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A phytotherapeutic extract of *Equisetum myriochaetum* is not genotoxic either in the *in vivo* wing somatic test of *Drosophila* or in the *in vitro* human micronucleus test

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Abstract

Equisetum myriochaetum is a Mexican plant used in folk medicine to treat kidney diseases and type 2 diabetes mellitus. The main constituents of the phytoextract are flavonol glycosides (kaempferol), phytoesterols and carbohydrates. In this study, phytotherapeutic extracts from *Equisetum myriochaetum* were investigated for genotoxicity in the *in vivo* wing spot test in *Drosophila melanogaster* and in the *in vitro* human micronucleus test. No acute toxicity of the phytoextract could be determined in *Drosophila* or in human lymphocytes in culture, ranging from 0.78 μg/ml to 3700 μg/ml for the wing assay and between 12.5 μg/ml and 500 μg/ml for the micronucleus test. The *Drosophila* wing somatic mutation and recombination test (SMART) was applied in the standard version with basal biotransformation activity as well as in a variant version with increased cytochrome P450-dependent bioactivation capacity. The ranges of exposure concentrations for these genotoxicity experiments were between 0.78 μg/ml and 500 μg/ml. The human micronucleus test *in vitro* was performed with cultured lymphocytes obtained from four healthy donors. The concentrations assayed for these experiments ranged from 12.5 μg/ml to 500 μg/ml. No statistically significant increase was observed between treated series when compared with a concurrent negative (water solvent) control series in either assay. The results demonstrate clearly that the phytotherapeutic extract from *Equisetum myriochaetum*, under the experimental conditions tested, is not genotoxic in the *in vivo* experiments or in the *in vitro* studies.

Keywords: *Equisetum myriochaetum* (Equisetaceae); Type 2 diabetes mellitus; Wing somatic assay; Human micronucleus test; Cytotoxicity; Genotoxicity

1. Introduction

Equisetum myriochaetum Schltdl. & Cham (Equisetaceae) is a plant distributed in South–central Mexico. Popularly known as "cola de caballo", it is traditionally used for the treatment of diabetes mellitus type 2, especially in the southern Mexican State of Guerrero (Andrade-Cetto et al., 2000). A decoction of the aerial parts of the plant is prepared and consumed as an herbal tea ("agua de uso"). Several flavonol glycosides (kaempferol derivatives) have been isolated from the butanol extract and the water extract of the dried herbal material by HPLC analysis, and the structures were elucidated by NMR (Wiedenfeld et al., 2000). Besides these, other compounds have been reported: pinocembrin, chrysin, β-sitosterol, β-D-glycosyl-sitosterol, β-D-glucose and fatty acids (Camacho et al., 1992).

The hypoglycaemic effect of the water extract (WE) and the butanol extract (BE) of the plant have been shown in streptozotocin-induced diabetic rats (Andrade-Cetto et al., 2000). In another pharmacological study, a water extract of the aerial part was administrated orally in a single dose in 11 diabetic type 2 patients. There was a reduction of blood glucose levels starting 90 min after administration, which was maintained for another 90 min. No stimulation of insulin secretion was observed (Revilla-Monsalve et al., 2002).

Several species of *Equisetum* (*Equisetum hymale* and *Equisetum laevigatum*), together with *Equisetum myriochaetum*, are traditionally used by healers to treat kidney diseases (Argueta,

Abbreviations: DER, ratio of the herbal drug to the native herbal drug preparation; BE, butanolic extract; WE, water extract

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1994). These species are sold without distinguishing between them in markets of the central part of Mexico (Andrade-Cetto and Heinrich, 2005). In view of the fact that the aerial parts of *Equisetum myriochaetum* are widely used in traditional medicine to treat diabetic patients and kidney problems, with a large volume of trade (Andrade-Cetto, 1999), and due to the lack of information about its genotoxicity, it is important to evaluate the effects of this complex mixture on genetic alterations. Over the last few years, genotoxicity assays have been developed that are able to detect several genetic endpoints. The *in vivo* multiple wing/flare wing somatic assay in *Drosophila* is based on the exposure of populations of mitotically growing cells in the wing imaginal disc of larvae. If a genetic alteration takes place in one of these cells, the descendent cells will form a clone of mutant cells than can be detected as a spot in adult tissue. The test can detect the genotoxic chemical compounds that produced a loss of heterozygosis by several chromosome-breakage mechanisms, such as mitotic recombination, deletion, point mutation and chromosomal loss (Graf et al., 1984). The assay also detects the activity of promutagens, using insecticide-resistant strains that are able to biotransform some compounds to their active metabolites (Graf and van Schaik, 1992). On the other hand, the micronucleus assay provides a readily measurable index of chromosome breakage and loss, due to the fact that micronuclei originate from acentric chromosome fragments or whole chromosomes that are not in the main nuclei following DNA replication and nuclear division (Fenech, 1997). The present study aims to gain information about the genotoxicity of a phytotherapeutic extract of *Equisetum myriochaetum* in two bioassays: the *in vivo* wing spot test in *Drosophila melanogaster* and the *in vitro* human micronucleus test.

2. Methodology

2.1. Plant material and concentrations employed

Extracts of the aerial parts of *Equisetum myriochaetum* were prepared as follows: the first extract was produced using dried powdered material extracted with water by refluxing for 4 h, and then the extract was lyophilized and stored in screw-cap vials at 4 °C (WE). The second extract was prepared with dried plant material by Soxhlet extraction. Defatting with *n*-hexane (24 h) was followed by MeOH (48 h) extraction. The methanolic extract was evaporated under reduce pressure for dryness. The residue was partitioned in a mixture of CCl_4/80% MeOH 1:1. The MeOH/H_2O phase was evaporated to dryness. The residue was dissolved in *n*-BuOH/H_2O 1:1 the BuOH layer was dried by lyophilization and stored (BE) (Andrade-Cetto et al., 2000). The phytochemical composition of the extracts was controlled by HPLC as described (Andrade-Cetto et al., 2000), and compared with the original samples.

Several concentrations were prepared from the extracts that were dissolved in distilled water. The concentrations of the phytotherapeutic extract of the plant employed were 0.78 μg/ml, 1.56 μg/ml, 3.12 μg/ml, 6.25 μg/ml, 12.5 μg/ml, 25 μg/ml, 50 μg/ml and 500 μg/ml for the wing test (1.56 μg/ml is the therapeutic dose used in the treatment of diabetic patients). For the micronucleus test, the concentrations assayed were 12.5 μg/ml, 25 μg/ml, 50 μg/ml and 500 μg/ml.

2.2. Somatic mutation and recombination test (SMART)

The SMART assay was performed using two different strains of flies, both carrying visible wing genetic markers on the third chromosome: multiple wing hairs (*mwh*, 3–0.3) and flare (flr^3, 3–38). The assay is based on the induction of genetic damage that gives rise to loss of heterozygosis (LOH) of these recessive markers due to several genetic end-points: mitotic recombination, point mutation, deletion and certain types of chromosome aberration. The standard (ST) and the high bioactivation (HB) crosses were performed according to Graf et al. (1984) and Graf and van Schaik (1992). For the ST cross, flr^3/*In* (*3LR*) *TM3*, *ri* p^p *sep l*(*3*)*89Aa* bx^{34e} *e* Bd^S females were mated with *mwh*/*mwh* males; for the HB cross ORR/ORR; flr^3/*In* (*3LR*) *TM3*, *ri* p^p *sep l*(*3*)*89Aa* bx^{34e} *e* Bd^S females were mated with *mwh*/*mwh* males. The ORR strain has chromosomes 1 and 2 from a DDT-resistant Oregon line (OR-R), which constitutively over-expressed CYP450 genes.

Eggs from both crosses were collected over 8 h in culture bottles containing a solid agar base (5%, w/v) covered with a layer of live fermenting yeast supplemented with sucrose. At 72(±3) h later, larvae were washed out of the culture bottles with a solution of 20% (w/v) sucrose and seeded in plastic vials (200 larvae/vial) containing 50 mg of cellulose wetted with 0.3 ml of the test solution. Concurrent negative controls were treated with the solvent alone (distilled water). Larvae were fed on the above medium for 6 h and transferred to fresh medium for the rest of their development. After eclosion, adult flies were collected and stored in 70% (v/v) ethanol. Wings of the transheterozygous flies from both crosses were mounted on slides and coded before scoring under a magnification of 400× for the presence of cell clones showing malformed wing hairs (Graf et al., 1984).

For evaluation of the genotoxic effects recorded, the frequencies of spots per fly of a treated series were compared to its concurrent negative control series. These statistical comparisons were done using a computer program written by Zordan (unpublished data), which employs the χ^2 test for proportions and followed by a multiple-decision procedure (Frei and Würgler, 1988, 1995). Statistical analyses were done for single, large, twin and total number of spots recovered.

2.3. In vitro cytokinesis-block micronucleus assay

Four healthy, non-smoking donors (two females and two males) aged between 24 years and 25 years were recruited after giving informed consent. Between 5 ml and 7 ml of blood was collected by venipuncture into Vacutainer® tubes containing 0.3 ml of heparin as anticoagulant. Blood was taken early in the morning before breakfast. The blood sample was centrifuged at 302 × *g* in a bench-top centrifuge for 30 min at room temperature. Approximately 1 ml of the isolated lymphocytes was added to 5 ml of McCoy's 5A culture medium supplemented with 10% (v/v) fetal bovine serum (Gibco), 2.8% (w/v) phytohaemagglutinin (Gibco) and 1% (w/v) penicillin/streptomycin (Gibco). All

cultures were prepared in duplicate. Cultures were incubated at 37 °C for 24 h. Four different concentrations of the phytotherapeutic extract were added (12.5 μg/ml, 25 μg/ml, 50 μg/ml and 500 μg/ml) as well as 0.2 ml (80 ng) of mitomycin C (MMC) as positive control. Negative control tubes were treated with the solvent (water) alone. Cultures were incubated at 37 °C for another 20 h and 0.3 ml of cytochalasin B (3 mg/ml) was added to each tube. Cultures were incubated at 37 °C for another 28 h. Cells were harvested at 72 h after the initiation of the culture. The cell suspensions were centrifuged at 302 × *g* for 10 min. The supernatant was aspirated to a minimal level, cells were resuspended, and a hypotonic solution at 37 °C was added. After 5 min, the cell suspensions were centrifuged at 302 × *g* for 10 min. The supernatant was aspirated to a minimal level, and fixed in 1 ml of methanol/glacial acetic acid (3:1 v/v). Resuspended fixed cells were centrifuged for 5 min at 100.66 × *g*. One additional change of the fixative was done. The slides were air-dried for 10 min and stained with 2.5% (w/v) Giemsa (45 ml of Sorensen solution and 5 ml of Giemsa) for 3 min. Excess stain was eliminated with water. All slides were coded before scoring. Scoring was carried out in a light microscope at a magnification of 1000× (Fenech, 1997; Fenech et al., 1999, 2003; Kirsh-Volders et al., 2003).

Determination of cell proliferation was done by calculating the cytokinesis-block proliferation index with cytochalasin-B (CBPI) by the formula:

$$\text{CBPI} = \frac{M_1 + 2M_2 + 3M_3 + 4M_4 + 5M_5}{N}$$

where M is the number of cells with one (M_1, mononucleated), two (M_2, binucleated), three (M_3, trinucleated), four (M_4, tetranucleated), five (M_5, pentanucleated) nucleus, and N is the total number of cells scored. For the evaluation of genotoxicity (MN and bridges) the Kruskall–Wallis test and the difference from proportions between two populations (Z statistics) were used (statistical Program version 6.0). Student's t-test was used for cytotoxicity (CBPI). The level of significance was set at $P < 0.05$.

3. Results

3.1. Phytochemical composition, and extract yield

Extraction yield; from the boiled in the traditional way filtrated and liophilizated (15 g), resulting in 1.5 g of dry extract (WE), DER native 10:1, from the butanolic extract aerial parts (30 g) resulting in 0.7 g of extract (BE), DER native 43:1.

The results of the HPLC analysis demonstrate that the extracts (similar to the traditionally used preparation) are composed mainly of flavonol glycosides.

3.2. Toxicity

In a pilot experiment, the concentrations chosen to assess the possible toxicity of the extract ranged from 0.78 μg/ml to 3700 μg/ml for the wing assay and between 12.5 μg/ml and 500 μg/ml for the micronucleus test. No acute toxicity of the phytoextract could be determined in *Drosophila* or

Table 1
Fly spot data obtained after exposure of trans-heterozygous larvae of *Drosophila melanogaster* to different concentrations of a phytoextract from *Equisetum myriochaetum*

Cross/concentration (μg/ml)	No. of flies	Spots per fly (no. of spots) statistical diagnoses[a]				Mean *mwh* clone	Clone induction frequency	
		Small single (1–2 cells)	Large single (>2 cells)	Twin spots	Total spots		Per 10^5 cells	Per cell division
Standard cross (ST)								
Control	60	0.3 (18)	0.06 (4)	0	0.36 (22)	2.09	0.8	0
0.78	60	0.23 (14) −	0.06 (4) −	0.01 (1) i	0.31 (19) −	2	0.6	−0.1
1.56	60	0.26 (14) −	0.06 (4) −	0.01 (1) i	0.35 (21) −	2.37	0.6	−0.1
3.12	60	0.41 (25) i	0.08 (5) −	0	0.5 (30) i	1.8	1	0.3
6.25	60	0.28 (17) −	0.06 (4) −	0	0.35 (21) −	2.14	0.7	0
12.5	60	0.46 (28) i	0.03 (2) −	0.03 (2) i	0.53 (32) i	1.79	1	0.2
25	60	0.25 (15) −	0.01 (1) −	0.03 (2) i	0.3 (18) −	2.39	0.6	−0.1
50	60	0.28 (17) −	0.03 (2) −	0.03 (2) i	0.35 (21) −	1.95	0.7	0
500	60	0.48 (29) i	0.1 (6) i	0.01 (1) i	0.29 (36) i	1.89	1.2	0.5
High-bioactivation cross (HB)								
Control	60	0.25 (15)	0.05 (3)	0.01 (1)	0.31 (19)	2.11	0.6	0
0.78	60	0.36 (22) i	0.08 (5) i	0	0.45 (27) i	1.73	0.9	0.2
1.56	60	0.33 (20) i	0.05 (3) i	0	0.38 (23) i	2	0.8	0.1
3.12	60	0.23 (14) −	0.06 (4) i	0	0.3 (18) −	2.06	0.6	2
6.25	60	0.18 (11) −	0.03 (2) −	0	0.21 (13) −	2.08	0.4	−0.2
12.5	60	0.2 (12) −	0.01 (1) −	0	0.21 (13) −	1.62	0.4	−0.2
25	60	0.28 (17) i	0.01 (1) −	0	0.3 (18) −	1.56	0.6	0
50	60	0.4 (24) i	0.06 (4) i	0.01 (1) i	0.48 (29) i	1.79	1	0.3
500	60	0.25 (15) −	0.03 (2) −	0.03 (2) i	0.31 (19) −	1.89	0.6	0

[a] Statistical diagnoses according to Frei and Würgler (1988, 1995): (+) positive; (−) negative; (i) inconclusive; (w+) weakly positive.

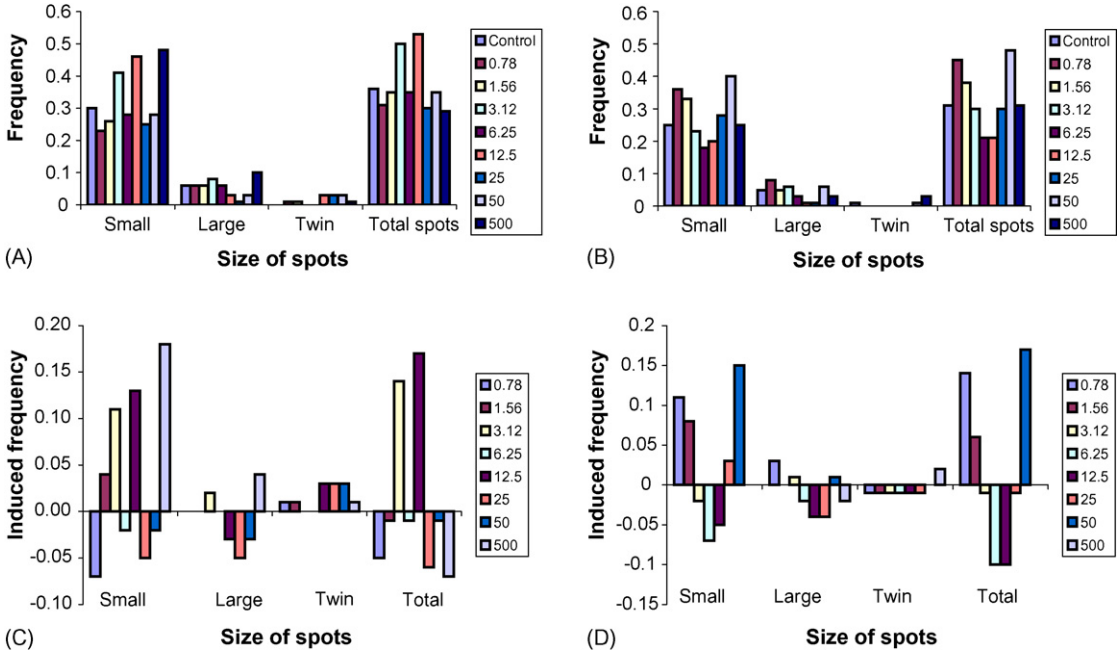

Fig. 1. (A) Frequency of spots obtained after acute treatment of ST larvae with a phytotherapeutic extract of *Equisetum myriochaetum*. (B) Frequency of spots obtained after acute treatment of HB larvae with a phtytotherapeutic extract of *Equisetum myriochaetum*. (C) Induced frequency of spots in the ST cross. (D) Induced frequency of spots in the HB cross

in human lymphocytes in culture. Therefore, different ranges of exposure concentrations were chosen for the genotoxicity experiments.

3.3. Somatic mutation and recombination test (SMART)

In the series of the wing somatic assay, the phytotherapeutic extract was assayed in three acute and independent experiments. The data from each experiment were not heterogeneous, as was shown clearly by a Kruskall and Wallis test at $P < 0.05$ (statistical Program version 6.0), so the data were pooled for statistical analysis.

Table 1 shows the pooled data recorded in the marker transheterozygous flies. The frequency of total spots per fly in the negative control of the ST cross was 0.36, and in the HB cross it was 0.31. The frequency of basal spots obtained in the ST crosses is, in general, slightly lower than those observed in the HB cross (Graf and van Schaik, 1992). The size distributions of single, large and twin spots for the negative control and the concentrations of the extract assayed are shown in Fig. 1. It should be noted that there are very few spots, and that those obtained for both crosses are small. No statistically significant difference between concurrent negative controls and the different concentrations of the phytotherapeutic extract was found in the ST cross (Fig. 1A) or in the HB cross (Fig. 1B) after acute treatment of larvae. The induced frequencies of all types of spots are shown in Fig. 1C and D.

3.4. In vitro cytokinesis-block micronucleus assay

The results obtained for the micronucleus assay are shown in Table 2.

A total of 2000 binucleated cells per concentration were analyzed for each individual (experiment and replicate). Also, the numbers of cells with one, two, three, or four nuclei were recorded. All concentrations assayed yielded a survival rate of 100%. The frequencies of micronuclei in binuclated cells obtained per donor are shown in Fig. 2A. It should be noted that, overall, the results are negative at all concentrations tested. Although in donor 2 the concentrations of 25 μg/ml and 50 μg/ml yielded a significant positive result for the frequency of MN when compared to the concurrent negative control. Lifestyle-based influences on individual variability in sensitivity to the complex mixture could explain these results. The frequencies and induced frequencies of micronucleus are shown in Fig. 2B and C. The proportion of binucleated cells was between 53% and 82%, while the CPBI varied between 1.5 and 2; thus, the phytotherapeutic extract was not cytotoxic.

4. Discussion

Ethnopharmacology involves inter- and multidisciplinary scientific research and collaboration between the investigators of pharmacology, toxicology, chemistry, anthropology and sociology (Holmstedt, 1991). Ethnopharmacological exploration has yielded highly valuable information about medicinal plants used in different cultures and many have been developed into drugs (Fabricant and Farnsworth, 2001). Thus, local and traditional knowledge has been the starting point of many successful drug development projects over the past few decades (Heinrich and Bremner, 2006).

The effects of phytotherapy are generally attributed to a plant as a whole, because phytotherapeutic preparations contain complex mixtures of active ingredients that are difficult to

Table 2
Number and distribution of micronuclei in binucleated cells, frequency of MN, distribution and frequency of cells according to the number of nuclei, percentage of binucleated cells and cytochalasin-block proliferation index obtained in the *in vitro* micronucleus assay with different concentrations of a phytotherapeutic extract of *Equisetum myriochaetum*

Donor/ sex[a]	Concentration (mg/ml)	Binucleated cells distribution of MN			Total of binucleated cells	Frequency of MN and Stat.Diag.[b]	Distribution (frequency) of cells according to the number of nuclei				Total no. of cells	Percentage of binucleated cells	CBPI
		0	1	2			Mononucleated	Binucleated	Trinucleated	Tetranucleated			
1 F	Control	2015	7	0	2022	0.003	233 (0.09)	2022 (0.82)	155 (0.06)	64 (0.03)	2474	81.73	1.99
	MMC	2008	52	1	2061	0.026	291 (0.11)	2061 (0.81)	125 (0.05)	71 (0.03)	2548	80.89	1.96
	12.5	2020	6	0	2026	0.003 −	356 (0.13)	2026 (0.76)	235 (0.09)	59 (0.02)	2676	75.71	1.98
	25	2061	10	0	2071	0.005 −	347 (0.13)	2071 (0.79)	143 (0.05)	63 (0.02)	2624	78.93	1.95
	50	2078	8	0	2086	0.004 −	258 (0.09)	2086 (0.78)	223 (0.08)	93 (0.03)	2660	78.42	2.02
	500	2018	10	1	2029	0.006 −	564 (0.21)	2029 (0.76)	51 (0.02)	19 (0.01)	2663	76.19	1.81
2 F	Control	2040	12	0	2052	0.006	409 (0.16)	2052 (0.81)	30 (0.01)	34 (0.01)	2525	81.27	1.86
	MMC	2021	73	1	2095	0.036	607 (0.22)	2095 (0.75)	46 (0.02)	39 (0.01)	2787	75.17	1.81
	12.5	2023	22	1	2046	0.012 −	786 (0.26)	2046 (0.69)	78 (0.03)	58 (0.02)	2968	68.94	1.78
	25	2049	48	11	2108	0.033 +	1912 (0.47)	2108 (0.52)	20 (0.00)	24 (0.01)	4064	51.87	1.54
	50	2173	50	4	2227	0.026 +	1076 (0.31)	2227 (0.65)	89 (0.03)	50 (0.01)	3442	64.7	1.73
	500	2031	10	0	2041	0.005 −	1840 (0.47)	2041 (0.53)	2 (0.00)	0 (0.00)	3883	52.56	1.53
3 M	Control	2133	25	0	2158	0.012	716 (0.23)	2158 (0.69)	148 (0.05)	117 (0.04)	3139	68.75	1.86
	MMC	2090	86	15	2191	0.053	728 (0.24)	2191 (0.72)	41 (0.01)	70 (0.02)	3030	72.31	1.8
	12.5	2056	15	0	2071	0.007 −	1248 (0.33)	2071 (0.56)	239 (0.06)	173 (0.05)	3731	55.51	1.78
	25	2046	12	2	2060	0.008 −	635 (0.21)	2060 (0.68)	213 (0.07)	140 (0.05)	3048	67.59	1.91
	50	2054	24	9	2087	0.020 −	489 (0.17)	2087 (0.73)	219 (0.08)	82 (0.03)	2877	72.54	1.93
	500	2119	21	2	2142	0.012 −	1295 (0.37)	2142 (0.62)	11 (0.00)	24 (0.01)	3472	61.69	1.64
4 M	Control	2045	6	0	2051	0.003	672 (0.23)	2051 (0.69)	107 (0.04)	131 (0.04)	2961	69.27	1.85
	MMC	2000	26	1	2027	0.014	750 (0.25)	2027 (0.68)	100 (0.03)	112 (0.04)	2989	67.82	1.82
	12.5	2106	4	0	2110	0.002 −	581 (0.20)	2110 (0.72)	205 (0.07)	40 (0.01)	2936	71.87	1.89
	25	2043	6	0	2049	0.003 −	569 (0.19)	2049 (0.69)	184 (0.06)	179 (0.06)	2981	68.74	1.93
	50	2006	6	0	2012	0.003 −	552 (0.18)	2012 (0.66)	207 (0.07)	266 (0.09)	3037	66.25	1.97
	500	2035	8	0	2043	0.004 −	533 (0.19)	2043 (0.75)	77 (0.03)	88 (0.03)	2741	74.53	1.87

[a] F: female; M: male.
[b] Satistical diagnoses according to a non-parametric 2×2 χ^2 tables, Fisher's exact test $P < 0.05$.

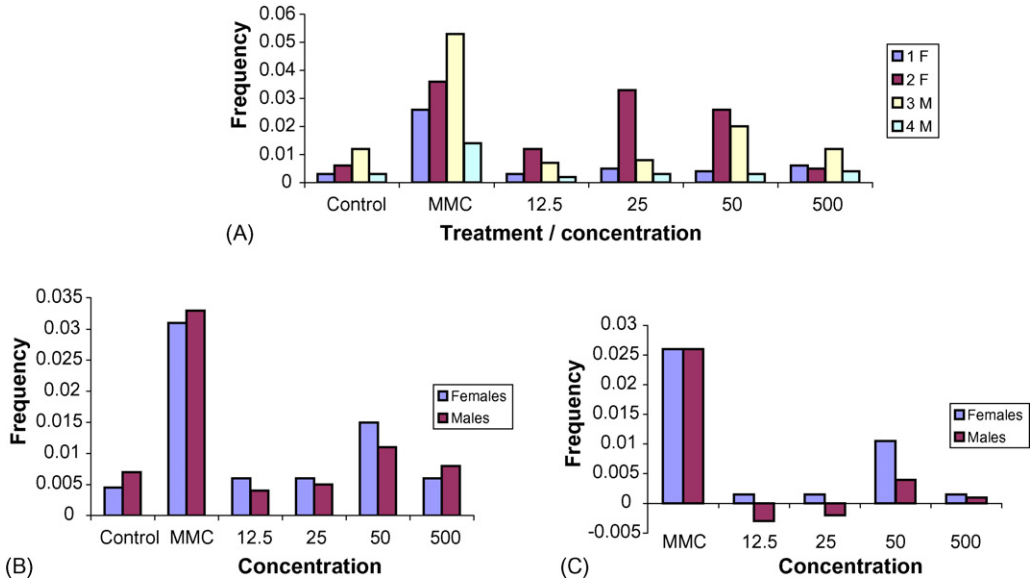

Fig. 2. (A) Frequency of micronuclei obtained in binucleated cells after exposure of human lymphocytes *in vitro* to a phyototherapeutic extract of *Equisetum myriochaetum*. (B) Frequency of micronuclei obtained by gender. (C) Induced frequency of micronuclei by gender.

characterize chemically and biologically (Maier, 2002). Pharmaceutical companies have an interest in investigating plants as sources for new lead molecular structures for the development of phytotherapeutic agents of proven efficacy, safety and quality (de Barros et al., 2005). The compounds and/or their complex mixture can be beneficial but can also be noxious or have other undesirable side-effects. Before a drug produced by the pharmaceutical industry is approved for sale on the open market, it is necessary to undertake several biological assays to assess potential toxicity and genotoxicity.

In this context, short-term genotoxicity tests can evaluate several genetic endpoints, *in vivo* and/or *in vitro*, during special stages of the life-cycle or during the cell-cycle. The wing spot assay in *Drosophila* is an efficient and versatile *in vivo* eukaryotic test that has been used during the last 20 years for the evaluation of the genotoxic activity of more than 400 different single compounds and several complex mixtures (Frei and Graf, unpublished data). The *in vitro* cytokinesis-block micronucleus assay has been employed for testing 34 compounds and is currently used as a screening test in the early stages of pharmaceutical development (Miller et al., 1998; Garriot et al., 2002).

The data obtained in the experiments assayed in this study showed that aqueous extracts of the aerial parts of *Equisetum myriochaetum* were practically non-toxic within the wide range of concentrations used in both assays. Furthermore, the phytotherapeutic extract of *Equisetum myriochaetum* did not induce somatic mutation and mitotic recombination in *Drosophila melanogaster* in both test crosses with basal (ST) or increased (HB) P450 biotransformation capacity, or micronucleus in the lymphocyte assay with concentrations ranging from 0.78 μg/ml to 500 μg/ml. This lack of genotoxicity indicates clearly that the complex mixture, at least under the experimental conditions examined, was not able to produce any chromosome breakage or loss-related effect.

Herb-based preparations may contain individual ingredients known to be toxic and even genotoxic. Among the main constituents of the phytotherapeutic extract of *Equisetum myriochaetum* are several structurally related flavonol glycosides (Wiedenfeld et al., 2000). Flavonoids are polyphenolic compounds found in edible portions of many fruits, vegetables, nuts, seeds, olive oil and red wine (Rueff et al., 1986), whose consumption has been associated with protection against heart disease and cancers (Hertog et al., 1995). Genotoxic and antigenotoxic effects of flavonoids have been reported. Kaempferol is a flavonoid that has been shown to be genotoxic to V79 cells in the absence of external metabolizing systems. The presence of an external metabolizing system, such as rat liver homogenates (S9 mix), leads to an increase in its genotoxicity, which is attributed to its biotransformation to the more genotoxic flavonoid quercetin, *via* the cytochrome P450 (CYP1A1) mono-oxygenase system (Silva et al., 1997). On the other hand, the antioxidant effect of flavonoids is well documented (Yuting et al., 1990; Yukiko et al., 1994; Stopper et al., 2005). Kaempferol reduced the DNA damage induced by mutagenic compounds in the human comet assay (Anderson et al., 1997); pre-treatment of human lymphocytes with kaempferol reduced the oxidative damage induced by hydrogen peroxide, and it has been shown that the glycogen kaempferol has a greater antioxidant capacity than that of the conjugate flavonoids (Noroozi et al., 1998). In addition to their mode of action as antioxidant, flavonoids may inhibit carcinogenesis by modulation of the metabolism of foodborne carcinogens through inhibition and/or induction of phases I and II biotransformation enzymes, and by suppression of the abnormal proliferation of early, preneoplastic lesions (Rietjens et al., 2005). In our study, we found a slightly non-significant reduction in the genotoxic effects induced by the extract, a result that can be due to chance variation alone or can be related to an antigenotoxic effect of the phytotherapeutic extract of *Equisetum myriochaetum*. We are currently doing experiments to

determine the true antioxidant and antigenotoxic properties of this complex mixture.

Other constituents of the phytotherapeutic extract are phytosterols, compounds that are structurally similar and functionally analogous to cholesterol in vertebrate animals. Phytosterols are cholesterol-lowering agents that, in the form of esters, are being incorporated into foods, such as margarine and salad dressings, in a number of countries (Moreau et al., 2002). The most abundant phytosterol in nature is β-sitosterol (Clifton, 2002), which can be oxidized by heating the plant. The β-sitosterol and β-sitosterol oxides did not induce genotoxic effects in mammalian cells in culture but caused a significant decrease in cell viability (Maguire et al., 2003).

In conclusion, our data demonstrate that, under the experimental conditions tested, the phytotherapeutic extract of *Equisetum myriochaetum* is not genotoxic either *in vivo* or *in vitro*. These results are relevant for diabetic patients, because this plant extract is very often used as a therapeutic modality to treat this metabolic incurable disease with or without biomedical medication. In the ethnopharmacological context, the results obtained are of particular relevance; the lack of toxicity and genotoxicity of a plant often used as a herbal tea (agua de uso) is important for the whole population. With this kind of study we hope to support the further development of the Mexican Legislation concerning the consumption of plants as teas and/or phytomedicines.

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