In vitro genotoxic evaluation of the medicinal plant *Chenopodium ambrosioides* L.

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Abstract

Chenopodium ambrosioides (Chenopodiaceae) is an anthelmintic herb used in Latin-America's folk medicine. The aim of this work is to evaluate genetic damage induced by decoction and infusion of this plant which were assayed in different concentrations (1, 10, 100, 1000 µg/ml), by addition of the extract to human lymphocyte cell cultures. The endpoints evaluated were chromosomal aberrations (CA), sister chromatid exchanges (SCE), cell proliferation kinetics (CPK) and mitotic indexes (MI). The repeated measure analysis of variance was used for statistic evaluation of the results. The results showed (a) a statistical increase in the percentage of cells with CA and in the frequency of SCE when cultures were exposed to both preparations of Paico, (b) a decrease in MI of both preparations assayed, although no modification in the CPK values either in the infusion or in the decoction was observed. These results suggest a possible genotoxic effect of both preparations, probably due to different active principles. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Medicinal plants; Paico; *Chenopodium ambrosioides*; Mitotic index; Genotoxicity

1. Introduction

The folk medicine of Argentina employs many herbs to counteract diverse diseases such as catarrh, bronchitis, pneumonia, ulcer and diarrhea (Ratera and Ratera, 1980; Toursarkissian, 1980; Martinez-Crovetto, 1981).

Chenopodium ambrosioides L. (Chenopodiaceae), popularly known as 'Paico' or 'Pazote', grows wild in Central and South America. It is one of the plants widely used in popular medicine as a vermifuge, emmenagogue and abortifacient (Curtin, 1965). It is a perennial, aromatic plant, more or less pubescent, with its branched stem often postrated. The leaves are oblong–lanceolate and serrate with small green flowers in dense terminal panicles, each with five sepals. The persistent calyx encloses the fruit and the seeds are black (less than 0.8 mm long).

The essential oil of *C. ambrosioides* is an irritant to the mucous membrane of the gastrointestinal tract, kidneys and liver. Overdoses of this oil have caused fatalities in both men and rats (De Pascual et al., 1980). The oil's main components are monoterpenes, with ascaridole, a terpenic peroxide, representing about 70%. Ascaridole is formed in the highest concentration in the seeds. Other components are limonene, transpinocarveol, ascaridole–glycol, aritasone, β-pynene, myrcene, phelandrene, alcanphor and α-terpineol (De Pascual et al., 1980; Sagrero-Nieves and Bartley, 1995). The primary symptoms during an acute intoxication are gastrointestinal, such as gastroenteritis with diffuse hyperemia at first, followed by alterations in the CNS, such as headache, facial flushing, impaired vision, vertigo, incoordination and paresthesis.

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The principal therapeutic uses of *Chenopodium* oil described in the literature are as an anthelmintic, abortifacient and emmenagogue (Conway and Slocumb, 1979). It is used for the treatment of digestive, respiratory, uro-genital, vascular and nervous disorders, metabolic disturbances such as diabetes, hypercholesterolemia, as a sedative, an antipyretic and an antirheumatic (De Feo and Senatore, 1993). Kishore et al. (1993) described the fungitoxicity against dermatophytes such as *Aspergillus fumigatus* and *Cladosporium trichoides*, while Lall and Meyer (1999) described the strong activity of the acetone extract of the plant against *Mycobacterium tuberculosis*.

C. ambrosioides extracts do not have any effect against *Vibrio cholerae* in vitro (Guevara et al., 1994) or against *Triatoma infestans* and intestinal worms. Kliks (1985) tested the infusion of *C. ambrosioides* (traditional anthelmintic plant) against intestinal worms, finding negative results.

The primary form by which the plant is used by the population is as an aqueous extract such as a decoction or infusion. Although ethnomedical uses of *Chenopodium* oil are known, there are very few reports on the pharmacological effects of this plant (Kliks, 1985). In view of its wide use and the lack of studies, the potential toxicity, in particular the genotoxic effect of the aqueous extract of this plant, was studied by in vitro exposure of lymphocyte cultures (derived from four healthy donors) to the extracts.

2. Materials and methods

2.1. Collection of plant

The aerial parts of *C. ambrosioides* used in this research were collected from plants growing around 'Luján' river, 'La Flora' island (Tigre), located 30 km north of Buenos Aires. The plant was identified in the field by one of the authors (*Gurni s.n.*, III-1997). Voucher specimens have been kept at the Museum of Pharmacobotany 'Juan A. Dominguez' School of Pharmacy and Biochemistry, University of Buenos Aires.

2.2. Preparation of C. ambrosioides aqueous extracts

Decoction and infusion of the aerial parts (extemporaneous aqueous pharmaceutical preparations) were prepared by boiling air-dried aerial parts (10 g) in water (100 ml) (100 °C) for 10 min in the case of decoction, while in the case of infusion, by adding boiling water to the plant material and leaving it standing for 10 min. Both preparations were made at a concentration of 10% w/v and were filtered off through a 0.22 μm filter, and stored at −20 °C.

2.3. Lymphocyte cultures and cell harvesting

Half-a-milliliter of each of heparinized blood samples, obtained from four healthy donors, was placed in a sterile flask containing 7 ml of HAM F-10 medium, supplemented with 1.5 ml of fetal bovine serum (GIBCO), 0.1 ml of phytohemmaglutinin (PHA) (GIBCO) and 32 μM of 5-bromo-2-deoxyuridine (BrdU) (Sigma). Then, *Chenopodium*'s preparations were added in four different concentrations (1, 10, 100 and 1000 μg/ml of the culture). Negative controls were developed by adding distilled water (100 μl) to each donor culture. The cultures were incubated for 72 h at 37 °C. Two hours before harvesting the cells, 0.2 ml of Colcemid (10 μg/ml; SIGMA) was added to each culture flask. Cells were centrifuged at approximately 800–1000 rpm for 10 min. The supernatant was removed and 5 ml of a prewarmed (37 °C) 0.075 M KCl hypotonic solution was added. Cells were resuspended and incubated at 37 °C for 45 min. The supernatant was removed by centrifugation, and 5 ml of a fixative (methanol:glacial acetic acid, 3:1) was added. The fixative was removed and the procedure was repeated twice. To prepare the slides, five drops of the fixed cell suspension were placed on clean glass slides and air-dried. Cells were stained following a modified Fluorescence plus Giemsa (FPG) technique (Perry and Wolff, 1974). Slides were stained for 20 min in a 0.05% w/v Hoechst 33258 solution, rinsed with tap water and placed under an ultraviolet lamp for 90 min. Then, the slides were covered with Sorensen's buffer (pH 6.8) and stained with a 3% Giemsa solution in phosphate buffer (pH 6.8) for 15 min.

2.4. Microscopic evaluation

2.4.1. Chromosomal aberrations (CA)

An analysis of cells with chromosomal aberrations was performed for each donor, preparation and concentration, in 100 consecutive first-division metaphases each with 46 centromeres. Chromatid and chromosomal breaks and fragments were the most frequent chromosomal aberrations found. Gaps were not taken into account. The criteria to classify the different types of aberrations were in accordance with the recommendation of EHC 46 for Environmental Monitoring of Human Populations (WHO, 1985).

The criteria for distinguishing between gaps and chromatid breaks were in agreement with Kauderer et al. (1991). Results were expressed as percentage of aberrant cells.

2.4.2. Sister chromatid exchanges (SCE)

The frequency of SCE was observed in 35–50 harlequin-stained metaphases each with 46 centromeres for each donor, preparation and concentration. The

results were expressed as the frequency of SCE per metaphase.

2.4.3. Mitotic index (MI)

The mitotic index was calculated as the proportion of metaphase for 2000 cells, in each preparation, donor and concentration.

2.4.4. Cell proliferation kinetics (CPK)

The proportion of first (M1), second (M2) and third (M3) dividing cells was scored from 100 consecutive metaphases from each duplicate 72-h-culture for each preparation, donor and concentration. The replication index (RI) was calculated according to the formula [RI = (M1 + 2M2 + 3M3)/100] (Rojas et al., 1993).

2.5. Fractionation and analysis of extracts

2.5.1. Thin layer chromatography

Preliminary analysis of the infusion and the decoction by thin layer chromatography on silicagel GF254 was performed. The chromatograms were developed in benzene, chloroform and *n*-hexane–diethyl ether (80:20), respectively, using ascaridole as reference, with the object of determining the absence of this compound in both extracts. Anisaldehyde/sulfuric acid was used as reagent (Sthal, 1969).

2.5.2. Partition of the aqueous extract

The aqueous extracts obtained as decoction and infusion were successively partitioned with methylene chloride (MC) and ethyl acetate (EA). The three fractions MC, EA and the residual aqueous layer (RA) were resuspended in distilled water. After removing the solvent under reduced pressure in a rotatory evaporator, dilutions were made to produce concentrations of 1, 10 and 100 µg/ml of culture. The fractions were screened

for cyto- and genotoxicity as determined by frequency of SCE and MI.

2.6. Statistical analysis

Repeated measures of analysis of variance (ANOVA) were applied in the experiments performed for MI, CPK, CA and SCE. One-way ANOVA was applied in the experiments performed for MI and SCE of decoction subfractions (MC, EA and residual aqueous layer) (Zar, 1974).

3. Results

When CAs were analyzed after treatment with different concentrations of decoction, significant changes could be detected in the percentage of aberrant cells ($P < 0.001$) (Table 1). The data obtained, in the analysis of 400 metaphases per treatment (100 metaphase cells/individual) after exposure to infusion showed a statistically significant increase of CA among the treatments as compared to controls ($P < 0.05$) (Table 1). At the same time, the analysis of chromosome aberrations showed that most of the breaks detected in the different treatments were of chromatid type.

Figs. 1 and 2 show the frequency of SCE/cell for decoction and infusion, respectively. For both extracts a statistically significant increase (decoction D: $P < 0.05$; infusion I: $P < 0.0005$) was detected, which became more evident at higher concentrations.

When the potential cytotoxicity of the extracts on lymphocyte cultures was analyzed, a decrease in the MI for both decoction and infusion was found (D: $P < 0.0001$; I: $P < 0.0005$) (Table 2). These results point to a greater significance for decoction than for the infusion. When CPK was studied for both extracts, no

Table 1
Percentage of aberrant cells in human lymphocyte cultures exposed to *C. ambrosioides* (decoction and infusion)

		Chromosomal aberrations						% cells with CA
		CB	ICB	AF	R	TRI	Quad	X ± SEM
Decoction	Neg. control	15	4	1				4.98 ± 0.33
	1 µg/ml	28	2	1				7.90 ± 0.79[a]
	10 µg/ml	26	9	1	1	5		10.39 ± 0.90[a]
	100 µg/ml	26	6			2		11.25 ± 0.48[a]
	1000 µg/ml	31	9					8.34 ± 0.92[a]
Infusion	Neg. control	15	4	1				4.87 ± 0.30
	1 µg/ml	16	5			1	1	7.53 ± 1.84[b]
	10 µg/ml	21	10	1				7.75 ± 1.93[b]
	100 µg/ml	26	13			2	1	9.78 ± 1.44[b]
	1000 µg/ml	24	5		1	1		6.75 ± 0.48[b]

CA, chromosomal aberration; CB, chromatid break; ICB, isochromatid break; AF, acentric fragment; R, ring; TRI, triradial; QUAD, quadriradial.
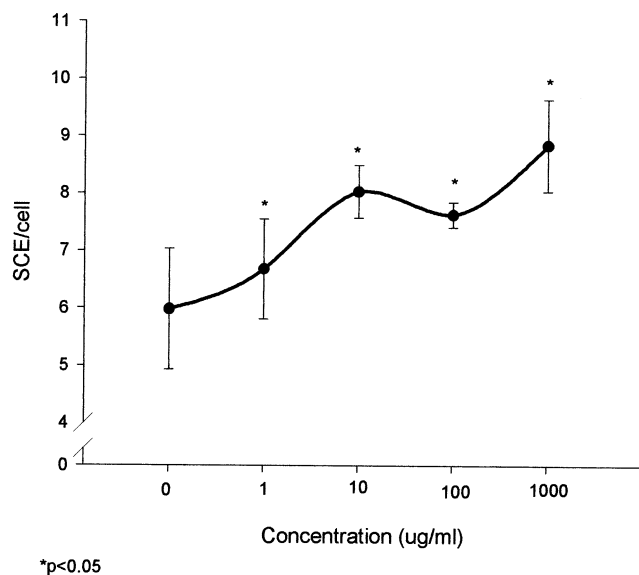[a] $P < 0.001$ ANOVA test.
[b] $P < 0.05$ ANOVA test.

Fig. 1. Effect of *C. ambrosioides* decoction on sister chromatid exchange frequency in human lymphocyte cultures

changes in RI could be detected. Interindividual variability appears to be related to a variety of physiological factors (Wen and Liew, 1993; Malaragno and Smith, 1990) (Table 2).

In the TLC assay, the identification of the active compounds such as ascaridole by way of R_f value could not be made, whether in the decoction or in the infusion, in the three different solvent systems assayed. In order to continue the characterization, three fractions were obtained from aqueous extracts. They were screened by mitotic index and sister chromatid exchange frequency, and a significant decrease in the MI was found in the methylene chloride fraction obtained from both extracts (D: $P < 0.05$; I: $P < 0.0005$) (Table 3).
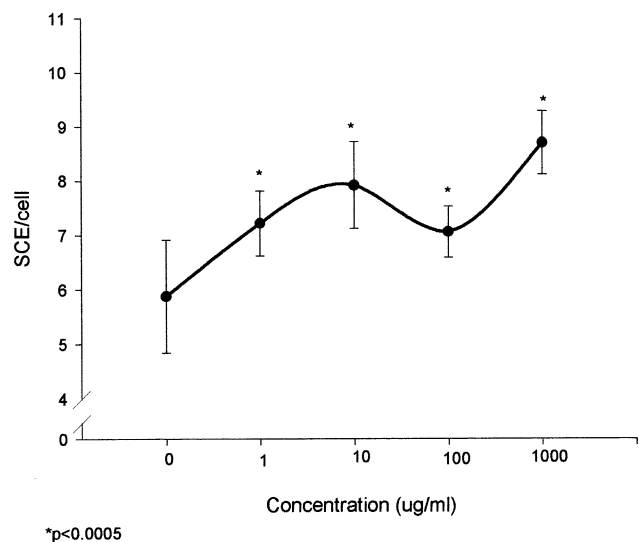

Fig. 2. Effect of *C. ambrosioides* infusion on sister chromatid exchange frequency in human lymphocyte cultures

4. Discussion and conclusions

Chromosomal aberrations and sister chromatid exchanges are extremely valuable and highly relevant endpoints for the detection of potential carcinogens (Swierenga et al., 1991). Chromosomal aberrations are changes in chromosome structure resulting from a break or exchange of chromosomal material. Most of the CA observed in cells are lethal, but there are many corresponding aberrations that are viable and can cause genetic effects, either somatic or reproductive. SCE represents a symmetrical exchange of complementary DNA strands between chromatids within a single chromosome (Swierenga et al., 1991)

The present data show an increase in the percentage of aberrant cells (Table 1), suggesting a strong interaction between active principles of both extracts and DNA, which could be responsible for the observed cytotoxicity. The increase of SCE frequency (Figs. 1 and 2) could be related to a potential carcinogenic effect of both extracts (Swierenga et al., 1991).

MI and RI are used as indicators of adequate cell proliferation biomarkers. Mitotic index measures the proportion of cells in the M-phase of the cell cycle and its inhibition could be interpreted as cellular death (Rojas et al., 1993). A cytotoxic effect of both extracts was observed, evidenced by the decrease of the MI (Table 2).

Subfraction analysis showed a cytotoxic effect of the MC fraction (Table 3). In spite of the increment in the sister chromatid exchange frequency in the same fraction, the statistical analysis shows a non-significant difference between controls and treated groups (Table 3). These results suggest that the active principles responsible for the cytotoxic effect of infusion and decoction could be present mainly in the MC fraction.

In a similar previous work, the genotoxic effect of another *Chenopodium* species (*C. multifidum*) was tested. It was shown that *C. multifidum* decoction and infusion increased the percentage of aberrant cells and SCE, and decreased the MI. The subfractions analysis showed that the MC fraction of decoction was the most active (Gadano et al., 2000), as also shown by the data in the present work.

The results obtained using biomarkers analysis of *C. ambrosioides*, strongly suggest an interaction between DNA and active principles of aqueous extracts. The data indicate that the MC fraction is the most active and is related with cellular damage (as an increase in the percentage of aberrant cells and sister chromatid exchange frequency) and cellular death (mitotic index decrease).

These results show that *C. ambrosioides* induces genetic damage in the experimental conditions assayed. Genetic biomarkers determination would help to estimate the potential toxicity of medicinal herbs in order to

Table 2
Replication index and mitotic index in human lymphocyte cultures exposed to *C. ambrosioides* aqueous extracts

	Decoction		Infusion	
	Mitotic index (X ± SEM)	Replication index (X ± SEM)	Mitotic index (X ± SEM)	Replication index (X ± SEM)
Control	5.04 ± 0.13	1.97 ± 0.10	5.04 ± 0.13	1.97 ± 0.10
1 μg/ml	3.66 ± 0.50[a]	1.98 ± 0.04	4.23 ± 0.12[b]	1.84 ± 0.26
10 μg/ml	3.19 ± 0.21[a]	2.06 ± 0.15	4.50 ± 0.17[b]	1.86 ± 0.14
100 μg/ml	3.15 ± 0.24[a]	1.95 ± 0.07	4.33 ± 0.13[b]	1.91 ± 0.12
1000 μg/ml	2.23 ± 0.19[a]	1.84 ± 0.15	3.96 ± 0.15[b]	1.65 ± 0.07

[a] $P < 0.0001$ ANOVA test.
[b] $P < 0.0005$ ANOVA test.

Table 3
Mitotic index and sister chromatid exchange frequency in human lymphocyte cultures, exposed to *C. Ambrosioides* subfractions

	Mitotic index (%) (X ± SEM)		SCE/cell (X ± SEM)	
	Decoction	Infusion	Decoction	Infusion
Neg. control	6.20 ± 0.01	6.31 ± 0.10	5.53 ± 0.44	5.53 ± 0.44
Ethyl acetate				
1 μg/ml	5.45 ± 0.95	5.09 ± 0.20	5.11 ± 0.44	4.70 ± 0.36
10 μg/ml	5.60 ± 1.10	5.45 ± 0.45	5.44 ± 0.69	5.35 ± 1.48
100 μg/ml	5.60 ± 1.10	5.55 ± 0.05	6.17 ± 1.26	6.61 ± 1.47
Methylene chloride				
1 μg/ml	3.70 ± 0.14[a]	3.99 ± 0.20[b]	7.27 ± 1.41	7.64 ± 1.73
10 μg/ml	3.30 ± 0.60[a]	3.45 ± 0.05[b]	7.53 ± 0.96	7.82 ± 0.99
100 μg/ml	2.51 ± 0.41[a]	3.05 ± 0.14[b]	7.64 ± 0.30	9.86 ± 0.99
Aqueous residual layer				
1 μg/ml	4.85 ± 0.75	5.20 ± 0.20	4.80 ± 1.17	5.42 ± 0.05
10 μg/ml	4.60 ± 0.71	5.70 ± 0.70	5.03 ± 0.40	4.97 ± 0.43
100 μg/ml	4.98 ± 0.45	5.90 ± 0.80	5.15 ± 0.75	5.17 ± 0.23

[a] $P < 0.05$ ANOVA test.
[b] $P < 0.0005$ ANOVA test.

establish a regulation over medicinal plant consumption, an important measure of protecting public health.

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