Genetics and Molecular Biology, 28, 1, 152-155 (2005)

www.sbg.org.br

Research Article

Evaluation of the genotoxic potential of the *Hypericum brasiliense* (Guttiferae) extract in mammalian cell system *in vivo*

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Abstract

Plants of the genus *Hypericum*, long used in folk medicine, contain active compounds which present, anti-septic, diuretic, digestive, expectorant, vermifugal, anti-depressive and other properties. The possible clastogenic effect of a *H. brasiliense* extract was tested in vivo on the bone marrow cells of Wistar rats. The extract was administered by gavage at doses of 50, 150 and 300 mg/kg body weight. Experimental and control animals were submitted to euthanasia 24 h after the treatment for micronucleus (MN) and chromosome preparations. *H. brasiliense* extract did not induce statistically significant increases in the average numbers of MN or chromosome aberrations in the test systems employed.

Key words: Hypericum brasiliense, micronucleus test, chromosome aberrations.

Received: March 4, 2004; Accepted: June 3, 2004.

Introduction

Herbs and botanical preparations are extensively used therapeutically in Brazil, the United States, India as well as other countries; in Europe they have been used for decades (Vogelzang, 2001). The genus *Hypericum* is represented by 350 species distributed throughout the world. Some species belonging to the Guttiferae family, may reach 12 m; others remain at 0.5 m (Robson, 1977). Some of its therapeutic properties have been described, and include actions: anti-septic, digestive, vermifugal, expectorant, etc. (Corrêa, 1979). Mitchel and Rook (1979) described its action against vitiligo. *H. perforatum* has been intensively studied and used as an anti-depressive (Suzuki *et al*., 1984; Gupta and Moller, 2003). The analgesic and anti-depressive actions of *Hypericum brasiliense* have been recently investigated (Rieli-Mendes *et al*., 2002). In Brazil, *H. brasiliense* has become popularly known as "erva de São João".

Prior to this work, *Hypericum perforatum* was the only specie from the genus *Hypericum* whose mutagenic potential had been evaluated. The genotoxicity of a standardized aqueous ethanolic *H. perforatum* extract was verified in different *in vivo* and *in vitro* test systems of mammalian cells. This extract contained hypericin and hypericin-like substances as well as flavonoids, among which quercetin in particular, has generated wide controversy regarding its mutagenic action (Okpanyi *et al*., 1990). Both in-vitro and in vivo tests using mouse cells and bone marrow cells from the Chinese hamster yielded negative results, giving completely no indication of the mutagenic potential of these extracts (Okpanyi *et al*., 1990).

The potential therapeutic use of *Hypericum brasiliense* extract and the absence of any data on their mutagenicity in eukaryotes, led to the present study, undertaken to evaluate possible clastogenic effects of this extract in terms of the induction of micronuclei and chromosome aberrations, in Wistar rat bone marrow cells in vivo.

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Material and Methods

Plant material

Plant material was collected in the mountainous region of the state of Rio de Janeiro, Brazil. A voucher specimen (n. 00237) has been deposited in the ICQBA, UNICAMP, Brazil. The extract was obtained from the leaves using as an extractor the solvent hexane, followed by ethanol percolation and standardized according to the technique described by Rocha *et al*. (1995, patent pending).

Animals and assay procedures

Experiments were carried out on six-week-old Wistar rats (*Rattus norvegicus*), weighing approximately 100 g. The animals were acquired from the Biotery of the Univer-

sity of Alfenas (UNIFENAS), kept in polyethylene boxes (n = 6), in a climate-controlled environment (25 ± 4 °C, 55 ± 5% humidity), light/dark controlled every 12 h (7 a.m. to 7 p.m.). Food and water were available *ad libitum*. Rats were divided into experimental groups of six animals each (three males and three females). The *Hypericum brasiliense* extract was administered in a single dose of 0.5 mL by gavage at concentrations of 50, 150 and 300 mg/kg body weight, chosen on the basis of the LD_{50} of 537 mg/kg as determined by Rieli-Mendes *et al.* (2002). The negative control group received distilled water and the positive control group received 30 mg of cyclophosphamide/kg. Animals were injected intraperitoneally with 0.5 mL of 0.16% colchicine 90 min before euthanasia, which occurred 24 h after experimental treatment. Both femur bones were then excised and their bone marrow flushed into test tubes using a syringe. For the micronucleus (MN) assay, the bone marrow cells were prepared as recommended by Schimid (1976). The slides were coded, fixed with methanol and stained by Giemsa solution. Two thousand polychromatic erythrocytes (PCE) from each animal were scored for MN presence. Bone marrow preparations for the analysis of chromosome aberrations in metaphase cells were obtained by the technique of Ford and Hamerton (1956). One-hundred metaphases per animal (600 metaphases per group) were analyzed in order to determine the number of chromosomal aberrations in a blind test. The chromosomal aberrations analyzed were gaps, breaks, deletions, fragments, rings and dicentric chromosomes. Gaps were not considered in the statistical analysis. The mitotic index (MI), was obtained by counting the number of mitotic cells in the 1000 cells analyzed per animal. The data obtained were submitted to the One-way analysis of variance test (ANOVA) and the Tukey-Kramer multiple comparison test using the GraphPad Instat® software (version 3.01). Results were considered statistically significant at $\alpha = 0.05$.

Results and Discussion

Tables 1 and 2 summarize the results of the analysis of micronucleus and chromosome aberrations respectively, in bone marrow cells of Wistar rats following treatment with different concentrations of the *H. brasiliense* extract and controls.

Administration of *H. brasiliense* extract did not result in a increase in the average number of polychromatic erythrocytes with micronuclei (MNPCE) (Table 1). Comparisons between different dose groups showed no significant differences between MNPCE mean numbers (Tukey-Kramer test, p 0.05).

The MI values obtained from the analysis of 1000 cells/animal for a sample of 30 animals (n = 6/group) ranged from 1.7 to 3.8% (means) and statistical analysis by the Tukey-Kramer test showed no significant differences ($p > 0.05$) between the different treatments with *H. brasiliense* extract, or between these treatments and their controls. These data indicate no cytotoxic effect of the *H. brasiliense* extract at the doses tested (Table 2).

The data obtained from 600 metaphases analysed per treatment (100 metaphase cells/animal), also showed that there were no statistically significant differences between the mean number of chromosome aberrations of treated groups and of the negative control. The most frequent types of aberrations of the treated groups were chromatid gaps, chromatid breaks and deletions.

The extract of the leaves and flowers of *H. brasiliense* was characterized by Rocha *et al.* (1995), who found, three known phloroglucinols (japonicine A, uliginosin A and isouliginosin B), a new phloroglucinol (hyperbrasiol A), and the flavonoids kaempferol, luteolin, quercitrin, isoquercitrin, hyperoside and guaijaverin. According to the authors, all four phloroglucinols presented antibacterial action against *Bacilus subtilis*. More recently, Rieli-Mendes *et al.* (2002), studying hydroalcoholic extracts from *H. brasiliense* and *H. cordatum*, observed that both species showed generalized depressant action on the central nervous system suggesting possible analgesic action.

A number of potentially positive health effects, including anti-cancer and anticarcinogenic effects, have been ascribed to the flavonoids, similar to that found in *H. brasiliense* extract, based on *in vitro* and *in vivo* studies

Table 1 - Mean of polychromatic erythrocytes with micronuclei (MNPCE) observed in bone marrow cells of female (F) and male (M) Wistar rats treated with a *Hypericum brasiliense* extract, and respective controls.

Treatments	Dose mg/kg	Number of MNPCE per animal						MNPCE (Mean ± SEM)
		F_1	F_2	F_3	M_1	M_2	M_3	
Negative control (Water)	0	9	10	9	10	11	8	9.50 ± 0.43
Hypericum extract	50	9	10	7	9	8	10	8.83 ± 0.48
Hypericum extract	150	9	10	9	8	9	9	9.00 ± 0.25
Hypericum extract	300	9	11	12	9	14	11	11.00 ± 0.77
Positive control (Cyclophosphamide)	30	29	23	22	27	25	25	25.17* ± 1.05

Two thousand cells were analyzed per animal, for a total of 12000 cells per group. SEM = standard error of the mean.
* Significantly different from negative control ($p < 0.05$).

Table 2 - Mitotic Index (MI) and distribution of the different types of chromosomal aberrations (CA) observed in female (F) and male (M) Wistar rat bone marrow cells treated with a *Hypericum brasiliense* extract, and respective controls.

Treatments	Sex	MI (%)	Chromosomal aberrations					Total (CA) without gaps
			Gaps		Breaks			
			C	IC	C	IC	OA	
Negative control	F_1	3.4	4	0	3	1	1del	5
(Water)	F_2	2.2	3	0	1	0	2del	3
	F_3	3.8	0	1	1	1	3del	5
	M_1	2.0	1	1	1	0	0	1
	M_2	3.2	1	2	2	0	3del	5
	M_3	2.8	1	0	1	0	3del	4
	mean ± SEM	2.90 ± 0.28						3.83 ± 0.65
H. brasiliense extract	F_1	4.2	3	1	1	0	3del/1r	4
(50 mg/kg)	F_2	2.4	1	0	1	1	3del	5
	F_3	5.2	1	0	1	0	1del	2
	M_1	5.5	6	3	3	2	4f	9
	M_2	3.3	2	0	2	0	2f/1dic	4
	M_3	2.2	0	0	0	0	1del/1r	1
	mean ± SEM	3.80 ± 0.57						4.16 ± 1.13
H. brasiliense extract	F_1	2.1	4	0	0	0	1del	1
(150 mg/kg)	F_2	2.3	4	2	1	0	2del	3
	F_3	2.3	3	1	2	0	3del	5
	M_1	4.1	2	0	6	1	2del	9
	M_2	2.8	1	0	1	0	1del	2
	M_3	2.5	4	5	1	0	0	1
	mean ± SEM	2.68 ± 0.29						3.50 ± 1.25
H. brasiliense extract	F_1	2.8	4	0	3	0	2del	5
(300 mg/kg)	F_2	2.9	3	0	1	0	1del	2
	F_3	2.7	2	2	0	0	1del	1
	M_1	2.4	2	0	3	0	3del	6
	M_2	2.2	0	0	7	0	1del	8
	M_3	2.3	5	1	0	0	1del	1
	mean ± SEM	2.55 ± 0.11						3.83 ± 1.19
Positive control	F_1	1.2	16	0	17	0	8del	25
(Cyclophosphamide)	F_2	1.0	8	1	13	1	8del/1r	22
(30 mg/kg)	F_3	1.4	9	0	8	0	9del	17
	M_1	2.7	9	1	12	1	7del	20
	M_2	2.0	10	3	9	1	14del	24
	M_3	2.0	9	1	12	0	8del	20
	mean ± SEM	1.71 ± 0.25						21.33* ± 1.20

One hundred cells were analyzed per animal, for a total of 600 cells per treatment. C, Chromatid-type; IC, isochromatid-type; OA, other aberrations: del = deletion; f = fragments; r = ring; dic = dicentric; SEM = standard error of the mean. * Significantly different from negative control ($p < 0.001$).

both in humans and in animals (Cody *et al.*, 1986; 1988; Sudheesh *et al.*, 1999). Chacon *et al.* (2002) observed an absence of genotoxic effects of a standardized extract of the medicinal plant *Solanum melongena*, rich in flavonoids, on peripheral blood and bone marrow cells of Wistar rats and Ferreira *et al.* (2003) related that the same extract presented protective effects against chromosomal aberrations induced by doxorubicin in Wistar rat cells.

The genotoxic effect of *H. brasiliense* extract on the bone marrow of Wistar rats was studied for the first time in the present work. The results indicated that the mixture of the compounds found in these extract did not induce a sig-

nificant increase in the mean number of cells with micronuclei or chromosome aberrations when given at the doses of 50, 150 and 300 mg/kg body weight. Although the results of the present study do not preclude the therapeutic consumption of *H. brasiliense* extract, caution regarding the indiscriminate use by the public of these, and other medicinal plants, continues to be necessary.

Acknowledgments

This investigation was supported by FAPEMIG (Rede Mineira de Ensaios Toxicológicos e Farmacológicos de Produtos Terapêuticos, EDT - 1879/02), CNPq and UNIFENAS.

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Associate Editor: Catarina S. Takahashi