

## ***In vitro* genotoxicity assessment of caffeic, cinnamic and ferulic acids**

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**ABSTRACT.** Phenols are a large and diverse class of compounds, many of which occur naturally in a variety of food plants; they exhibit a wide range of biological effects, including antibacterial, anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, anticarcinogenic, and vasodilatory actions. We examined the genotoxic and clastogenic potential of three phenolic compounds: caffeic, cinnamic and ferulic acids, using the comet and micronucleus assays *in vitro*. Drug-metabolizing rat hepatoma tissue cells (HTCs) were used. Three different concentrations (50, 500 and 1500  $\mu$ M) of these phenolic acids were tested on the HTCs for 24 h. The caffeic, cinnamic and ferulic acids were not genotoxic by the comet assay ( $P > 0.05$ ). However, the micronucleus test showed an increase in the frequency of micronucleated cells for the three compounds, indicating that these substances have clastogenic effects in HTC.

**Key words:** Caffeic acid; Cinnamic acid; Ferulic acid; Comet assay; Micronucleus test; Clastogenic effects

## INTRODUCTION

Phenolic acids are plant metabolites that are widely found throughout the plant kingdom. They are essential for the growth and reproduction of plants, and are produced as a defence mechanism against pathogens. Phenolic compounds are considered to be potential therapeutic agents against a wide range of ailments including neurodegenerative diseases, cancer, diabetes, coronary heart dysfunction, inflammatory diseases, and ageing (Ferguson, 2001; Soobrattee et al., 2005), and there is growing interest in their strong antioxidant activity (Srinivasan et al., 2007).

Caffeic acid (CA) is a naturally occurring phenolic compound, and its derivative caffeic acid phenethyl ester is found in many fruits, vegetables, and plants, including coffee, although the amounts vary greatly depending on the plant (Chung et al., 2004). An important source of these substances is propolis from bee hives, which contain, as prominent constituents, two distinct series of polyphenols, i.e., flavonoids and caffeic acid esters (Greenaway et al., 1987). Sestili et al. (2002) reported that the most prominent activity of CA is that it prevents DNA single-strand breakage and cytotoxicity. It also has the ability to chelate iron. Caffeic acid has antiviral, anti-inflammatory and immunomodulatory properties (Grunberger et al., 1988) and has been shown to inhibit the growth of different types of transformed cells (Burke Jr. et al., 1995). The mutagenicity of CA was evaluated by the Ames test with negative results for both, with and without metabolizing S9 fractions (Fung et al., 1988). On the other hand, Hanham et al. (1983) reported clastogenic activity in Chinese hamster ovary cells and Hirose et al. (1997) observed that CA slightly increased incidences of forestomach papillomas in rats.

Cinnamic acid (CI) is a key intermediary in the shikimate and phenylpropanoid pathways. Phenylpropanoids are a class of plant metabolites based on phenylalanine. Phenylalanine is first converted into cinnamates, coumarines, caffeic acids, ferulic acids, and sinapic acids. CI, which is the precursor of these acids, is the parent compound of its esters, which are more volatile enabling them to be transported to other parts more easily. Cinnamic acid and its derivatives, including esters and carboxylic functional derivatives, are important components in flavorings, perfumes, synthetic dyes, and pharmaceuticals (Natella et al., 1999; Bickers et al., 2005). CI is not acutely toxic by the dermal route of exposure, while cinnamaldehyde has low toxicity by the dermal route (RIFM, 1976, 1997). The mutagenicity of CI was evaluated in *Salmonella typhimurium*, *Escherichia coli* and by the Rec assay in *Bacillus subtilis*, and no evidence of mutagenicity was found (Sekizawa and Shibamoto, 1982; Kuroda et al., 1984; Azizan and Blevins, 1995).

Ferulic acid (FA) is a phenolic acid plant constituent that is the result of the metabolism of phenylalanine and tyrosine. It occurs primarily in the seeds and leaves, both in its free form and is covalently linked to lignin and other biopolymers. In wheat, FA is an ester, which is linked to cell wall carbohydrates and occurs in higher concentrations in the alcurone, pericarp and embryo cell walls (Fulcher, 1983). FA is also a major constituent of fruits (e.g., orange), certain vegetables (e.g., tomato, carrot), and sweet corn (Sri et al., 2003). Due to its phenolic nucleus and an extended side chain conjugation, it readily forms a resonance stabilized phenoxy radical, which accounts for its potent antioxidant potential. Ultraviolet (UV) absorption by FA catalyzes stable phenoxy radical formation, thereby giving it the ability to terminate free radical chain reactions. By virtue of effectively scavenging deleterious radicals and suppressing radiation-induced oxidative reactions, FA may have an important antioxidant

function in preserving the physiological integrity of cells exposed to both air and impinging UV radiation. FA has similar photoprotection properties, offering skin protection when dissolved in cosmetic lotions. When added to foods, it inhibits lipid peroxidation and subsequent oxidative spoilage. By the same mechanism, FA may protect against various inflammatory diseases. A number of other industrial applications are based on the antioxidant potential of FA (Graf, 1992; Kanski et al., 2002).

Despite the commercial and therapeutic potential of the caffeic, cinnamic and ferulic acids, so far there have very little studies on the possible genotoxic effects of these natural compounds on mammalian cells. Therefore, the objective of the present study was to evaluate the genotoxic and clastogenic potential of these three phenolic compounds in cultured mammalian cells, where genetic damage was measured by the comet and micronucleus assays in the presence of drug-metabolizing rat hepatoma tissue cells (HTCs).

## MATERIAL AND METHODS

### Chemical agents

Caffeic, cinnamic and ferulic acids were purchased from Acros Organics (Geel, Belgium), and diluted in dimethyl-sulfoxide (DMSO; CAS: 67-68-5, Merck Chemicals, SP, Brazil) for use in the experiments. The final concentration of DMSO did not exceed 1% in any of the protocols performed. The indirect adduct forming agent benzo[a]pyrene was used as the DNA damaging agent (positive control) (CAS: 50-32-8, Fluka). It was diluted in DMSO and used at a concentration of 20  $\mu\text{M}$  (concentration established in a pilot experiment - data not shown). Cytochalasin-B (Cyt-B; CAS: 14930-96-2, Sigma) was diluted in DMSO to obtain a stock solution of 300  $\mu\text{g}/\text{mL}$ , which was kept at 4°C in the dark, until use. The other main chemicals used were obtained from the following suppliers: normal melting point (NMP) agarose: Roth, Karlsruhe, Germany; low melting point (LMP) agarose (CAS: 9012-36-6, FMC, Rockland, MD, USA); sodium *N*-lauroyl sarcosine (CAS: 7631-98-3), and ethylenediamine-tetraacetic acid (EDTA) (CAS: 60-00-4, Sigma).

### Cell viability

Cell viability was determined by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay (Mosmann, 1983) as reflected by the activity of succinate dehydrogenase. HTCs ( $3 \times 10^4$  per well) were plated in 96-well microplates. Following incubation with phenolic acids (0-1500  $\mu\text{M}$ ) for 24 h, MTT (100  $\mu\text{L}$ , 0.5 mg/mL) was added to each well, and the plate was incubated at 37°C for 4 h. The medium was then removed and replaced with 100  $\mu\text{L}$  DMSO to dissolve the formazan crystals. The product was quantified spectrophotometrically, by measuring absorbance at 570 nm using a microplate reader. Cells without phenolic acid treatment served as negative control and doxorubicin as positive control (Figure 1).

### Culture and cell treatments

The hepatoma cell line from *Rattus norvegicus*, HTC, which expresses phase I and phase II drug-metabolizing enzymes (henceforth called the drug-metabolizing cell line), was

obtained from the Rio de Janeiro Cell Bank (RJCB). The stability of the HTC ploidy is periodically checked by RJCB and by several studies of our research group. Cells were grown in 5.0 mL DMEM/F12 culture medium (Gibco®, Brazil), supplemented with 10% fetal bovine serum (Gibco®). Cells were cultivated as monolayers in 25-cm<sup>2</sup> flasks, at an initial concentration of  $1.0 \times 10^6$  cells per flask in a BOD-type incubator at 37°C. Under these conditions, the length of the cell cycle was 24 h. The cells were cultivated for one complete cycle prior to treatment with fresh complete medium containing the test substances. Caffeic, cinnamic and ferulic acids were tested at concentrations of 50, 500 and 1500 µM. Benzo[a]pyrene was used as the positive control treatment at a final concentration of 20 µM, and one culture treated with the solvent DMSO served as the negative control in each experiment. Treatment with the substances was carried out for 24 h. The cells were washed twice with PBS after each treatment. All cell treatments were repeated three times.

### Comet assay

The general procedure was carried out by the method described by Uhl et al. (1999, 2000), based on the premises proposed by Tice et al. (2000). HTCs in the early log-phase were trypsinized (0.1%) and seeded  $2 \times 10^5$  cells/well in plates of 24 wells (TPP), and incubated for another 24 h after performing the treatments with the test substance, as described above. At the end of the treatments, the cells were washed twice with 5 mL ice-cold PBS, pH 7.4, and trypsinized with 0.2 mL in a 0.025% solution. After about 2 min, this process was terminated by adding complete culture medium. The cells were collected by centrifugation at 900 rpm for 5 min, and the pellet was gently resuspended in 1 mL culture medium. A 20-µL aliquot of the cell suspension was mixed with 100 µL 0.5% LMP agarose at 37°C, and immediately spread onto microscope slides pre-coated with 1.5% NMP agarose. Coverslips were added and the slides were allowed to gel at 4°C for 20 min. 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 nM Tris, pH set to 10.0 with ~8 g solid NaOH, 890 mL distilled water and 1% sodium lauroyl sarcosine), plus 1 mL Triton X-100 (Merck) and 10 mL DMSO. The slides were left to stand for 1 h at 4°C, protected from light. They were then placed in the gel box, positioned at the anode end, and left for 20 min at 4°C, prior to electrophoresis, 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 EDTA, pH 10.0) to allow the DNA to unwind. Electrophoresis was performed in an ice bath (4°C) for 20 min at 25 V and 300 mA (0.722 V/cm). The slides were then submerged in a neutralization buffer (0.4 M Tris-HCl, pH 7.5) for 15 min, dried at room temperature and fixed in 100% ethyl alcohol for 10 min. The slides were dried and stored at least overnight before staining.

For staining, the slides were briefly rinsed in distilled water, covered with 30 µL 1X ethidium bromide-staining solution prepared from a 10X stock (200 µg/mL) and covered with a coverslip. The material was evaluated immediately at 400X magnification, using a fluorescence microscope (Olympus) with a 515-560-nm excitation filter and a 590-nm barrier filter.

### Cytokinesis-micronucleus assay

The cytokinesis-block micronucleus assay was performed according to the protocol of

Natarajan and Darroudi (1991). Test agents were added to 5 mL fresh medium at 24 h (one cell cycle) after initiating the culture of HTC<sub>1</sub>9, which had been previously seeded in the complete culture medium and stabilized in 25-cm<sup>2</sup> flasks, as described. Each of the substances used in the treatments cited above (negative control, positive control, phenolic acids tested at concentrations of 50, 500 and 1500 µM) were applied for 24 h. At the end of the treatments, the cells were washed twice with 5 mL ice-cold PBS, pH 7.4, and Cyt-B was added to fresh complete culture medium to obtain a final concentration of 3 µg/mL. It was continually used for 28 h to block cytokinesis and yield binucleated cells. All assays described here were repeated independently three times.

The procedures used for harvesting, slide preparation and staining were based on Salvadori et al. (1993). At harvest time, the cells were rinsed twice with 5 mL PBS, pH 7.4, trypsinized (0.025% trypsin) and centrifuged for 5 min at 900 rpm. The pellet was resuspended in ice-cold hypotonic solution (1% sodium citrate) plus one drop 40% formaldehyde and then carefully homogenized with a Pasteur pipette. This cell suspension was centrifuged under the same conditions and the pellet resuspended in methanol:acetic acid (3:1), and once again homogenized with a Pasteur pipette. The cell suspension was then centrifuged and resuspended in 1.5 mL methanol:acetic acid (3:1). The fixed cells were then dropped onto slides previously cleaned and covered with a film of ice-cold distilled water. They were stained in 2% Giemsa dissolved in phosphate buffer (0.06 M Na<sub>2</sub>HPO<sub>4</sub> and 0.039 M KH<sub>2</sub>PO<sub>4</sub>, pH 7.0) for 10 min, washed with water, dried and kept at 4°C until microscopic analysis.

### **Frequencies, scoring procedures and data evaluation**

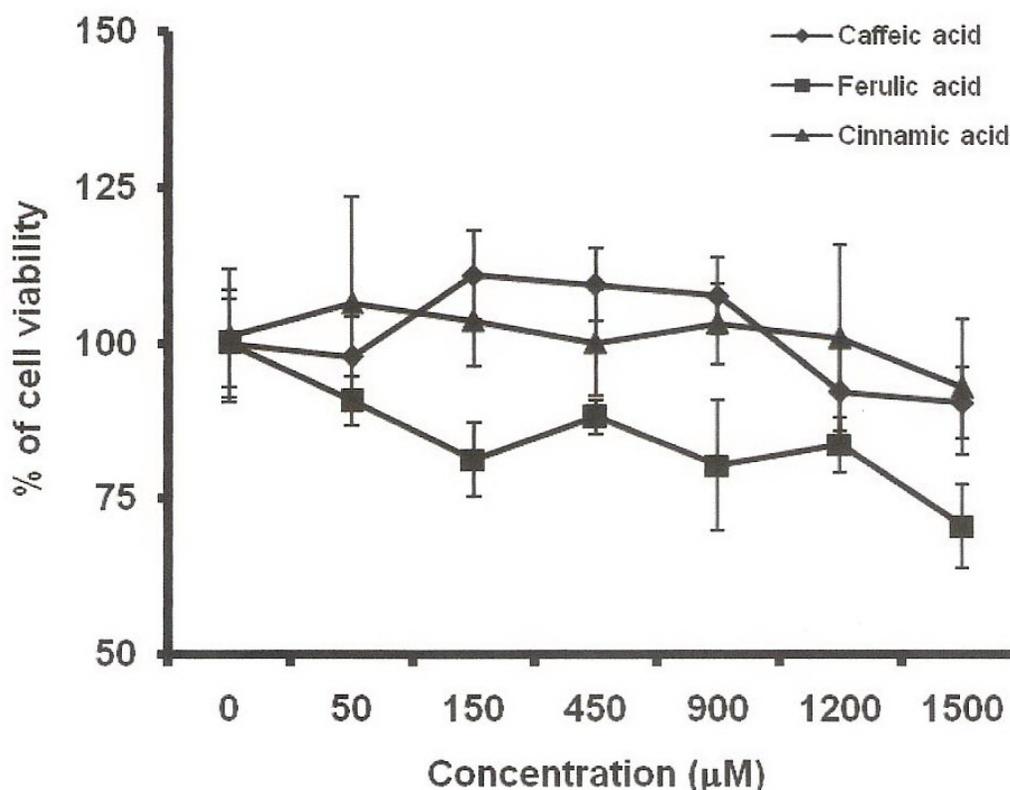
The extent and distribution of DNA damage indicated by the comet assay were evaluated by examining at least 100 randomly selected and non-overlapping cells on the slides, per treatment. These cells were scored visually according to tail size into four classes: 1) class 0: undamaged, without a tail; 2) class 1: with a tail shorter than the diameter of the head (nucleus); 3) class 2: with a tail length one or twice the diameter of the head, and 4) class 3: with a tail longer than twice the diameter of the head (Kobayashi et al., 1995). Apoptotic cells with totally fragmented nuclei were not considered in the analysis (Speit and Hartmann, 2005). The total score for 100 comets was obtained by multiplying the number of cells in each class by the damaged class, which ranged from 0 (all undamaged) to 300 (all maximally damaged).

To evaluate micronucleus induction, one thousand binucleated cells with well-preserved cytoplasm were scored per coded slide in each experimental repetition. The criteria for the identification of binucleated cells and micronuclei (Titenko-Holland et al., 1997) were as follows: a) both nuclei and micronuclei should be round; b) micronuclei should be smaller than one-third of the main nuclei; c) micronuclei must not touch the main nuclei; d) micronuclei must not be refractive and should be of the same color and intensity as the main nuclei. To avoid the influence of cytotoxic effects in the determination of micronuclei, the nuclear division index (NDI) was calculated for each experimental point. Five hundred viable cells were scored for each culture to determine the average of cells with 1, 2, 3, and 4 nuclei and calculate the NDI using the formula:  $NDI = (M1 + 2(M2) + 3(M3) + 4(M4)) / N$ , where M1-M4 represent the number of cells with one to four nuclei and *N* is the total number of viable cells scored (Eastmond and Tucker, 1989). The results of the three independent tests were totaled and statistically analyzed.

The data obtained from the comet and micronucleus assays were submitted to the one-way analysis of variance (ANOVA) and the Tukey multiple comparison test (Sokal and Rohlf, 1995), using the GraphPad Instat<sup>®</sup> software (version 3.01). The results were considered to be statistically significant at  $P < 0.05$ .

## RESULTS

Regarding the ability of phenolic acid to influence cell viability by the MTT test, none of the compounds tested presented significant toxic effect in the concentrations assayed (1 to 1500  $\mu\text{M}$ ) (Figure 1).



**Figure 1.** Cell viability indices for the three phenolic acids.

The results obtained for the genotoxicity assessment of caffeic, cinnamic and ferulic acids in HTC<sub>3</sub> by the comet assay are shown in Table 1. None of the three phenolic acids tested showed statistically significant increases in the total damaged cells and scores in the doses tested, compared with the negative control group. An increase in the total damaged cells was observed for the higher dose (1500  $\mu\text{M}$ ) of the compounds tested, but without statistical significance.

**Table 1.** DNA migration in the comet assay for genotoxicity assessment of caffeic, cinnamic and ferulic acids in hepatoma tissue cells *in vitro*.

| Treatments (total repetitions)               | Total damaged cells | Comet class |      |      |   | Scores       |
|--|---------------------|-------------|------|------|---|--------------|
|  |                     | 0           | 1    | 2    | 3 |              |
| DMSO<br>(negative control)                   | 10.6 ± 3.78         | 89.3        | 10.3 | 0.3  | 0 | 11.0 ± 4.3   |
| Benzo[a]pyrene (20 µM)<br>-positive control) | 89.3 ± 6.11*        | 10.6        | 69.3 | 18.0 | 2 | 111.3 ± 3.7* |
| Caffeic acid                                 |                     |             |      |      |   |              |
| 50 µM  | 6.33 ± 3.05         | 93.6        | 6.3  | 0    | 0 | 6.33 ± 3.0   |
| 500 µM                                       | 5.0 ± 2.0           | 95.0        | 5.0  | 0    | 0 | 5.0 ± 2.0    |
| 1500 µM                                      | 16.0 ± 5.29         | 84.0        | 13.6 | 2.3  | 0 | 18.3 ± 6.8   |
| Cinnamic acid                                |                     |             |      |      |   |              |
| 50 µM  | 5.66 ± 3.21         | 94.3        | 5.6  | 0    | 0 | 5.66 ± 3.2   |
| 500 µM                                       | 7.66 ± 5.50         | 92.3        | 7.6  | 0    | 0 | 7.66 ± 5.5   |
| 1500 µM                                      | 18.0 ± 13.8         | 82.0        | 16.3 | 1.6  | 0 | 19.6 ± 16.7  |
| Ferulic acid                                 |                     |             |      |      |   |              |
| 50 µM  | 15.3 ± 11.0         | 84.6        | 15.0 | 0.3  | 0 | 15.6 ± 11.5  |
| 500 µM                                       | 8.66 ± 2.51         | 91.3        | 8.6  | 0    | 0 | 8.6 ± 2.5    |
| 1500 µM                                      | 23.0 ± 3.6*         | 77.0        | 20.6 | 2.3  | 0 | 25.3 ± 4.0   |

Data are reported as means ± SD. DMSO = dimethyl-sulfoxide. \*Significantly different from the negative control (P < 0.05).

The results of experiments designed to assess the clastogenicity of the three phenolic acids in HTC are shown in Table 2. The effects of phenolic compounds on nuclear division showed that only exposure to concentration of 1500 µM caffeic and ferulic acids resulted in some decrease in the cell division; however, the differences were not statistically significant (P > 0.05). The results obtained in the micronucleus test demonstrated a dose-related increase in the number of the micronucleated cells for the three substances tested, which was at least three times higher than that observed in the negative control group, for all the doses tested. This increase was not statistically significant at the dose of 50 µM for caffeic and ferulic acids.

**Table 2.** Micronucleus frequencies in hepatoma tissue cells *in vitro* for the assessment of mutagenicity of caffeic, cinnamic and ferulic acids.

| Treatments                                   | NDI/1500 cells<br>(mean ± SD) | Number of cells analyzed | MN     |      |
|--|-------------------------------|--------------------------|--------|------|
|  |                               |                          | Number | %    |
| DMSO<br>(negative control)                   | 1.48 ± 0.02                   | 3000                     | 22     | 0.73 |
| Benzo[a]pyrene (20 µM)<br>(positive control) | 1.50 ± 0.02                   | 3000                     | 75*    | 2.50 |
| Caffeic acid                                 |                               |                          |        |      |
| 50 µM  | 1.46 ± 0.05                   | 3000                     | 50     | 1.66 |
| 500 µM                                       | 1.44 ± 0.01                   | 3000                     | 98*    | 3.26 |
| 1500 µM                                      | 1.15 ± 0.03                   | 3000                     | 119*   | 3.96 |
| Cinnamic acid                                |                               |                          |        |      |
| 50 µM  | 1.57 ± 0.05                   | 3000                     | 68*    | 2.26 |
| 500 µM                                       | 1.39 ± 0.08                   | 3000                     | 77*    | 2.56 |
| 1500 µM                                      | 1.48 ± 0.03                   | 3000                     | 78*    | 2.60 |
| Ferulic acid                                 |                               |                          |        |      |
| 50 µM  | 1.60 ± 0.01                   | 3000                     | 63     | 2.10 |
| 500 µM                                       | 1.37 ± 0.01                   | 3000                     | 66*    | 2.20 |
| 1500 µM                                      | 1.54 ± 0.03                   | 3000                     | 95*    | 3.16 |

NDI = nuclear division index; DMSO = dimethyl-sulfoxide; MN = micronucleus. \*Significantly different from the negative control (P < 0.05). The increased frequencies of micronuclei represent cells with only a single micronuclei.

## DISCUSSION

In this study, the possibility of the DNA damage and micronucleus induction by CA, CI and FA phenolic acids, i.e., genotoxic and clastogenic activity, were investigated. Regarding the ability of phenolic acid to influence cell viability, none of the compounds tested presented significant toxic effect in the concentrations assayed (1 to 1500  $\mu\text{M}$ ). Viability was assayed as the ability of mitochondrial enzyme succinate dehydrogenase to reduce MTT to its corresponding formazan. The determination of cell viability after treatment is of importance when further assaying DNA damage, in order to avoid DNA fragmentation due to apoptotic events. With this in mind, we further studied the effect of CA, CI and FA phenolic acids, in DNA damaging, using the micronucleus test and the comet assay as genotoxic end-points.

According to the data obtained in the comet assay, the three acids did not present genotoxicity at any of the concentrations used. In relation to the CA acid, these results are in accordance with the data that one of the major activities of CA is to prevent DNA single-strand breakage and cytotoxic residues (Sestilli et al., 2002). On the other hand, the micronucleus test showed that these same acids present clastogenic effects.

Some assays using bacterial studies in the absence and presence of metabolic activation did not find any evidence of mutagenicity of the CI acid in *Salmonella typhimurium* (Lijinski and Andrews, 1980; Azizan and Blevins, 1995), some *Escherichia coli* strains (Kuroda et al., 1984; Yoo, 1986) and by the rec assay test in *Bacillus subtilis* (Sekizawa and Shibamoto, 1982; Yoo, 1986). There have been no reported mutagenicity and genotoxicity mammalian studies on the CI acid, but there have been several on its precursor, cinnamaldehyde, some of which show positive results (see Bickers et al., 2005, for review).

CA presented a dose-related increase of the micronucleated cells. It is possible that these mutagenic effects may be responsible for the enhancing of carcinogenicity effects observed in a rat medium-term multi-organ model by Hirose et al. (1997). This idea should be investigated in further studies. Clastogenic activity of CA was also reported by Hanham et al. (1983). There have been no previously reported studies evaluating the mutagenic potential of FA in eukaryotic cells. However, there have been some studies that show that FA exhibits anticarcinogenic and anti-ageing effects, which are specifically attributed to its antioxidant potential (Lesca, 1983; Graf, 1992; Kawabata et al., 2000). In our study, we observed clastogenic effects of CA and FA.

There are substances that can present mutagenic and antimutagenic action. Tavares et al. (2006) evaluated the mutagenic and antimutagenic effects of Brazilian propolis, and observed that this complex mixture presents mutagenicity at higher concentrations, while at lower concentrations, it displays a chemopreventive effect on doxorubicin-induced genotoxicity. Comparative chemical analysis of *Baccharis dracunculifolia* and green propolis demonstrated that *Baccharis* is the main plant source of propolis produced in the *cerrado* or savannah region in the south of Minas Gerais, Brazil (Park et al., 2004). Flavonoids have been suggested as the components largely responsible for both the mutagenic and antimutagenic effects of propolis, since these compounds can act either as pro-oxidants or as free radical scavengers, depending on their concentration (Tavares et al., 2006). *Baccharis* and propolis have similar constituents (Park et al., 2002, 2004) and between them there are some phenolic compounds, as well as FA and CI acids and their derivatives, including CA. According to Munari et al. (2010), studies designed to isolate, identify and characterize the compounds of *B. dracuncu-*

*lifolia* and propolis should provide a better understanding of the genotoxic and antigenotoxic mechanisms observed on both complex extracts. Therefore, the results obtained in our study enabled us to correlate the compounds analyzed here with the mutagenic effects observed in *Baccharis* and propolis (Tavares et al., 2006; Resende, 2008; Munari et al., 2010), e.g., once these extracts present CA, CI and FA on their composition and also showed mutagenic effects only in higher doses.

Despite the fact that phenols constitute one of the widest marketed groups of dietary supplements, and the lack of studies on their genotoxic effects in higher organisms, some have shown mutagenic effects in mammalian cells, such as tannic acid (Ferguson et al., 1985), coumarin, vanillin, caffeine, tannic acid, and cinnamaldehyde (Sanyal et al., 1997). In the present study, the three phenolic acids tested showed no genotoxicity in HTC cells by the comet assay. However, in the micronucleus test, clastogenicity was observed for all the compounds. The comet assay detected primary DNA damage. While the formation of DNA adducts or strand breakage is often the first step in mutation induction, this damage can also be repaired (Brendler-Schwaab et al., 2005). On the other hand, the micronuclei observed in the micronucleus assay are usually formed from loss of chromosomal fragments during the division of the nucleated precursor cells (clastogenic effects) (Salamone et al., 1980). Micronuclei may also be formed if whole chromosomes are lost (aneugenic effects), and this type of mutation is not detected by the comet assay. Our micronucleus data indicate that CA, FA and CI phenolic acids produce clastogenic effects on HTC cells. Our NDI analysis evidenced no cytotoxic effects on HTC cells of the phenolic acids tested. Since the phenolic acids studied here exhibit a wide range of therapeutic properties, caution is needed with the intake of high or cumulative doses of these compounds and the risk of lower doses to human health needs to be better established.

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