



No clastogenic activity of *Caesalpinia ferrea* Mart. (Leguminosae) extract on bone marrow cells of Wistar rats

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Abstract

Extracts of the fruits of *Caesalpinia ferrea* Mart. (Leguminosae) are widely consumed in folkloric medicine in Brazil and several other countries without any genetic toxicity evaluation. In this study we investigated, the clastogenic and cytotoxic potential of the crude aqueous extract of the fruits of *C. ferrea* in Wistar rat bone marrow cells using the micronucleus and chromosomal aberration test systems. The animals were treated by gavage with 3 concentrations of the extract, 500, 1000 and 1500 mg/kg, and cyclophosphamide 30 mg/kg. Bone marrow cells were collected 24 h after the treatment. There was no statistically significant difference in the mean of micronucleated polychromatic erythrocytes (MNPCE), mean number of chromosomal aberrations or mitotic index (MI) for the 3 concentrations compared with negative control suggesting that the crude aqueous extract from the fruits of the *C. ferrea* has no clastogenic and cytotoxic effect in Wistar rat bone marrow cells.

Key words: *Caesalpinia ferrea*; micronucleus test; chromosome aberrations.

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Many plant extracts and their active principles have been described and utilized as therapeutic agents. There is considerable interest in determining the risks that these products may pose to health, because many of these plants contain compounds which are known to cause diseases or even death in animals and humans. Thus, an assessment of their cytotoxic and mutagenic potential is necessary to ensure a relatively safe use of medicinal plants (Surh and Ferguson, 2003).

Caesalpinia ferrea Mart. (Leguminosae) popularly known as "Pau-Ferro" is a large tree that grows throughout Brazil, primarily in Pernambuco and Ceará (Alzugaray, 1984). Some of its therapeutic properties have been described, and include treatment of wounds and bruises, alleviation of chronic cough and asthma (Braga, 1976). The root has been used as an antipyretic agent and the fruit has been used for treatment of diabetics. An infusion of the stem bark has been used for treating enterocolitis and diarrhea (Balbach, 1972). Bacchi and Sertie (1988) described the anti-gastric ulcer effect of an aqueous crude extract of *C. ferrea*, and its anti-inflammatory and analgesic activity also was reported (Thomas *et al.*, 1988; Carvalho *et al.*, 1996).

In this study, three different concentrations of a crude aqueous extract of the fruits of *C. ferrea* were tested for

acute clastogenicity *in vivo* in Wistar rat bone marrow cells, using the chromosome aberration analysis and, as a comparison parameter the micronucleus test.

Plant material was collected in the Icoaracy region, Belém, Pará, Brazil. A voucher specimen (no. 3221) collected by Dr. Antonio Barioni Guzman has been deposited in the herbarium (SPFR) of the Biology Department of the University of São Paulo, Campus Ribeirão Preto, São Paulo, Brazil.

The air-dried fruits were ground and 100 g of the powder was macerated with water at 60 ± 10 °C for 2 h. After filtration, the extract was lyophilized to yield 27.2 g of crude aqueous extract.

Experiments were carried out on six-week-old Wistar rats (*Rattus norvegicus*), weighing approximately 100 g. The animals were acquired from the animal house of the University of Alfenas (UNIFENAS), kept in polyethylene boxes ($n = 6$), in a climate-controlled environment (25 ± 4 °C, $55 \pm 5\%$ humidity) with a 12 h, light/dark cycle (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). *Caesalpinia ferrea* extract was administered in a single dose of 0.5 mL by gavage at concentrations of 500, 1000 and 1500 mg/kg body weight, chosen on the basis of the LD₅₀ of 2200 mg/kg as determined by Carvalho (1993). 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 excised and the bone marrow flushed into test tubes using a syringe. For the micronucleus (MN) assay, the bone marrow cells from one femur were prepared as recommended by Schmid (1976). The slides were coded, fixed with methanol and stained with Giemsa solution. Two thousand polychromatic erythrocytes (PCE) from each animal were scored for micronucleus (MN) presence. Bone marrow preparations from the other femur were used for the analysis of chromosome aberrations in metaphase cells 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. Since the cytogenetic significance of the gaps still is not well established, the statistical analyses were made including and excluding gaps. The mitotic index (MI), was obtained by counting the number of mitotic cells in the 1000 cells analyzed per animal. The Animal Bioethical Committee of the UNIFENAS approved the present study on January 10, 2004. The data obtained were submitted to the One-way analysis of variance test (ANOVA) and the Tukey-Kramer multiple comparisons test using the GraphPad Instat[®] software (version 3.01). Results were considered statistically significant at $p < 0.05$.

Table 1 shows the micronucleus test results obtained for female and male Wistar rats treated with *C. ferrea* extract: the number of micronucleated polychromatic erythrocytes (MNPCE) of each animal and means, for untreated controls and treated animals. The clastogenicity test revealed no enhancement in the mean number of micronucleated polychromatic erythrocytes at any of the tested doses. In contrast, as was expected, cyclophosphamide administered intraperitoneally as positive control at 30 mg/kg showed a significant increase in the mean number of micronuclei ($p < 0.001$).

Table 2 shows data on the induction of chromosome aberrations (CA) in dividing bone marrow cells of Wistar rats following in vivo exposure to different doses of *C. ferrea* extract. The table shows the Mitotic Index (MI) and number of different types of CA, mean CAs/treatment including and excluding gaps, for a total of 600 metaphases per dose.

The MI values obtained from the analysis of 1000 cells/animal for a sample of 30 animals ($n = 6/\text{group}$) ranged from 1.85 to 2.65% (means) and statistical analysis by the Tukey-Kramer test showed no significant differences ($p > 0.05$) between the different treatments with *C. ferrea* extract, or between these treatments and their controls. These data indicate that *C. ferrea* extract has no cytotoxic effects at the tested doses (Table 2).

Treatment of mice infected with *Listeria* and tumor-bearing mice with 500 and 1000 mg/kg of *C. ferrea* extract significantly stimulated myelopoiesis, whereas no effects were observed with 250 mg/kg dose. Similar results were obtained in normal mice (Queiroz et al., 2001). The authors demonstrate that *C. ferrea* extract acts as a positive regulator of white cells, and suggest that therapeutic effects of *C. ferrea* may be partially mediated by this action. In the present work, *C. ferrea* extract did not stimulate cell division.

The anti-tumor promoting effects of fruits of *C. ferrea* were tested in the *in vitro* Epstein-Barr virus early antigen and showed potent inhibitory activity (Nakamura et al., 2002a). Gallic acid and methyl gallate isolated from fruits of *C. ferrea* decreased significantly the average number of papilloma mouse showing chemopreventive effects against skin carcinogenesis (Nakamura et al., 2002b).

The data obtained from 100 metaphase cells/animal also showed that there were no statistically significant differences between the mean number of chromosome aberrations (including and excluding gaps) of treated groups and of the negative control ($p > 0.05$). The most frequent types of aberrations of the treated groups were chromatid breaks, chromatid gaps and deletions.

The crude aqueous extract of the fruits of *C. ferrea* was characterized by Carvalho (1993). Contains alkaloids,

Table 1 - Polychromatic erythrocytes with micronuclei (MNPCE) observed in bone marrow cells of female (F) and male (M) Wistar rats treated with a *Caesalpinia ferrea* extract, and respective controls. Two thousand cells were analyzed per animal, for a total of 12000 cells per group. SEM = standard error of the mean.

Treatments	Dose (mg/kg)	Number of MNPCE per animal						MNPCE (Mean \pm SEM)
		F ₁	F ₂	F ₃	M ₁	M ₂	M ₃	
Negative control (water)	0	1	0	0	2	0	1	0.66 \pm 0.33
<i>C. ferrea</i> extract	500	0	4	1	0	1	0	1.00 \pm 0.63
<i>C. ferrea</i> extract	1000	3	0	0	0	0	1	0.66 \pm 0.49
<i>C. ferrea</i> extract	1500	0	0	0	1	2	2	0.83 \pm 0.40
Positive control (Cyclophosphamide)	30	7	5	5	5	6	7	5.83* \pm 0.40

* Significantly different from negative control ($p < 0.001$).

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 *Caesalpinia ferrea* extract, and respective controls. 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; r = ring; SEM = standard error of the mean.

Treatments	Sex	MI (%)	Chromosomal aberrations					Total (CA) without gaps	Total (CA) with gaps
			Gaps		Breaks		OA		
			C	IC	C	IC			
Negative control (water)	F ₁	2.2	3	1	1	0	0	1	5
	F ₂	3.4	1	1	2	0	1 del	3	5
	F ₃	2.8	2	0	1	0	0	1	3
	M ₁	1.6	2	0	1	0	0	1	3
	M ₂	2.9	3	0	1	0	0	1	4
	M ₃	3.0	3	0	1	0	1 del	2	5
	mean ± SEM	2.65 ± 0.26						1.5 ± 0.34	4.16 ± 0.40
<i>C. ferrea</i> extract (500 mg/kg)	F ₁	1.9	2	0	1	0	0	1	3
	F ₂	1.5	0	0	1	0	0	1	1
	F ₃	2.3	0	1	2	1	0	3	4
	M ₁	2.3	2	0	1	0	0	1	3
	M ₂	2.8	2	0	1	0	0	1	3
	M ₃	3.1	1	0	0	0	0	0	1
	mean ± SEM	2.32 ± 0.24						1.1 ± 0.40	2.5 ± 0.50
<i>C. ferrea</i> extract (1000 mg/kg)	F ₁	1.6	0	0	1	0	0	1	1
	F ₂	1.2	1	0	0	0	0	0	1
	F ₃	1.7	0	0	1	0	1 del/ 1 r	3	3
	M ₁	1.8	0	0	2	0	0	2	2
	M ₂	2.8	2	0	2	0	1 del/ 2 r	5	7
	M ₃	2.0	0	0	3	2	5 del/ 1 r	11	11
	mean ± SEM	1.85 ± 0.22						3.6 ± 1.62	4.17 ± 1.64
<i>C. ferrea</i> extract (1500 mg/kg)	F ₁	2.4	1	0	0	0	0	0	1
	F ₂	1.2	0	0	1	0	0	1	1
	F ₃	1.6	0	0	1	0	0	1	1
	M ₁	1.7	1	0	1	0	0	1	2
	M ₂	1.6	1	0	2	0	0	2	3
	M ₃	2.6	1	0	1	0	1 del	2	3
	mean ± SEM	1.85 ± 0.22						1.2 ± 0.31	1.83 ± 0.40
Positive control Cyclophosphamide (30 mg/kg)	F ₁	1.4	3	0	12	0	2 del	14	17
	F ₂	1.6	2	2	4	1	0	5	9
	F ₃	1.8	2	0	12	0	3 del	15	17
	M ₁	1.8	6	1	14	4	9 del	27	34
	M ₂	2.5	6	7	12	3	6 del	21	34
	M ₃	2.4	2	3	14	4	8 del	26	31
	mean ± SEM	1.92 ± 0.18						18.0* ± 3.4	23.6* ± 4.36

* Significantly different from negative control ($p < 0.001$).

anthraquinones, sugars, depsides, depsidones, flavonoids, tannins, saponins, sesquiterpene lactones and triterpenes. Tannins are the majority compounds of the extract and can be considered responsible by the activity against diabetics (Balbachas, 1988; Carvalho, 1993). The analgesic and anti-inflammatory effects of the crude extract were confirmed in rodents (Carvalho *et al.*, 1996). Other parts of this plant

have been used for other diseases in popular medicine, but their efficiencies need to be further investigated.

The crude aqueous extract from fruits of *C. ferrea* had its clastogenic potential evaluated for the first time in the present work. The results indicated that the mixture of compounds found in these extracts did not induce a significant increase in the mean number of cells with micronuclei or

chromosome aberrations when given at the doses of 500, 1000 and 1500 mg/kg body weight, respectively. Thus, these data permit us to conclude that, under the experimental conditions employed in the present study, the *C. ferrea* extract had no clastogenic effect in Wistar rat bone marrow cells.

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