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Bronchodilator activity of *Mikania glomerata* Sprengel on human bronchi and guinea-pig trachea

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Abstract

The effects of aqueous extracts and hydro-alcoholic extract (HAE), and of a dichloromethane fraction (MG1) obtained from the HAE of *Mikania glomerata* leaves on isolated respiratory and vascular smooth muscle have been investigated. Aqueous extracts and HAE induced a significant inhibition on the histamine contractions on the isolated guinea-pig trachea. HAE extract induced a concentration-dependent relaxation on guinea-pig trachea pre-contracted with histamine (IC50 0.34 (0.29–0.39) mg mL^{-1}), acetylcholine (IC50 0.72 (0.67–0.77) mg mL^{-1}) or K^+ (IC50 1.41 (1.18–1.64) mg mL^{-1}) and on isolated human bronchi precontracted with K^+ (IC50 0.34 (0.26–0.42) mg mL^{-1}). The dichloromethane fraction induced a concentration dependent relaxation in guinea-pig trachea precontracted with K^+ (IC50 0.017 (0.012–0.022) mg mL^{-1}). The dichloromethane fraction had also a small vasodilator effect on the isolated mesenteric vascular bed and on the isolated rat aorta, and a significant reduction of the oedema induced by subplantar injections of *Bothrops jararaca* venom in mice. When tested on plasmid DNA, MG1 did not damage the DNA. Chromatographic analysis showed the presence of 11.4% w/w coumarin in MG1. The results supported the indication of *M. glomerata* products for the treatment of respiratory diseases where bronchoconstriction is present.

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Introduction

Mikania glomerata Sprengel (Compositae), popularly known as ''guaco'', is a medicinal plant extensively used in Brazilian folk medicine, mainly in the treatment of respiratory diseases such as asthma (Martins et al 1995). It is also present in many commercial phytotherapeutic preparations. Bronchoconstriction plays a very important role on the physiopathology of asthma and compounds that relax respiratory smooth muscles such as β_2-agonists, theophylline and cholinergic antagonists are usually used in symptomatic treatment of that disease. Despite the widespread use of ''guaco'' by the Brazilian population, there is little information about its pharmacological properties. As the isolated guinea-pig trachea is a well accepted pharmacological model for the study of bronchospasmolytics (Gilani et al 2001), we have studied the effect of an aqueous and a hydro-alcoholic extract (HAE), and a dichloromethane fraction of the HAE on human and guinea-pig respiratory smooth muscle. We have investigated the effect of *M. glomerata* on rat vascular smooth muscle, on mouse hind-paw oedema and its genotoxicity, to establish the pharmacological effect of this plant.

Materials and Methods

Plant material

Leaves of *M. glomerata* were collected in Petropolis, Rio de Janeiro, Brazil. The plant was identified by Dr M. Vernie Lane, Department of Botany, Universidade Federal Fluminense. A voucher specimen (N° 83.002) was deposited at Herbarium Bradeanum, Universidade do Estado do Rio de Janeiro.

Preparation of *M. glomerata* extracts and fractions

Aqueous extracts

Dried leaves of *M. glomerata* (15 g) were cut into tiny pieces, infused in 100 mL distilled boiling water (infusion) or boiled in 100 mL distilled water for 15 min (decoction). After cooling at room temperature, the infusion and the decoction were filtered and assayed on the respiratory smooth muscle on the same day of preparation.

Hydro-alcoholic extract (HAE)

Fresh leaves of *M. glomerata* were cut into small pieces and macerated at room temperature with ethanol for 15 days. The extract was separated and kept in dark bottles. The macerated leaves were again extracted with ethanol for two days. The 2-day extracts were combined with the 15-day extract and the final volume corrected to a final concentration of 10% w/v (yield: *M. glomerata* 16 g kg^{-1} dry weight). The HAE was kept in dark bottles at 4°C until use. On the day of the experiment, 20 mL HAE was evaporated at 40°C yielding 17.1 ± 0.3 mg (n = 6) of evaporated residue mL^{-1}. The residue was diluted in ethanol/water (50%) for pharmacological tests.

Dichloromethane fraction

The HAE was fractionated by liquid–liquid extraction using dichloromethane as the organic solvent 10 times and two fractions were obtained. The organic phase (MG1) represented 17.3% w/w HAE. MG1 was dissolved in ethanol/water (50%) and the aqueous phase (MG2) was dissolved in saline on the day of the pharmacological test.

Chromatographic analysis and coumarin estimation

Chromatography analysis

MG1 was analysed by high-resolution gas chromatography (HRGC) using flame ionization detection (FID) and by HRGC interfaced with a mass spectrometer (MS). The HRGC/FID analysis were performed on a Shimadzu CGS-14A equipped with a 25 m × 0.3 mm i.d. column coated (0.25-μm film thickness) with 5% phenyl 1% vinyl methyl silicone. A sample of MG1 fraction (1 μL injection volume) was injected in split mode (split ratio 1:25) with detector temperature at 280°C and injector at 250°C. Column temperature was programmed from 140°C (held for 10 min) at 8°C min^{-1} to 280°C. Helium was used as the carrier gas. Data were processed using a C-R6A chromatopac integrator.

HRGC/MS analysis was performed using a Hewlett-Packard (HP 5972 GC) coupled to an HP 5985 mass selective detector (electron impact 70 eV). The column used was a PONA (25 m × 0.22 mm; 0.5-μm film thickness) of 100% polydimethylsiloxane. MG1 was injected in splitless mode with the injector temperature and GC-MS interface both held at 280°C. The column temperature followed the same programme as for HRGC/FID analysis. Hydrogen was used as the carrier gas. The MS scan range was 40–450 a.m.u. Identifications were carried out by computer-aided fragmentation comparison using MS software.

The coumarin content in the dichloromethane fraction was obtained using HRGC/FID detection following the same programme as above. The samples were injected in triplicate and the quantification of coumarin in the MG1 and MG2 fractions were determined by area interpolation in the curve area × coumarin concentrations.

Isolated guinea-pig trachea and human bronchi

The Ethical Committee for Use of Experimental Animals of the State University of Rio de Janeiro and the Ethical Committee of the Hospital Universitário Pedro Ernesto approved the experimental protocol. Male guinea-pigs (250–350 g) were killed with CO_2. The trachea was gently and rapidly dissected and all fat and connective tissue were removed. Small segments of human lungs (left-overs) were obtained from patients with lung cancer undergoing pneumectomy at the Hospital Universitário Pedro Ernesto, Universidade do Estado do Rio de Janeiro. Shortly after the pneumectomy, segments of third order were gently dissected and all adjacent tissue removed. Segments of the guinea-pig trachea and human bronchus were cut into rings approximately 0.5-cm long. The rings were mounted for tension recording under a 2-g tension and were allowed to equilibrate for 1–2 h in 30-mL chambers containing Krebs–Henseleit solution (composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2,

$NaHCO_3$ 2.5, EDTA 0.026 and glucose 11) bubbled with 95% O_2 and 5% CO_2 at 37°C. In solutions with elevated K^+, the Na^+ concentration was simultaneously decreased to maintain isosmolarity.

After the equilibration period, the cumulative concentration–response curves to histamine were constructed in absence and in the presence of aqueous extract and/or HAE. Control concentration–effect curves were also obtained on rings treated with saline or ethanol/water solution.

Following the equilibration period, rings of guinea-pig trachea or human bronchi were contracted with a single effective concentration of histamine 10 μM (in the presence of atropine 0.1 μM, propranolol 0.1 μM, nitro^G-L-arginine methyl ester (L-NAME) 100 μM and indometacin 0.3 μM) or acetylcholine 10 μM (in the presence of mepyramine (pyrilamine) 0.3 μM and propranolol 0.1 μM) or K^+ 60 mM (in the presence of atropine 0.1 μM, mepyramine 0.3 μM and propranolol 0.1 μM). Once a stable contraction had been obtained, concentration–relaxation curves for HAE were constructed by increasing the concentration in the organ chamber. Concentration–relaxation curves for MG1 and MG2 were also constructed in rings of guinea-pig precontracted with K^+. The relaxation induced by HAE or MG1 were expressed as percentage changes from the contraction induced by the constrictor agents and as the IC50 obtained for the HAE and MG1, according to the constrictor agent. Control concentration–effect curves were also obtained on rings treated with ethanol/water solution.

Isolated rat superior mesenteric vascular bed and rat aorta

The rat superior mesenteric vascular bed was isolated according to McGregor (1965). Male Wistar rats (250–350 g) were killed with CO_2. The mesenteric bed was perfused at a flow rate of 4 mL min^{-1} with Krebs–Henseleit solution bubbled with 95% O_2 and 5% CO_2 at 37°C and the mesenteric perfusion pressure was measured with a Hewlett-Packard pressure transducer on a Hewlett-Packard 7702B recorder. Preparations were left to equilibrate for 30–60 min.

Noradrenaline (norepinephrine) 1 μM was perfused to raise the basal tonus of the mesenteric vascular smooth muscle and a cumulative dose–response curve to MG1 was constructed. The vasodilator effect of MG1 was expressed as a percentage reduction of the pressor effect of noradrenaline.

Rat thoracic aorta rings approximately 0.5-cm long were suspended for tension recording in organ chambers containing 30 mL Krebs–Henseleit solution bubbled with 95% O_2 and 5% CO_2 at 37°C. Following stabilization, the rat aorta was contracted with noradrenaline 1 μM and relaxation–concentration curves were constructed by increasing the concentration of MG1 in the organ bath. The vasodilator effect of MG1 was estimated as a percentage decrease on the noradrenaline vasoconstrictor effect.

Mouse paw oedema

Paw oedema was induced in male Swiss mice (20–30 g) by sub plantar injection of 0.05 mL saline containing 50 ng g^{-1} of *Bothrops jararaca* venom in the hind footpads, under light ether anaesthesia. At different intervals after the injection of venom the volume of the paw was measured by plethysmography (Ferreira 1979). The oedema was estimated as the area enclosed by the Δ increase in paw volume during 180 min calculated by the trapezoid method (Lesser et al 1980). The oedema induced by *B. jararaca* venom was studied in mice (n = 6) pre-treated intraperitoneally with MG1 (150 μg kg^{-1}) or MG1 vehicle (control groups n = 6) injected 90 min before the venom injection.

Evaluation of potential genotoxicity

Potential genotoxicity of MG1 was performed on plasmid DNA using an alkaline lyses procedure (Sambrock et al 1989). Plasmid DNA pUC 9.1 (200 ng) was treated with $SnCl_2$ $2H_2O$ (200 μg mL^{-1}), MG1 fraction (1, 10 and 90 μg mL^{-1}) or $SnCl_2$ $2H_2O$ (200 μg mL^{-1}) plus MG1 (90 μg mL^{-1}). To evaluate the role of reactive oxygen species in DNA breakage, two samples (MG1 90 μg mL^{-1} and $SnCl_2$ $2H_2O$ 200 μg mL^{-1} plus MG1 90 μg mL^{-1}) were prepared adding sodium benzoate (100 mM), a hydroxyl radical scavenger. In all cases, reaction mixtures were incubated for 60 min at room temperature.

Drugs

Acetylcholine hydrochloride, histamine phosphate, mepyramine (pyrilamine), propranolol hydrochloride, indometacin, L-NAME, agarose gel and all chemical reagents were purchased from Sigma Chemical Co (St Louis, MO). *B. jararaca* venom was a gift from Instituto Vital Brazil, Niterói, Brazil. Stock solutions of these compounds were prepared in distilled water

(except indometacin which was dissolved in ethanol) on the day of the experiment.

Statistical analysis

The results are reported as mean of n observation ± s.e.m. The mean IC50 for HAE and MG1 fractions were calculated by interpolation from semi-logarithmic plots Microcal Origin software and reported as means, with 95% confidence limits. For the comparison of data, the Student's paired or un-paired *t*-test was used where applicable. Differences were considered to be significant when $P < 0.05$.

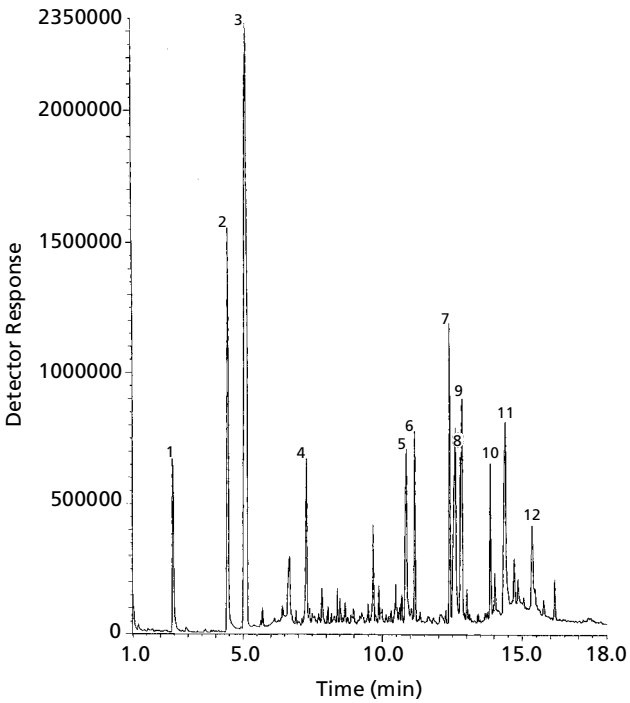

Figure 1 GC-FID chromatogram of the MG1 fraction.

Results

Chromatographic analysis and coumarin estimation

The HAE was fractionated by liquid–liquid extraction with dichloromethane (CH_2Cl_2), yielding two phases. The CH_2Cl_2-soluble organic phase (MG1) and the insoluble material (MG2) corresponded to 17.3% and 82.7% of HAE, respectively.

The chromatogram of the MG1 fraction is shown in Figure 1. The main constituents of MG1 fraction were identified by comparison with literature data (Table 1). The concentrations of coumarin in MG1 and MG2 fractions, calculated by interpolation of the area in the calibration curve, were 11.4% w/w and 0.3% w/w, respectively.

Effects of aqueous extract, HAE and MG1 on isolated guinea-pig trachea and human bronchi

Exposure of guinea-pig trachea to the aqueous extract of *M. glomerata* (4 mL of infusion (n = 6) or decoction (n = 6) in 30 mL Krebs solution) did not affect basal tension, but reduced the histamine-evoked contraction

Table 1 Identities of peaks of figure 1, and relevant MS data (EI,70 eV) of the identified compounds from MG1.

Tr (min)	Relevant MS fragments (m/z and intensity)	Identification
2, 45	91(100, 0), 120(58, 0), 65(17, 4), 51(10, 6), 121(5, 3), 77(4, 2), 45(0, 5), 122(0, 1)	Coumaric acid
4, 47	91(100, 0), 120(91, 8), 148(91, 3), 51(30), 63(20, 8), 106(9, 7), 149(8, 7), 78(6, 3)	Dihydrocoumarin
5, 12	118(100, 0), 146(67, 8), 90(48, 1), 89(46, 2), 63(33, 2), 51(10, 6), 147(7, 2), 74(4, 3)	Coumarin
6, 68	120(100, 0), 91(55, 8), 77(48, 6), 148(42, 8), 166(26, 4), 107(24, 5), 51(16, 3), 65(14, 4)	1-Ethoxy-1-phenylethanol
7, 31	182(100, 0), 181(66, 8), 167(15, 9), 139(13, 9), 111(21, 6), 96(20, 2), 65(17, 3), 79(14, 4)	4-Hydroxy-3,5-dimethoxybenzaldehyde
10, 84	52(100, 0), 73(88, 9), 60(76, 9), 129(33, 7), 83(27, 4), 256(25, 2), 97(21, 6)	Hexadecanoic acid
11, 17	88(100, 0), 101(53, 9), 55(32, 2), 70(21, 6), 157(16, 1), 241(8, 2), 284(8, 1), 143(4, 3)	Ethyl hexadecanoate
12, 41	71(100, 0), 55(31, 3), 81(26, 4), 123(24, 5), 95(13, 5), 111(7, 7), 137(2, 4), 196(1, 4)	Phytol
12, 59	79(100, 0), 67(84, 1), 55(57, 7), 95(45, 7), 108(26, 9), 209(15, 9), 121(12, 0), 135(8, 2)	Instaturated hydrocarbon
12, 79	67(100, 0), 81(83, 7), 55(64, 4), 95(52, 9), 109(25, 0), 123(13, 9), 150(12, 0), 262(9, 6)	Ethyl linoleoate
12, 85	79(100, 0), 67(62, 5), 95(52, 9), 55(50, 0), 108(37, 0), 121(24, 0), 135(17, 8), 173(7, 2)	Ethyl linoleoate
13, 88	105(100, 0), 91(92, 8), 135(64, 9), 79(58, 7), 119(49, 0), 257(47, 1), 148(44, 7), 55(50, 0)	Kaurenol
14, 40	91(100, 0), 135(83, 7), 105(83, 7), 122(68, 3), 79(62, 0), 302(60, 0), 55(45, 2), 67(35, 6)	Isomer of kaurenoic acid
15, 37	91(100, 0), 79(75, 5), 131(62, 0), 105(59, 6), 55(48, 6), 67(44, 7), 119(38, 5), 259(30, 8)	Kaur-16-en-18-oic acid

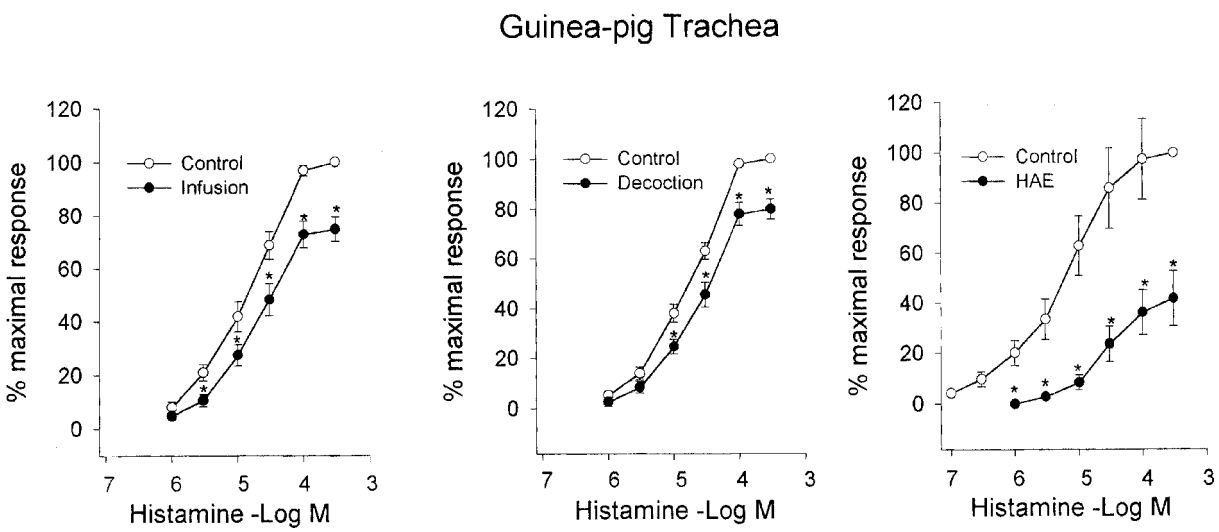

Figure 2 Inhibition of histamine-stimulated smooth muscle contraction on the isolated guinea-pig trachea preparations. Concentration–response curves of histamine alone (○) and in the presence (●) of infusion 13.3% v/v (left panel), decoction 13.3% v/v (central panel) and hydro-alcoholic extract (HAE) 1 mg mL^{-1} (right panel) of *Mikania glomerata*. Each point represents mean of n ⩾ 6, vertical bars s.e.m. *$P < 0.05$, comparison between control and treated groups.

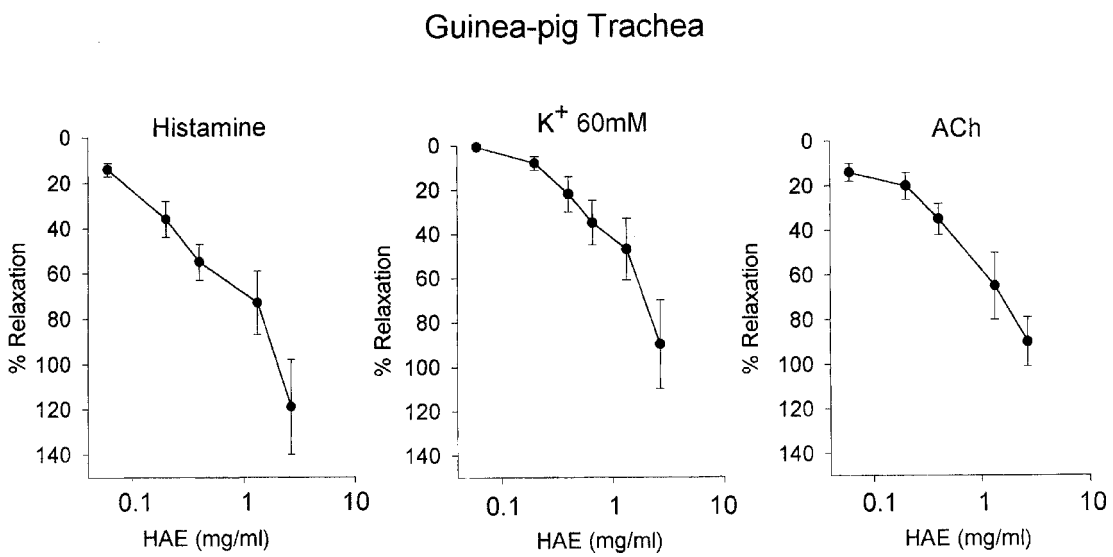

Figure 3 Relaxant effect induced by hydro-alcoholic extract (HAE) of *Mikania glomerata* on isolated guinea-pig trachea precontracted with histamine, K^+ or acetylcholine. Each point represents mean of n ⩾ 6 animals, vertical bars s.e.m.

and induced a depression of the maximum response (Figure 2). HAE, 1 mg mL^{-1} (n = 7) induced a decrease in basal tension (0.61±0.1 g) and significantly reduced the histamine-evoked contraction at all concentrations and depressed the maximum response (Figure 2). During sustained contractions induced by histamine (10 μM, n = 7), K^+ (60 mM, n = 6) and acetylcholine (10 μM, n = 6), addition of HAE induced a progressive concentration-dependent relaxation (IC50: 0.34 (0.29–0.39) mg mL^{-1}; 1.41 (1.18–1.64) mg mL^{-1} and 0.72 (0.67–0.77) mg mL^{-1}, respectively; Figure 3). In human bronchi (n = 5) contracted with histamine (10 μM), addition of HAE induced a progressive concentration-dependent relaxation (IC50: 0.34 (0.26–0.42) mg mL^{-1};

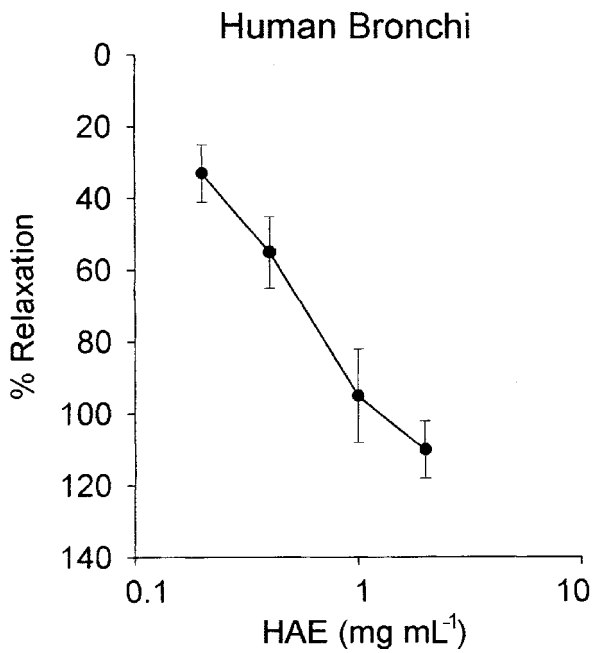

Figure 4 Antispasmodic effect induced by hydro-alcoholic extract (HAE) of *Mikania glomerata* on isolated human bronchi precontracted with histamine (10 μM). Each point represents the mean of rings from five patients, vertical bars s.e.m.

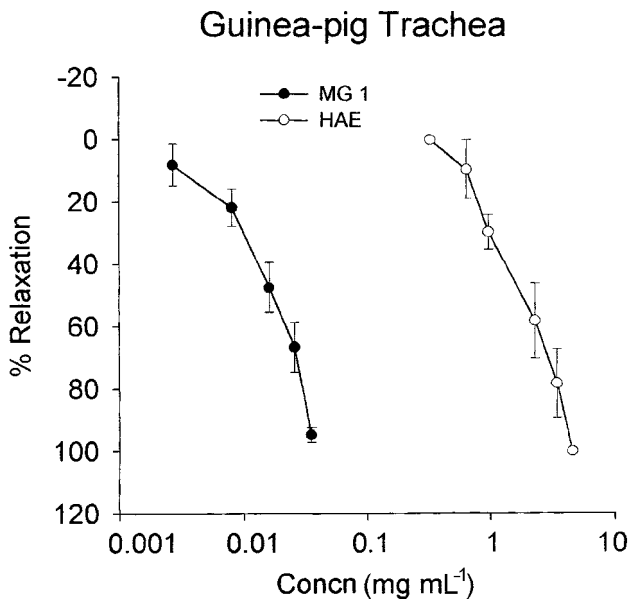

Figure 5 Relaxant effects of MG1 and hydro-alcoholic extract (HAE) of *Mikania glomerata* on guinea-pig trachea precontracted with KCl. Each point represents the mean of n $\geqslant$ 6 animals, vertical bars s.e.m.

Figure 4). In guinea-pig trachea contracted with K^+ (20 mM) addition of MG1 (n = 7) or HAE (n = 6) induced a progressive concentration-dependent relaxation (IC50: 0.017 (0.012–0.022) mg mL^{-1} and 1.80 (0.62–2.98) mg mL^{-1}, respectively; Figure 5). MG2 and ethanol/water mixture did not induce relaxation in guinea-pig rings precontracted with K^+ (data not shown).

Effects of MG1 on isolated mesenteric vascular bed and aorta of the rat

In the isolated mesenteric vascular bed (n = 8) and in the isolated aorta (n = 6) of the rat, precontracted with noradrenaline (1 μM), administration of MG1 induced a dose-dependent vasodilator effect. In the rat aorta the IC50 was 0.249 (0.233–0.264) mg mL^{-1} and in the isolated mesenteric vascular bed the IC50 was higher than 0.3 mg. This suggested a very low affi nity for the vascular smooth muscle when compared with the respiratory smooth muscle.

Effects of MG1 on mouse hind-paw oedema

In the MG1 vehicle- or in MG1-treated mice, subplantar injections of *B. jararaca* venom induced an increase in the paw volume. However, the increase in paw volume, estimated as the area enclosed by the Δ increase in paw volume during the 180 min after the injection of *B. jararaca* venom, in mice pre-treated with MG1 vehicle or 150 μg kg^{-1} MG1 were significantly different (134.2$\pm$14 and 84.8$\pm$6% increase, respectively).

Effect of MG1 on plasmid DNA

MG1 at all concentrations tested had no effect on DNA, and did not change the effects of stannous chloride and sodium benzoate.

Discussion

In popular medicine, plants are usually used as infusions, decoctions or alcoholic beverages. Therefore to be closer to the therapeutic conditions of phytotherapy we must study first the pharmacodynamic effects of the plant aqueous extracts. Since an infusion or decoction of *M. glomerata* leaves are used mainly in the treatment of bronchitis, where bronchoconstriction is always present, we decided to evaluate the effect of the aqueous extract on the basal tonus of the isolated guinea-pig trachea and on the contractile effect induced by histamine, which is an important autacoid related to the physiopathology of bronchial hyper-reactivity that is observed in patients with respiratory diseases (Kay 1986).

Our results have shown that the aqueous extract of *M. glomerata* induced a non-competitive reduction of the contractile effect of histamine on the respiratory smooth muscle of the guinea-pig. The results indicated the presence of an active principle in the leaves of *M. glomerata*. The low activity of the aqueous extracts observed may be due to the low capacity of the boiling water to extract the active principle(s) present in the leaves of *M. glomerata*. Therefore, we decided to improve the extraction method and fresh leaves of *M. glomerata* were exhaustively extracted with ethanol for a long period (17 days).

Our results demonstrated that the HAE produced a decrease of the basal tonus of the isolated respiratory smooth muscle of the guinea-pig trachea. When the respiratory smooth muscle was contracted with either histamine, acetylcholine or high K^+, in the presence of diverse pharmacological agents such as propranolol, atropine, mepyramine or L-NAME, addition of HAE induced a significant concentration-dependent relaxation under all experimental conditions. This result suggested that the inhibitory effect of *M. glomerata* was not dependent on inhibition of muscarinic or histaminergic receptors, activation of β_2-adrenoceptor, release of nitric oxide and/or prostanoids or activation of K^+ channels. As the contraction induced by high K^+ (60 mM) was reduced significantly by *M. glomerata*, a calcium-channel-blockade action may play an important role on the bronchodilator effect of *M. glomerata*. It is worth noting that the bronchodilator effect of HAE was observed in human respiratory smooth muscle, suggesting a therapeutic potential of *M. glomerata* on respiratory diseases where bronchoconstriction is present.

The inhibitory effect of HAE on the human and guinea-pig respiratory smooth muscle was obtained only with a fairly high concentration. To increase the inhibitory effect of *M. glomerata* on isolated respiratory smooth muscle, HAE was partitioned into two fractions using dichloromethane. Our results demonstrated that the active dichloromethane soluble fraction (MG1) was more active than HAE on the isolated guinea-pig trachea, since the IC50 for HAE and MG1 obtained in rings pre-contracted with 20 mM K^+ were 1.8 (0.62–2.98) and 0.017 (0.012–0.022) mg mL^{-1}, respectively.

Chromatographic studies performed with the dichloromethane fraction confirmed the findings of Lucas (1942) and Oliveira et al (1984), that showed the presence of coumarin in leaves of *M. glomerata*. Experiments performed in our laboratory (Soares de Moura et al 1990) have shown that coumarin has a significant inhibitory effect on guinea-pig isolated tracheal rings pre-contracted with histamine, acetylcholine or K^+. Since the concentration of coumarin in the dichloromethane fraction was very high (11.4% w/w in MG1), coumarin probably had a very important role in the relaxant effect of *M. glomerata* on respiratory smooth muscle. The potency (IC50) of unadulterated coumarin in guinea-pig tracheal preparation contracted with potassium ions was 3.6 μg mL^{-1} (Soares de Moura personal observations), but the actual concentration of coumarin that contributed towards the IC50 value of this extract was approximately 1.9 μg mL^{-1}. Therefore, it was likely that other active participants contributed towards the bronchodilator activity of MG1.

The vasodilator effect (potency) was lower than the bronchodilator effect of MG1. This suggested that the compounds present in the extracts of *M. glomerata* were more active on the respiratory smooth muscle than on vascular smooth muscle. Thus, when *M. glomerata* extracts are used in the treatment of respiratory disease, the probability of a large reduction in arterial blood pressure would appear to be remote.

The beneficial effects of *M. glomerata* in the treatment of respiratory diseases such as asthma may not only comprise a direct relaxation of the respiratory smooth muscle but also an anti-inflammatory effect. The latter may be important since it is well documented that asthma is an inflammatory disease. Experiments performed in our laboratory have demonstrated an anti-allergenic effect of MG1 in rats (Fierro et al 1999). Pereira et al (1993) demonstrated that coumarin, extracted from *M. glomerata*, had a protective effect on the lethality induced by *B. jararaca* venom. Since our results have demonstrated an inhibitory effect of the dichloromethane fraction on the mouse hind-paw oedema induced by release of inflammatory agents by *B. jararaca* venom (Trebien & Calixto 1989), an anti-inflammatory action of *M. glomerata*, as demonstrated by Ruppelt et al (1991), seems to exist.

The present results have shown that MG1 is devoid of genotoxicity since this fraction did not damage DNA either directly or by producing reactive oxygen species (at least the hydroxyl radical).

Conclusion

A significant inhibitory action of *M. glomerata* on the isolated human bronchus and on tracheal rings of the guinea-pig has been demonstrated. The inhibitory action on the respiratory smooth muscle seemed to be independent of any action on β-adrenoceptors, muscarinic or histaminergic receptors, activation of potassium channels, release of prostanoids or nitric oxide. A calcium-channel blockade has to be taken in consideration

when considering the mechanism of the bronchodilator effect of *M. glomerata*. Probably, the inhibitory effect of *M. glomerata* on respiratory smooth muscle is due to the presence of both coumarin and non-coumarin compounds. Our results suggested that products obtained from *M. glomerata* leaves might have potential beneficial effects in the treatment of bronchoconstrictive diseases.

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