Acta Farm. Bonaerense **13** (1): 35-40 (1994)
Recibido el 4 de noviembre de 1993
Aceptado el 22 de diciembre de 1993

A Comparative Study of Antispasmodic Activity of Hydroalcoholic 80% (V/V) Extracts of *Achyrocline satureioides* (Lam.) DC. *(Asteraceae)* with Papaverine and Atropine on Rat Isolated Jejunum *

Lucimar FILOT DA SILVA and Augusto LANGELOH

Departamento de Farmacologia, Instituto de Biociências - UFRGS
Rua Sarmento Leite, 500/202, 90046-900, Porto Alegre, RS, Brasil.

SUMMARY. In order to evaluate antispasmodic potencies the hydroalcoholic (80%, V/V) extracts of *Achyrocline satureioides* inflorescences, stems and leaves were compared with papaverine and atropine on rat isolated jejunum. All extracts antagonized muscle contractions induced by acethylcoline (ACh) and barium chloride via a non-specific mechanism. The three extracts and papaverine promoted a dose-dependent flattening of concentration-responses curves (CRC) obtained to ACh and Ba^{+2} on jejunum strips. Besides, inflorescences extract and atropine induced a dose-dependent, rightward shifts of the CRC to ACh. Inflorescences, leaves and stems extracts shown to be 16.7-26.6-fold less potent than papaverine in non-competitive antagonism. On the other hand, inflorescences extract shown to be 5.87 x 10^4-fold less potent than atropine in competitive antagonism on CRC obtained for ACh. The concentration-response curves for $CaCl_2$ obtained on potassium-depolarized rat jejunum were shifted to the right by inflorescences extract. In addition, inflorescences extract induced a reduction of the maximum effect of CRC for $CaCl_2$.

RESUMEN. "Estudio comparativo de la Actividad Antiespasmódica de Extractos Hidroalcohólicos al 80% (v/v) de Extractos de *Achyrocline satureioides* (Lam.) DC. *(Asteraceae)* con Papaverina y Atropina sobre Yeyuno Aislado de Rata". La infusión de inflorescencias, hojas y tallos de *Achyrocline satureioides*, conocida como "marcela", ha sido utilizada como antiespasmódico. El presente trabajo fue realizado con el objetivo de comparar la actividad espasmolítica de extractos hidroalcohólicos al 80% (V/V) de las inflorescencias (EHI), hojas (EHF) y tallos (EHC) de *A. satureioides* obtenidos por maceración, con antiespasmódicos clásicos como papaverina y atropina en el yeyuno aislado de rata. Los tres extractos ejercen un antagonismo no-competitivo inespecífico en las contracciones inducidas por acetilcolina (ACh) y cloruro de bario. El EHI también mostró antagonismo del tipo competitivo siendo 5.87 x 10^4 veces menos potente que la atropina. En relación al antagonismo no-competitivo, el EHI, EHF y EHC se mostraron 16,7 a 26,6 veces menos potentes que la papaverina. El EHI promovió desplazamiento hacia la derecha y reducción del efecto máximo de las curvas concentración-respuesta obtenidas con $CaCl_2$. Los resultados muestran que los extractos hidroalcohólicos al 80% (V/V) de *A. satureioides* poseen menor actividad antiespasmódica que atropina y papaverina y sugieren que el efecto pueda estar relacionado con el antagonismo de calcio.

* Trabajo presentado en el Primer Congreso de la Federación Farmacéutica Sudamericana y II Congreso de Ciencias Farmacéuticas del Cono Sur, Montevideo, Uruguay, 4-7 de noviembre de 1993.

KEY WORDS: Antispasmodic activity; *Achyrocline satureioides* (Lam.) DC. *Asteraceae;* Atropine; Papaverine; Smooth Muscle.
PALABRAS CLAVE: Actividad antiespasmódica, *Achyrocline satureioides* (Lam.) DC. *Asteraceae;* Atropina; Papaverina; Músculo liso.

ISSN 0326-2383

INTRODUCTION

Infusions of inflorescences, stems and leaves of *Achyrocline satureioides* (Lam.) DC. *Asteraceae,* known as "marcela", have been employed in South of Brazil as an antispasmodic remedy. The infusion is most frequently prepared with inflorescences and is used in folk medicine for the treatment of human ailments, particularly those of gastrointestinal tract. Externally it is utilized as an antiinflammatory [1,2], and the optimized hydroalcoholic (80%, V/V) extract showed antiinflammatory activity in carrageenan-induced rat hind paw edema [3]. It has previously been reported that aerial parts contain essential oils [4-6], aglycones and glycosides of flavonoids [7-11], Kawapirone derivatives [12], terpenes derivatives [13] and caffeic acid [8,10]. In 1982, Langeloh & Schenkel [14,15], using alcoholic and hydroalcoholic inflorescences extracts of "marcela" have demonstrated its antispasmodic activity. In 1984 Simoes *et al.* showed the same effect in aqueous extracts of *A. satureioides* leaves/stems [16].

The aim of the present work was: (a) to compare antispasmodic activity among extracts of inflorescences, leaves and stems of *A. satureioides* obtained by maceration; (b) to compare these extracts with atropine and papaverine; and (c) evaluate if the effect of hydroalcoholic inflorescences extract may be related to the amount of calcium available for make contraction possible.

MATERIAL AND METHODS

Plant Material

Inflorescences, leaves and stems of *A. satureioides* were collected at Morro Santana in April, 1992. A voucher specimen has been deposited at ICN Herbarium, UFRGS - Porto Alegre, RS, Brazil (MSOBRAL 7440).

Preparation of the plant extract

Dried and powdered *A. satureioides* inflorescences, leaves and stems were extracted by maceration with 80:20 (V/V) alcohol:water ratio (7,5% plant:solvent) at room temperature.

The three extracts were filtered and concentrated under reduced pressure in a rotary evaporator at 45-55 °C, resulting in an aqueous fraction. Next, the aqueous fraction was used to determine the "dried residue", wich was then utilized as concentration in bath fluid.

The extracts plus standard substances, quercetin, 3-*O*-metilquercetin, luteolin and caffeic acid were chromatographed on celulose. The chromatograms were developed with acetic acid at 40% (v/v) and revealed with $AlCl_3/UV_{360nm}$.

Evaluation of antispasmodic activity

Jejunum strips removed from male and female Wistar rats (200-250g) were isolated, cleaned and placed in baths containing continuously aerated Tyrode solution at 37 °C. Isotonic contractions were recorded on smoked drums with tangential levers (6-fold amplification) under a load of 1g.

After an initial equilibration of 30 min, cumulative concentration-responses curves (CRC) for ACh and Ba^{+2} were obtained at 20 min intervals by the "bracket-

ing" method proposed by Furchgott [17]. After achieving stable responses, CRC were obtained in the presence of the extracts, atropine or papaverine.

The results are expressed as percentages of the maximal contraction (E_{max}) observed in the control CRC obtained prior to the addition of the antagonists. Apparent affinities for agonists were stimated graphycally from individual experiments as the concentration inducing 50% of E_{max} and are expressed as the mean logarithm of the EC_{50} (pD_2). pD'_2 values expressed the cologarithm of the concentration of the spasmolytic substance which reduced the maximal response of the agonist by 50%. pA_2 values expressed the cologarithm of the concentration of the spasmolytic substance which induced a displacement of two times towards the right in the CRC.

Since the molecular weight of *A. satureioides* active principle is unknown, pD'_2 and pA_2 values for the extracts, papaverine and atropine were calculated using g/ml as the unit of concentration.

Calcium antagonism in depolarized tissue

In order to evaluate the effect of hydroalcoholic inflorescences extract on calcium-induced contraction, jejunum strips were exposed to high-potassium calcium-free depolarizing solution. After equilibration, cumulative CRC to $CaCl_2$ were obtained at 20 min intervals in the presence or absence of three concentrations of the extract (98.4; 164.0 and 328.0 $\mu g.ml^{-1}$).

Statistical analysis

The data were given as mean SEM, with differences between groups evaluated statistically using Student's unpaired t-test.

RESULTS

Evaluation of antispasmodic activity

Inflorescences hydroalcoholic extract (IHE), atropine and papaverine induced a rightward displacement of ACh concentration-response curves resulting in a reduction of sensitivity ranging from 2.0-9.3 times (Table 1). Like papaverine, inflorescences (IHE), leaves (LHE) and stems (SHE) of hydroalcoholic extracts of A. satureioides induced a dose-dependent unspecific flattening of the CRC on rat isolated jejunum to both ACh and Ba^{+2} (Figure 1).

Inflorescences and stems extracts at concentrations of 98.4 and 122.1 $\mu g.ml^{-1}$, respectively, produced a similar inhibition of ACh and Ba^{+2} maximum contraction (Table 1). Papaverine at 3.76 $\mu g.ml^{-1}$ induced a 45 and 65% inhibition of ACh and Ba^{+2} CRC respectively (Table 1). From the differencés between the calculated pD'_2 values, IHE was shown to be 16.7-fold and 18.8-fold less potent than papaverine in non-competitively antagonizing ACh- and Ba^{+2}-induced contractions respectively, while LHE and SHE were shown to be 21.0- and 26.6-fold less potent than papaverine on the same agonists. From the differences between the calculated pA_2 values, 9.2 for atropine and 4.3 for inflorescences extract, IHE was shown to be 5.87 x 10^4-fold less potent than atropine in competitive antagonizing ACh-induced contraction.

Antagonist or extract (µg/ml)	Agonist	(N)	% inhibition of maximal response	pD'$_2$[a]	pD$_2$	ΔpD$_2$	Reduction of sensitivity
Atropine	ACh	(8)	-3.7 ± 4.0ns	-	6.2 ± 0.2	0.97++	9.3
(0.002)	Ba	(4)	-5.8 ± 7.0ns	-	4.0 ± 0.1	-0.01ns	-
Papaverine	ACh	(13)	45.0 ± 5.9**	5.0	6.6 ± 0.1	0.56++	3.6
(3.76)	Ba	(6)	66.8 ± 8.9**	5.1	3.3 ± 0.2	0.45ns	2.8
IHE	ACh	(6)	52.3 ± 11**	4.2	6.8 ± 0.1	0.39+	2.5
(98.4)	Ba	(7)	69.1 ± 8.0**	4.2	3.4 ± 0.1	0.30+	2.0
(164.0)	ACh	(8)	81.9 ± 5.6**	4.2	6.3 ± 0.1	0.92++	8.3
	Ba	(4)	95.5 ± 1.4**	4.2	2.8 ± 0.3	0.96++	9.1
LHE	ACh	(7)	71.3 ± 7.6**	4.1	6.6 ± 0.1	0.28ns	1.9
(185.7)	Ba	(8)	79.8 ± 4.3**	4.0	3.6 ± 0.1	0.17ns	1.5
SHE	ACh	(7)	60.4 ± 3.6**	4.0	6.7 ± 0.1	0.28ns	1.9
(122.1)	Ba	(8)	57.8 ± 5.9**	4.1	3.8 ± 0.1	0.04ns	1.1

Table 1. Effects of hydroalcoholic extracts of *A. satureioides* (Lam.) DC., atropine and papaverine on maximum effects and pD2 values of ACh and Ba^{+2} CRC on rat isolated jejunum. Two or three control cumulative concentration-responses curves (CRC) were obtained at 20 min intervals by the "bracketing" method for ACh and Ba^{+2}. Then the extracts or antagonists were added to the fluid bath before beginning the next CRC. The pD2 values were determined graphically as the - logarithm of the concentration inducing 50% of maximal contraction. Reduction of sensitivity = cologarithm of pD_2. Data are presented as mean SEM. ΔpD_2 = pD_2 control - pD_2 experimental. ** $p < 0.01$ significantly inhibited; + ($p < 0.05$), ++($p < 0.01$) significantly different from control pD_2, ns = not significant; a = calculated by linear regression.

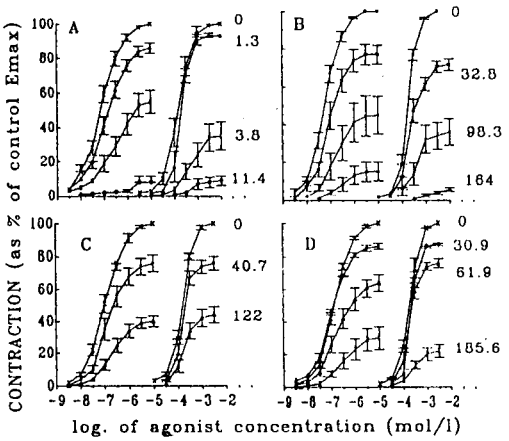

Figure 1. Mean concentration-response curves (CRC) obtained to ACh and Ba^{+2} on rat isolated jejunum in the absence and presence of papaverine (A), or IHE (B), SHE (C) and LHE (D). The same concentrations (µg.ml^{-1}) of each drug were used for ACh and Ba^{+2} CRC and are indicated near the Ba^{+2} curve. Each point represents the mean of 6 to 9 determinations and vertical bars indicate the SEM. Note the non-competitive effect of each drug in relation to either ACh or Ba^{+2}. A slight shift to the right was also observed for the curves for both agonists when IHE was used (B).

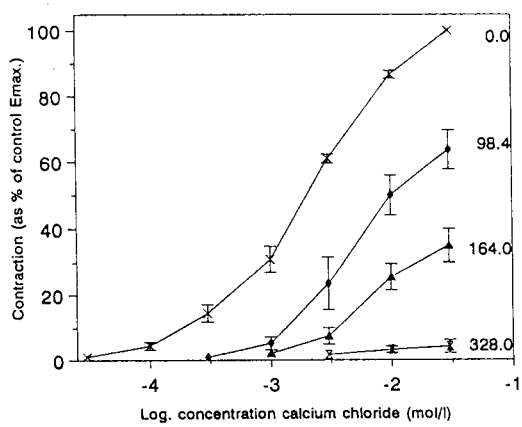

Figure 2. Mean concentration-response curves obtained to $CaCl_2$ on isolated potassium-depolarized rat jejunum in the absence or presence of IHE. The concentrations of IHE (µg.ml^{-1}) are indicated on the right side of the figure. Each point represents the mean of 6 determinations and the vertical bars indicate the SEM.

Calcium antagonism in depolarized tissue

The IHE induced a parallel, dose-dependent, rightward shifts of the CRC to $CaCl_2$, and addition induced a dose-dependent non-competitive antagonizing of contraction elicited by $CaCl_2$ (Figure 2).

DISCUSSION

Infusions of inflorescences, leaves and stems of *A. satureioides* have been used by the population of South Brazil as antispasmodic. The chromatogram revealed that IHE contain quercetin, 3-*O*-methylquercetin and luteolin (flavonoids), while LHE and SHE contain caffeic acid.

The three extracts, like papaverine, exerted a non-competitive antagonism of contraction induced by ACh and Ba^{+2} on rat isolated jejunum (Figure 1). This effect of extracts were reversed upon washing the preparation tested.

It has been proposed that antispasmodic action of papaverine is at least partially due to its ability to competitively antagonize calcium entry to the smooth cell [18, 19]. Since the profile of the antispasmodic effects of IHE and papaverine were strikingly similar, we examined the possibility that this drugs share a common mechanism of action. Indeed, the IHE was found to competitively antagonize calcium-induced contractions in high-potassium-depolarized rat jejunum strips (Figure 2). In addition, the IHE was shown to be non-competitive antagonist.

Furthermore, our present findings indicate that the IHE studied exerted a non-specific antispasmodic action on smooth muscle that may be related to the amount of calcium available, and show that *A. satureioides* hydroalcoholic extracts despite being a usefull antispasmodic drug in popular medicine have a weaker activity than classical antispasmodic drugs.

REFERENCES

1. Simões, C.M.O. (1988) *Fitoterapia* **5**: 419-21
2. Petrovick, P.R. and M.T. Knorst (1991) *Phytother. Res.* **5**: 237-8
3. Sonaglio, D. (1987) "Padronização de extrato hidroalcoólico das sumidades floridas de *Achyrocline satureioides* (Lam.) DC. *Compositae* (marcela)". Master's thesis, UFRGS, Porto Alegre
4. Lamaty, G., C. Menut, J.M. Bessiére, E.P. Schenkel, M.A. Santos and V.L. Bassani (1991) *J. Essential Oil Res.* **3**: 317-21
5. Ricciardi, A.I.A., A.E. Cassano and J.L. Burgos (1965) *Rev. Fac. Ing. Quim.-Litoral Santa Fe,* Argentina **36**: 37
6. Bauer, L., G.A.A.B. Silva, N.C.S. Siqueira, C.T.M. Bacha and B.M.S. Sant'ana (1979) *Rev. Bras. Farm.* **69**: 97-100
7. Hansel, R. and D. Ohlendorf (1971) *Arch. der Pharm.* **304**: 893-6
8. Ferraro, G.E., C. Norberdo and J.D. Coussio (1981) *Phytochemistry* **20**: 2053
9. Simões, C.M.O. and L. Bauer (1983) In: Anais do II Simpósio Nacional de Farmacologia e Química de Produtos Naturais, p: 415-9

10. Simões, C.M.O. (1984) "Investigação químico-farmacológica de *Achyrocline saturei-oides* (Lam.) DC. *Compositae* (marcela)". Master's thesis, URFGS, Porto Alegre
11. Lima, C.S.A., L.W. Bieber and J.F. Mello (1990) In: Anais do XI Simpósio de Plantas Medicinais do Brasil p. 2.05
12. Kaloga, M., R. Hansel and E.M. Cybulski (1983) *Planta Medica* **48**: 103-4
13. Hirschmann, G.S.(1984) *Rev. Latinoamer. Quím.* **15**: 134-5
14. Langeloh, A. and E.P. Schenkel (1982) *Oréades* **8**: 454-8
15. Langeloh, A. and E.P. Schenkel (1982) In: Anais do I Congresso Brasileiro de Farmacologia e Terapêutica Experimental p. 136
16. Simões, C.M.O., N. Rech and A.J. Lapa (1986) *Caderno de Farmácia* **2**: 37-54
17. Furchgott, R.T. (1972) In: "Catecholamines, Handbook of Experimental Pharmacology", XXXIII: 283-335
18. Takayanagi, I., A. Karasawa and Y. Kasuya (1980) *Japan. J. Pharmacol.* **30**: 405-12
19. Imai, S. and T. Kitagawa (1981) *Japan. J. Pharmacol.* **31**: 193-9