Evaluation of the genotoxic potential of *Austroplenckia populnea* (Reiss) Lundell chloroform fraction from barkwood extract in rodent cells in vivo

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Abstract

The genotoxic effect of the *Austroplenckia populnea* chloroform fraction from barkwood extract was tested in vivo on peripheral blood cells of Swiss mice with the comet assay (SCGE), and the clastogenic effect was investigated on peripheral blood cells of Swiss mice and bone marrow cells of Wistar rats, with the micronucleus and chromosome aberrations tests. The animals were treated by gavage with 3 concentrations of the extract: 300, 600 and 900 mg.kg^{-1}. Peripheral blood cells of Swiss mice were collected 4 and 24 hours after the treatment to the SCGE assay and 48 and 72 hours to the micronucleus test. Bone marrow cells of Wistar rats were collected 24 hours after the treatment to the micronucleus and chromosome aberration tests. The results showed that the *A. populnea* chloroform fraction induced an increase in the average number of DNA damage in peripheral blood cells at the three concentrations tested, but this increase was not statistically significant. In the micronucleus and chromosome aberrations test, no significant increase was observed in the mean number of micronucleated polychromatic erythrocytes (MNPCE) of Swiss mice or MNPCE or chromosome aberrations for the rat bone marrow cells, for any of the tested doses. Our findings enable us to conclude that by the comet assay, *A. populnea* chloroform fraction from barkwood extract showed no genotoxic effects, and by the micronucleus and chromosome aberration tests, the extract fraction showed no clastogenic/aneugenic effects on the rodent cells tested.

Keywords: *Austroplenckia populnea*, celastraceae, micronucleus test, single cell gel electrophoresis, chromosome aberrations.

Avaliação do potencial genotóxico da fração clorofórmica de cascas do caule de *Austroplenckia populnea* em células de roedores in vivo

Resumo

O possível efeito genotóxico da fração clorofórmica de cascas de caule de *Austroplenckia populnea* foi testado in vivo em células do sangue periférico de camundongos Suíços pelo ensaio cometa, e o seu possível efeito clastogênico foi investigado em células de sangue periférico de camundongos Suíços e células da medula óssea de ratos Wistar, respectivamente pelos testes do micronúcleo e de aberrações cromossômicas. Os animais foram tratados por via oral com três concentrações do extrato: 300, 600 e 900 mg.kg^{-1} de peso corpóreo. Células do sangue periférico dos camundongos foram coletadas 4 e 24 horas após o tratamento para a realização do ensaio cometa, e 48 e 72 horas para o teste do micronúcleo. Células da medula óssea dos ratos Wistar foram coletadas 24 horas após o tratamento para os testes do micronúcleo e de aberrações cromossômicas. Os resultados mostraram que a fração clorofórmica do extrato de *A. populnea* induziu um pequeno aumento no número médio de danos ao DNA nas células sanguíneas nas três concentrações testadas, mas tal aumento não foi estatisticamente significativo. Nos testes do micronúcleo e de aberrações cromossômicas não houve um aumento significativo no número médio de eritrócitos policromáticos micronucleados (EPM) dos camundongos, bem como nos EPM e aberrações cromossômicas nas células da medula óssea dos ratos, em

nenhuma das doses testadas. Nossos resultados nos permitem concluir que, pelo ensaio cometa, a fração clorofórmica de cascas do caule de *A. populnea* não apresentou efeito genotóxico, e, pelos testes do micronúcleo e de aberrações cromossômicas, o extrato não mostrou efeitos clastogênicos e/ou aneugênicos nas células analisadas dos roedores.

Palavras-chave: Austroplenckia populnea, celastraceae, teste do micronúcleo, ensaio cometa, aberrações cromossômicas.

1. Introduction

There is considerable interest in determining the risks that plant extracts may pose to health, since many of these extracts contain compounds known to cause diseases or even death to animals and humans by acting as natural mutagens and carcinogens (Araújo et al., 1999; Burim et al., 1999; Chacon et al., 2002; Espósito et al., 2005, Maistro et al., 2005).

Austroplenckia populnea (Reiss) Lundell is a plant of the Brazilian Cerrado or Savannah region, which belongs to the Celastraceae family. This botanical family includes several plant species that have been widely used in traditional medicine for their antiulcerogenic, analgesic, male antifertility and antiinflammatory properties, among others (Corrêa, 1985). *A. populnea* is commonly known in Portuguese as "marmelinho do campo, mangabeira-brava, mangabarana" and "vime", and is a folk medicine used as an anti-dysenteric and anti-rheumatic (Corrêa, 1985). Antiulcerogenic and analgesic effects of the leaf extracts have been reported in mice (Seito et al. 2002), and Mazaro et al. (2000) reported a decrease in sperm count following treatment of rats with this extract. Studies involving chemical aspects of leaf preparations revealed the presence of friedelane and oleanane triterpenes and sesquiterpenes (Vieira-Filho et al., 2001).

The phytochemical study of the *A. populnea* crude barkwood extract resulted in isolation and/or identification of steroids (stigmasterol, campesterol, β-sitosterol) and triterpenes (populnoic acid, methyl populnonate, epitaraxerol, β-amirine, lupeol acetate, β-friedalanol and friedelin) (Andrade, 2005). These mixed compounds showed anti-inflammatory, analgesic and antiulcer activities, but their hexane, chloroform and ethyl acetate fractions showed a decrease in these activities (Andrade, 2005).

Zanoni et al. (2005) evaluated, for the first time, the mutagenic potential of *A. populnea* extract and observed that crude hydroalcoholic barkwood extract showed a slight but statistical significant clastogenic effect in the bone marrow cells of Wistar rats. On the other hand, *A. populnea* leaf extract did not induce clastogenicity in the bone marrow cells of Wistar rats (Pugliesi et al., 2007).

Considering the phytotherapeutic potential of the *A. populnea* extracts and the mutagenic antecedent of one barkwood extract, we are developing several studies to further evaluate the genotoxicity of some *A. populnea* extracts/fractions. In this report we determined the mutagenic potential of the *A. populnea* chloroform fraction from crude barkwood extract in peripheral blood and bone marrow cells in vivo in rodents, using the Single Cell Gel Electrophoresis (SCGE), micronucleus and chromosome aberration tests.

2. Material and Methods

2.1. Plant material

The plant material of *Austroplenckia populnea* was collected in the Cerrado, at Botucatu, in the state of São Paulo, Brazil. The plant was identified, and a voucher specimen (n° 20415) was deposited in the BOTU herbarium of the Bioscience Institute of Paulista State University (IB, UNESP). The hydro-alcohol extract was obtained from the bark wood (3,248.55 g), which was dried and pulverized, to yield 405.77 g, and cold macerated with 96% ethanol for 48 hours (three times). The final filtrate was concentrated (rota-evaporator) to yield the hydro-alcoholic extract. The hydro-alcoholic (EtOH) crude extract was partitioned between hexane, CHCl$_3$ and ethyl acetate (EtOAC), to yield 8.0, 9.5 and 98.17 g of fractions, respectively.

2.2. Chemicals

The agent N-nitroso-N-ethylurea (ENU, CAS No. 759-73-9) was used as the DNA damaging agent in the SCGE and micronucleus assays using Swiss mice. It was dissolved in phosphate-buffer at pH 6. The alkylating agent cyclophosphamide (CPA, CAS: C 0768, Sigma) was used as the clastogenic agent in the micronucleus and chromosome aberrations assay using Wistar rats. The other principal chemicals used were obtained from the following suppliers: normal melting point (NMP) agarose (Cat. No. 15510-019: Invitrogen); Low melting point (LMP) agarose (Cat. No. 15517-014: Invitrogen); Sodium salt N-lauroyl sarcosine (L-5125: Sigma) and EDTA (Merck).

2.3. Animals and assay procedures

Experiments were carried out using six-week-old male Wistar rats (*Rattus norvegicus*), weighing approximately 100 g, and twelve-week-old male albino Swiss mice (*Mus musculus*) weighing 25-30 g. The animals were acquired from the animal house of the Jose do Rosario Vellano University (UNIFENAS), and kept in polyethylene boxes (*n* = 6), in a climate-controlled environment (25 ± 4 °C, 55 ± 5% humidity) with a 12 hours, light/dark cycle (7:00 AM to 7:00 PM). Food (LABINA – PURINA) and water were available ad libitum. The rats and mice were divided into experimental groups of six

animals each. *Austroplenckia populnea* chloroform fraction was administered at a single dose of 0.5 mL by gavage, at concentrations of 300, 600 and 900 mg.kg⁻¹ body weight, selected on the basis of acute toxicity studies in mice using *A. populnea* leaf extract (Seito et al. 2002). The negative control groups (both mice and rats) received distilled water. The positive control group using Swiss mice received 50 mg of N-nitroso-N-ethylurea/kg (ENU), and the Wistar rats received 50 mg of cyclophosphamide/g. The rats were injected intraperitoneally with 0.5 mL of 0.16% colchicine 90 minutes, prior to euthanasia 24 hours after the experiments. 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. 2 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, using the technique of Ford and Hamerton (1956). 100 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 were classified according to Savage (1976), as gaps, breaks, deletions, fragments, rings and dicentric chromosomes. Since the cytogenetic significance of the gaps is still not well established, the statistical analyses were carried out excluding gaps. The mitotic index (MI) was obtained by counting the number of mitotic cells in the 1,000 cells analyzed per animal. For the micronucleus (MN) assay on Swiss mice, peripheral blood was collected from the orbital vein, 48 and 72 hours after the treatment, then blood smear slides were prepared. All the slides were coded, fixed with methanol and stained with Giemsa solution. For micronucleus (MN) presence, two thousand polychromatic erythrocytes were scored from each Wistar rat and four thousand peripheral erythrocytes from each Swiss mouse (2,000 cells from the 48 hours blood sample and 2,000 cells from the 72 hours blood sample) (MacGregor et al., 1980).

The Single cell gel electrophoresis test (SCGE) was carried out using the method described by Speit and Hartmann (1999), which is based on the original work of Singh et al. (1988) and includes modifications introduced by Klaude et al. (1996), with some additional modifications. 4 and 24 hours after the treatment, peripheral blood cells from Swiss mice were taken. A 10 μL aliquot of the peripheral blood was mixed with 120 μL of 0.5% low melting point agarose at 37 °C, and rapidly spread onto microscope slides pre-coated with 1.5% normal melting point agarose. Coverslips were added and the slides were allowed to gel at 4 °C, for 20 minutes. The coverslips were gently removed and the slides were then immersed in cold, freshly prepared lysing solution consisting of 89 mL of a stock solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, pH set to 10.0 with ~8 g

solid NaOH, 890 mL of distilled water and 1% sodium lauryl sarcosine), plus 1 mL of Triton X-100 (Merck) and 10 mL of DMSO. The slides, which were protected from light, were left to stand at 4 °C for 1 hour and then placed in the gel box, positioned at the anode end, and left in a high pH (>13) electrophoresis buffer (300 mM NaOH per 1 mM EDTA, prepared from a stock solution of 10 N NaOH and 200 mM, pH 10.0, EDTA) at 4 °C for 20 minutes before electrophoresis, to allow the DNA to unwind. The electrophoresis run was performed in an ice bath (4 °C) for 20 minutes at 25 V and 300 mA. The slides were then submerged in a neutralization buffer (0.4 M Tris-HCl, pH 7.5) for 15 minutes, dried at room temperature and fixed in 100% ethyl alcohol for 10 minutes. The slides were dried and stored at least overnight before staining. For the staining process, the slides were briefly rinsed in distilled water, covered with 30 μL of 1× ethidium bromide staining solution prepared from a 10× stock (200 μg.mL⁻¹), and covered with a coverslip. The material was evaluated immediately at 400× magnification, using a fluorescence microscope (Nikon) with a 515-560 nm excitation filter and a 590 nm barrier filter. The Animal Bioethical Committee of the UNIFENAS, Brazil, approved the present study on November, 2004 (protocol number 11A/2004), in accordance with the Federal Government legislation on animal care.

2.4. Scoring procedures and data evaluation

The extent and distribution of DNA damage indicated by the SCGE assay was evaluated by examining at least 200 randomly selected and non-overlapping cells on the slides per each animal. These cells were visually scored according to tail size, into four classes, as follows: (1) class 0: undamaged, with no tail; (2) class 1: with a tail shorter than the diameter of the head (nucleus); (3) class 2: with a tail length of 1 to 2× the diameter of the head; and (4) class 3: with a tail longer than 2× the diameter of the head. Comets with no heads and images with nearly all DNA in the tail, or with a very wide tail, were excluded from evaluation because they probably represent dead cells (Hartmann and Speit 1997). The total score for 200 comets was obtained by multiplying the number of cells in each class by the damage class, ranging from 0 (all undamaged) to 600 (all maximally damaged).

2.5. Statistical Analysis

The data obtained from the micronucleus and SCGE assays were submitted to One-way analysis of variance test (ANOVA) and the Tukey-Kramer multiple comparison test (Sokal and Rohlf 1995), using the GraphPad Instat® software (version 3.01). The results were considered statistically significant at $p < 0.05$.

3. Results

Table 1 shows the effects of a 4 and 24 hours treatment with the extract on DNA migration in peripheral blood leukocytes from Swiss mice in the comet as-

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Table 1. DNA migration in the comet assay for the assessment of genotoxicity of *Austroplenckia populnea* chloroform fraction in peripheral blood leukocytes (collected 4 and 24 hours after the treatment) from male Swiss mice (n = 6 per group) in vivo.

Treatments	Comet class (Mean ± SD)					Scores
	Total[1]	0	1	2	3	
Control						
4 hours	12.1 ± 2.4	187.8 ± 2.48	11.3 ± 2.16	0.8 ± 0.40	0.0	13.0 ± 2.8
24 hours	11.3 ± 3.5	188.6 ± 3.55	11.0 ± 3.34	0.3 ± 0.81	0.0	11.6 ± 3.9
A. populnea (300 mg.kg^{-1})						
4 hours	20.5 ± 3.9	179.5 ± 3.93	17.8 ± 3.60	2.6 ± 1.50	0.0	23.1 ± 4.7
24 hours	18.5 ± 7.7	181.5 ± 7.74	18.6 ± 7.65	0.3 ± 0.51	0.0	19.0 ± 8.0
A. populnea (600 mg.kg^{-1})						
4 hours	16.3 ± 7.3	183.6 ± 7.36	15.3 ± 6.31	0.8 ± 0.98	0.16 ± 0.40	17.5 ± 8.8
24 hours	15.0 ± 7.3	185.0 ± 7.37	14.1 ± 7.52	0.6 ± 0.81	0.16 ± 0.40	16.3 ± 7.3
A. populnea (900 mg.kg^{-1})						
4 hours	20.3 ± 4.7	179.6 ± 4.76	19.5 ± 5.08	0.6 ± 0.81	0.16 ± 0.40	21.3 ± 4.6
24 hours	17.8 ± 9.6	182.1 ± 9.64	17.6 ± 9.87	0.0	0.16 ± 0.40	18.1 ± 9.2
ENU (50 mg.kg^{-1})						
4 hours	180.5** ± 5.3	19.5** ± 5.3	166.0** ± 9.01	12.8** ± 4.4	1.6** ± 1.03	196** ± 4.6
24 hours	158.6** ± 13	41.3** ± 13.5	149.0** ±13.2	8.3** ± 1.36	1.3** ± 0.81	170** ± 12.9

*Significantly different from the negative control (p < 0.05); **Significantly different from the negative control (p < 0.001); and [1]Total number of damaged cells (class 1 + 2 + 3)

Table 2. Number of micronucleated polychromatic erythrocytes (MNPCEs) observed in bone marrow cells of male (M) Wistars rats and in the peripheral blood cells of male (M) Swiss mice treated with an *Austroplenckia populnea* chloroform fraction, and respective controls. For each time period (24, 48 and 72 hours) 2000 cells were analysed. SDM = standard deviation of the mean.

Treatments	Animal species	Cell collect time	Number of MNPCEs per Animal						MNPCE (Mean ± SDM)
			M1	M2	M3	M4	M5	M6	
Control	Wistar rat	24 hours	5	3	2	5	7	3	4.1 ± 1.83
	Swiss mice	48 hours	1	1	1	0	2	3	1.3 ± 1.0
	Swiss mice	72 hours	1	2	0	1	1	0	0.8 ± 0.75
A. populnea (300 mg.kg^{-1})	Wistar rat	24 hours	3	3	4	2	2	4	3.0 ± 0.89
	Swiss mice	48 hours	2	2	1	1	2	2	1.6 ± 0.51
	Swiss mice	72 hours	2	1	1	3	2	0	1.5 ± 1.0
A. populnea (600 mg.kg^{-1})	Wistar rat	24 hours	3	4	6	6	4	4	4.5 ± 1.22
	Swiss mice	48 hours	1	2	1	2	2	3	1.8 ± 0.75
	Swiss mice	72 hours	1	2	0	0	2	2	1.1 ± 0.98
A. populnea (900 mg.kg^{-1})	Wistar rat	24 hours	7	5	4	6	6	6	5.6 ± 1.03
	Swiss mice	48 hours	3	1	3	1	2	3	2.1 ± 0.98
	Swiss mice	72 hours	3	1	2	2	2	2	2.0 ± 0.63
Cyclophosphamide	Wistar rat	24 hours	21	19	23	27	15	23	21.3* ± 4.08
ENU	Swiss mice	48 hours	6	7	8	9	12	8	8.3* ± 2.0
ENU	Swiss mice	72 hours	7	6	6	9	10	9	7.8* ± 1.7

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

say. As expected, N-nitroso-N-ethylurea (ENU) agent used as positive control led to some fragmentation and migration of the fragments in the peripheral blood cells, in the SCGE assay. No statistically significant effects on DNA migration were found at the three extract concentrations tested in blood cells (p > 0.05). When exposed to the test extract, most of the cells examined on the slides were undamaged (class 0), a few cells showed minor

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damage (class 1), and a very few showed a large amount of damage (class 2 and 3). Furthermore, there was no significant difference in DNA migration among the three extract concentrations tested.

Table 2 summarises the results of the analysis of micronucleus in bone marrow cells of Wistar rats and in peripheral blood cells of Swiss mice following treatment with different concentrations of the *A. populnea* chloroform fraction and controls.

Administration of the *A. populnea* chloroform fraction did not result in an increase in the average number of polychromatic erythrocytes with micronuclei (MNPCE)

in the bone marrow cells of Wistar rats or MNPCE on peripheral blood cells of Swiss mice (Table 2). Comparisons between different dose groups showed no significant differences between the mean MNPCE (Tukey-Kramer test, $p > 0.05$).

The MI values obtained from the analysis of 1,000 cells/animal for a sample of 30 animals (n = 6 / group) ranged from 1.7 to 4.4% (means) and statistical analysis by the Tukey-Kramer test showed no significant differences ($p > 0.05$) between the different treatments with the tested extract, or between these treatments and their controls (Table 3).

Table 3. Mitotic Index (MI) and distribution of the different types of chromosomal aberrations (CA) observed in males (M) Wistar rat bone marrow cells treated with a *Austroplenckia populnea* chloroform fraction, and respective controls. One hundred cells were analysed per animal, for a total of 600 cells per treatment. C, Chromatid-type; IC, isochromatid-type; OA, other aberrations: del = deletion, f = fragment; SDM = standard deviation of the mean.

Treatments	Animal	MI (%)	Chromosomal aberrations					Total (CA)
			Gaps		Breaks			without gaps
			C	IC	C	IC	OA	
Control	M_1	2.9	0	0	1	0	0	1
(Water)	M_2	2.4	0	0	1	0	0	1
	M_3	2.0	0	0	0	0	1 del	1
	M_4	1.9	1	0	1	0	0	1
	M_5	2.7	0	0	1	0	0	1
	M_6	2.9	1	0	0	0	1 del	1
	mean ± SDM	2.4 ± 0.44	-	-	-	-	-	1.0 ± 0.0
A. populnea	M_1	2.4	0	1	0	0	0	0
(300 mg.kg^{-1})	M_2	3.6	0	0	1	0	0	1
	M_3	1.7	0	1	0	0	0	0
	M_4	1.8	0	0	1	0	0	1
	M_5	2.2	0	0	0	0	0	0
	M_6	2.4	0	0	1	0	0	1
	mean ± SDM	2.3 ± 0.68	-	-	-	-	-	0.5 ± 0.54
A. populnea	M_1	2.4	1	0	1	0	0	1
(600 mg.kg^{-1})	M_2	2.4	0	0	1	0	0	1
	M_3	3.4	0	0	1	0	0	1
	M_4	3.6	0	0	0	0	0	0
	M_5	3.9	0	1	0	0	0	0
	M_6	2.8	1	0	1	0	0	1
	mean ± SDM	3.0 ± 0.64	-	-	-	-	-	0.66 ± 0.51
A. populnea	M_1	2.5	0	0	1	0	0	1
(900 mg.kg^{-1})	M_2	1.9	0	0	0	0	2 del	2
	M_3	3.2	0	0	0	0	0	0
	M_4	3.4	0	0	1	0	1 del	2
	M_5	2.0	0	0	0	0	0	0
	M_6	3.1	0	0	1	0	0	1
	mean ± SDM	2.6 ± 0.64	-	-	-	-	-	1.0 ± 0.89
Cyclophosphamide	M_1	2.2	0	3	0	0	0	3
(50 mg.kg^{-1})	M_2	3.5	4	0	3	0	2 f	5
	M_3	4.4	3	0	6	0	3del/1f	10
	M_4	3.9	1	1	3	1	1 del	5
	M_5	3.2	0	1	3	2	1 del	6
	M_6	3.3	1	1	4	1	1 del	6
	mean ± SDM	3.4 ± 0.74	-	-	-	-	-	5.83* ± 2.31

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

The data obtained from 100 metaphase cells/animal also showed that there were no statistically significant differences between the mean number of chromosome aberrations for the treated groups and the negative control (p > 0.05). The most frequent types of aberrations in the treated groups were chromatid breaks, deletions, and isochromatid gaps (Table 3).

4. Discussion

In this study, the animals were submitted to acute treatment via gavage of the *Austroplenckia populnea* chloroform fraction, obtained from its hydroalcoholic barkwood extract. This treatment regimen and the administration method were considered the most suitable because they were closer to the form in which *A. populnea* extracts are ingested by the population.

The comet assay (SCGE) has been used as a rapid and sensitive tool for detecting primary DNA damage in individual cells. This technique enables the detection of several classes of DNA injury, such as double-strand breaks, single strand-breaks, alkali-labile sites, incomplete repair of a-basic sites and cross-links (Singh et al., 1988; Rojas et al., 1999; Brendler-Schwaab et al., 2005). In the present study, the comet assay was used as an additional comparison method with micronucleus and chromosome aberration assays and showed a slight increase in total damaged cells and the scores in animal groups treated with the *A. populnea* chloroform fraction, but the results did not differ significantly from the negative control group, indicating no genotoxic effects of this extract fraction.

The micronucleus (MN) assay in rodents is the best-documented in vivo test on clastogenic effects (chromosome aberrations), in relation to the number of tested chemicals (Mavournin et al., 1990; Morita et al., 1997) and chromosome aberration test is an important cytogenetic endpoint that is routinely used to evaluate genotoxicity (Preston et al., 1987; Krishna et al., 1991). In the present study, it was demonstrated that three high doses of *A. populnea* chloroform fraction from crude barkwood extract showed no increase in the mean number of micronucleated erythrocytes in the peripheral blood of mice, and the bone marrow of rats, and no chromosomal aberrations in the bone marrow cells of Wistar rats. The mitotic index values obtained indicate that this fraction extract has no cytotoxic effects at the tested doses. These data contrast with the results obtained by Zanoni et al. (2005), in which crude barkwood extract from *A. populnea* induced a significant dose-related increase in mean chromosomal aberrations at the 600 and 900 mg.kg^{-1} doses, and also a dose related increase in the mean MNPCE at the 300, 600 and 900 mg.kg^{-1} doses in the bone marrow cells of Wistar rats. On the other hand, Pugliesi et al. (2007) observed that the leaf extract of *A. populnea* did not induce a decrease in the mitotic index, neither did it induce a statistically significant increase in the mean number of MNPCE or chromosome aberrations in the bone marrow cells of Wistar rats at doses of 300, 600 and 900 mg.kg^{-1}. The quantitative differences in chemical composition between the stalk and leaf extracts obtained from *A. populnea* (the barkwood extract is richer in triterpenes than the leaf extract; S. F. Andrade, personal communication) could explain the differences in clastogenic potential observed between these 2 extracts. Similarly, qualitative and quantitative differences between *A. populnea* crude barkwood extract and their chloroform fraction could explain the differences in micronucleus induction observed between these two extracts.

In conclusion, analysing the available data on the genotoxic/clastogenic potential of *A. populnea* extracts obtained by Zanoni et al. (2005), Pugliesi et al. (2007), and in the present work, all developed in our laboratory, we observe that *A. populnea* crude bark wood extract shows a slight but significant increase in clastogenicity in the bone marrow cells of Wistar rats (Zanoni et al., 2005), however, the chloroform fraction of this extract shows no genotoxic or clastogenic/aneugenic effects in the bone marrow cells of Wistar rats and in the peripheral blood cells of Swiss mice (present study). *A. populnea* leaf extract does not present any clastogenic/aneugenic effects on Wistar rats cells (Pugliesi et al., 2007). These data do not preclude the therapeutic consumption of *A. populnea* extracts, but demonstrate that caution is needed in their indiscriminate use, until the other extract fractions (hexane and ethyl acetate) have been studied and the relationship between the qualitative and quantitative analyses of each extract/fraction and their mutagenic potential can be better investigated.

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