Biological and chemical study of paico (*Chenopodium chilense*, Chenopodiaceae)

R. García [a,*], I. Lemus [a], P. Rivera [b], S. Erazo [a]

[a] *Facultad de Ciencias Quimicas y Farmacéuticas, Departamento de Química Farmacológica y Toxicológica, Universidad de Chile, P.O. Box 233, Santiago-1, Chile*

[b] *Facultad de Ciencias, Universidad de Chile, P.O. Box 653, Santiago-1, Chile*

Abstract

The methanolic extract of the aerial portion of *Chenopodium chilense* Schrad., used in Chilean traditional medicine as a remedy for stomach-ache, has been found to exert the major spasmolytic activity in acetylcholine contracted rat ileum. This extract, with a complex flavonoid patterns on thin layer chromatography (TLC) analysis, is practically non-toxic both for rats and brine shrimp *Artemia salina* in acute toxicity test. © 1997 Elsevier Science Ireland Ltd.

Keywords: Traditional medicine; Toxicity; *Chenopodium chilense*

1. Introduction

Chenopodium (Chenopodiaceae) is represented by 24 species in Chile; 18 them are endemic (Marticorena and Quezada, 1985). Locally, the aromatic species of Chenopodium are known under the vernacular name of 'paico' (*C. ambrosioides* L., *C. chilense* Schrad., and *C. multifidum* L.), and the leaves are used in traditional medicine as a remedy for stomachache. The aqueous extract of the roots of *Chenopodium* together with *Peumus boldus* Mol. are used to treat diarrhoea in children, while mixed with *Artemisia vulgaris* L. it is taken as a purgative (San Martín, 1983; Mösbach, 1992).

C. ambrosioides L. essential oil has been clinically used as a vermifuge since 1913, but its use was discontinued due to its high toxicity (Reynolds, 1982). However, no adverse effects were observed with plant decoction of endemic *C. ambrosioides* L. (Calcagno, 1925).

Chemical and biological assessments on *C. chilense* Schrad. extracts are not existent since this species was merged until recently as subspecies of *C. ambrosioides* L. (Navas, 1976). Of the latter, many substances have been isolated, such as alka-

* Corresponding author.

loids, terpenes, flavonoids, carotenes and sterols (Bahrman et al., 1985).

We studied the antispasmodic activity of *C. chilense* Schrad. in order to verify whether its folk medical use has a scientifically justified basis.

2. Materials and methods

2.1. Plant material

Chenopodium chilense Schrad. used in this investigation was collected at Baños Morales, near Santiago in a mountain climate site. A voucher specimen (SQF 17277) has been identified by R. Peña and deposited at the Herbarium of Escuela de Química y Farmacia, Universidad de Chile.

The air-dried ground material was extracted sequentially with n-hexane, chloroform, ethyl acetate and methanol. The infusion was prepared in situ at a ratio of 100 g plant powder per liter of boiling water. The essential oil was obtained by steam distillation from the fresh aerial parts of the plant (0.38% v/w).

2.2. Antispasmodic activity

Chenopodium chilense Schrad. extracts were evaluated for antispasmodic activity on isolated rat ileum by Magnus's method (Guerra, 1946). The muscle strips were suspended under a tension of 1 g in a 25-ml organ bath filled with Tyrode solution, aerated with a mixture of oxygen (95%) and carbon dioxide (5%), maintained at 37°C. Changes in contractions were registered on a Kymograph.

The extracts were dried in vacuo and were redissolved in water. Those water insoluble dry extracts were tested as a 1% glyceryl monostearate emulsions. Contractions obtained with acetylcholine chloride (ACh) were compared with responses to the same concentration of spasmogen after the tissue had been incubated for 5 min with the different test solutions. The smooth muscle relaxing effect (inhibition of contraction) was expressed as a percentage of the maximum response in the control curve induced by acetylcholine.

2.3. Toxicity assays

Acute toxicity assays of the infusion, extracts and essential oil of *C. chilense* were determined on both brine shrimp *Artemia salina* (Meyer et al., 1982) and Wistar rats of either gender, using oral and intraperitoneal route for this mammals (Williams and Burson, 1985).

2.4. Statistical analysis

Results are expressed as the means ± S.E.M. and data were analyzed by Student's t-test. Differences below 0.05 probability level ($p < 0.05$) were considered to be statistically significant.

2.5. Phytochemical analysis.

Preliminary studies on *C. chilense* showed the presence of sterols and/or triterpenes, flavonoids, coumarins and alkaloids traces. Furthermore, β-carotene and lutein were isolated from the hexane extract.

The essential oil, as analyzed by gas–liquid chromatography (GLC), shows a peak for one major component and at least five minor components. The major component was isolated using high-performance liquid chromatography (HPLC) and its structure was determined by spectroscopic methods (UV, IR, ^{1}H-NMR, ^{13}C-NMR and Mass).

3. Results

Contractions induced by acetylcholine (ACh) were reduced significantly by pretreatment with methanolic extract of *C. chilense* (37.35% at 2.24 mg/ml organ bath) with a doses-related behavior (Fig. 1).

A 10% infusion was practically without antispasmodic activity. Glyceryl monostearate, chloroform and ethyl acetate extracts have neither contractile nor relaxant effects. The lipophilic plant constituents, hexane extract and the essential oil, present a poor antispasmodic activity at low concentration (0.2 mg/ml), and were too toxic for the smooth muscle to higher concentrations (0.4 mg/ml) to detect any antispasmodic effects.

The essential oil of *Chenopodium* were lethal for rats at i.p. doses of 0.06 ml/kg and oral doses of 0.6 ml/kg of body weight in the acute toxicity test. This test reveals that the infuse and the methanolic extract were devoid of toxic effects with no deaths observed even at 12 g/kg of body weight.

The sensitivity of shrimp (*Artemia salina*) toward *Chenopodium* essential oil is greater than the reference standard hexachlorocyclohexane (gamma isomer, 20 ppm), with a median lethal concentration (LC_{50}) of 17.5 ppm. The more polar *Chenopodium* samples resulted less toxic for the shrimp than the apolar fractions, with an LC_{50} of 224 ppm for the dried methanol extract and without toxic effects for infusion under 1000 ppm.

The structure of the major component of the essential oil was determined as 2-*p*-mentene-1,4-peroxide (ascaridol). It was also observed that ascaridol decomposes to produce a non-oxygenated homologue, *p*-cymene, detected mainly in the NMR spectra (Table 1 and Table 2). Ascaridol shows IR absorption maximal at 3023 cm^{-1} (=CH=) and 1378 cm^{-1} (gem-dimethyl). The mass spectrum shows characteristic fragments at *m/z* 169 (M^{+1}), 136 ($M^{+}-O_2$), 134 ($M^{+}-2OH$) and 119 (134-CH_3) base peak.

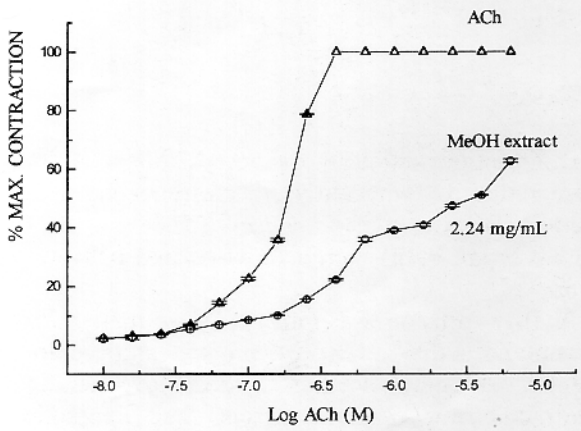

Fig. 1. Antispasmodic activity of methanolic paico extract. Log dose–response curves for acetylcholine (Ach), and acetylcholine pretreated with a dry methanolic paico extract (incubated for 5 min) dissolved in water (2.24 mg/ml). Each point is the mean of three observations.

Table 1
^{1}H-NMR, 200 MHz ($CDCl_3$): chemical shifts in ppm

H-Carbon	Ascaridol	*p*-Cymene
2	6.47 d	7.10 s
3	6.42 d	7.10 s
5	1.4–1.8 m	7.10 s
6	1.4–1.8 m	7.10 s
7	1.37 s	2.30 s
8	1.80 m	2.66 m
9–10	1.00 d	1.22 d

s, singlets; d, doublets; m, multiplets.

4. Discussion and conclusions

The screening of *C. chilense* for antispasmodic activity shows that the methanolic extract exhibits a significant ($P < 0.05$) relaxing effect on the acetylcholine contracted smooth muscle with a very low acute toxicity. This extract presents a complex flavonoid pattern on TLC, from which we could not isolate any metabolite.

The dilute lipophilic fraction of this plant present a poor spasmolytic activity and were toxic to the smooth muscle at higher concentration level. The other solvent fractions did not have biological significance. The 10% infusions show a low antispasmodic activity, contrasting with its traditional widespread use for stomachache.

The isolation of ascaridol as the major component of the essential oil of *C. chilense* is not striking, because it is the most characteristic chemical compound of the genus *Chenopodium* with scarce exceptions (De Pascual, 1983). The

Table 2
^{13}C-NMR ($CDCl_3$): chemical shifts in ppm

Carbon	Ascaridol	*p*-Cymene
1	74.3	135.0
2	136.4	128.9
3	133.1	126.2
4	79.7	145.8
5	25.6	126.2
6	29.6	128.9
7	21.3	20.9
8	32.2	33.7
9–10	17.2	24.0

presence of *p*-cymene as decomposition product of ascaridol was detected earlier on boldo (*Peumus boldus* Mol.) essential oil by Craveiro and Araújo (1984), suggesting a possible interconversion between this two substances.

Acknowledgements

This work was granted by DTI (Departamento Técnico de Investigación, Universidad de Chile), grant B 2747–9244 to R. García and grant Q 3069–9244 to Dr. A.P. Rivera. The authors wish to thank Hochstetter Cia. and grant Etnobotánico Caritas/Chile for financial support.

References

Bahrman, N., Jay, M., Gorenflot, R., 1985. Contribution to the chemosystematic knowledge of some species of genus *Chenopodium*. Lett. Bot. 2, 107–113.

Calcagno, L., 1925. Farmacología y Formas Farmacéuticas del Paico en Estudio. Tesis Químico Farmacéutico. Universidad de Concepción, Chile, 22 pp.

Craveiro, A.A., Araújo, Y.B.M., 1984. Plants with ascaridol modifications induced by light. Rev. Latinoam. Quím. 14, 135–138.

De Pascual, T., 1983. Dehidro-1-hydroxycarvomenthols from the essential oil of *C. multifidum*. Phytochemistry 22, 2749–2752.

Guerra, F., 1946. Farmacología Experimental. Editorial Utecha, México, 544 pp.

Marticorena, C., Quezada, M., 1985. Flora vascular de Chile. Gayana Botánica 42 (1-2), 28.

Meyer, B., Ferrigni, N., Putnam, L., Jacobsen, L., Nichols, D., McLaughlin, L., 1982. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 45, 31–34.

Mösbach, E.W., 1992. Botánica Indígena de Chile. In: Villagrán, C., Aldunate, C. (Eds.), Editorial Andrés Bello, 140 pp.

Navas, L.E., 1976. Flora de la Cuenca de Santiago de Chile. Ediciones de la Universidad de Chile, Vol. II, 549 pp.

Reynolds, J., 1982. Martindale, The Extra Pharmacopoeia, 28th Edition. Pharmaceutical Press, London.

San Martín, J., 1983. Medicinal plants in central Chile. Econ. Bot. 37, 216–227.

Williams, P., Burson, J. (Eds.), 1985. Industrial Toxicology—Safety and Health Applications in the Work Place. Van Nostrand Reinhold, New York, 193 pp.