, Zeiger RS, Mauger DT, Boehmer SJ, Szefler SJ, et al. Long-term inhaled corticosteroids in preschool children at high risk for asthma. N Engl J Med. 2006;354(19):1985-97.
14. Chaudhuri R, Livingston E, McMahon AD, Thomson L, Borland W, Thomson NC. Cigarette smoking impairs the therapeutic response to oral corticosteroids in chronic asthma. Am J Respir Crit Care Med. 2003;168(11):1308-11.
15. Sankar J, Lodha R, Kabra SK. Doxofylline: the next generation methylxanthine. Indian J Pediatr. 2008;75(3):251-4.
16. Salami SA, Salahdeen HM, Rahman OC, Murtala BA, Raji Y. Oral administration of Tridax procumbens aqueous leaf extract attenuates reproductive function impairments in L-NAME induced hypertensive male rats. Middle East Fertil Soc J. 2017;22(3):219-25.
17. Janssen LJ, Tazzeo T, Zuo J, Pertens E, Keshavjee S. KCl evokes contraction of airway smooth muscle via activation of RhoA and Rho-kinase. Am J Physiol Lung Cell Mol Physiol. 2004;287(4):L852-8.
18. Ouedraogo N, Roux E. Physiology of airway smooth muscle contraction: an overview. J Pulm Respir Med. 2014;4(6):221.
19. Buels KS, Fryer AD. Muscarinic receptor antagonists: effects on pulmonary function. Handb Exp Pharmacol. 2012;208:317-41.
20. Boskabady MH, Krachian MA. Mechanisms of bronchodilatory effect of Carum copticum on isolated guinea pig tracheal chains. Physiol Pharmacol. 2000;4:103-9.
21. Boskabady MH, Sheiravi N. Inhibitory effect of nigella sativa on histamine (H1) receptors of isolated guinea pig tracheal chains. Pharm Biol. 2002;40(8):596-602.
22. Boskabady MH, Shahabi M. Bronchodilatory and anticholinergic effects of Nigella sativa on isolated guinea pig tracheal chains. Iran J Med Sci. 1997;22(3-4):127-33.
23. Emami B, Shakeri F, Gholamnezhad Z, Saadat S, Boskabady M, Azmounfar V, et al. Calcium and potassium channels are involved in curcumin relaxant effect on tracheal smooth muscles. Pharm Biol. 2020;58(1):257-64.
24. Shakeri F, Boskabady MH. A review of the relaxant effect of various medicinal plants on tracheal smooth muscle, their possible mechanism(s) and potency. J Ethnopharmacol. 2015;175:528-48.
25. Chedi BAZ, Mandawari FK. In vitro evaluation of antiasthmatic activity of ethanol leaf extract of *Guiera senegalensis* j. F. GMEL (Combretaceae). Afr J Pharm Pharmacol. 2018;12(9):112-20.
26. Dare A, Salami SA, Kunle-Alabi OT, Akindele OO, Raji Y. Comparative evaluation of the aphrodisiac efficacy of sildenafil and Carpolobia lutea root extract in male rabbits. J Intercult Ethnopharmacol. 2015;4(4):302-7.
27. Shi R, Xu JW, Xiao ZT, Chen RF, Zhang YL, Lin JB, et al. Naringin and naringenin relax rat tracheal smooth by regulating BK_{Ca} activation. J Med Food. 2019;22(9):963-70.
28. Ikewuchi CC, Ikewuchi JC, Ifeanacho MO. Phytochemical composition of *Tridax procumbens* Linn leaves: potential as a functional food. Food Nutr Sci. 2015;6(11):992-1004.
29. Gosens R, Gross N. The mode of action of anticholinergics in asthma. Eur Respir J. 2018;52(4):1701247.