PharmacologyOnLine
August 30, 2012
Archives • 2012 • vol.2 • 82 - 86

Relaxant effects of *Lippia microphylla* Cham. (Verbenaceae) on isolated rat aorta and trachea

Antunes R.O.G.[1], Silva APS[1], Silva A.C.S.[1], Oliveira P.R.[1], Tavares J.F.[2], Nouailhetas V.L.A.[3], Silva J.L.V.[1]
[1] Farmácia-Bioquímica, Universidade Nove de Julho, UNINOVE, São Paulo, SP, Brazil
[2] Laboratório de Tecnologia Farmacêutica, Centro de Ciências da Saúde, Universidade Federal da Paraíba, UFPB, João Pessoa-PB, Brazil
[3] Depto. Biofísica, Universidade Federal de São Paulo, UNIFESP, São Paulo, SP, Brazil

Abstract

Lippia microphylla is known as "alecrim-pimenta" and has been used to treat respiratory disease in Northeast Brazil. Thus, we decide to investigate whether the crude ethanol extract from aerial parts of *L. microphylla* (LM-EtOH) induces relaxant effects on the rat aorta and trachea. LM-EtOH (3 - 243 µg/mL) relaxed in a concentration-dependent and equipotent manner the aorta pre-contracted with either phenylephrine (EC_{50}= 52.1±0.3 µg/mL) or KCl (EC_{50}=70.7±12.4 µg/mL). It also relaxed the trachea pre-contracted (n=3) with either carbachol (EC_{50}= 53.6±7.8 µg/mL) or KCl (EC_{50}= 28.5±1.0 µg/mL). The observation that LM-EtOH relaxed both organs pre-contracted by agonists (phenylephrine and carbachol) is suggestive that *Lippia microphylla* is an unspecified smooth muscle relaxant. In addition, the extract induced relaxation of both organs contracted by KCl is indicative of Ca^{2+} influx blockade. Conclusions, the aerial parts of Lippia microphylla have smooth muscle relaxant properties supporting its use in folk medicine against respiratory disease.

Key words: *Lippia microphylla*, relaxation, smooth muscle

Introduction

The genus *Lippia* (Verbenaceae) includes approximately 200 species of herbs, shrubs and small trees. The species are mainly distributed throughout the South and Central America countries, and Tropical Africa territories [1]. Most of them are utilized in folk medicine as gastrointestinal and respiratory remedies [2]. Nevertheless, their pharmacological properties are still very scarce [3,4]. Essential oils and phenol compounds obtained from the plant extracts are the active principles responsible for their use [5].

Lippia microphylla Cham. is found in Guyana and Brazil [6], which is locally called as "alecrim-pimenta" and used to treat respiratory disease [7]. There is only an antimicrobial activity reported to an essential oil from *L. microphylla* [6]. Essentials oil obtained from *L. alba*, *L. graveolens* and *L. dulcis* present vasorelaxant, spasmolytic and antispasmodic effects, respectively [8-10], while aqueous extract from *L. multiflora* has vascular actions [11]. Monoterpenes and sequiterpenes were isolated from aerial parts [6] and naphthoquinones [12] of the *L. microphylla* roots.

Thus the aim of this study was to investigate the effects of the crude ethanol extract obtained from aerial parts of *L. microphylla* on the rat aorta and trachea.

Methods

Plant material and preparation of extract: aerial parts from *L. microphylla* were macerated in ethanol followed by concentration and evaporation resulting in a crude ethanol extract (LM-EtOH). This procedure was realized by Dr. Josean Fechine Tavares, in Laboratório de Tecnologia Farmacêutica of the Universidade Federal da Paraíba. The LM-EtOH was dissolved in cremofor (3%) and diluted in MiliQ water to obtain store solution (10mg/mL) maintained to 0 °C.

Animals: 10 male Wistar rats (250-300g) were purchased from the Centro de Desenvolvimento de Modelos Experimentais of Universidade Federal de São Paulo (CEDEME). The animals were housed into groups of five on standard laboratory conditions at temperature (22±1°C) in a 12 h light–dark cycle with free access to food and water. The experiments were performed during the light portion (8-17h) of the light-diary cycle. The research procedure was approved by the Ethics Committee in Research of Universidade Federal de São Paulo (CEP 0038/10).

Preparation of rat aorta and trachea: The aorta and trachea were isolated from rats [13] and suspended in glass cube baths (5mL) containing Krebs physiological solution with the following composition (mM): NaCl 118.0, KCl 4.6, $CaCl_2.2H_2O$ 2.5, KH_2PO_4 1.1, $MgSO_4$ 5.7, glucose 11.0, $NaHCO_3$ 25; pH 7.4. Tissues (aorta or trachea) preparations were maintained under 1g basal tension, bubbled continuously with O_2 and at 37°C [14]. Tissue contractile responses were recorded through acquisition and analogy system AQCD (AVS Projetos, Brazil). The contractions were induced either by phenylephrine (0.3µM) or KCl (60mM) on the aorta rings. The integrity of the endothelium was accessed by the addition of acetylcholine (1µM) on the phenylephrine-induced maintained contraction [15]. Aorta rings were considered endothelium-functional when relaxation higher than 50% was observed [16]. Before control response LM-EtOH was applied. Addition also of LM-EtOH (3 - 243 µg/mL) was done on the steady trachea tonic contraction in response to either carbachol (1µM) or KCl (60mM). The relaxing effect was accessed by normalized the percent of relaxation relative to the maximum contraction by the indicated stimulants and EC_{50} was obtained by adjusting data to non-linear regression.

Drugs and chemicals: acetylcholine HCl, carbamylcholine HCl (carbachol), NaCl, $NaHCO_3$, KH_2PO_4, $CaCl_2.2H_2O$, KCl, $MgSO_4.7H_2O$ and glucose were purchased from Merck (Brazil), and phenylephrine from Pfizer (USA).

Statistics: All data are represented as mean ± S.E.M. values and were performed using the GraphPad Prism 5.0 software. Data were analyzed by Student *t*-test. The level of statistical significance adopted was P <0.05.

Results

Isolated rat aorta: After endothelium removal the vasorelaxation effect of LM-EtOH (3 - 243 µg/mL) was concentration-dependent either by phenylephrine or KCl contractions (Fig. 1A and 2). EC50 values were similar for both stimulants phenylephrine (EC50= 52.1±0.3 µg/mL) and KCl (EC50=47.0±10.8 µg/mL) and it completely blocked either by phenylephrine- or KCl-induced contractile response at 81 µg/mL. The initial contractile response to both stimulants was sustained throughout the whole experimental time (not shown.

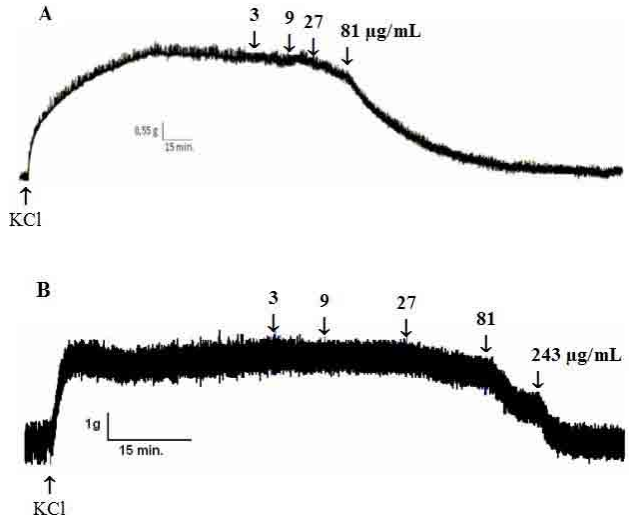

Figure 1: Select example of original tracing showing LM-EtOH-induced relaxation on the KCl (60mM)-induced contraction in rat aorta (A) and trachea (B)

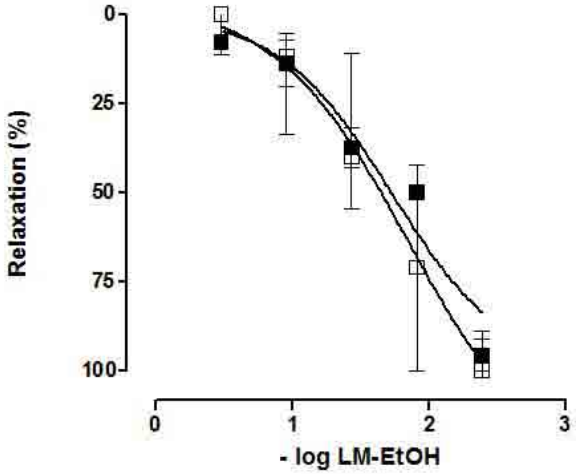

Figure 2: Concentration-relaxation curves to LM-EtOH (g/mL) on pre-contracted aorta rings in response to phenylephrine 0.3 M (ÿ, n=3) or KCl 60mM (ÿ, n = 5).

Isolated rat trachea: Similar effects were observed for the effects of LM-EtOH on isolated rat trachea. LM-EtOH (3 - 243 µg/mL) relaxed the trachea pre-contracted either by carbachol (EC_{50}= 53.6±7.8 µg/mL) or KCl (EC_{50}= 30.8±2.4 µg/mL) (Figures 1B e 3).

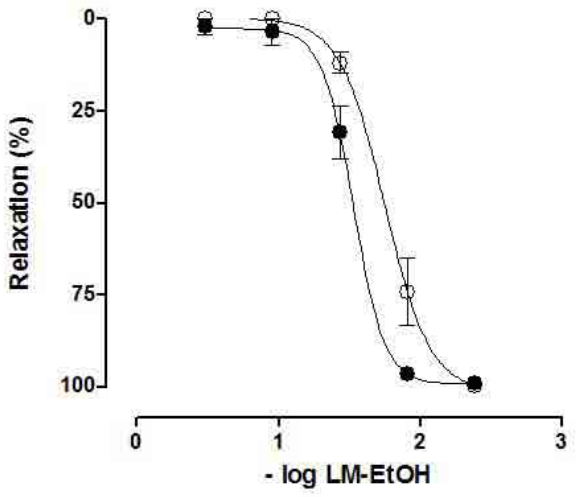

Figure 3: Concentration-relaxation curves to LM-EtOH (g/mL) on pre-contracted trachea either by carbachol 1µM (ÿ, n = 4) or KCl 60mM (ÿ, n = 3).

Discussion

We provide evidence for the relaxation effects of crude ethanol extract from aerial parts of *L. microphylla* on both rat aorta and trachea, once *Lippia microphylla* is used to treat respiratory diseases popularly [7].

We observed that LM-EtOH relaxed both rat aorta and trachea pre-contracted by either phenylephrine or carbachol with a same potency and efficacy, respectively (Fig. 1 and 2). Considering that contraction induced to either by phenylephrine or carbachol in these two tissues were due to G-protein coupled receptor alpha-1 adrenergic and muscarinic type 3 activation [19,20] is reasonable to conclude that LM-EtOH induced relaxant effects non-selective. Similar effects have been already reported for essential oil from *L. dulcis* and aqueous extract from *L. multiflora* on porcine bronchial bioassay and rabbit central ear artery, respectively [10,11], even though the crude extract was used.

In addition, the LM-EtOH could be interfering with a common pathway of the contraction mechanism such as extracellular Ca^{2+} entrance due to changes in the membrane potential or when stimulated by agonists able of opening L type voltage-dependent calcium channels ($Ca_V1.2$) [21,22]. So, in order to access this hypothesis, the crude extract was assayed on contraction induced by depolarizing agent (KCl). Indeed, we observed that LM-EtOH fully relaxed both aorta and trachea pre-contracted by KCl (Figs. 1, 2 and 3), thus strongly arguing that LM-EtOH is a voltage-dependent calcium blocker, most likely due the presence of flavonoids in other similar plant species [23-32]. However, on electrophysiological prove in order to confirm this possibility.

References

1. Terblanché FC, Kornelius G. Essential oil constituents of the genus Lippia (Verbenaceae)-A literature review. J Essent Oil Res 1996;8:471-485.
2. Morton. Atlas of Medicinal Plants of Middle America, vol. I. Illinois: Springfield, 1981:745–750.
3. Oliveira FP, Lima EO, Siqueira-Júnior JP, Souza EL, Santos BHC, Barreto HM. Effectiveness of Lippia sidoides Cham. (Verbenaceae) essential oil in inhibiting the growth of Staphylococcus aureus strains isolated from clinical material. Rev Bras Farmacogn 2006;16:510-516.
4. Sena-Filho JG, Melo JGS, Saraiva AM et al. Antimicrobial activity and phytochemical profile from the roots of Lippia alba (Mill.) N.E. Brown. Rev Bras Farmacogn 2006;16:506-509.
5. Pascual M, Slowing K, Carretero E, Sánchez MD, Villar A. Lippia: traditional uses, chemistry and pharmacology: a review. J Ethnopharmacol 2001;76:201-214.
6. Lemos TLG, Monte FJQ, Matos FJA et al. Chemical composition and antimicrobial activity of essential oils from Brazilian plants. Fitoterapia 1992;63:266-268.
7. Matos, FJA. Plantas medicinais – guia de seleção e emprego de plantas usadas em fitoterapia no nordeste do Brasil. 2nd ed. Fortaleza: Imprensa Universitária/Edições UFC, 2000:344.
8. Maynard LG, Santos KC, Cunha OS et al. Chemical composition and vasorelaxant effect induced by the essential oil of Lippia alba (Mill.) N.E. Brown. (Verbenaceae) in rat mesenteric artery. Indian J Pharmacol. 2011;43(6):694-698.
9. Rivero-Cruz I, Duarte G, Navarrete A et al. Chemical composition and antimicrobial and spasmolytic properties of Poliomintha longiflora and Lippia graveolens essential oils. J Food Sci 2011;76(2):C309-317.
10. Görnemann T, Nayal R, Pertz HH, Melzig MF. Antispasmodic activity of essential oil from Lippia dulcis Trev. J Ethnopharmacol 2008;117(1):166-169.
11. Noamesi BK, Adebayo GI, Bamgbose SOA. The Vascular Actions of Aqueous Extract of Lippia multiflora. Planta Med 1985;51(3):256-258.
12. Santos HS, Costa SM, Pessoa OD et al. Cytotoxic naphthoquinones from rootts of Lippia microphylla. Z Naturforsch [C] 2003;58(7-8):517-520.
13. Fedan JS, Van Scott MR, Johnston RA. Pharmacological techniques for the in vitro study of airways. J Pharmacol Toxicol Methods 2001;45(2):159-174.
14. Altura BM, Altura BT. Differential effects of substrate depletion on drug-induced contractions of rabbit aorta. Am J Physiol 1970;219(6):1698-1705.
15. Furchgott RF, Zawadki JV. The obligatory role of endothelium cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980;288(5789):373-376.
16. Ajay M, Gilani AH, Mustafaa MR. Effects of flavonoids on vascular smooth muscle of the isolated rat thoracic aorta. Life Sci 2003;74(5):603-612.
17. Billington CK, Penn RB. Signaling and regulation of G protein-coupled receptors in airway smooth muscle. Respir Res 2003;4:2.
18. Bruce GA, Michael PW. The biochemical basis of the regulation of smooth muscle contraction. TIBS 1994;19:362-368.
19. Wilson SJ. Peripheral alpha-1 and alpha-2 adrenergic receptors in three models of hypertension in rats: an in vitro autoradiography study. J Pharmacol Exp Ther 1991;256:801–810.
20. Ehlert FJ, Ostrom RS, Sawyer GW. Subtypes of the muscarinic receptor in smooth muscle. Life Sci 1997;61:1729-1740.
21. Neild T, Kote-Cha N. Relation between membrane potential and contractile force in smooth muscle of the rat tail artery during stimulation by norepinephrine, 5-hydroxytryptamine, and potassium. Circ Res 1987;60:791-795.
22. Schramm M, Thomas G, Towart R, Franckowiak G. Novel dihydropyridines with positive inotropic action through activation of Ca2+ channels. Nature 1983;303(5917):535-537.
23. Rodríguez-Ramos F, González-Andrade M, Navarrete A. Gnaphaliin A and B relax smooth muscle of guinea-pig

trachea and rat aorta via phosphodiesterase inhibition. J Pharm Pharmacol 201;63(7):926-935.
24. Joskova M, Franova S, Sadlonova V. Acute bronchodilator effect of quercetin in experimental allergic asthma. Bratisl Lek Listy 2011;112(1):9-12.
25. Liu B, Yang J, Wen Q, Li Y. Isoliquiritigenin, a flavonoid from licorice, relaxes guinea-pig tracheal smooth muscle in vitro and in vivo: role of cGMP/PKG pathway. Eur J Pharmacol 2008;587(1-3):257-66.
26. Ghayur MN, Khan H, Gilani AH. Antispasmodic, bronchodilator and vasodilator activities of (+)-catechin, a naturally occurring flavonoid. Arch Pharm Res 2007;30(8):970-975.
27. Macêdo CL, Vasconcelos LHC, Correia ACC, Martins IR, Lira DP, Santos BVO, Silva BA. Spasmolytic effect of galetin 3,6-dimethyl ether, a flavonoid obtained from Piptadenia stipulacea (Benth) Ducke. J Smoth Muscle Res 2011;47(5): 123-134.
28. Dong X, Wang Y, Liu T et al. Flavonoids as vasorelaxant agents: synthesis, biological evaluation and quantitative structure activities relationship (QSAR) studies. Molecules 2011;16(10):8257-72.
29. Lapa Fda R, Soares KC, Rattmann YD et al. Vasorelaxant and hypotensive effects of the extract and the isolated flavonoid rutin obtained from Polygala paniculata L. J Pharm Pharmacol 2011 Jun;63(6):875-81.
30. Tep-Areenan P, Sawasdee P, Randall M. Possible mechanisms of vasorelaxation for 5,7-dimethoxyflavone from Kaempferia parviflora in the rat aorta. Phytother Res 2010;24(10):1520-5.
31. Sasaki Y, Suzuki M, Matsumoto T et al. Vasorelaxant activity of Sappan Lignum constituents and extracts on rat aorta and mesenteric artery. Biol Pharm Bull 2010;33(9):1555-1560.
32. Song MJ, Baek I, Seo M et al. Effects of 3',4'-dihydroxyflavonol on vascular contractions of rat aortic rings. Clin Exp Pharmacol Physiol 2010;37(8):803-10