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Remedies

The contribution of solubilizers to Mikania laevigata extracts pharmacological effects: A traditional bronchodilator plant

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Abstract

Introduction

Mikania laevigata Schultz Bip. Ex Baker, popularly known as guaco, had its use approved as a phytomedicine bronchodilator by Brazilian regulatory agency, since exerts a trachea relaxation which is attributed to coumarin, the main biomarker of guaco. The role of diaphragm muscle in the respiration face to guaco extract was investigated comparatively to salbutamol and coumarin, aiming to verify the better extract from nonpolar to polar extracts of M. laevigata.

Methods

A validated experimental model of mouse phrenic nerve-diaphragm (PND) preparation was used. The diaphragm aids in breathing contracting muscle fibers, so we sought a facilitatory effect on these different extracts based on their polarity. An inconvenience for studying substances of different polarities is become them soluble in water, since the biological preparation is maintained in a nutritive solution. We selected polyethylene glycol 400 (PEG400) and dimethyl sulfoxide (DMSO) as solubilizers, while coumarin and salbutamol were used as reference in diaphragm muscle preparation.

Results

Among hexane, dichloromethane, ethyl acetate and methanolic extracts from guaco only hexane and ethyl acetate showed a facilitatory effect. Between the solubilizing agents, PEG400 causes lesser influence on contractile response than DMSO, although the latter has major absorptivity to coumarin than PEG400.

Discussion

In this study, we argue the influence of solubilizers on the coumarin and salbutamol roles, in PND preparation, while the better extracts from guaco were selected for explaining the involvement of diaphragm muscle in the use of guaco as bronchodilator.

Conclusion

Under these perspectives we conclude that hexane and ethyl acetate extracts of M. laevigata contains substances, including coumarin, that participate in therapeutical efficacy of guaco on the pulmonary diseases.

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Introduction

Mikania glomerata Sprengel and Mikania laevigata Schultz Bip. Ex Baker are plants popularly known as guaco which promote among several medicinal properties a bronchodilatator effect.[1] The use of guaco in Brazil is as an alternative to allopathic medicine, as salbutamol.

Two questions arise from the medicinal use of guaco. First, how is the respiratory muscle physiology? Second, which are the guaco's active phytochemicals that act as bronchodilators? It is well known that there are two main types of skeletal muscles responsible in respiration: pump muscle as diaphragm, that serves as an inspiratory pump for lung ventilation; and regulators, that control the caliber of the conduits for air movement into and out of the lungs, being: the nose, mouth and pharynx the upper airways, larynx, trachea and primary bronchi the middle airways, and distal bronchi, bronchioles, alveolar ducts, alveolar sacs and alveoli, the lung airways.[2]

On the mechanic of respiratory muscles, the guaco acts inducing a concentration-dependent relaxation in guinea pig trachea (composed by smooth muscle), effect attributed to coumarin (1, 2-benzopirone), that is the main biomarker of guaco.[3] Coumarin and o-coumaric acid are found in hexane and dichloromethane extracts of guaco[4] and in its ethanolic and aqueous extracts[5] demonstrating that these metabolites are ubiquitous in this plant used to treat lung allergic inflammation and pneumonitis.[6,7]

Considering that respiration involves skeletal and smooth muscles, where the diaphragm contracts and the trachea relaxes to conduct the air to the lungs, we hypothesized that the nonpolar to polar extracts of M. laevigata leaves could be assayed using an experimental model of mouse phrenic nerve-diaphragm preparation, to test the better extract in ameliorating the diaphragm activity, comparatively to salbutamol. As solubility from nonpolar to polar extracts varies, were selected other solubilizers such as polyethylene glycol 400 (PEG400) and dimethyl sulfoxide (DMSO). Hence this work can contribute to understand the benefit mechanisms of guaco on the pulmonary diseases.

Materials and Methods

Mikania laevigata extracts

Fresh leaves of M. laevigata were collected in May 2011 from the University of Sorocaba – UNISO (Sorocaba, SP,

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Brazil) orchard. A voucher specimen was deposited at the Biology Institute from University of São Paulo – São Paulo, SP, Brazil, after identification by B. Loeuille.

The leaves was dried after 4 days using a forced air circulation apparatus at 42ºC (Marconi® dryer) then grinding to 10 mesh (Wiley type Marconi, MA 340 model macromill).

Resultant powder was used as described:

a) To obtain aqueous extract, 1 g of leaves powder was infused in 100 mL water at 90°C, evaporated (Büchi R-215 rotatory evaporator), and lyophilized (Thermo Electron Corporation - ModuleD Freezer Dryer), getting a yield of 14.57%.

b) Soxhlet vessels containing 20 g of leaves powder were filled successively with 300 mL of different solvents ranging, from nonpolar to polar, as follows: hexane, dichloromethane, ethyl acetate (all from Synth®, Brazil) and methanol (Ecibra®, Brazil). Then, the solvents were evaporated to dryness and the yield of dried extract powders were: 3.01% for hexane extract; 1.94% for dichloromethane extract; 0.68% for ethyl acetate extract; and 11.52% for methanol extract. All extracts were stored at room temperature and protected from light and humidity until the assays were performed.

Thin layer chromatography (TLC)

Aliquots of M. laevigata extracts were spotted onto a plate with 200 µm thickness of silica gel Alugram® UV 254, and exposed to hexane:ethylacetate (8:2) solvent system and two revelators, sulfuric anisaldehyde (0.5 mL anisaldehyde, 10 mL glacial acetic acid, 85 mL methanol and 5 mL sulfuric acid) and KOH 10% m/v. The extracts were solubilized in its own solvents, while the phytochemical standards (Sigma-Aldrich®) β-sitosterol 1% m/v in hexane and coumarin (1-2 benzopyrone, 1% m/v) in 50% ethanol. The retention factor (Rf) of extract spots were visualized (under UV light at 254nm) and compared with the Rf of each standard.

Animals

Male Swiss white mice (20-30 g) were supplied by the Anilab - Animais de Laboratório (Paulínia, São Paulo, Brazil). The animals were housed at 22±3°C on a 12h light/dark cycle with access to food and water ad libitum. This study was approved (protocol number 028/2012) by the Committee for Ethics in Research from the Federal University of São Carlos (UFSCAR) and all experiments were performed according to the guidelines of the Brazilian College for Animal Experimentation.

This work conforms to the values laid down in the Declaration of Helsinki (1964). The protocol of this study has been approved by the relevant ethical committee related to our institution in which it was performed. All subjects gave full informed consent to participate in this study.

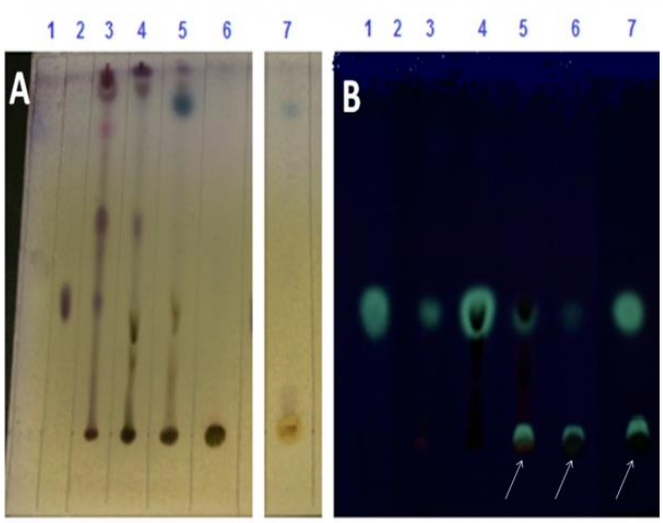

Figure 1: Chromatophic profile of extracts from Mikania laevigata spotted onto a plate with 200 µm thickness of silica gel alugram UV 254 and exposed to hexane:ethylacetate (8:2) solvent system. Revelators: A, Sulfuric anisaldeyde and B, KOH. Phytochemical standards: coumarin (1) and β-sitosterol (2). Extracts: (3) hexane (Hex), (4) dichloromethane (DCM), (5) ethyl acetate (EAc), (6) methanol (MeOH), and (7) aqueous extract. Arrows: indicative of o-coumaric acid presence. Retention factors (Rfs) are described in the text. Note that all extracts express commonly the coumarin (B).

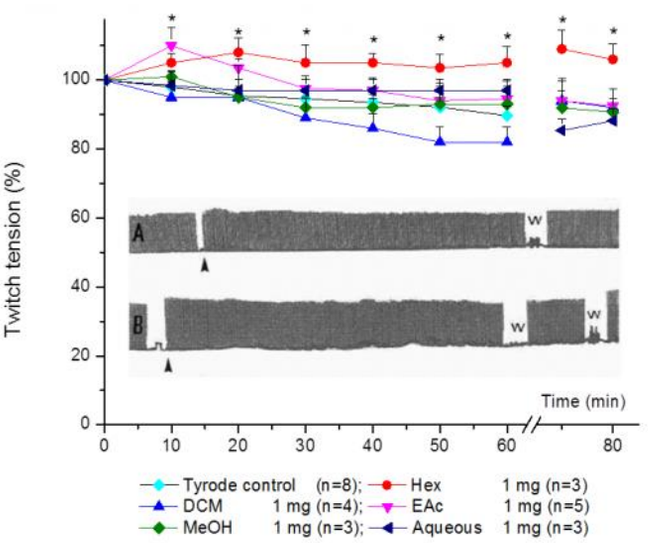

Figure 2: Mouse phrenic nerve-diaphragm preparation, indirect stimuli. The graphic shows the pharmacological profile of extracts (1 mg) from Mikania laevigata added into the bath containing the isolated neuromuscular preparation. The points are the means ± SEM of 3-8 experiments. * $p<0.05$ vs. control. Hex, hexane extract; DCM, dichloromethane extract; EAc, ethyl acetate extract; MeOH, methanol extract; Aqueous, aqueous extract. Extracts solubilized in 15 µL polyethylene glycol (PEG400): Hex and EAc. Extracts solubilized in 30 µL of dimethyl sulfoxide (DMSO): DCM and MeOH. Inserted to figure, two myographical registers show no influence of 15 µL of PEG (A) and 30 µL of DMSO (B), respectively, according to Cintra-Francischinelli et al. standardization.9 solubilizing agent addition, W, washing.

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Mouse phrenic nerve-diaphragm muscle (PND) preparation

Mice were killed by exsanguination after halothane anesthesia and the phrenic nerve-diaphragm preparations were removed and mounted under a resting tension of 5 g placed in a 5 mL organ bath containing Tyrode solution (composition, in mM: NaCl 137, KCl 2.7, CaCl2 1.8, MgCl2 0.49, NaH2PO4 0.42, NaHCO3 11.9 and glucose 11.1; pH 7.4, 37 ^{0}C), aerated with 95% O2 and 5% CO2 (adapted for mice from Bülbring, 1946).[8]

Muscle contractions were evoked by indirect stimulation with supramaximal pulses (3 V, 0.1 Hz, 0.2 ms) delivered from an ESF-15D stimulator and applied to the phrenic nerve by a bipolar electrode. The muscle twitches were recorded using a force displacement transducer (Ugo Basile cat. nº 7003) coupled to a basic preamplifier (cat. nº 7080, Ugo Basile) linked to a two-channel Gemini flatbed recorder (cat. nº 7070). The preparations were allowed to stabilize for at least 20 min before adding M. laevigata extracts or coumarin (main indicator of M. laevigata), solubilized in polyethylene glycol (PEG400) or dimethyl sulfoxide (DMSO), according to Cintra-Francischinelli et al.[9] Experiments were performed using a bronchodilator, salbutamol (0.1; 0.5; 2.5 and 5.0 mg), as reference.

UV spectral study

An UV spectral study of coumarin was performed to study the possible spectroscopic changes in the structure of coumarin in presence of different solubilizing agents (PEG400 or DMSO), and to interpret the influence of them on the absorptivity parameter.[10]

Statistical analysis

In the pharmacological assays each experimental protocol was repeated three times. All data are presented as mean ± S.E.M. The number of experiments (n) is indicated in the legend of the figure. Statistical comparison of data were made by using Student's t-test and the confidence level was set as 5% (alpha=0.05).

Results and Discussion

Mikania laevigata and Mikania glomerata popularly known as guaco are indistinctly used in Brazilian folk medicine for treating respiratory diseases involving inflammatory and allergic conditions.[11,12,13,14,15]

In order to supply phytochemical information with quality control purpose, related to the nonpolar to polar Mikania laevigata extracts from leaves used in this study, a chromatographical profile was obtained (Figure 1).

Note that the sulfuric anisaldehyde revelator shows β-sitosterol (Rf 0.28-0.30) and other nonpolar substances (mainly terpenes), but not coumarin; whereas KOH revelator shows coumarin (Rf 0.30), but not β-sitosterol. In the chromatoplate A, the Rfs of Hex were: 0.33; 0.45; 0.52; 0.90 and 0.95; Rfs of DCM were: 0.52; 0.90; and 0.95; Rfs of EAc were: 0.85 and 0.95. In MeOH extract nothing was revealed, whereas in aqueous extracts Rf was 0.85. On the other hand, note in the chromatoplate B the coumarin expression in all — nonpolar to polar — extracts and o-coumaric acid was visualized in extracts of major polarity such as Eac, MeOH and aqueous (arrows).

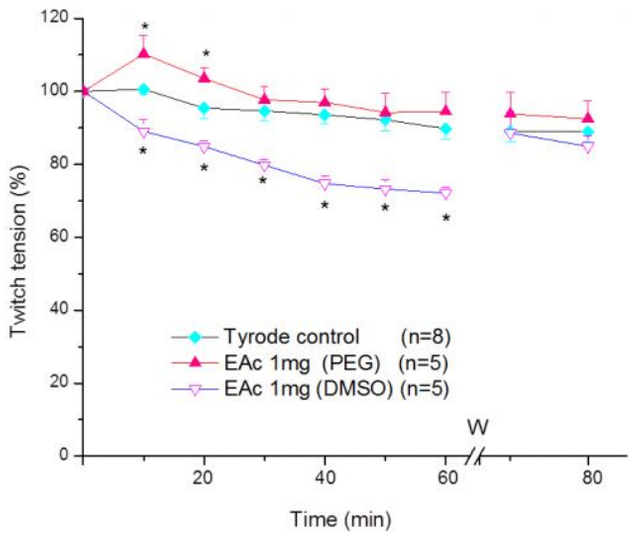

Figure 3: Mouse phrenic nerve-diaphragm preparation, indirect stimuli. Ethyl acetate extract (EAc, 1 mg) was taken as example (among the extracts) to show the solvent influence (PEG400 or DMSO) on the pharmacological response, major than 20% one each other, at the end of experiment. Note that the extract PEG-solubilized reached the basal response at 60 min. The points are the means ± SEM of 5-8 experiments. *p<0.05 vs. control. W, washing. PEG400, polyethylene glycol 400. DMSO, dimethyl sulfoxide.

Gasparetto et al.[15] published a review about the chemical constituents of Mikania glomerata and M. laevigata, a reason by which is unnecessary to deep this subject here. Authors declare "despite the relevance of the described metabolites, the benefits of guaco have been attributed mainly to the presence of coumarin". Coumarin, an anticoagulant and antithrombotic agent, is the main biomarker of guaco and[16] as shown here, present in all extracts.

Respiratory diseases are related to respiratory muscles, which serve to implement the primary function of the lung (to supply O2 and to remove CO2 from the blood stream), via pump muscles (ventilation) and airway muscles that control the caliber of upper (nose, mouth and pharynx); middle (larynx, trachea and primary bronchi); and the lung airways (distal bronchi, bronchioles, alveolar ducts, alveolar sacs and alveoli). The upper airways are comprised of both skeletal whereas trachea and bronchi are comprised of smooth muscles.[17]

Relaxation of smooth muscle (an involuntary non-striated muscle) from respiratory tract is the expected therapeutic effect from guaco, producing the air passage as result and relief of respiratory diseases symptoms. In addition to that, coumarin seems to act also in endothelium that overlies trachea, a reason by which the bronchodilator effect of coumarin is partly due to an endothelium-dependent tracheal relaxation, and may be mediated through a non-specific tracheal relaxation.[18]

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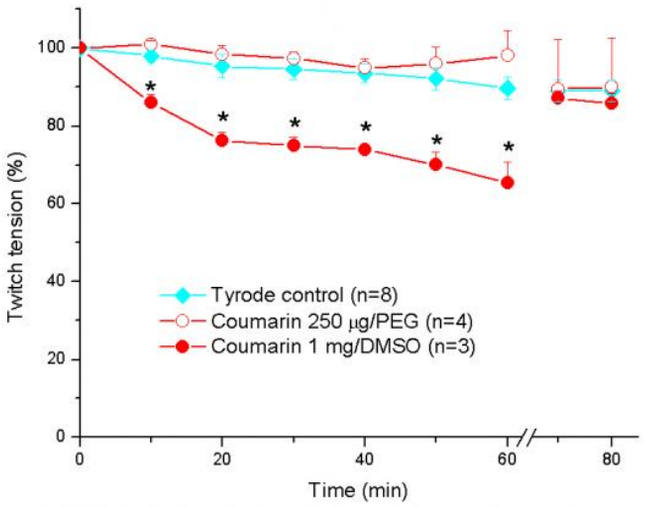

Figure 4: Mouse phrenic nerve-diaphragm preparation, indirect stimuli. Coumarin 1 mg solubilized in DMSO and Coumarin 250 µg solubilized in PEG expressed different response in the same experimental model, showing the solvent influence major than 30% at the end of experiment. The points are the means ± SEM of 3-8 experiments. * $p<0.05$ vs. control. W, washing. PEG, polyethylene glycol 400. DMSO, dimethyl sulfoxide.

Unlike the smooth muscles the diaphragm muscle exhibits an activation pattern which function can be described by force generation and change in length. Thus our premise is that, in this muscle the guaco did not block the twitch-tension and different nonpolar to polar extracts from M. laevigata can be studied in this experimental model.

It is known that "abundant organic solvents used are problematic in the extraction/separation of biological active compounds from the herb because of their toxicity, volatility and flammability".[19] Therefore all obtained extracts need to be lyophilized to evaporate the used solvents avoiding any interference on biological models. However, the different polarities of compounds require the use of different solubilizers depending on the experimental model. Since the neuromuscular preparation is maintained in nutritive Tyrode solution, in this work we used two solubilizers, polyethylene glycol 400 (PEG400) and dimethyl sulfoxide (DMSO), as previously standardized by Cintra-Francischinelli et al.[9]

Figure 2 shows the pharmacological profile of hexane (Hex), dichloromethane (DCM), ethyl acetate (EAc), methanol (MeOH) and aqueous extracts (all 1 mg) from M. laevigata leaves, on the isolated PND preparation, a traditional experimental model which anatomically represents the nerve-muscle synapse; physiologically, the muscular contraction; and pharmacologically is used for testing therapeutic and toxic compounds.[20]

The Hex extract revealed a facilitatory effect (20 to 60 min, $p<0.05$) when compared to nutritive Tyrode solution, which persisted after washing and the replacement by a fresh Tyrode solution. Ethyl acetate extract also showed an initial facilitatory effect, visualized at 10 min, but declining to basal levels at the end of experiment. It would be this effect due the presence of apolar compounds as shown in chromatoplate A, like do the triterpenoids in Dipteryx alata[21]? Facilitatory effect was also found in hydroalcoholic extract of Casearia sylvestris[20,22] in Camellia sinensis hydroalcoholic extract,[23] an effect attributed to theaflavin, a polar compound;[24] and also previously in M. laevigata hydroalcoholic extract.[25] In these examples of plants showing the facilitatory effect were used the same experimental model. As nonpolar and polar compounds can cause facilitation, such phenomena seem to depend of each substance.

Curiously, the facilitatory effect was only observed in extracts PEG-solubilized, Hex and EAc, using a low amount as 15 µL to solubilize 1 mg of these extracts; whereas 30 µL DMSO also solubilizes 1 mg of DCM or MeOH extracts, which did not exhibit the same facilitatory effect. Figure 2A and 2B are myographic recordings showing no influence on the basal response when 15 µL PEG or 30 µL DMSO was added into the bath containing the biological preparation, respectively, validated by Cintra-Francischinelli et al.[9] These concentration criteria were rigorously applied here, since PEG also causes a facilitation concentration-dependent.[26] Obviously, aqueous extract was soluble in Tyrode solution not needing the use of PEG nor DMSO.

Solubilizers make hydrosoluble substances with low solubility in water, but it must be stable and cause no interference in the stability or effectiveness of the active substance[27,28]. Using each 1 mg EAc extract PEG-solubilized or DMSO-solubilized, the pharmacological effect was different one each other in around 20%, an effect more accentuated when EAc was solubilized with DMSO ($p<0.05$) than PEG ($p>0.05$), in comparison with nutritive Tyrode solution at 60 min (Figure 3).

Based on the profile of PEG that exhibited a normal pattern ($p>0.05$) at the end of experiment using EAc, our attention was addressed to DMSO. Explaining, PEGs are on the Compounds Generally Recognized as Safe (GRAS) list of

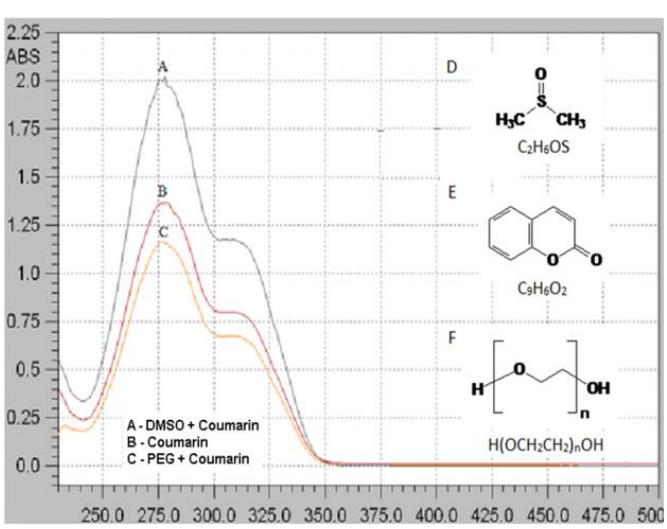

Figure 5: UV spectral profile of the coumarin (B) solubilization by dimethyl sulfoxide (DMSO, A) and polyethylene glycol monomer (PEG, C). Chemical structures of DMSO (D). coumarin (E) and PEG (F).

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Food and Administration's (FDA's), and have been approved by the FDA for internal consumption, due its good biocompatibility and low immunogenicity. Besides, PEGs are stable to acid, base, high temperature, high oxidation systems and reduction systems; they have good miscibility with water and organic solvents, as well as good solubility for various organic compounds.[19]

Our rationale experimental design was to reproduce the same protocol using 1 mg coumarin (that are predominantly poorly water-soluble), the main biomarker of guaco, solubilized with 30 μL DMSO. Coumarin is a natural product occurring in plants and has many important biological activities[29,30], including myelin-binding properties.[31,32] Coumarin derivatives were binding to human serum albumin at sub-domain IB with the hydrophobic interactions and also with hydrogen bond interactions.[33]

Figure 4 shows the same pharmacological profile of coumarin from that exhibited by EAc extract (Figure 3). For excluding the PEG interaction, some experiments using 250 μg were shown in the Figure 4. A maximum of 500 μg coumarin solubilized in PEG also had no statistical difference from control (data not shown).

Notice that at the end of experiment, after the washing of preparation and the fresh Tyrode replacement, the total recovery of the contractile response showing to be any effect of DMSO on coumarin, a transitory and not persistent event on the neuromuscular preparation. On this respect, as medicinal perspective PEG would be a preference than DMSO.

Soni et al.[34] described about the increase of solubilizers concentration and the increase of indomethacin solubility leading to a toxic effect enhance of the solubilizing agents. In our case, the neuromuscular blockade was caused by a coumarin-DMSO interaction, since 30 μL of DMSO is a concentration that alone did cause no change in the basal response.

In order to clarify the different pharmacological response face to both solubilizers, an UV spectral of the mixture DMSO-coumarin (Figure 5A) and PEG-courmarin (Figure 5C) was run, comparatively to coumarin alone (Figure 5B). At the right side, the chemical structures of DMSO (Figure 5D), coumarin (Figure 5B) and PEG (Figure 5C) are exhibited. PEG has a lipophilic alkyl tail[35] that decreases the coumarin solubility when compared to DMSO.[36]

The poor water-solubility of coumarin undergoing to the use of different solubilizers (PEG or DMSO) resulted in different pharmacological response on the target, frequently the plasma membrane, and the first barrier in the organism. The difference obtained in the pharmacological results of coumarin, a blockade of 30% compared to control, point out to the major bioavailability of coumarin on membranes when solubilized in DMSO than PEG, a parameter based on the influence of physicochemical properties on the dissolution of drugs. The amphipathic nature of DMSO molecule with a highly polar domain and two apolar groups, making it soluble in both aqueous and organic media[37] can constitute an explanation for its pharmacological action (Figure 4) and also for the increasing of coumarin/DMSO absorbance (Figure 5).

In order to confirm the antagonic effect of drugs on skeletal and smooth muscles was assayed the commercial salbutamol (or albuterol), a short-acting β-2 adrenergic agonist that is primarily used as a bronchodilator agent to treat asthma.[38] Figure 6 shows a dose-response curve of 0.1–5 mg of salbutamol in the same biological preparation. Note that salbutamol, that is soluble in water, exerts a facilitatory effect, only declining at high concentration (5 mg), an effect that can be caused by saturation of nutritive medium, which salt concentration equilibrium is vital for preparation functionality.[39]

Conclusion

Taken these results together, the comparison with salbutamol allowed to conclude that hexane and ethyl acetate were the better extracts of M. laevigata (at 1 mg concentration solubilized in PEG400) on the activated pattern of neuromuscular junction due the facilitatory effect, which in turn, is not related to coumarin alone, as showed in this study.

References

1.Oliveira MJR, Simões MUS, Sassi CRR. Fitoterapia no sistema de saúde pública (SUS) no estado de São Paulo, Brasil. Rev. Bras. Plantas Med. 2006; 8:39-41.

2.Mantilla CB, Sieck GC. Invited review: Mechanisms underlying motor unit plasticity in the respiratory system. J. Appl. Physiol. 2003; 94:1230-1241.

3.Brasil. ANVISA. Instrução normativa no 5, de 11 de dezembro de 2008- Determina a lista de registro simplificado de fitoterápicos no Brasil. 2008. Revoga o disposto da Resolução RE no 89, de 16 de março de 2004. D.O.U. , Brasília – DF. p. 5.

4.Oliveira F, Alvarenga MA, Akisue G, Akisue MK. Isolamento e identificação de compostos químicos de Mikania glomerata Sprengel e de Mikania Laeviagata Schultz Bip ex Baker. Rev. Farm. Bioquim. Univ. 1984; 20:169-183.

5.Cabral LM, dos Santos TC, Alhaique F. Development of a profitable procedure for the extraction of 2-H-1-benzopyran-2-one (coumarin) from Mikania glomerata. Drug Dev. Ind. Pharm. 2001; 27:103-106.

6.Santos SC. Caracterização cromatográfica de extratos medicinais de guaco: Mikania laevigata Shultz Bip. Ex Baker e Mikania glomerata Sprengel e ação da Mikania laevigata na inflamação alérgica pulmonar. MSc Dissertation, Universidade do Vale do Itajaí, Itajaí, Brazil. 2005. p. 81.

7.Santos SC, Krueger CL, Steil AA, Krueger MR, Biavatti MW, Wisnewski Junior A. LC characterization of guaco medicinal guaco, Mikania laevigata and Mikania glomerata, and their effects on allergic pneumonitis. Planta Med. 2006; 72:679-684.

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All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

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8.Bülbring E. Observation on the isolated phrenic nerve diaphragm preparation of the rat. Br. J. Pharmacol. 1946; 1: 38-61.
9.Cintra-Francischinelli M, Silva MG, Andreo-Filho N, Cintra ACO, Leite GB, Cruz-Höfling MA, Rodrigues-Simioni L, Oshima-Franco Y. Effects of commonly used solubilizing agents on a model nerve-muscle synapse. Lat. Am. J. Pharm. 2008; 27: 721-726.
10.Solanki SS, Soni LK, Maheshwari RK. Study on mixed solvency concept in formulation development of aqueous injection of poorly water soluble drug. J Pharm. 2013; 2013:1-8.
11.Teske M, Trentine AMM. Herbárium Compêndio de Fitoterapia. Herbarium, Curitiba. 1997; pp. 73-76.
12.Moraes MD, Monteiro R.. A família Asteraceae na planície litorânea de Picinguaba município de Ubatuba, São Paulo. Hoehnea. 2006; 33: 41-78.
13.Freitas TP, Silveira PC, Rocha LG, Rezin GT, Rocha J, Citadini-Zanette V, Romão PT, Dal-Pizzol F, Pinho RA, Andrade VM, Streck EL. Effects of Mikania glomerata Spreng. and Mikania laevigata Schultz Bip. ex Baker (Asteraceae) extracts on pulmonary inflammation and oxidative stress caused by acute coal dust exposure. J. Med. Food. 2008; 11:761-766.
14.Duke JA, Godwin MB, Ottesen AR. Duke´s handbook of medicinal plants of Latin America. CCR Press, Boca Raton. 2009; p 449.
15.Gasparetto JC, de Francisco TMG, Pontarolo R. Chemical constituents of Mikania glomerata Spreng and Mikania laevigata Sch. Bip. ex Baker. 2013; 7: 753-765.
16.Brasil. ANVISA. Instrução normativa no 02 de 13 de maio de 2014 – Publica a "Lista de medicamento fitoterápicos de registro simplificado" e a "Lista de produtos tradicionais fitoterrápicos de registro simplificado". 2014. Revoga o disposto na Instrução Normativa nº 5, de 11 de dezembro de 2008. DOU de 14/05/2014 (nº 90, Seção 1, pág. 58).
17.Gransee HM, Mantilla CB, Sieck GC. Respiratory muscle plasticity. Compr. Physiol. 2012; 2:1441-1462.
18.Ramanitrahasimbola D, Rakotondramanana DA, Rasoanaivo P, Randriantsoa A, Ratsimamanga S, Palazzino G, Galeffi C, Nicoletti M. Bronchodilator activity of Phymatodes scolopendria (Burm.) Ching and its bioactive constituent. J. Ethnopharmacol. 2005;102: 400-407.
19.Zhou T, Xiao X, Li G, Cai ZW. Study of polyethylene glycol as a green solvent in the microwave-assisted extraction of flavone and coumarin compounds from medicinal plants. J. Chromatogr. A. 2011; 1218: 3608-3615.
20.Cintra-Francischinelli M, Silva MG, Andréo-Filho N, Gerenutti M, Cintra AC, Giglio JR, Leite GB, Cruz-Höfling MA, Rodrigues-Simioni L, Oshima-Franco Y. Antibothropic action of Casearia sylvestris Sw. (Flacourtiaceae) extracts. Phytother. Res. 2008; 22: 784-790.
21.Ferraz MC, Parrilha LAC, Moraes MSD, Amaral Filho J, Cogo JC, dos Santos MG, Franco LM, Groppo FC, Puebla P, San Feliciano A, Oshima-Franco Y. The effect of lupane triterpenoids (Dipteryx alata Vogel) in the in vitro neuromuscular blockade and myotoxicity of two snake venoms. Curr. Org. Chem. 2012; 16: 2717-2723.
22.Oshima-Franco Y, Alves CMV, Andréo Filho N, Gerenutti M, Cintra ACO, Leite GB, Rodrigues-Simioni L, Silva MG. Neutralization of the neuromuscular activity of bothropstoxin-I, a myotoxin from Bothrops jararacussu snake venom, by a hydroalcoholic extract of Casearia sylvestris Sw. J. Venom. Anim. Toxins incl. Trop. Dis. 2005; 11:465-478.
23.de Jesus Reis Rosa L, Silva GA, Filho JA, Silva MG, Cogo JC, Groppo FC, Oshima-Franco Y. The inhibitory effect of Camellia sinensis extracts against the neuromuscular blockade of Crotalus durissus terrificus venom. J Venom Res. 2010;1:1-7.
24.Basu S, Chaudhuri T, Chauhan SPS, Das Gupta AK, Chaudhury L, Vedasiromoni JR. The theaflavin fraction is responsible for the facilitatory effect of black tea at the skeletal myoneural junction. Life Sci. 2005; 76: 3081–3088.
25.Collaço RdeC, Cogo JC, Rodrigues-Simioni L, Rocha T, Oshima-Franco Y, Randazzo-Moura P. Protection by Mikania laevigata (guaco) extract against the toxicity of Philodryas olfersii snake venom. Toxicon. 2012; 60: 614-622.
26.Oshima M, Leite GB, Rostelato-Ferreira S, da Cruz-Höfling MA, Rodrigues-Simioni L, Oshima-Franco Y. Insights of the effects of polyethylene glycol 400 on mammalian and avian nerve terminals. Muscle Nerve. 2010; 41:540-546.
27.Prista LN, Alves AC, Morgado R. Tecnologia farmacêutica. Fundação Calouste Gulbenkian, Lisboa. 1995.
28.Ansel HC, Popovich NG, Aleen-Jr LV. Farmacotécnica: formas farmacêuticas e sistema de liberação de fármacos. Editorial Premier, São Paulo. 2000.
29.Casley-Smith JR. Benzopyrones in the treatment of lymphedema. Intern. Angiol. 1999; 18:41976–41990.
30.Luszczki JJ, Andres-Mach M, Cisowski W, Mazol I, Glowniak K, Czuczwar SJ. Osthole suppresses seizures in the mouse maximal electroshock seizure model. Eur. J. Pharmacol. 2009; 607:107–109.
31.Wang C, Popescu DC, Wu C, Zhu J, Macklin W, Wang Y. In Situ fluorescence imaging of myelination. J. Histochem. Cytochem. 2010; 58:611–621.
32.Wang C, Wu C, Zhu J, Miller RH, Wang Y. Design, synthesis, and evaluation of coumarin-based molecular probes for imaging of myelination. J. Med. Chem. 2011; 54: 2331-2340.
33.Garg A, Manidhar DM, Gokara M, Malleda C, Reddy CS, Subramanyam R. Elucidation of the Binding Mechanism of Coumarin Derivatives with Human Serum Albumin. PLoS One. 2013; 8: e63805.
34.Soni LK, Solanki SS, Maheshwari RK. Solubilization of poorly water soluble drug using mixed solvency approach for aqueous injection. Br. J. Pharmac. Res. 2014; 4: 549-568.
35.Yuandong M, Zheng Y, Liu K, Tian G, Tian Y, Xu L, Yan F, Huang L, Mei L. Nanoparticles of poly(lactide-co-glycolide)-d-a-tocopheryl polyethylene glycol 1000 succinate random

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copolymer for cancer treatment. Nanoscale Res. Lett. 2010 ;5: 1161–1169.
36.Mercer DK, Robertson J, Wright K, Miller L, Smith S, Stewart CS, O'Neil DA. A prodrug approach to the use of coumarins as potential therapeutics for superficial mycoses. PLoS One. 2013; 8: e80760.
37.Santos NC, Figueira-Coelho J, Martins-Silva J, Saldanha C. Multidisciplinary utilization of dimethyl sulfoxide: pharmacological, cellular, and molecular aspects. Biochem. Pharmacol. 2003; 65:1035-1041.
38.MeSH, Medical Subject Headings. Salbutamol, in http:www.ncbi.nlm.nih.gov/mesh/?term=salbutamol. 2014. Accessed in 30/10/2014.
39.Rubem-Mauro L, Rocha-Jr DS, Barcelos CC, Varca GH, Andreo-Filho N, Barberato-Filho S, Oshima-Franco Y, Vila MMDC. Phenobarbital pharmacological findings on the nerve-muscle basis. Lat. Am. J. Pharm. 2009; 28: 211-218.

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