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Journal of Ethnopharmacology 106 (2006) 90–96
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Cardiotonic and sedative effects of *Cecropia pachystachya* Mart. (ambay) on isolated rat hearts and conscious mice

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Received 5 August 2005; received in revised form 1 December 2005; accepted 2 December 2005
Available online 18 January 2006

Abstract

Cecropia pachystachya Mart. is popularly called "ambay" and extensively used in herbal medicine of South America for cough and asthma. In Argentina it grows in neotropical rainforest (*Ntr C.p.*) and in a temperate region (*Tp C.p.*). In a previous work we showed their hypotensive properties with different potency and toxicity, and now we studied the *Tp C.p.* effects in isolated heart from rats and central effects of both plants on the open-field test for mice. *Tp C.p.* produced a positive inotropic effect on isolated rat hearts, which was not affected by 1 μM propranolol, suggesting that it is not due to a β-adrenergic effect. In contrast, it was prevented by pretreatment with high $[K]_o$ media, which stimulates the Na,K-ATPase pump, suggesting an inhibition of the pump by "ambay", as digital do. In the open-field test, both *Ntr C.p.* and *Tp C.p.* similarly decreased the spontaneous locomotion and exploratory behavior of mice at doses between 180 and 600 mg/kg. *Ntr C.p.* potentiated the effect of 3 mg/kg diazepam to one similar to 10 mg/kg diazepam, but was not antagonized by 0.5 mg/kg flumazenil. Amphetamine at 5 mg/kg prevented the sedative effect of *Ntr C.p.* Chromatographic analysis showed that both plants have a qualitatively similar fingerprint but quantitatively differed in at least three components. Although the purpose was not to identify them, both plants have at least 10 compounds. Two of them were in higher amount in *Tp C.p.* than in *Ntr C.p.*, and then, they could be responsible for the cardiovascular toxicity of *Tp C.p.* In conclusion, the results suggest that ambay has cardiotonic and sedative properties. The sedative effect could be useful in cough treatment. The extract resulted additive to benzodiazepines but it did not bind to the same site on GABA-A receptor, and it was interfered by the dopamine release produced with amphetamine.

Keywords: *Cecropia pachystachya*; Ambay; Cardiotonic; Sedative; Open-field; HPLC-fingerprint; TLC

1. Introduction

Cecropia pachystachya Mart. (syn.: *Cecropia adenopus* Mart., Moraceae) is a plant that grows in the South American rainforests. It is popularly known in Argentina as "ambay", "ambaí", "amba-hu", "ambaiba" or "ambay-guazú" because it comes from guaranies indigenous medicine (Gupta, 1995). It is used for treating cough and asthma, and was cited as diuretic and cardiotonic (Soraru and Bandoni, 1978). It was incorporated to the Argentinian National Pharmacopeia (1978) because of its extensive popular use and it is prepared as a 5% infusion or a 4% decoction (Alonso, 1998). Nevertheless, not all its popular uses were pharmacologically assayed. In this way, we recently have demonstrated that it has not diuretic activity on rats (Consolini and Migliori, 2005). Another species of *Cecropia* has been used in America by indigenous from Mexico (*Cecropia obtusifolia*). This one has been demonstrated to be antihypertensive (Salas et al., 1987), diuretic (Vargas Howell and Ulate Montero, 1996), hypoglycemic (Roman-Ramos et al., 1991), analgesic and central depressor (Perez-Guerrero et al., 2001).

The species from South America (*Cecropia pachystachya* = *Cecropia adenopus*) has been early studied by a qualitative description on dogs and rabbits (Domínguez and Soto, 1925), suggesting weak broncodilator activity and cardiac toxicity. More recently, we have studied and demonstrated a hypotensive effect in two plants of the same species, which growed in

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doi:10.1016/j.jep.2005.12.006

different climatic and phytogeographical regions of Argentina, the neotropical forest (*Ntr C.p.*) and the temperate central hilly region (*Tp C.p.*) (Consolini and Migliori, 2005). The one from neotropical region is accepted in herbal medicine of several South America countries as dietary supplement for treating cough and as cardiotonic, and the other is used by local people for the same illnesses. Both plants produced hypotensive effect in rats associated to autonomic blockade, while only *Tp C.p.* produced tachycardia in vivo (Consolini and Migliori, 2005). This difference and the higher toxicity of *Tp C.p.* suggested a difference in composition of the extracts.

As the cardiotonic or cardiotoxic effect of ambay could have been masking in vivo, the aim of this work was to study the *Tp C.p.* effects on an isolated cardiac preparation. Also, having in account the autonomic depression produced by the plants, they were studied for central in vivo effects on locomotive and exploratory behavior in mice. This effect could also explain the effectivity on cough. Also, there were compared the chromatographic qualitative fingerprint of both plant populations of *Cecropia pachystachya* using HPLC and TLC to evaluate whether both plants which grow in different climates have differences in composition.

2. Materials and methods

2.1. Preparation of the extracts

Leaves of *Cecropia pachystachya* Mart. (syn.: *Cecropia adenopus*, Moraceae) were collected either from the neotropical forest of Misiones province (*Ntr C.p.*) and from the temperate hilly grasslands of Cordoba province (*Tp C.p.*) both in Argentina. The plants were authenticated by Prof. Dr. Etile Spegazzini. Voucher herbarium specimens (No.: 1000 and 1001, respectively) are kept in the Herbarium Museum of Botany and Pharmacognosy, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Argentina. The leaves were dried at 40 °C. For biological experiments, aqueous crude extract of both samples were prepared by boiling 20 g dried leaves in 100 ml distilled water for 20 min. After filtration and/or centrifugation the decoction was liophylized, obtaining a 9–10% (w/w) yield of the dried leaves. The liophylized extract was diluted in Krebs or in saline solutions for in vitro or in vivo experiments, respectively, the day of each experiment.

For analytical chromatographic purposes, the liophylized and methanolic extracts were used. The methanolic extract was prepared from 5 g of the dried and powdered aerial parts of both *Ntr C.p.* and *Tp C.p.* that were refluxed in 50 ml of methanol during 15 min. After filtration the extracts were stored in a refrigerator until analysis.

2.2. Cardiac contractility measurements

Wistar rats of either sex, weighing 220–280 g, were heparinized (2000 U) and anesthetized with pentobarbital sodium overdose. The beating hearts were rapidly excised and retrograde perfusion by Langendorf method was initiated with control perfusate (Consolini and García Sarubbio, 2002). Both atria and right papillary muscles were dissected to prevent spontaneous contractions. A latex balloon was placed into the left ventricle and connected to a Bentley 800 pressure transducer for measuring pressure development during isovolumic contractions. The muscles were electrically stimulated with pulses of 5 V for 5 ms at 1 Hz. All muscles were perfused by aorta and coronary arteries at a constant rate (6 ml/min/g) at 30 °C with a Krebs solution containing (in mM): 1 $MgCl_2$, 120 NaCl, 0.5 NaH_2PO_4, 6 KCl, 2 $CaCl_2$, 25 $NaHCO_3$, and 6 dextrose. The solution was bubbled with 95% O_2–5% CO_2 to achieve a pH of 7.3–7.4. With this method it was possible to continuously record left intraventricular pressure on a Beckman R511A polygraph (USA) and calculate its maximal value (*P*). Once the muscle was cannulated, a 30-min equilibration period with Krebs solution was allowed to elapse before any experimental intervention. Optimal diastolic pressure was obtained by steps until *P* reached a steady maximum value and it was kept throughout the experiment. After the equilibration period, one of the following protocols were done in each muscle:

(a) Perfusion of Krebs with *Tp C.p.* at concentrations of 0.015, 0.05, 0.15, 0.30, 1.5 and 9 mg liophylized by 100 ml Krebs;
(b) Perfusion of Krebs with 1 μM propranolol during 10 min and then adding *Tp C.p.* at concentrations of 0.015, 0.05, 0.15, 0.30, 1.5 and 9 mg liophylized by 100 ml Krebs to the perfusate in the presence of propranolol;
(c) Perfusion of Krebs-high $[K]_o$ (Krebs solution in which $[K]_o$ was isotonically increased to 25 mM) and, after 10 min addition of *Tp C.p.* at concentrations of 0.015, 0.05, 0.15, 0.30, 1.5 and 9 mg liophylized by 100 ml Krebs-high $[K]_o$.

2.3. Open-field test in mice

This test was used to evaluate the spontaneous locomotive and exploratory activity of mice. The open-field apparatus consists of a 30 cm × 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. The animals (20–30 g of weight, normally feeded) received one of the following: extract of *Ntr C.p.* or *Tp C.p.* (180, 320 and 600 mg/kg), diazepam (Roche, Argentina) (3, 10 and 30 mg/kg), flumazenil (Richmond, Argentina) (0.5 mg/kg), amphetamine (Sigma, USA) (5 mg/kg) or saline solution (control) by i.p. injections in a volume of 0.1 ml by 10 g of weight. After 40 min, each animal was placed at his time in the same corner of the field, and during 5 min there were observed the number of squares crossed (with the two front paws), rearing, grooming and other signs (Choleris et al., 2001; Molina-Hernández et al., 2004). This routine was repeated for every mouse at 80, 120 and 160 min of administration.

2.4. Chromatographic qualitative analysis

The high performance liquid chromatography analysis was done by using an HPLC system consisted of a Konik KNK 500G Chromatographer with a double piston serial pump, a programmer for the microprocessor KNK 029-375 (Konik, Spain), a

Rheodyne 7125 sample injector with a fixed loop of a 20 μl capability (Rheodyne, USA) and an helium bubbling degasificator KNK 029-254. There were used a Lichrocart RP 18 reverse phase column of 250 mm × 4 mm i.d. with a particle size of 5 μm (Merck, Germany). A variable wavelength UV–vis detector model 204 (Linear, USA) was used, and the integrator was a Datajet model SP 4600 (Spectra Physics, USA).

For the preparation of samples, Eppendorf research micropipettes of 100–1000 μl were used (Eppendorf, Alemania). Drugs and reagents were weighed on an analytical balance (Mettler Toledo AG 204, Switzerland). Millipore membranes type HV, with diameters of 47 mm (Millipore, USA) and 25 mm (MSI, USA) and a pore size of 0.45 μm were used to filter the mobile phase and the samples, respectively. All reagents used were HPLC grade.

The HPLC analysis was performed using two chromatographic systems both in an isocratic elution mode, a technique, which was accepted for accuracy in many reports of other authors (Raffaelli et al., 1997; Schoefs, 2003; Nishitani and Sagesaka, 2004; Reginatto et al., 2004):

(A) The mobile phase was composed by water:methanol:acetic acid (65:35:5) at pH 2.5 and flow rate of 0.8 ml/min. The components were monitored at 254 nm, which is usual to detect flavonoids, and the injection volume was 20 μl. All instruments and the column were operated at room laboratory temperature (25 °C).
(B) The mobile phase was orthophosphoric acid 0.04 M: acetonitrile:methanol (80:10:10) at pH 2.0 and flow rate of 1.0 ml/min. The UV detection was done also at 254 nm, the injection volume was 20 μl and the temperature was 25 °C.

The methanolic extracts were diluted in mobile phase (1:50 v/v) for each chromatographic system, filtered and injected for triplicate into the HPLC system. The aqueous liophylized extracts were used in a concentration of 1 mg/ml for each chromatographic system, filtered and injected for triplicate into the HPLC system.

The thin layer chromatography (TLC) analysis was performed on Silica Gel 60 GF_{254} of 0.25 mm thickness aluminium sheets 20 × 20 (Merck, Germany). Aliquots of 100 μl of each methanolic extract were applied on the plates in narrow bands and developed in a TLC chamber previously saturated using dichloromethano:acetone (80:20) as the mobile phase, at a development length of 80 mm. After development, plates were dried and the components were first detected under UV light (254 and 366 nm) and then, by spraying the chromatogram with diphenyl boric acid 2-amino-ethyl ester and PEG 400 in methanol (Sigma, USA) which is an specific reagent for flavonoids detection.

2.5. Statistics

Results of the "in vitro" experiments were expressed as mean ± S.E.M., and paired Student's *t*-test was used to evaluate differences between two means, considering $P < 0.05$ for significance. For in vivo mice experiments, multiple comparisons by the one-way analysis of variance (ANOVA) test were done followed by "a posteriori" all paired Bonferroni tests, considering $P < 0.05$ for significance.

2.6. Animals

The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as was established by US guidelines (NIH, 1985).

3. Results

3.1. Effects of Cecropia pachystachya aqueous extract on rat isolated ventricles

Rat ventricles developed a basal maximal intraventricular pressure (*P*) of 205.8 ± 19.7 mmHg ($n = 5$). Perfusion of *Tp C.p.* in Krebs produced a positive inotropic effect at each concentration that remained for about 6 min before return-

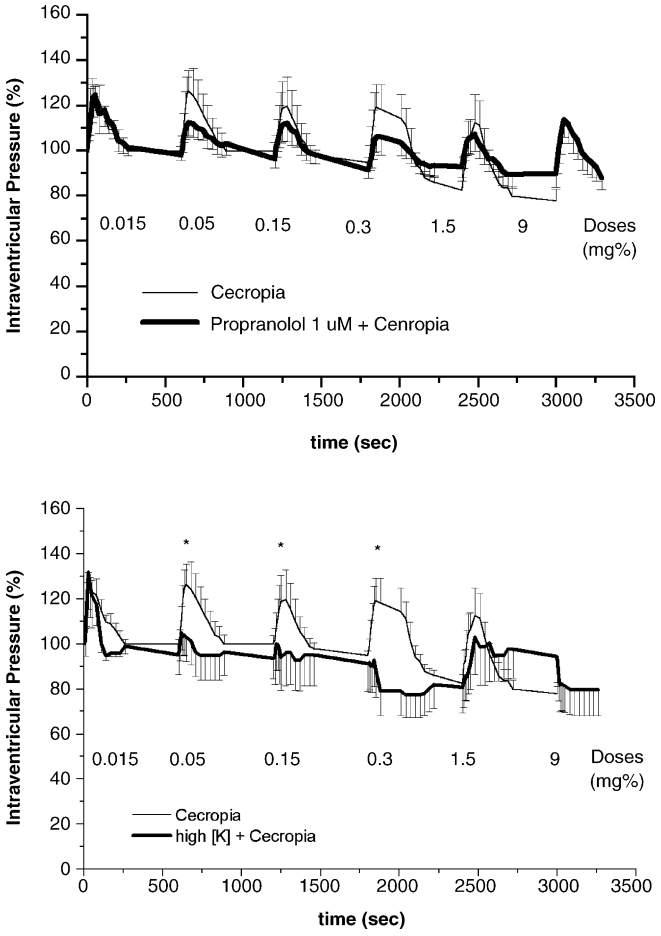

Fig. 1. Maximal intraventricular pressure development (*P*) of rat heart contractions as a percentage of initial value, after perfusing *Tp C.p.* at the doses indicated in the graph in the absence (thin line) and in the presence of 1 μM propranolol (thick line) (upper) or in the absence (thin line) and in the presence of Krebs-high $[K]_o$ (thick line) (lower). Results are expressed as mean ± S.E.M ($n = 5$ to 7). $^{*}P < 0.05$ between maximum values of both curves.

Table 1
Exploratory behavior (number of rearings in 5 min) produced by "ambay" from the neotropical region (*Ntr C.p.*) and from the temperate region (*Tp C.p.*) in comparison with diazepam and flumazenil

Treatment	40 min	80 min	120 min	160 min
Saline ($n=11$)	75.3 ± 4.5	54.9 ± 6.8	34.4 ± 6.6	37.4 ± 6.8
Ntr C.p. 180 mg/kg ($n=8$)	$24.9 \pm 6.4^{*}$	$19.7 \pm 8.4^{*}$	14.9 ± 5.5	$12.9 \pm 4.5^{*}$
Ntr C.p. 320 mg/kg ($n=11$)	$27.6 \pm 5.5^{*}$	$18.9 \pm 4.5^{*}$	$10.6 \pm 1.6^{*,\#}$	$8.8 \pm 2.1^{*}$
Ntr C.p. 600 mg/kg ($n=9$)	$19.3 \pm 3.3^{*}$	$14.5 \pm 3.8^{*,\#}$	$5.2 \pm 2.0^{*,\#}$	$3.8 \pm 1.1^{*}$
Tp C.p. 180 mg/kg ($n=8$)	$27.6 \pm 7.2^{*}$	$24.7 \pm 4.9^{*}$	$7.0 \pm 3.4^{*,\#}$	$7.1 \pm 3.7^{*}$
Tp C.p. 320 mg/kg ($n=8$)	$13.6 \pm 5.6^{*}$	$11.2 \pm 4.9^{*,\#}$	$6.7 \pm 5.3^{*,\#}$	$6.2 \pm 4.0^{*}$
Tp C.p. 600 mg/kg ($n=8$)	$22.1 \pm 5.2^{*}$	$21.2 \pm 4.2^{*}$	$7.1 \pm 1.9^{*,\#}$	$2.9 \pm 1.1^{*}$
Diaz 3 mg/kg ($n=10$)	$37.8 \pm 8.0^{*}$	41.2 ± 8.5	29.9 ± 4.6	$19.4 \pm 5.6^{*}$
Diaz 3 mg/kg + *Ntr C.p.* 320 mg/kg ($n=8$)	$11.7 \pm 2.4^{*,\#}$	$5.4 \pm 1.4^{*,\#}$	$2.2 \pm 0.7^{*,\#}$	$2.0 \pm 0.6^{*}$
Diaz 10 mg/kg ($n=11$)	$13.4 \pm 1.7^{*,\#}$	$13.8 \pm 1.9^{*}$	$8.5 \pm 1.5^{*,\#}$	$4.6 \pm 1.0^{*}$
Diaz 10 mg/kg + *Ntr C.p.* 320 mg/kg ($n=6$)	$16.5 \pm 3.2^{*}$	$6.2 \pm 2.3^{*,\#}$	$1.7 \pm 0.8^{*,\#}$	$2.0 \pm 0.6^{*}$
Flumazenil 0.5 mg/kg + *Ntr C.p.* 320 mg/kg ($n=8$)	$20.6 \pm 5.2^{*}$	$24.7 \pm 7.7^{*}$	19.7 ± 6.2	$10.9 \pm 4.1^{*}$
Flumazenil 0.5 mg/kg + *Tp C.p.* 320 mg/kg ($n=6$)	$16.5 \pm 1.7^{*}$	$13.7 \pm 2.0^{*}$	$7.3 \pm 2.7^{*,\#}$	$5.3 \pm 2.1^{*}$
ANOVA	$F=12.34$, $P=0.0001$	$F=6.86$, $P=0.0001$	$F=6.99$, $P=0.0001$	$F=7.55$, $P=0.0001$

[*] $P<0.05$ vs. saline.
[#] $P<0.05$ vs. diazepam 3 mg/kg by Bonferroni test.

ing to basal *P*. This effect was independent on the extract concentration between 0.015 and 9 mg liophylized (%, w/v) (Fig. 1).

To analyze if such effect was due to a β-adrenergic effect we perfused seven hearts with propranolol 1 μM by 10 min. The developed *P* under this condition was 125.9 ± 19 mmHg, but under its presence *Tp C.p.* produced a similar transitory positive inotropism than in its absence (Fig. 1a).

To analyze whether the inotropic effect of *Tp C.p* could be due to a cardiotonic effect, by inhibition of the Na, K-ATPase pump, we treated six hearts with Krebs-high $[K]_o$, which enhance the pump activity (Ponce-Hornos et al., 1992). Under this condition, the contraction can be obtained by application of a high electrical stimulus (30 V for 30 ms) because the inhibition of sodium channels. Under Krebs-high $[K]_o$ *P* was 48.1 ± 8.5 mmHg and *Tp C.p.* did not induce positive inotropism at concentrations between 0.05 and 9 mg (%, w/v) (Fig. 1b).

3.2. Effects of Cecropia pachystachya on open-field test of mice

The exploratory behavior and the spontaneous locomotion of mice were measured in the open-field. The exploratory behavior, measured by the number of rearings in 5 min, was severely decreased by both *Cecropia pachystachya*, from neotropical forest (*Ntr C.p.*) and from the temperate region (*Tp C.p.*) during the 160 min of testing at all concentrations studied (Table 1). The spontaneous locomotion of mice was measured by the number of crossed lines in the open-field during 5 min. Table 2 shows that *Ntr C.p.* significantly decreased locomotion at the higher dose (600 mg/kg) during 80 min, and *Tp C.p.* significantly decreased the spontaneous locomotion of mice at 320 and 600 mg/kg during 80 and 160 min, respectively. There were not significant effects in grooming, which was poor still in control mice (results not shown).

Table 2
Locomotive behavior (number of crossed lines in 5 min) produced by *Ntr C.p.* and from the temperature region (*Tp C.p.*) in comparison with diazepam and flumazenil

Treatment	40 min	80 min	120 min	160 min
Saline ($n=11$)	114.3 ± 16.2	$64.8 \pm 10.8^{\#}$	62.4 ± 16.9	48.5 ± 11.6
Ntr C.p. 180 mg/kg ($n=8$)	$60.6 \pm 17.0^{\#}$	$24.1 \pm 5.4^{\#}$	32.0 ± 9.2	23.75 ± 6.0
Ntr C.p. 320 mg/kg ($n=11$)	$64.4 \pm 8.0^{\#}$	$34.2 \pm 6.1^{\#}$	26.7 ± 11.8	20.2 ± 9.9
Ntr C.p. 600 mg/kg ($n=9$)	$57.4 \pm 10.6^{*,\#}$	$25.6 \pm 7.3^{\#}$	$17.75 \pm 6.3^{\#}$	8.75 ± 2.7
Tp C.p. 180 mg/kg ($n=8$)	$58.5 \pm 15.5^{\#}$	$31.3 \pm 8.8^{\#}$	19.4 ± 8.2	14.1 ± 8.3
Tp C.p. 320 mg/kg ($n=8$)	$23.7 \pm 6.3^{*,\#}$	$14.0 \pm 3.9^{*,\#,a}$	$15.2 \pm 11.4^{\#}$	15.4 ± 12.1
Tp C.p. 600 mg/kg ($n=8$)	$40.2 \pm 9.3^{*,\#}$	$37.5 \pm 12.5^{\#}$	$9.2 \pm 4.3^{*,\#}$	$4.1 \pm 1.7^{*}$
Diaz 3 mg/kg ($n=10$)	122.3 ± 11.5	$110.7 \pm 8.4^{*}$	69.0 ± 13.5	47.2 ± 15.6
Diaz 3 mg/kg + *Ntr C.p.* 320 mg/kg ($n=8$)	$41.7 \pm 8.5^{*,\#}$	$22.6 \pm 6.5^{*,\#}$	$8.5 \pm 1.6^{*,\#}$	6.4 ± 1.6
Diaz 10 mg/kg ($n=11$)	$36.6 \pm 3.4^{*,\#}$	$30.8 \pm 4.4^{\#}$	18.2 ± 3.2	9.9 ± 1.25
Diaz 10 mg/kg + *Ntr C.p.* 320 mg/kg ($n=6$)	$46.2 \pm 14.1^{*,\#}$	$5.2 \pm 2.1^{*,\#,a}$	$2.0 \pm 1.3^{*,\#}$	$3.7 \pm 1.0^{*}$
Flumazenil 0.5 mg/kg + *Ntr C.p.* 320 mg/kg ($n=8$)	$60.9 \pm 11.5^{\#}$	$63.9 \pm 12.1^{\#}$	39.4 ± 12.3	56.5 ± 15.1
Flumazenil 0.5 mg/kg + *Tp C.p.* 320 mg/kg ($n=6$)	67.5 ± 9.9	$49.2 \pm 12.1^{\#}$	20.3 ± 8.2	8.0 ± 2.7
ANOVA	$F=6.27$, $P=0.0001$	$F=10.9$, $P=0.0001$	$F=3.72$, $P=0.0001$	$F=3.63$, $P=0.0001$

[a] $P<0.05$ vs. *Ntr C.p.* flumazenil by Bonferroni test.
[*] $P<0.05$ vs. saline.
[#] $P<0.05$ vs. diazepam 3 mg/kg.

Table 3
Exploratory and locomotive behavior produced by amphetamine without and with *Ntr C.p.* 320 mg/kg

	40 min	80 min	120 min	160 min
Number of rearings				
Amphetamine 5 mg/kg ($n = 10$)	61.0 ± 10.9	32.8 ± 6.5	21.6 ± 5.1	23.0 ± 6.2
Amphetamine 5 mg/kg + *Ntr C.p.* 320 mg/kg ($n = 10$)	48.9 ± 9.6	32.7 ± 6.8	12.6 ± 4.0	4.0 ± 1.9[#]
Number of crossed lines				
Amphetamine 5 mg/kg ($n = 10$)	114.7 ± 25.4	106.6 ± 19.7	64.6 ± 20.8	75.5 ± 21.6
Amphetamine 5 mg/kg + *Ntr C.p.* 320 mg/kg ($n = 10$)	138.8 ± 33.5	84.9 ± 18.6	43.0 ± 4.0	34.1 ± 13.8

[#] $P < 0.05$ respect to amphetamine treatment.

For evaluating the mechanism of the sedative effect, we administrated *Ntr C.p.* at 320 mg/kg 5 min before either diazepam (3 and 10 mg/kg) or amphetamine (5 mg/kg). As it is shown in Tables 1 and 2, diazepam at 3 mg/kg significantly reduced the exploration at 40 and 160 min but increased locomotion at 80 min (anxiolytic dose). At 10 mg/kg it decreased both, locomotion and exploration, during 80 and 160 min, respectively (sedative dose). The sedative effects of both, *Ntr C.p.* and *Tp C.p.* were higher than those of diazepam 3 mg/kg and similar to those of diazepam 10 mg/kg. *Ntr C.p.* 320 mg/kg potentiated the sedation of 3 mg/kg diazepam until obtaining a similar effect to 10 mg/kg diazepam. In order to elucidate whether that effect was due to binding to the benzodiazepine receptor, we treated another group of mice with the antagonist flumazenil at 0.5 mg/kg 5 min previous to *Ntr C.p.* 320 mg/kg. As Tables 1 and 2 show, flumazenil did not significantly change the effect of *Ntr C.p.* 320 mg/kg, suggesting that they both do not act at the same site. The comparison of results from Table 3 with those from Tables 1 and 2 shows that amphetamine 5 mg/kg did not change mice behaviors respect to saline (it only increased the stereotyped movements) but avoided the decrease in locomotion and exploration produced by *Ntr C.p* except at 160 min., suggesting a functional antagonism.

3.3. Qualitative chromatographic analysis of extracts

The qualitative comparison in composition between the extracts of both plants of *Cecropia pachystachya* was made by comparing the retention times under the same chromatographic conditions. The HPLC chromatograms showed a good separation profile with a total running time for the assay within 20 min for system A and 30 min for B (Fig. 2). There were no qualitative changes in the chromatographic profiles of the methanolic and aqueous extracts of *Ntr C.p.* and *Tp C.p.* under both chromatographic systems. However, it was observed in all profiles obtained with *Tp C.p.* that two peaks (t_R 14.05 and 19.21 in system A, and 7.49 and 14.89 in system B) were increased in height and area respect to the ones from *Ntr C.p.*, whereas *Ntr C.p.* exhibited another peak (t_R 10.89 in system A and 4.79 in system B) higher than *Tp C.p.* Results of the TLC analysis were summarized in Table 4. Almost identical resolution and R_f values were observed with both *Ntr C.p.* and *Tp C.p* extracts at the different ways of revelation. There were detected at least 10 different compounds, and the specific reagent shows that there were distinguished six spots of

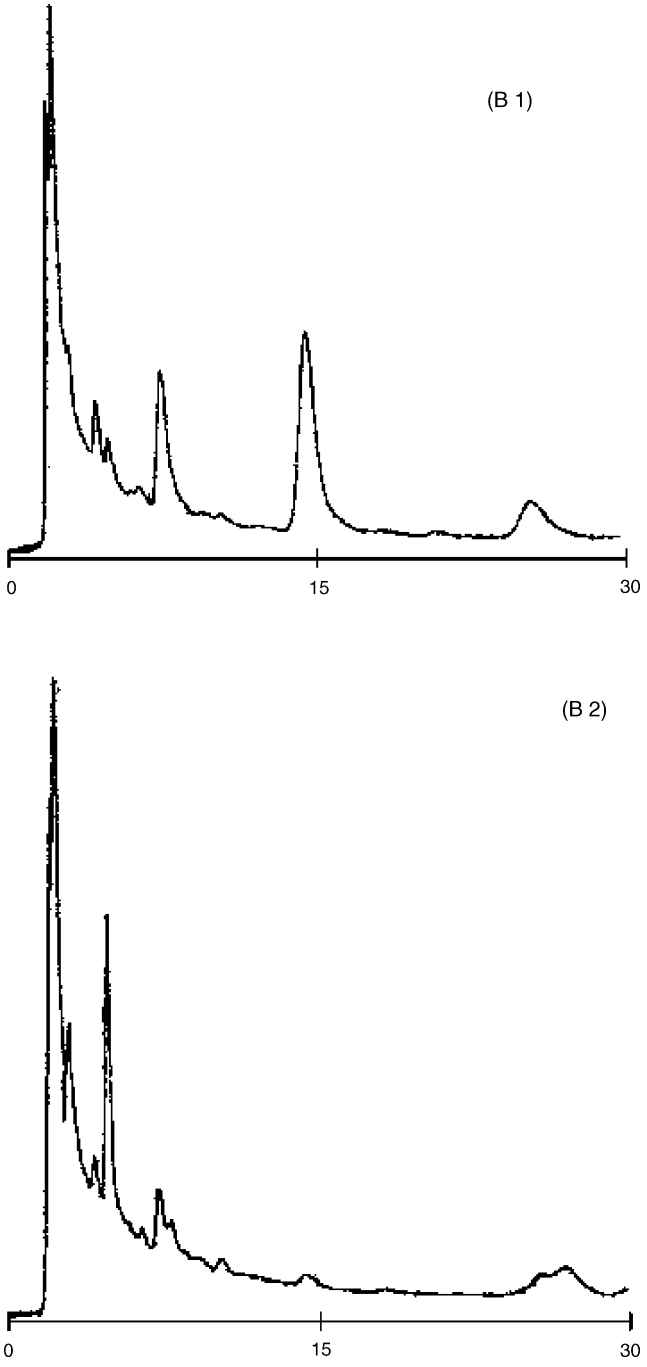

Fig. 2. HPLC chromatograms of aqueous liophylized extracts from *Tp C.p.* in system B (B_1) and from *Ntr C.p.* in system B (B_2).

Table 4
Intensities of absorbance and color of *Ntr C.p.* and *Tp C.p.* on TLC plates

R_f	UV_{254}		UV_{366}[a]		Reagent	
	Tp C.p.	*Ntr C.p.*	*Tp C.p.*	*Ntr C.p.*	*Tp C.p.*	*Ntr C.p.*
0.06	**	*	–	–	**	*
0.15	*	–	–	–	–	–
0.31	*	–	Orange	–	**	–
0.45	****	–	Orange	–	****	–
0.74	***	*	–	–	**	**
0.88	*	–	Orange	–	–	–
0.92	*	**	Yellow	Yellow	*	*
0.95	***	***	Orange	Orange	***	***

(–) Not detected by TLC. Color intensity of the spots was expressed by the increasing number of asterisk; i.e. (****) represents the highest and (*) represents the weakest absorbance and color intensity.
[a] Color of the fluorescence.

flavonoids in *Tp C.p* and four of them were also present in *Ntr C.p.*

4. Discussion

Cecropia pachystachya Mart. (Moraceae) is widely used in herbal medicine of our country and other ones from South America for treating cough and asthma, and was proposed also as cardiotonic (Soraru and Bandoni, 1978; Gupta, 1995). As it was shown for the other species *Cecropia obtusifolia* (Vidrio et al., 1982) we demonstrated a hypotensive effect of *Cecropia pachystachya* in a previous work (Consolini and Migliori, 2005). We have found that the plant which growed in the temperate region (*Tp C.p.*) produced tachycardia as it was described for *Cecropia obtusifolia* (Vidrio et al., 1982), while the one from the neotropical region (*Ntr C.p.*) did not (Consolini and Migliori, 2005). Then, we studied in this work the effect of *Tp C.p.* on an isolated heart, measuring contractility at constant rate. We found a transitory positive inotropic effect that was concentration-independent under the assayed range. The absence of blockade by propranolol suggests that it was not due to a β-adrenergic effect. By the other hand, the increase in $[K]_o$ to 25 mM in order to stimulate the Na, K-ATPase activity (Ponce-Hornos et al., 1992; Consolini et al., 1997) prevented the inotropic effect of the extract. This result suggests that at least one compound of the extract would be an inhibitor of the Na, K-pump, as the cardiotonic plant *Digitalis* is. This cardiotonic effect had not been evident in the previous in vivo experiments in which inotropism had been evaluated by the differential blood pressure (systolic minus diastolic) (Consolini and Migliori, 2005). It could be possible that the cardiotonic effect had been balanced by the negative inotropism consequent to tachycardia in rat. Since this "negative staircase phenomenon" is not present in humans, the cardiotonic properties could be manifested in therapeutics. The early studies had suggested the presence of two glycosides, which were named ambaina and ambainina (Domínguez and Soto, 1925; Gupta, 1995). Nevertheless, the global effects of "ambay" are different to those of digitalic glycosides, since these compounds produce bradycardia by stimulating cardiac cholinergic endings (Smith, 1988) while *Tp C.p.* produced tachycardia by inhibition of cholinergic innervation (Consolini and Migliori, 2005).

In this work, we studied the central effects of *Cecropia pachystachya* because we have observed respiratory depression during the cardiovascular assays, and considering the possibility that the antitusive effect could be related to central depression. We again compared the plant that grows in the neotropical forest (*Ntr C.p.*) with the plant that grows in a temperate region (*Tp C.p.*). The first is accepted in the National Pharmacopeia of our country, and the second is used by local people for the same diseases. Both plants produced a similar sedative effect (evidenced by the decrease in spontaneous locomotion and exploratory behavior), suggesting that the compound responsible for that effect is present in both plant populations of *Cecropia pachystachya*. In contrast, the hypotensive effect had been stronger for *Ntr C.p.* than for *Tp C.p.* (Consolini and Migliori, 2005). Then, the sedative and hypotensive activities are possibly associated to different compounds. The decrease in exploration was found at oral doses between 180 and 600 mg liophylized/kg, which were hypotensive in rats. These doses are respectively equivalent to about 1.8 and 6 g dried leaves/kg, which are much higher than the ethnotherapeutic one calculated as 340 mg dried leaves/kg (Consolini and Migliori, 2005). Nevertheless, extrapolation of doses from animals to humans or between other two species is generally not possible. For instance, although *Tp C.p.* has produced a delayed mortality in rats at intravenous doses of 320 mg liophylized/kg by respiratory paralysis, this toxic effect was not observed in mice by intraperitoneal injection, suggesting either differences in absorption or greater sensitivity to cardiorespiratory toxicity in rats.

When we studied the mechanism of central effect, *Ntr C.p.* at 320 mg/kg potentiated the sedation of diazepam 3 mg/kg (anxiolytic dose) but not that of diazepam 10 mg/kg (sedative dose). Furthermore, the sedative effect of both, *Ntr C.p.* and *Tp C.p.* were not significantly antagonized by flumazenil, which is used to selectively inhibit the benzodiazepine binding site (Clénet et al., 2005; Shinomiya et al., 2005). These results suggest that the extract would act in an inhibitory central via, which could be the GABA-A receptor, but it is not bound to the alosteric site of benzodiazepines. Also, the sedative effect of *Ntr C.p.* seems to be interfered by amphetamine (Table 3), which may release dopamine in a stimulatory central via. Although 5 mg/kg amphetamine could increase neither locomotion nor exploration, it produced stereotyped movements in mice, which are dependent on dopamine release. Then, these results suggest that the sedative effect of *Cecropia pachystachya* could be functionally interfered by dopamine release.

The chromatographic profiles showed that the extracts of both plant populations are qualitatively similar. Nevertheless, by comparing the HPLC peaks and the similarities and differences among biological activities it could be hypothetized that the highest two peaks (t_R 7.49 and 14.89 min in system B) from *Tp C.p.* could be responsible for the higher toxicity, while the other peak (t_R 4.79) higher in *Ntr C.p.* could be responsible for the higher hypotensive effect of this plant. The TLC profiles show also similarities in at least ten spots with R_f correspondent to different compounds. Since they were detected by the

diphenyl boric acid ethanolamine complex, six of the spots are flavonoids present in *Tp C.p.* and four of them were also present in *Ntr C.p.* at relatively similar amounts. It could be possible that these compounds, which are present in both *Ntr C.p.* and *Tp C.p* plants, were responsible for the sedative activity. In contrast, the flavonoids with R_f 0.31 and 0.45 are more abundant and only present in *Tp C.p.* and could be associated with the higher toxicity of this plant, as it was suggested from the HPLC fingerprints comparison. Since there are not previous reports about composition of *Cecropia pachystachya* extracts and it was not the purpose of this work, we did not identify these compounds. It would need a further extensive work to compare these chromatograms with standards of at least six different flavonoids and other types of compounds in order to identify the composition of the extract.

In conclusion, *Cecropia pachystachya* ("ambay") demonstrated to have several pharmacological activities as follows: cardiotonic, which was not evident in rats in vivo because of a tachycardic anticholinergic effect, a strong sedation comparable to diazepam 10 mg/kg evidenced by decrease in spontaneous locomotion and exploration, and the previously shown hypotension. The sedative effect was similar for the two plant populations, and could contribute to its therapeutic use in cough and asthma. These four activities could be associated to different compounds, while there have been qualitatively found at least ten compounds in the respective chromatographic fingerprints.

Acknowledgments

This work was supported by a grant from Universidad Nacional de La Plata (11-X-408, 2005) and from Colegio de Farmacéuticos de la Provincia de Buenos Aires, Argentina. We thank to Prof. Dra. Etile Spegazzini for identification of the species and to Mr. Ruben Greco for his technical assistance.

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