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INTESTINAL ANTISPASMODIC EFFECTS OF THREE ARGENTINIAN PLANTS: *HYPERICUM CONNATUM, BERBERIS RUSCIFOLIA* AND *CECROPIA PACHYSTACHYA*: MECHANISMS OF ACTION AND COMPARISON WITH THE EFFECTS OF *BRUGMANSIA ARBOREA*.

Matera, S.; Bruno, F.; Bayley, M.; Pérez, V.; Querini, C.; Ragone, M.I.; Consolini, A.E.*

Cátedra de Farmacología, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina.

*dinamia@biol.unlp.edu.ar

Abstract

Some medicinal plants are used in Argentina to reduce gastrointestinal symptoms as antispasmodics, but not all these effects were validated. This work studied the effects of three native plants as the ethanolic tinctures (T) of *Hypericum connatum* and *Berberis ruscifolia,* as well as the acqueous crude extracts (A.c.e) of *Cecropia pachystachya,* and compared the mechanisms with those of the peruvian plant *Brugmansia arborea* in isolated rat intestines. The first three plants demonstrated to have antispasmodic effect mainly due to the non-competitive blockade of the agonist-induced contraction and calcium influx to the smooth muscle, which was associated to the presence of flavonoids. *H. connatum* also induced the release of prostaglandins which cause intestinal contraction, and NO which cause peristalsis, in a way that the blockade of both mechanisms potentiated the relaxant effect of the T-H.c. In contrast, the A.c.e of *Brugmansia arborea* showed to be a competitive antagonist of muscarinic receptors in the isolated intestine, in agreement with the presence of tropane alkaloids. Moreover, *H. connatum* and *C. pachystachya* also produced certain sedation, which could contribute to the antispasmodic effect. The tincture of *Hypericum connatum,* but not that of *Berberis ruscifolia,* reduced the spontaneous locomotion and exploration of mice in the open-field test, at doses of 200 mg leaves/Kg. Results suggest that the three native plants exhibited an important antispasmodic effect mainly due to non-competitive antagonism of the agonist and of Ca^{2+}-influx to smooth muscle.

Key words: *Hypericum connatum*, *Berberis ruscifolia*, *Cecropia pachystachya*, *Brugmansia arborea*, antispasmodic, Ca^{2+}-blockade, sedative

Introduction

In Latin America, and particularly in Argentina, there are several native plants traditionally used for gastrointestinal disorders, such as diarrhea or spasms, but few of them were scientifically studied. In this work we aimed to evaluate the gastrointestinal effects of three plants used in the temperate central regions of Argentina, near the hills and fields bathed by small rivers and the great basin of the Rio de la Plata. The antispasmodic effect is generally associated to a direct effect on the smooth muscle, but in other case it is also due to a central sedative effect which reduce the autonomic tone on the peristaltism [1,2]. Then, both properties were studied in these three plants in order to validate their use in gastrointestinal disorders. One of the plants was *Hypericum connatum* Lam. (Clusiaceae) known as "cabotoril", which has been preliminary described to have cardiotonic effects [3] and antiviral, antibacterial and antifungic properties [4,5,6]. In spite of belonging to the same genus than *H. perforatum*, it was not found the antidepressant effect in "cabotoril" but is used as eupeptic [7]. The chemical identification of secondary metabolites in the leaves of *H. connatum* showed the presence of tannins and flavonoids in the leaves such as quercitrin and chlorogenic acid, but only traces of rutine which is important in *H. perforatum* [4]. Also, there are terpens in the essential oil [5,6]. Other native plant is *Berberis ruscifolia* Lam. (Berberidaceae) known as "quebrachillo" which belongs to a genus with about 500 species in the world. This plant grows in the temperate and tropical regions of the Nordeast and center of Argentina and also in Uruguay [7, 6]. It is used against diarrhea, Malaria and hepatic diseases, as well as antiinflammatory and eupeptic [8]. Other species native from Irak, *Berberis vulgaris*, has demonstrated to be anticholinergic, antihystaminic [9] and positive cardiac inotropic by the presence of berbamine [10]. Also, the edible fruits of *Berberis aristata* native from Pakistan evidenced inotropic effects [11]. We also studied the gastrointestinal effects of *Cecropia pachystachya* Mart. (known as "ambay") which is native from the Nordeast of Argentina near the Iguazu river and falls. This plant is used for health problems as cough, asthma, and gastrointestinal problems [7]. Some years ago we studied the properties of *C. pachystachya* from different regions, and found that the plants from tropical region had more intensive hypothensive and cardiotonic effects than plants from the temperate region [12]. Also this plant showed sedative effects in the open-field test of mice and cardiotonic effects [13]. More recently, the same plant from Brazil showed antidepressant-like effects in a cronic stress model [14]. The evoked sedative effects could be at least in part responsible for the relaxant effect on bronquial and intestinal spasms. The phytochemical reports for the genus *Cecropia* include C-glycosylflavonoids, proanthocyanidins, terpenoids and steroids [15]. The antiinflammatory effect was well described and related to the presence of chlorogenic acid [16]. Nevertheless, there are not reports about direct effects of this plant on the smooth muscle, which were evaluated here. Moreover, we evaluated the effects of a peruvian plant whose flowers are also used in our country to obtain central stimulant effects, *Brugmansia arborea* L. Lagerhein (Solanaceae), known as "floripondio". The decoction of its leaves and flowers is used externally as analgesic, antirheumatic, vulnerary, decongestant and antispasmodic. Several tropane and nicotinic alkaloids were found in this plant, and three derivatives of tropane reduced the morphine withdrawal in guinea-pig ileon [17]. But the mechanism as intestinal antispasmodic has not been studied. Then, the aim of this work was to evaluate the effects of different extracts traditionally used for these plants on the *in vitro* intestinal contractility and their mechanisms by using concentration-response curves. Also, the effects on the behavior of mice with the open-field test were evaluated in order to validate their properties on diseases such as diarrhea or intestinal spasms.

Methods

Plant material

Commercial herboristery samples of *Cecropia pachystachya* Mart., *Hypericum connatum* Lam., and *Berberis ruscifolia* Lam. leaves and *Brugmansia arborea* L. flowers were identified in the Museum Carlos Spegazzini (LPE) from the Facultad de Ciencias Exactas, Universidad Nacional de La Plata (UNLP) (vouchers LPE-1000, LPE-1190 and LPE-1191, and 1012, respectively). Alcoholic extracts or tinctures (T, obtained by maceration at 10% w/v in 70^{o} ethanol) from the first two plants, and aqueous crude extracts (A.c.e, obtained by decoction and lyophilization, yield about 10%) from the last two plants were prepared. For the biological protocols, the T and A.c.e were diluted in Tyrode solution.

Animals

The research was conducted in accordance with the internationally accepted principles for the laboratory animal use and care as was established by US

guidelines (NIH publication # 85-23 revised in 1985 and 2006).

Gastrointestinal effects

Biological preparation and contractile measurements

Sprague-Dawley rats (200-250g) were subjected to a 24 hours fasting with free access to water before experimentation. The animals were full anaesthetized by pentobarbital overdose and then quickly sacrificed by the opening of torax and abdomen. Duodenums and ileons (about 2 cm long were prepared and mounted in organ baths of 20 mL containing Tyrode solution at 37º C constantly oxygenated with air (pH 8.2) as in other works (18, 19, 20). The preparations were equilibrated for at least 45 minutes at 1g of pre-load. Tissues were connected to isometric transducers WPI (USA). The signals of 4 organs were simultaneously amplified by a 4-channels preamplifier (WPI, USA) and acquisited to a computer by Eagle Program.

Solutions and drugs

The used solutions had the following composition:

Tyrode (Tyr): 150 mM NaCl, 2.7 mM KCl, 2 mM $MgCl_2$, 12 mM $NaHCO_3$, 0.4 mM PO_4H_2Na, 1.8 mM $CaCl_2$, bubbled with air (pH 8.2).

Tyrode-0Ca: by eliminating $CaCl_2$

Tyrode-0Ca-40 mM K^+: by adding 0.6 ml KCl 10% to 20 ml Tyrode-0Ca in the chamber.

The concentration-response curves (CRC) were done with the agonists carbachol chloride (CbCh, Sigma, USA), or acetylcholine bromide (ACh, Sigma, USA) in rat intestine, and with hystamine (H, Sigma, USA) in guinea-pig ileum. The agonists were diluted in water to obtain solutions of 1, 2, 7, 20, 70, 200, 700 and 1000 µg/mL which were cumulatively added to the chamber. The CRC to Ca were done by preparing $CaCl_2$ anhydro (Mallinckrodt, Germany) at concentrations of 0.882, 1.764, 5.3, 18.5, 53, 185.22 and 530 mg/mL which were cumulatively added to the chamber. The extracts were diluted in the respective vehicle (water or 70º ethanol) to the series 1, 3, 10 and 30 in different and respective concentractions. All the solutions were added at 0.2 ml in 1/100 dilution to the chamber.

Concentration-response curves to agonists

At least two CRC to acetylcholine (ACh), carbachol (CbCh) or hystamine (H) were done after a 45 minutes period of stabilization in the respective intestinal portions (rat duodenum and ileums for Ach and CbCh, and guinea-pig ileums for H). Previous tests in the laboratory demonstrated that the CRC of duodenal and ileal portions to Ach were not significantly different. Then, another control CRC of the respective agonist in the presence of vehicle (70º ethanol) was done to compare the respective CRC that have the same dilution of vehicle. The agonist concentrations were cumulatively added to the bath (to reach from 0.01 to 10 (µg/mL) in the absence (control CRC) and the presence of a unique concentration of the respective extract, which was added 5 min before the CRC and remained during it in the bath. A growing order of extract concentrations was used for making the several CRC in each preparation.

Concentration-response curves to calcium

After stabilization during 45 min in Tyrode and testing the muscle response with ACh, the external Ca^{+2} was eliminated with Tyrode-0Ca. To do the CRC of Ca^{2+}, muscles were depolarized with Tyrode-0Ca-40 mM K+ and there were cumulatively added the successive aliquots of $CaCl_2$ solutions to reach concentrations from 0.0195 to 17.5 mmol/L. At least two CRC-Ca^{2+} were done as control, before those done in the presence of one concentration of the respective extract, in growing order of concentrations. These solutions were added 5 min before the depolarization with high-K^+ in Tyrode-0Ca and remained in the bath during the CRC.

Pharmacological and statistical analysis

From the CRC there were calculated the pEC50 of the agonist (as –log EC50, in mol/L) [21]. For the extracts, the pattern of CRC with reduction in the maximal effect (E_{max}) suggested that they act as non-competitive antagonists, while running to the right maintaining E_{max} indicated competitive antagonism [21]. The inhibitory concentration to 50% (IC50) of extracts was calculated by extrapolating to 50% the individual inhibition curves, which were obtained at a constant and given [agonist]. For non-competitive blockers was by plotting E_{max} of the agonist from the respective CRC curves vs. [extract], and expressed as mg leaves by mL. All results are expressed as media ± SEM. The statistical analysis of each protocol of CRC was done by two-way ANOVA with the following variables: treatment and log [agonist] (being the agonist either, Ach, CbCh, H or Ca^{2+}) followed by "a posteriori" Bonferroni paired tests to compare the CRC in the presence of extract with the CRC without extract or with vehicle (control or vehicle) from the same preparation. The level of significance was always $p<0.05$. For statistical analysis it was used the program Graph Pad Prisma v. 4.0.

Behavioral effects:

Open-field test in mice

The spontaneous locomotion and the exploratory activity of mice were evaluated on the open field, consisted of a 30 x 50 cm white box with walls of 27 cm height divided in 15 squares of 10 cm^2 by black lines. It was placed in a light and sound-attenuated room, and done as previously described [13]. Mice were divided in 6 groups, respectively for the following treatments: saline solution (negative control), 0.6 mg/kg diazepam (positive control) (Roche, Argentina), and groups treated with tinctures equivalent to 200 mg leaves/Kg of *H. connatum* and *B. ruscifolia*, respectively. All drugs were administered by i.p. injections in a volume of 0.05 ml by 25 g of weight. After 30 minutes, each animal was placed in the same corner of the field, and during 5 minutes there were counted the number of crossed lines (CL), rearings (Re), grooming and other signs as previously described [13]. This rutine was repeated for every mouse at 60, 90 and 120 min of administration and the experiment was repeated until reach a number of experiments in each group. The statistical analysis was done by two-way ANOVA with the following variables: treatment and time followed by "a posteriori" Bonferroni paired tests to compare each treatment with control group.

Results

Gastrointestinal effects of Hypericum connatum

Figure 1a shows that the tincture of *Hypericum connatum* (T-H.c) inhibited the concentration-response curves of CbCh in the rat intestine with a non-competitive pattern. In order to evaluate the mechanism of action, other CRC were done in the presence of 0.1 µmol/L indomethacine to inhibit the cycloxigenase (Fig. 1b), and with 30 µmol/L L-NAME (Fig. 1c) to inhibit the NO-synthase. Moreover, to evaluate whether this plant interferes directly with the contraction of smooth muscle, the effect of T-H.c were evaluated on the hystamine CRC in guinea-pig ileons (Fig. 2a) and in a CRC of calcium in rat duodenum and ileum (Fig. 2b). In all of them T-H.c showed a non-competitive inhibition of the CRC. Table 1 shows the 50%-inhibitory concentration (IC50) of T-H.c in the different protocols, where both indomethacine and L-NAME significantly reduced the IC50 of T-H.c in the CbCh-CRC. The IC50 of the Ca^{2+}-CRC was similar to that obtained for the CbCh-CRC and for the H-CRC.

Gastrointestinal effects of Berberis ruscifolia

The tincture of *Berberis ruscifolia* (T-B.r) also inhibited the concentration-response curves of CbCh in a non-competitive way in the rat small intestine. For the mechanism of action, other CRC were done in the presence of 30 µmol/L L-NAME (Fig. 3a) to inhibit the NO-synthase, and the T-B.r was also evaluated in CRC of calcium (Fig. 3b). In all of them, the T-B.r showed a non-competitive inhibition of the CRC of the respective agonist. Table 2 shows the 50%-inhibitory concentration (IC50) of T-B.r in the different protocols. L-NAME did not significantly change the IC50 of T-B.r in the CbCh-CRC, but the IC50 in the Ca^{2+}-CRC was reduced with respect to that obtained for the CbCh-CRC.

Gastrointestinal effects of Cecropia pachystachya

Fig. 4a shows that the A.c.e of *Cecropia pachystachya* inhibited the CRC of acethylcholine in a non-competitive way (IC50 of 0.68±0.14, n=9) , as well as the CRC of calcium (Fig. 4b). But, since the CRC of Ca^{2+} was not inhibited to 50%, the IC25% were compared in Table 3.

Gastrointestinal effects of Brugmansia arborea

Fig. 4c shows that the A.c.e of flowers from *Brugmansia arborea* showed a different behavior against the CRC of acethylcholine (pEC50: 6.61 ± 0.12), to which inhibited in a competitive way since it runs the CRC to the right (IC50 of 20.1±7.4 µg/mL, n=11) (two-way ANOVA results: by treatment: F= 101.8, p<0.0001; by log[Ach]: F= 133.4, p<0.0001). Results suggest that the active principles would block the muscarinic receptors in the bowel.

Effects of Hypericum connatum and Berberis ruscifolia on mice in the open field test

Table 4 shows that the T-H.c reduced the number of rearings (Re) (spontaneous exploration) during all the period of the open-field test, at the doses of 200 mg leaves/Kg of the tincture, but the T-B.r did not significantly change the Re. The number of crossed lines (CL) (spontaneous locomotion) was also reduced by T-H.c but not by T-B.r with respect to saline group (Table 5).

Discussion

Results show that the three plants traditionally used for gastrointestinal diseases have antispasmodic effect, associated to a non-competitive inhibition on the cholinergic contraction in isolated small bowel. Contrarily, *Brugmansia arborea* inhibited in a competitive way the acethylcholine contraction curves. The tincture of *Hypericum connatum* was also a non-competitive inhibitor against the hystaminergic contraction developed in the guinea-pig ileons, with

a lower IC50 than that obtained against carbachol in the rat intestine. When the effects of T-H.c were done on the CbCh-CRC in the presence of indomethacine to inhibit the prostaglandin synthesis by cycloxigenase, the IC_{50} was significantly reduced. This synergism suggested that the T-H.c would be releasing prostaglandins that have a contractile role in the small intestine. This result agrees with a report that show that flavonoids induce the synthesis of contractile prostaglandins as PGE2 in the intestinal preparations [22, 23]. This behavior is different to that of the arterial smooth muscle, in which cycloxigenase catalyzes the synthesis of the vasorelaxant prostacyclin. The presence of flavonoids was demonstrated in the leaves of *Hypericum connatum*, particularly quercitrin and chlorogenic acid [4]. The IC_{50} of T-H.c was also potentiated by inhibition of the nitric oxide synthase by L-NAME. The NO has been demonstrated to be a transmitter of inhibitory neurons of the enteric nervous system [24] and to mediate relaxation of the gut [25]. Nevertheless, it was described that nanomolar quantities of NO produced by a calcium-dependent nNOS play a physiological role in peristalsis of the intestine, in a way that decreased nNOS can result in aperistalsis and obstructive sphincters [26]. So, this effect could explain the potentiation of L-NAME on the T-H.c relaxation. Finally, the effect of T-H.c on the calcium CRC showed a non-competitive blockade of the calcium influx in a depolarizing medium, in which the contraction depends on the activation of Ca^{2+} channels. This behavior shows differences with the dihydropiridines which are competitive blockers of L-channels, but was similar to the pattern of the non-competitive inhibition of Ca^{2+} channels showed by verapamil [27]. This result agrees with previously described studies of other plants rich in flavonoids as those of *Aloysia polystachya* and *A. gratissima* [19, 20] or rich in essential oil with terpens as *Lippia alba* [27]. In fact, the presence of flavonoids was described in the leaves of *H. connatum,* especially chlorogenic acid and quercitrin [4], as well as terpens in the essential oil [5,6]. The tincture of *Berberis ruscifolia* leaves also showed non-competitive inhibition of the carbachol CRC, but it did not activated the NO synthesis since L-NAME did not change the IC50. The effect also was related to non-competitive inhibition of calcium influx to the muscle. At a difference of *H. connatum*, the phytochemistry of the leaves from *B. ruscifolia* was not yet described. But other species of *Berberis* as *B. vulgaris, B. rigidifolia* and the argentinian *B. buxifolia* have the inotropic alkaloids berbamine and berberine [9,10,11, 28, 29, 30], as well as tannins [7], and the last could produce intestinal relaxation. The described presence of flavonoids as vitexin, isovitexin and rutin in the leaves of *Crecropia pachystachya* [16, 31] could explain the antispasmodic effect in the intestinal smooth muscle. We have previously described that vitexin and isovitexin inhibited the cholinergic contractility in a non-competitive way, and vitexin also non-competitively blocked the CRC of calcium [19]. This mechanism agrees with the hypotensive effect of *C. pachystachya* seen in rats [12].

While the three native plants traditionally used for gastrointestinal diseases induced a non-competitive blockade of cholinergic contraction and calcium influx as discussed above, the exotic plant used for recreative purposes *Brugmansia arborea* showed a competitive antagonism of muscarinic receptors. This mechanism could cause constipation when the aerial parts of the plant are smoked or in other way of consumption. This result agrees with the reports about the presence of at least three tropane alkaloids in the aerial parts: atropine, scopolamine and nor-hyoscine [17], which are known antimuscarinic drugs. It has been described that *B. arborea* reduces the morphine withdrawal in guinea-pig ileum [17] and the cocaine abuse symptoms [32].

On the other hand, it was reported that the A.c.e of *Cecropia pachystachya* had central effects as benzodiazepine-type sedation [13], and the A.c.e of *Brugmansia arborea* inhibits the dopamine receptors D1 and D2 [33]. In this work it is showed that the tincture of *Hypericum connatum* reduces the spontaneous locomotion and exploration of mice in the open-field test, at a doses of 200 mg/Kg, activity which could be due to the essential oil. Contrarily, the tincture of *Berberis ruscifolia* did not have any effect in the open-field test. The sedative or anxiolitic effects could contribute to reduce the gastrointestinal spasms by reducing the parasympathetic stimulation. In summary, this work validates the antispasmodic effects of *Hypericum connatum*, *Berberis ruscifolia*, *Cecropia pachystachya* and *Brugmansia arborea*. While the effect in *B. arborea* was due to the antimuscarinic effect of tropane alkaloids, the effect of the other plants was mainly due to the non-competitive blockade of agonist-induce contraction and of calcium influx to the smooth muscle, generally associated to the presence of flavonoids. *H. connatum* and *C. pachystachya* also produced certain sedation, which contribute to the antispasmodic effect.

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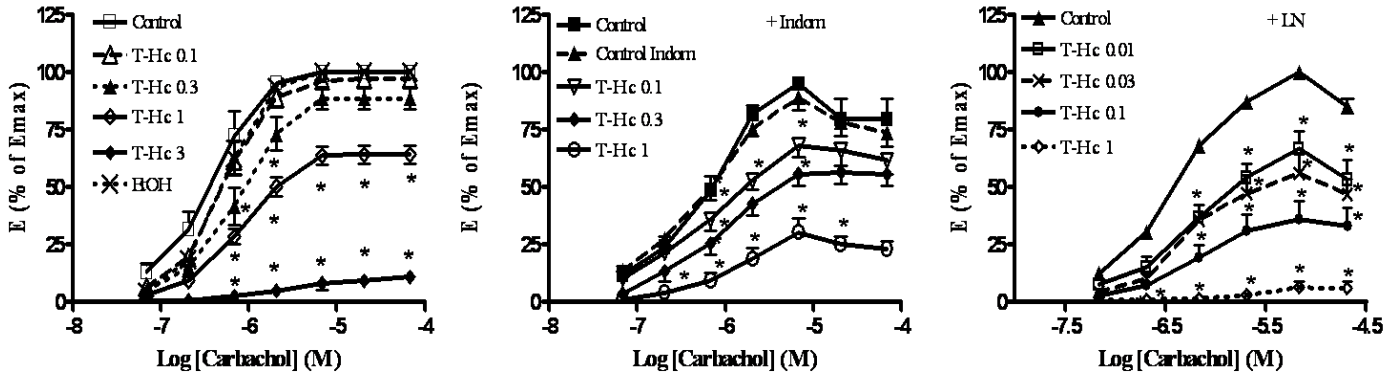

Figure 1. Effects of the ethanolic tincture of Hypericum connatum on the CRC of carbachol (CbCh) in rat intestine, in the absence (a) and the presence of 0.1 µmol/L indomethacine (b) and 30 µmol/L L-NAME (c). Results as mean ± SEM. The tincture concentrations (mg leaves/mL) are in labels. Table 1 shows thye two-way ANOVA results, and the parameters of pEC50 and IC50 (in mg leaves/mL). Bonferroni post-tests: *p<0.05 vs control.

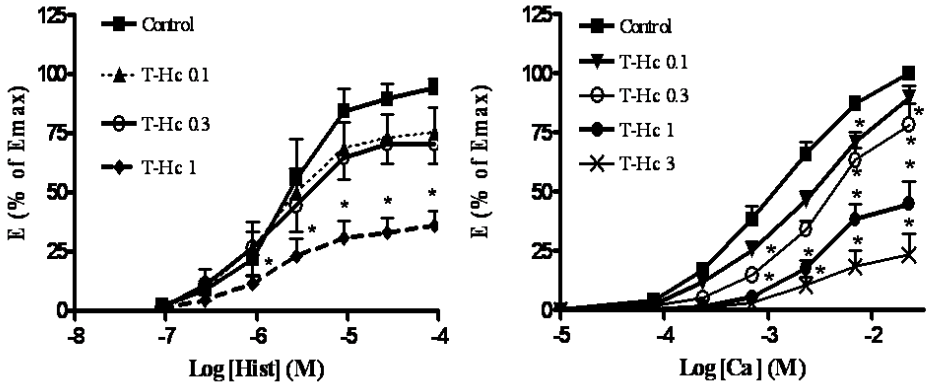

Figure 2. Effects of the ethanolic tincture of Hypericum connatum on the CRC of hystamine (H) on the guinea-pig ileons (a) and on the CRC of calcium in rat intestine (b). Results as mean ± SEM. Tincture concentrations (mg leaves/mL) are in labels. Table 1 shows the two-way ANOVA results, and the parameters of pEC50 and IC50 (in mg leaves/mL). Bonferroni post-tests: *p<0.05 vs control.

Table 1. Effects of Hypericum connatum on the CRC of different agonists and protocols. Parameters of the agonist (pEC50) and the inhibition (IC50), and statistical results of each CRC protocol.

Protocol and agonist	pEC50	IC50 H.c (mg/mL) (n)	Two-way ANOVA	
			By treatment	by log [agonist]
Carbachol (CbCh)	6.40±0.11	1.50±0.12 (8)	F= 365.6 **	F= 237.0 **
Indometh + CbCh	6.20±0.07	0.38±0.09 (7)*	F= 96.25 **	F= 114.0 **
L-NAME + CbCh	6.51±0.03	0.09±0.05 (5)*	F= 137.0 **	F= 80.36 **
Hystamine (H)	5.60±0.19	0.73±0.14 (6)*	F= 25.82 **	F= 49.97 **
Calcium (Ca^{2+})	2.96±0.11	0.94±0.27 (6)	F= 63.27 **	F= 239.6 **
ANOVA		F= 14.63, p<0.0001		

*: p<0.05 vs CbCh-CRC; **: p<0.0001; [T-H.c] was expressed in mg dried leaves/mL.

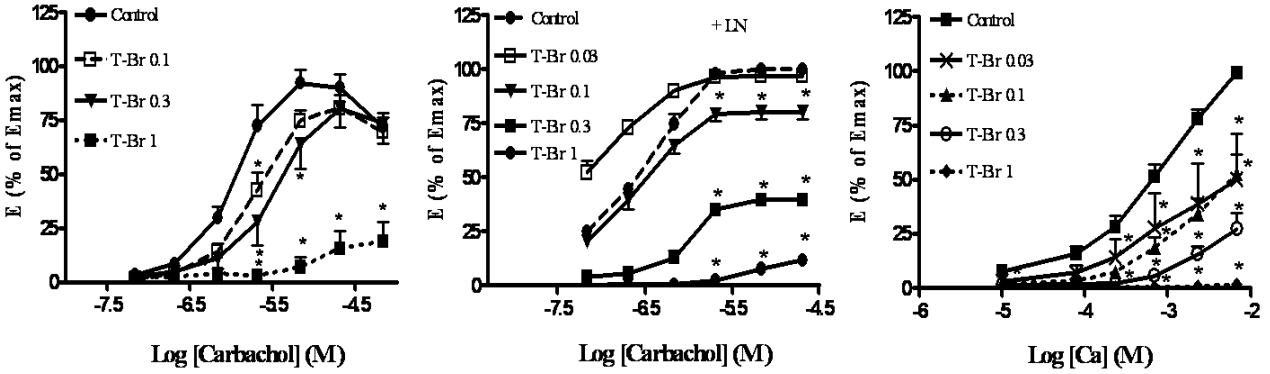

Figure 3. Effects of the ethanolic tincture of Berberis ruscifolia (T-B.r) on the CRC of carbachol (CbCh) in rat intestine, in the absence (a) and the presence of 30 µmol/L L-NAME (b), and on the CRC of calcium (c). Results as mean ± SEM. Tincture concentrations (mg leaves/mL) are in labels. Table 2 shows the two-way ANOVA results, and the parameters of pEC50 and IC50 (in mg leaves/mL). Bonferroni post-tests: *p<0.05 vs control.

Table 2. Effects of Berberis ruscifolia on the CRC of different agonists and protocols. Parameters of the agonist (pEC50) and the inhibition (IC50), and statistical results of each CRC protocol.

Protocol and agonist	pEC50	IC50 B.r (mg/mL) (n)	Two-way ANOVA	
			By treatment	by log [agonist]
Carbachol (CbCh)	5.90±0.12	0.43±0.09 (6)	F= 68.83 **	F= 80.90 **
L-NAME + CbCh	6.59±0.05	0.24±0.01 (4)	F= 1098 **	F= 278.1 **
Calcium (Ca^{2+})	3.23±0.08	0.10±0.03 (10)*	F= 54.35 **	F= 47.24**
ANOVA	F= 10.13, p=0.0013			

*: p<0.05 vs CbCh-CRC; **: p<0.0001; [T-B.r] was expressed in mg dried leaves/mL.

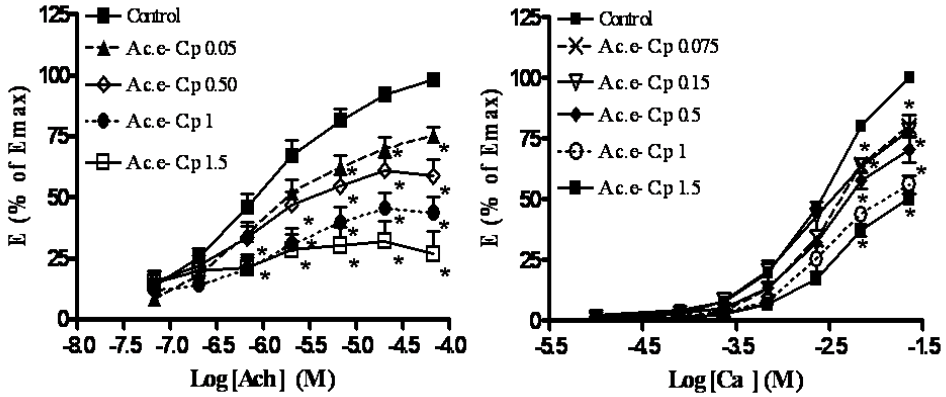

Figure 4. Effects of the acqueous crude extract of Cecropia pacystachya (A.c.e-C.p) on the CRC of acetylcholine (ACh) in rat intestine (a) and on the CRC of calcium (b). Results as mean ± SEM. A.c.e concentrations (mg liophilized/mL) are in labels. Table 3 shows the two-way ANOVA results, and the parameters of pEC50 and IC50 (in mg liophilized/mL). Bonferroni post-tests: *p<0.05 vs control.

Table 3. Effects of Cecropia pachystachya on the CRC of different agonists and protocols. Parameters of the agonist (pEC50) and the inhibition (IC50), and statistical results of each CRC protocol.

Protocol and agonist	pEC50	IC25 C.p (mg/mL) (n)	Two-way ANOVA	
			By treatment	by log [agonist]
Acetilcholine (ACh)	6.03±0.10	0.095±0.029 (10)	F= 54.91 **	F= 89.71 **
Calcium (Ca^{2+})	2.59±0.03	0.34±0.12 (4)*	F= 71.21 **	F= 861.6**

*: p<0.05 vs ACh-CRC; **: p<0.0001; [A.c.e-C.p] was expressed in mg lyophilized/mL.

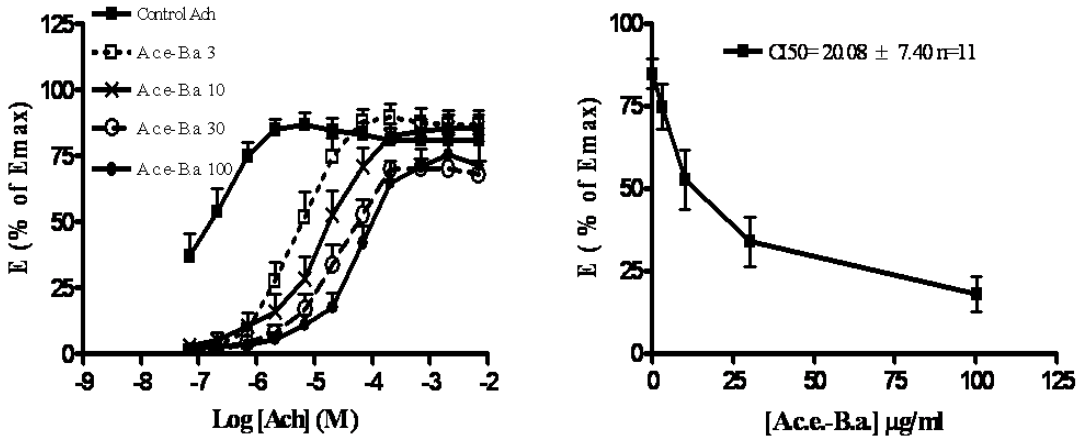

Figure 5. Competitive antagonism of the A.c.e from Brugmansia arborea on the CRC of acetylcholine (ACh) in rat intestine (a) and inhibitory curve built from %E obtained at log [Ach]= -5 to calculate the IC50. Results as mean ± SEM. A.c.e concentrations (mg liophilized/mL) are in labels See the statistics and parameters in the text.

Table 4. Effects of the tincture of Hypericum connatum and Berberis ruscifolia on the exploration (number of rearings) of mice in the open-field test.

Treatment (n)	30 min	60 min	90 min	120 min
Saline (7)	27.86±5.50	26.43±4.51	16.29±5.05	23.0±8.22
Ethanol 70° (7)	17.0±9.48	11.14±5.48	10.57±6.30	8.29±5.24
H. connatum 200 mg/Kg (7)	6.71±4.92*	1.00±0.58*	1.57±0.68*	1.43±0.48*
B. ruscifolia 200 mg/Kg (6)	15.5±7.48*	7.67±6.68	7.67±5.12	8.16±4.45
Diazepam 0.6 mg/Kg (6)	11.00±4.37	7.50±2.99	6.83±3.24	3.00±2.03*
Two-way ANOVA:	by treatment F= 8.99, p<0.0001; by time F= 1.89, p= 0.134			

Initial values: 40.79±2.15 (33). *p<0.05 by a posteriori Bonferroni test vs Saline group at the respective time.

Table 5. Effects of the tincture of Hypericum connatum and Berberis ruscifolia on the locomotion (number of crossed lines) of mice in the open-field test.

Treatment (n)	30 min	60 min	90 min	120 min
Saline (7)	89.0±7.3	72.8±8.4	44.4±7.5	55.6±15.4
Ethanol 70° (7)	59.8±27.7	33.4±14.2	24.4±11.3	21.6±10.8
H. connatum 200 mg/Kg (7)	45.8±18.3	6.9±2.2*	7.0±2.7	8.7±3.5*
B. ruscifolia 200 mg/Kg (6)	63.8±25.3	27.7±15.1	22.0±9.6	27.2±11.0
Diazepam 0.6 mg/Kg (6)	49.7±15.6	30.0±8.9	36.3±16.1	27.8±10.6
Two-way ANOVA:	by treatment F= 8479, p<0.0001; by time F= 8690, p= 0.0002			

Initial values: 130.7±6.1 (33). *p<0.05 by a posteriori Bonferroni test vs Saline group at the respective time.