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ACTIVITY-DIRECTED ISOLATION OF SPASMOLYTIC (ANTI-CHOLINERGIC) ALKALOIDS FROM *BRUGMANSIA ARBOREA* (L.) LAGERHEIM

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ABSTRACT

This paper examined the effects of the extracts MeOH and H_2O, chromatographic fractions and three pure alkaloids from Brugmansia arborea *(L.) Lagerheim (Solanaceae) on the electrically- and acetylcholine (Ach)-induced contractions of isolated guinea-pig ileum. The results indicate that both MeOH and H_2O extracts dose-dependently reduced the electrical contractions of guinea-pig ileum. Furthermore, the partially purified fractions 2 and 3 from MeOH extract and three pure compounds obtained from the fraction 2 of MeOH extract significantly and dose-dependently reduced the electrical and the Ach-induced contractions of the ileum. The chemical identification, performed by spectroscopic methods, indicate that the three active substances — atropine, scopolamine, nor-hyoscine — were tropane alkaloids.*

INTRODUCTION

There are no data in literature about the pharmacological properties of *Brugmansia arborea* (L.) Lagerheim (Syn: *Datura arborea* L.) (Solanaceae), whereas some tropane alkaloids (Roses *et al.*, 1987) and cuscohygrine (Ghani, 1985) have been isolated from the plant.

Keywords: Ach-induced contractions, atropine, *Brugmansia arborea* (L.) Lagerheim, electrical-induced contractions, guinea-pig ileum, nor-hyoscine, scopolamine, tropane alkaloids.

List of the binomials: Brugmansia arborea (L.) Lagerheim.

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The South American species of the genus *Brugmansia* (often included under *Datura*) are known with the vernacular names "floripondio", "campanchu", "yerba del diablo" and are used in the traditional therapeutic and magical practices of the folkloric Peruvian medicine to reach altered states of consciousness (De Feo, 1992).

Species of *Brugmansia* have been reported to be used also by other peoples during ritual practices for magical and curative purposes (Schultes and Hoffman, 1973). Previous studies identified tropane and nicotinic alkaloids (Roses *et al.*, 1987, 1988a; Gambaro and Roses, 1989) in the genus and studies on the activity of a hydro-alcoholic decoction of the flowers of *B. candida* on mouse behaviour are reported (Roses *et al.*, 1988b).

B. arborea is known in the northern Peruvian Andes also as "misha oso", "misha toro" and "misha galga" and, in addition to the ritual use, the decoction of its leaves and flowers is used topically as an analgesic, anti rheumatic, vulnerary, decongestant and antispasmodic (De Feo, 1992).

The present study was carried out to examine the effects of the extracts (MeOH and H_2O), some chromatographic fractions of MeOH extract and the pure compounds isolated from *B. arborea* on the electrically induced contractions of isolated guinea-pig ileum, widely used to study the activity of substances which interact with the cholinergic system (Johnson *et al.*, 1978). The influence of these extracts, chromatographic fractions, and alkaloids on Ach-induced contractions of guinea-pig ileum was also evaluated.

Table 1. Inhibitory effects of the extracts, chromatographic fractions, and alkaloids isolated from *B. arborea* on the electrically-induced contractions of guinea-pig ileum.

	IC_{50}	Confidence limits	
		Lower	Upper
MeOH extract	0.28 µg/ml	0.22	0.35
H_2O extract	6.3 µg/ml	4.6	8.6
Fr. 2 of MeOH ext.	0.054 µg/ml	0.044	0.065
Fr. 3 of MeOH ext.	0.52 µg/ml	0.44	0.62
Atropine	7.3×10^{-8} M	4.1×10^{-8} M	1.0×10^{-7} M
Scopolamine	8.4×10^{-6} M	6.7×10^{-6} M	1.0×10^{-5} M
Nor-hyoscine	3.1×10^{-7} M	2.4×10^{-7} M	4.0×10^{-7} M

The IC_{50} and 95% confidence intervals were computed from dose-response curves by the method of Litchfield and Wilcoxon with the aid of a computer program.

Table 2. Effects of the alkaloids isolated from *B. arborea* on the Ach-induced contractions of guinea-pig ileum.

Concentration of compounds	*Ach contraction (% inhibition)
Atropine (1.0×10^{-7} M)	82.0 ± 6.7
Atropine (5.0×10^{-8} M)	51.6 ± 5.5
Atropine (1.0×10^{-8} M)	30.3 ± 4.6
Scopolamine (1.0×10^{-5} M)	85.0 ± 7.2
Scopolamine (5.0×10^{-6} M)	47.3 ± 6.3
Scopolamine (1.0×10^{-6} M)	33.2 ± 5.2
Nor-hyoscine (5.0×10^{-7} M)	82.0 ± 5.5
Nor-hyoscine (1.0×10^{-7} M)	53.1 ± 6.1
Nor-hyoscine (5.0×10^{-8} M)	29.7 ± 4.1

* Values shown are % inhibition of Ach-induced contractions (mean ± SEM; $n = 6$).

MATERIALS AND METHODS

Plant Material

Leaves and flowers of *B. arborea* were collected in September, 1994 near Huancabamba, Piura Department, northern Peru. The plant was identified by Dr. V. De Feo. A voucher specimen of the plant (DF 94/298) is deposited at the herbarium of the School of Pharmacy, University of Salerno (Italy).

Extraction and Isolation

Air-dried and powdered leaves and flowers of *B. arborea* (480 g) were sequentially extracted at room temperature with methanol and water, to give 13.04 and 7.03 g of residues, respectively. Part of the methanol extract (4.2 g), in 2 g lots, was chromatographed on Sephadex LH-20 column, eluting with methanol. 72 fractions of 8 ml were collected and combined by TLC similarly in *n*-butanol:acetic acid:water (60:25:15) (BAW) and in $CHCl_3$:MeOH:H_2O (80:18:2), in 4 main fractions, 1 (mg 345), 2 (mg 477), 3 (mg 547), and 4 (mg 274). Fraction 2, which exhibited the greater biological activity, was chromatographed on TLC and the alkaloids revealed by the Dragendorff reagent, showing the presence of three compounds with R_f values of 0.2, 0.4 and 0.5, respectively, in BAW.

Fraction 2 was dried and extracted with chloroform, obtaining the pure nor-hyoscine. Atropine and scopolamine were obtained by PLC eluting with BAW.

The obtained compounds were then treated with aqueous NH_4OH (pH 10) and extracted with diethyl ether, giving free atropine (mg 7.9), scopolamine (mg 5.3) and nor-hyoscine (mg 8.2).

The identification of the compounds was performed by comparison of spectral data (^{1}H NMR, ^{13}C NMR and ^{13}C DEPT NMR) with those reported in literature (Stenberg *et al.*, 1977; Sarazin *et al.*, 1991) and, for atropine and scopolamine, with those obtained from standard compounds (Sigma, Milano, product number A9547 and S 1875, respectively).

Animals

Male Charles River guinea-pigs (180–200 g) were used for all the experiments. The animals were housed in colony cages (four guinea-pigs each) under standard light (light on from 7.00 a.m. to 7.00 p.m.), temperature (22 ± 1 °C) and room humidity (60% ± 10%) conditions for at least 1 week before the experimental sessions. Food and water were available *ad libitum*.

Transmurally Stimulated Guinea-pig Ileum Test

The animals were sacrificed by cervical dislocation. Guinea-pig ileum was prepared as previously described (Okpako and Taiwo, 1984). Pieces of ileum, 2–3 cm long, were set up in 10 ml organ bath containing Tyrode solution with 5% CO_2 in 95% oxygen; the solution was maintained at 37°C. Whole ileum preparation was

placed between platinum electrodes and connected to a 85/2/50 M.A.R.B. Stimulator (Ditta M.A.R.B., Chiesina Uzzanese, Pistoia, Italy). A force-displacement transducer and unirecord model Polygraph was used for measurement of isotonic contraction (Ditta Ugo Basile, Italy). A resting tension of 0.5 g was applied. After a 30 min equilibration period, the preparation was stimulated for 5 msec pulse delivered transmurally at a frequency of 10 sec at supramaximal voltage (25V). In these conditions, the preparation showed a contraction mean of 60 mm ± 0.57. The inhibition of ileal contractions by drugs was expressed as percentage of basal value (mean ± SEM).

Experimental Procedure

The extracts used were dissolved in distilled water as well the partially purified fractions and pure compounds. The effects of the extracts, chromatographic fractions and compounds on the electrically-induced contractions of guinea-pig ileum (E.C.I.) were investigated according to the following experimental schedule:

a) MeOH (0.7, 0.35 and 0.07 µg/ml organ bath) and H_2O extract (10, 5, 1 µg/ml organ bath): 15 min contact period.

b) Partially purified fractions 1 and 4 up to 500 µg/ml organ bath; fraction 2 (0.1, 0.05 and 0.025 µg/ml organ bath); fraction 3 (1, 0.5, 0.25 µg/ml organ bath): 15 min contact period.

c) Pure alkaloids from 10^{-7} M to 5×10^{-5} M: 15 min contact period.

Ach-induced Contractions of Guinea-pig Ileum

It was performed as previously described (Capasso *et al.*, 1991). Briefly, guinea-pig isolated ileum was placed in a 10 ml organ bath and connected to an isotonic transducer; the resting tension was 0.5 g and maintained throughout the experiment. Ach was added to the bathing fluid and allowed to remain in contact with the tissue until the maximal effect occurred in 2 min and then washed out. After at least three control contractions, alkaloids isolated from fraction 2 of the methanol extract were added to the bath 5 min before the next addition of the agonist.

Statistical Analysis

In the electrically-stimulated guinea-pig ileum (E.C.I.), regression methods were used for statistical analysis and critical significance set at $P < 0.05$. In the Ach-induced contractions of guinea-pig ileum, the inhibition of ileal contraction by alkaloids was expressed as a percent of the corresponding control (mean ± SEM); a paired two-tailed Student's t test was used for statistical analysis of corresponding values.

IC_{50} values (Concentrations producing 50% inhibition) were calculated according to Lichfield and Wilcoxon (Tallarida and Murray, 1987).

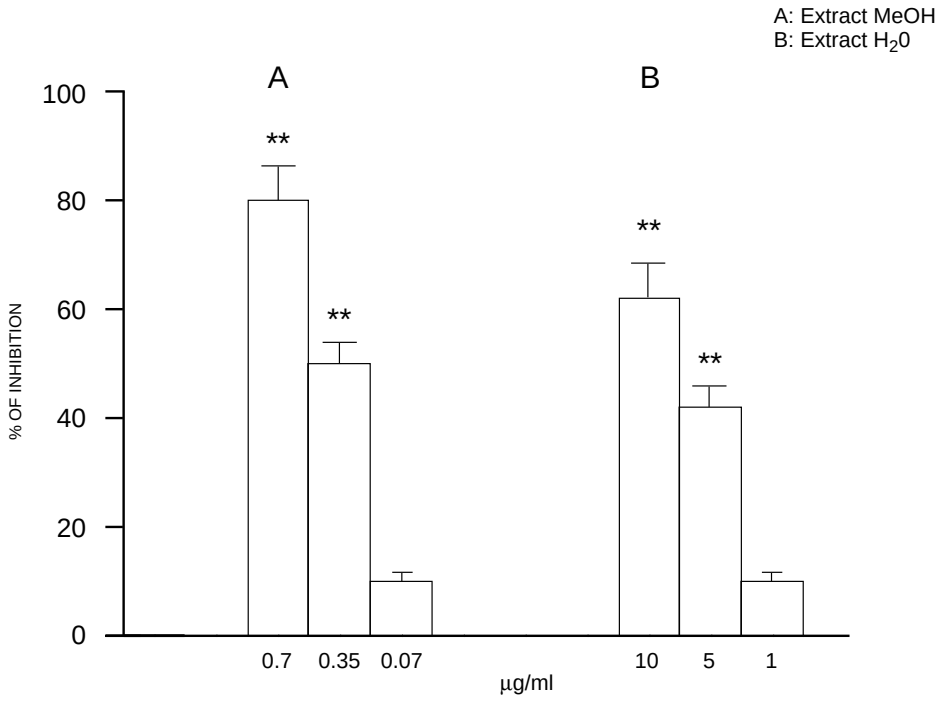

FIG. 1. Dose-related inhibition by MeOH and H_2O extracts from *B. arborea* on the electrically-induced contractions of guinea-pig ileum. Results are expressed as mean ± SEM, ($n = 6$). ** $P < 0.01$.

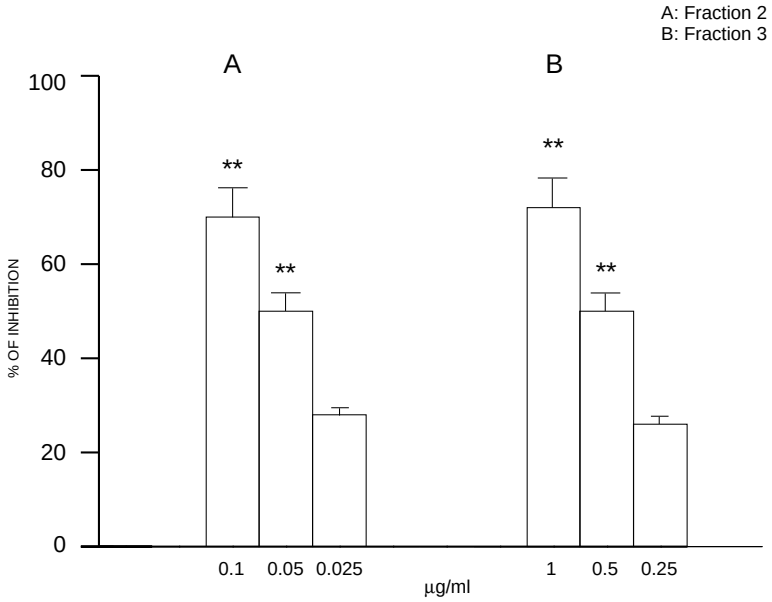

FIG. 2. Concentration-dependant inhibitory effects of the partially purified fractions 2 and 3 from the MeOH extract from *B. arborea* on the electrically-induced contractions of guinea-pig ileum. Results are expressed as mean ± SEM, (n = 6). ** $P < 0.01$.

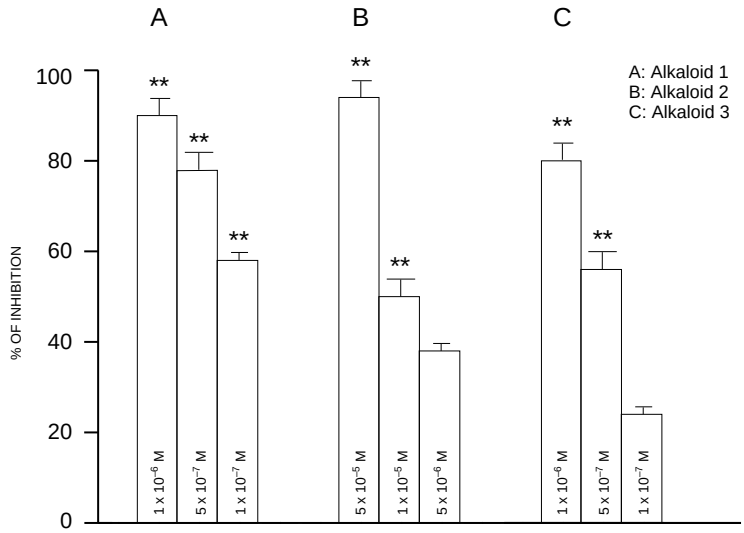

FIG. 3. Concentration-dependant inhibitory effects of the alkaloids isolated from *B. arborea* on the electrically-induced contractions of guinea-pig ileum. Results are expressed as mean ± SEM, (n = 6). * $P < 0.05$; ** $P < 0.01$.

RESULTS

Effects of the Extracts on the Electrically-induced Contractions of Guinea-pig Ileum (E.C.I.)

Figure 1 shows that both MeOH and H_2O extracts, at the concentrations used, dose-dependently (0.07 – 0.7 µg/ml) reduced the E.C.I. The inhibition began 2–4 min after the extracts administration, and it was enhanced with time and lasted for the whole recording period (15 min). MeOH extract was more potent in E.C.I. inhibition than H_2O extract; in Table 1, the IC_{50} calculated for the extracts were reported.

Effects of the Partially Purified Fractions 1–4 on the E.C.I.

As the MeOH extract was more active in inhibiting the ileum contractions, it was submitted to a further purification by Sephadex LH 20 column and four main fractions were collected and tested for E.C.I. activity. Only fractions 2 and 3 were able to reduce significantly the E.C.I. (Fig. 2), whereas fractions 1 and 4 did not produce significant modification on the preparation contractions (data not shown). Fraction 2 was more potent than fraction 3, in causing inhibition of E.C.I.-induced contraction of guinea-pig ileum.

Also in this case, the inhibition appeared 2–4 min after the administration, and it was enhanced with time and lasted for all the recording period (15 min). Table 1 showed the IC_{50} calculated for the active fractions.

Effects of the Pure Compounds on the E.C.I.

Figure 3 shows that the three compounds purified from the fraction 2 of methanol extract, exert a strong inhibitory activity on the E.C.I. Atropine was able to reduce dose-dependently the E.C.I. of guinea-pig ileum at concentrations of 10^{-7}, 5×10^{-7} and 10^{-6} M; scopolamine, at concentrations of 5×10^{-6}, 5×10^{-5} and 10^{-5} M; and nor-hyoscine at concentrations of 10^{-7}, 5×10^{-7} and 10^{-6} M. Also in this case, the inhibition appeared 2–4 min after the administration, it was enhanced with time and lasted for entire recording period (15 min); in Table 1, the IC_{50} calculated for the active compounds were reported.

Effect of the Alkaloids on Ach-induced Contractions of Guinea-pig Isolated Ileum

Results presented in Table 2 show that the three alkaloids after 5 min contact with ileum, reduced dose-dependently (atropine: 1.0×10^{-7}, 5.0×10^{-7} and 1.0×10^{-8} M; scopolamine: 1.0×10^{-5}, 5.0×10^{-6} and 1.0×10^{-6} M; nor-hyoscine: 5.0×10^{-7}, 1.0×10^{-7} and 5.0×10^{-8} M) the contractions induced by a submaximally effective concentration of Ach (10^{-6} M). All inhibitory effects of the alkaloids were completely lost after washing out six times in 6 min after the bath.

DISCUSSION

Although *B. arborea* is empirically used in folk medicine to reach an altered states of consciousness (De Feo, 1992), there are no data in the literature on the possible pharmacological effects exerted by extracts, fractions, and pure compounds isolated from this plant.

The results of the present study indicate that MeOH and H_2O extracts, some chromatographic fractions of the methanol extract and three tropane alkaloids from *B. arborea* are able to reduce the electrically-stimulated contractions of guinea-pig ileum. Furthermore, the alkaloids were also able to reduce the Ach-induced contractions of guinea-pig ileum, thus indicating their anticholinergic activity.

Of the tested extracts, MeOH extract was more active than H_2O extract in inhibiting the ileum electrical contractions; therefore the MeOH extract was submitted to a further purification by Sephadex LH-20 column, to yield four main fractions, which were tested under the same experimental conditions. All the fractions tested showed a different potency in inhibiting the E.C.I. The relative order of potency was: fraction 1 and 4 inactive, fraction 3 $<$ fraction 2. In comparison to the whole methanol extract, its fraction 2 and 3 were much more potent, thus indicating that the fractions contain a mixture of the active components which caused a marked inhibitory activity on the E.C.I. In order to identify the compounds responsible for the activity, the fractions were submitted to a purification and three components (atropine, scopolamine and nor-hyoscine) were isolated. The experiments performed indicated that all the tested pure alkaloids were able to reduce the E.C.I.

In this respect, it is well known that tropane alkaloids antagonize the muscarinic actions of Ach and they are known as antimuscarinic or muscarinic cholinergic blocking agents. Because the major effects of most members of this class of drugs are quantitatively similar to those of its best known member, the term atropine-like is also used (Brown, 1987).

The relative order of potency was atropine $>$ nor-hyoscine $>$ scopolamine. Therefore, in order to assess their ability to antagonize the muscarinic actions of Ach, they were tested on Ach-induced contractions of guinea pig isolated ileum. In these experiments, all three alkaloids were able to reduce the Ach-induced contractions.

Taken together, the above results indicate that the inhibitory activity of MeOH extract, and of its fraction 2, was due to the presence of a combination of active principles. This is confirmed by the experiments performed with this fraction which resulted more active when compared to the fraction 3 (less active) or to fractions 1 and 4 (completely inactive). Furthermore, the results of the present study also show that the inhibitory activity on the E.C.I. is due to the alkaloids, whose ability in inhibiting the Ach-induced contractions indicate an anticholinergic activity related to the presence of the tropane nucleus. The presence of anti-cholinergic constituents in this plant may explain the antispasmodic use (De Feo, 1992) of the plant.

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