



Cytotoxic and mutagenic effects of iodine-131 and radioprotection of acerola (*Malpighia glabra* L.) and beta-carotene *in vitro*

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ABSTRACT. The radioisotope iodine-131 [¹³¹I] can damage DNA. One way to prevent this is to increase the amount of antioxidants via dietary consumption. The goal of this study was to evaluate the radioprotective effect of fresh acerola pulp and synthetic beta-carotene in *Rattus norvegicus* hepatoma cells (HTC) in response to [¹³¹I] exposure *in vitro*. Cellular DNA damage was subsequently assessed using a cytokinesis block micronucleus assay. The mutagenic and cytotoxic activities of doses of [¹³¹I] (0.1, 0.5, 1, 5, and 10 µCi), acerola (0.025, 0.125, and 0.25 g acerola pulp/mL), and beta-carotene (0.2, 1, and 2 µM) were evaluated. Radioprotective tests were performed

by simultaneous treatment with acerola (0.25 g/mL) plus [^{131}I] (10 μCi) and beta-carotene (0.2 μM) plus [^{131}I] (10 μCi). Acerola, beta-carotene, and low concentrations of [^{131}I] did not induce micronucleus formation in HTC cells; in contrast, high concentrations of [^{131}I] (10 μCi) were mutagenic and induced DNA damage. Moreover, neither acerola nor beta-carotene treatment was cytotoxic. However, acerola reduced the percentage of [^{131}I]-induced damage, although beta-carotene did not show a similar effect. Thus, our results suggest that acerola diet supplementation may benefit patients who are exposed to [^{131}I] during thyroid diagnostics and therapy.

Key words: Antioxidants; Antimutagenic; Cell culture; Ionizing radiation; Radiotherapy

INTRODUCTION

It is well known that exposure to certain environmental substances can cause genetic mutations. Because mutations are often associated with the development of cancer and teratogenesis, information regarding the mutagenic potential of various agents, especially those used in medicine, is crucial (Loureiro et al., 2002).

Nuclear medicine uses radioisotopes to diagnose and treat various diseases. The radioisotope iodine-131 [^{131}I] is currently used to diagnose thyroid dysfunction and to treat hyperthyroidism and differentiated thyroid cancer (Thrall and Ziessman, 2003). This isotope emits beta particles (β) ($E = 0.61$ MeV) and gamma rays (γ) ($E = 0.36$ MeV) and has a half-life of eight days (Turgut et al., 2006).

[^{131}I] interacts with the surrounding environment and causes the formation of oxidizing free radicals via water radiolysis; free radicals may then react with intracellular macromolecules and alter cellular metabolism. Ionizing radiation also acts directly on nuclear DNA and may result in single- or double-strand breaks (Calegari, 2007). Previous studies assessed the impact of various doses of radioiodine in several systems and indicated that isotope exposure increases the frequency of chromosomal aberrations (Puerto et al., 2000; da Silva et al., 2008; Düsman et al., 2011) and micronuclei (MN) (Watanabe et al., 2004; Joseph et al., 2009).

One way to prevent DNA damage and potential disease development is to avoid exposure to mutagenic agents. Alternatively, the administration of chemoprotective agents may increase resistance to mutagens/carcinogens and/or inhibit disease progression (De Flora and Fergurson, 2005).

Antioxidants are substances that can bind free radicals and significantly reduce or prevent oxidation of the substrate (Hosseinimehr, 2007). Specifically, antioxidants protect against free radical damage by preventing free radicals from attacking lipids, protein amino acids, and the double bond of polyunsaturated fatty acids and DNA bases, thereby preventing cellular damage (Ratnam et al., 2006). Free radicals can be generated by a number of sources, such as cellular metabolism or radioisotopes like [^{131}I].

Antioxidant compounds can be obtained by the consumption of fruits and vegetables. Acerola (*Malpighia glabra* L.), from the family Malpighiaceae, is a native fruit of Central America that has high concentrations of the antioxidant vitamin C, a high amount of vitamins

A, B1, and B2, as well as carotenoids, anthocyanins, proteins, fats, carbohydrates, and minerals (Mezadri et al., 2006; Marques et al., 2007). One of the most potent antioxidant carotenoids is beta-carotene, which is a natural pigment present in numerous fruits and vegetables (Ben-Amotz et al., 1998).

The goal of this study was to evaluate the cytotoxic and mutagenic effects of [¹³¹I] and the radioprotective effect of fresh acerola fruit pulp and synthetic beta-carotene in *Rattus norvegicus* hepatoma cells (HTC) *in vitro*.

MATERIAL AND METHODS

Cell line - HTC

HTC cells derived from a *R. norvegicus* hepatoma were provided by Dr. Mario Sergio Mantovani (Universidade Estadual de Londrina, PR, Brazil). The cells were grown at 37°C in 25-cm² culture flasks containing 5 mL DMEM (Gibco) supplemented with 10% fetal bovine serum (Gibco). Under these conditions, the cell cycle time was approximately 24 h.

Evaluated substances

Fresh acerola was prepared at the Medicinal Garden “Irenice Silva” at the State University of Maringá. Fifty grams of macerated fruit pulp was diluted in 10 mL phosphate-buffered saline (PBS) to facilitate filtration on filter paper and a 0.22-μm cellulose acetate filter (Sartorius), and the concentrations 0.025, 0.125, and 0.25 g/mL culture medium (DMEM supplemented with 10% fetal bovine serum). Beta-carotene (Sigma) was dissolved in dimethylsulfoxide (DMSO, Merck) and added at a final concentration of 0.2, 1, and 2 μM beta-carotene per milliliter of cell growth medium. [¹³¹I] was obtained from the Institute of Nuclear and Energy Research (IPEN, São Paulo, Brazil). The isotope was diluted to a final concentration of 0.1, 0.5, 1, 5, and 10 μCi per 5 mL culture medium. For the negative control, 20 μL PBS was added per milliliter of culture medium. A treatment with DMSO was performed at final a concentration of 10 μL/mL culture medium. The positive control was doxorubicin (DXR, Sigma) at a final concentration of 0.2 μg/mL culture medium, which in previous studies (Almeida et al., 2011) was shown to be optimal for the induction of DNA damage and showed responsiveness in the cell line used.

Cytokinesis block micronucleus assay (MNCtB)

Cells were incubated for 24 h with the various compounds to evaluate their mutagenicity and radioprotection. Radioprotective tests were performed by simultaneous treatment with acerola (0.25 g/mL) plus [¹³¹I] (10 μCi) and beta-carotene (0.2 μM) plus [¹³¹I] (10 μCi). Cell cultures were incubated with cytochalasin B (Sigma, 3 μg/mL culture medium) to obtain binucleated cells. The cells were harvested according to a previously published protocol by Martins de Oliveira et al. (2002). Briefly, the cells were trypsinized [500 μL trypsin-0.025% EDTA (Gibco) at 37°C], centrifuged (5 min at 1000 rpm), hypotonized (1.5 mL 1% sodium citrate), and fixed (5 mL 3:1 mixture of methanol:acetic acid). Doxorubicin (Sigma) was incubated with the cells for 24 h to serve as a positive control for DNA damage.

All of the experiments were performed in triplicate; 3000 binucleated cells were analyzed per treatment to assess the frequency of micronuclei (MN), and 1500 cells were counted to determine the nuclear division index (NDI). The process for selecting binucleated cells, identifying micronuclei, and calculating the NDI was followed as described by Fenech (2000). The percentage reduction of damage was calculated using the following formula: Reduction (%) = $(AB / BC) \times 100$, where A is the average number of cells with MN observed with the damage inducing agent [^{131}I], B is the number of cells with MN observed with the radioprotective treatment (simultaneous treatment [^{131}I] + acerola or beta-carotene), and C is the number of cells with MN in the negative control test.

Statistical analysis was carried out using ANOVA and the Tukey test ($\alpha = 0.05$).

Obtaining extracts of acerola for physico-chemical analysis

The acerolas were washed with running potable water, and the edible part, pulp, and rind were separated manually. The pulp was disintegrated manually, prepared, and frozen in a household refrigerator, protected from light until further analysis.

Treatments were prepared with three different extracts of acerola pulp and were performed with the addition of dilution water. The methanol extract 50%/70% acetone was prepared according to the methodology described by Rufino et al. (2007). Besides these extracts, aqueous extracts (80% methanol and 80% acetone) were prepared using the solvent extractor with 2.0 g pulp agitation in the dark for 60 min. The extracts were not lyophilized.

Determination of total phenols

The total phenols of the four extracts were determined using the Folin-Ciocalteu reagent and a standard curve of gallic acid as a reference according to the methodology described by Wettasinghe and Shahidi (1999). The total phenol content was expressed as mg gallic acid/100 g pulp. The statistical calculation was performed using the Tukey test ($N = 3$; $\alpha = 0.05$).

Determination of antioxidant activity by DPPH scavenging

The free radical scavenging activity of four extracts obtained from acerola pulp was determined using the method using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and a series of dilutions, as described by Brand-Williams et al. (1995) and modified by Miliauskas et al. (2004). The absorbance was read at 515 nm after the reaction of extract and DPPH solution had been incubated at room temperature for 30 min.

BHT (2,6-di-*tert*-butyl-4-methylphenol) was used as a reference substance for free radical scavenging activity (positive control). Antioxidant activity (AA), expressed as a percentage, was calculated in relation to the control according to the expression below:

$$AA\% = (A_{\text{control}} - A_{\text{sample}}) \times 100 / (A_{\text{control}})$$

The extract concentration required for 50% capture of free radical DPPH (IC_{50}) was calculated by linear regression. The statistical calculation was performed using the Tukey test ($N = 3$; $\alpha = 0.05$).

Determination of ascorbic acid levels

For the determination of ascorbic acid content, the methodology described by Horwitz (2000) and modified by Benassi and Antunes (1998) was used. Briefly, pulp solution was extracted with 2% oxalic acid and titration with 0.01% 2,6-dichlorophenolindophenol to obtain a clear pink color. The results were expressed as mg ascorbic acid/100 g sample.

Determination of total carotenoids

The determination of total carotenoids was performed according to the methodology described by Higby (1962) in which the acerola pulp was extracted with isopropyl alcohol:hexane (3:1). The absorbance was read at 450 nm, and the results were expressed as mg/100 g pulp, as calculated using the following formula:

$$\text{Total carotenoids} = (A_{450} \times 100) / (250 \times L \times W),$$

where: A = absorbance, L = cuvette width in cm, W = quotient between the mass of the original sample in g and the final volume of dilution in mL.

Determination of total anthocyanins and yellow flavonoids

The determination of total anthocyanins followed the method of Francis (1982). The extracting solution used was 95% ethanol + 1.5 N HCl (85:15) proceeding reading the absorbance at 535 nm for anthocyanins and 374 nm for yellow flavonoids. The results were expressed as mg/100 g pulp, as calculated using the formulas:

$$\text{Total anthocyanins} = \text{dilution factor} \times \text{absorbance} / 98.2$$

$$\text{Yellow flavonoids} = \text{dilution factor} \times \text{absorbance} / 76.6$$

RESULTS

The average number of MN (Figure 1) indicates that only the highest dose of [¹³¹I] tested (10 μCi) resulted in significantly different data compared to the negative control ($P < 0.001$); in contrast, the lower doses tested (0.1, 0.5, 1, and 5 μCi) were not significantly different from the negative control. Moreover, the different concentrations of [¹³¹I] had a statistically similar average number of MN according to the Tukey test.

The NDI of the groups that were treated with [¹³¹I] (Table 1) indicates that radioiodine (at any dose) was not significantly different from the negative control. However, all of the concentrations tested caused an increase in the NDI.

Results indicate that synthetic beta-carotene and acerola fruit pulp were not mutagenic; the average number of MN was similar to the negative control, as shown in Figure 2. Furthermore, neither of the treatments was cytotoxic, and the NDI data were similar to the negative control (Table 2).

The results of the radiation protection test showed that simultaneous treatment with acerola and [¹³¹I] was statistically similar to the negative control, similar to treatment with only

acerola and with only [^{131}I] (10 μCi) (Figure 3). When beta-carotene was administered with the radioisotope, the average number of MN was significantly different than the negative control ($P < 0.01$), the treatment with DMSO ($P < 0.05$), and the treatment that included only beta-carotene (0.2 μM) ($P < 0.001$); however, these data were similar to treatment with only [^{131}I] (10 μCi) (Figure 3).

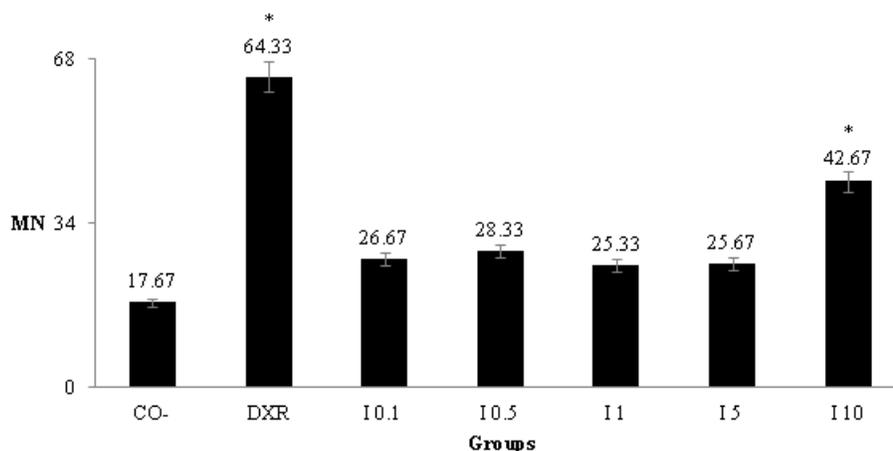


Figure 1. Average number of micronuclei (MN) and standard deviation for the negative (CO-) and positive (DXR-doxorubicin) control groups compared to the groups exposed to 0.1 to 10 μCi of [^{131}I]. *The results are significantly different compared to the CO-.

Table 1. Mean nuclear division index (NDI) and standard deviation of 1500 control cells compared to cells that were exposed to five concentrations of [^{131}I] (μCi).

Group	Total cells analyzed	NDI (means \pm SD)
CO-	1500	1.46 \pm 0.07
DXR	1500	1.43 \pm 0.12
Iodine 0.1	1500	1.70 \pm 0.13
Iodine 0.5	1500	1.67 \pm 0.10
Iodine 1	1500	1.57 \pm 0.12
Iodine 5	1500	1.63 \pm 0.01
Iodine 10	1500	1.65 \pm 0.02

CO- = negative control; DXR = doxorubicin.

As is shown in Table 3, simultaneous treatment with beta-carotene or acerola with [^{131}I] significantly decreased the NDI ($P < 0.001$ for beta-carotene and $P < 0.05$ for acerola) compared to [^{131}I] exposure. Furthermore, treatment with beta-carotene plus [^{131}I] resulted in lower NDI values than that observed for the beta-carotene only treatment.

The results of physic-chemical analysis show that acerola pulp is as excellent antioxidant (Table 4), with IC_{50} values to 50% methanol /70% acetone, water and 80% methanol extracts about 257.1 to 290.33 $\mu\text{g}/\text{mL}$. In addition, acerola pulp was found to contain high levels of antioxidant compounds (ascorbic acid, anthocyanins, carotenoids, total phenols, and yellow flavonoids) (laboratory data published in Düsman et al., 2012a).

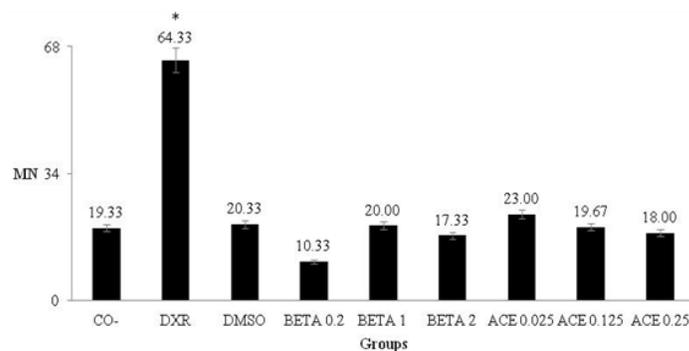


Figure 2. Average number of micronuclei (MN) and standard deviation for the negative (CO-) and positive (DXR-doxorubicin) control groups compared to groups treated with 10 μ L of DMSO/mL (dimethylsulfoxide), 0.2, 1, and 2 μ M of beta-carotene (BETA) and 0.025, 0.125 and 0.25 g of acerola pulp/mL (ACE). *The results are significantly different from the CO-.

Table 2. Mean nuclear division index (NDI) and standard deviation of 1500 control cells compared to cells that were treated with three concentrations of beta-carotene (μ M) and acerola (g/mL).

Group	Total cells analyzed	NDI (means \pm SD)
CO-	1500	1.50 \pm 0.05
DXR	1500	1.43 \pm 0.12
DMSO	1500	1.38 \pm 0.01
BETA 0.2	1500	1.57 \pm 0.07
BETA 1	1500	1.55 \pm 0.04
BETA 2	1500	1.42 \pm 0.03
ACE 0.025	1500	1.37 \pm 0.04
ACE 0.125	1500	1.68 \pm 0.10
ACE 0.25	1500	1.67 \pm 0.10

CO- = negative control; DXR = doxorubicin; DMSO = dimethylsulfoxide; BETA = beta-carotene; ACE = acerola.

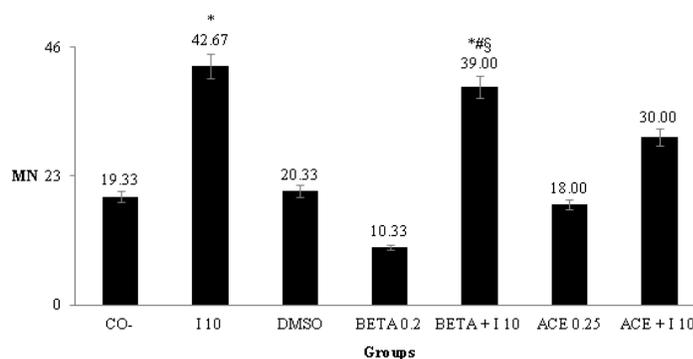


Figure 3. Average number of micronuclei (MN) and standard deviation for the negative control group (CO-) and groups treated with 10 μ Ci of 131 Iodine (I), 10 μ L of DMSO/mL (dimethylsulfoxide), 0.2 μ M of beta-carotene (BETA), and 0.25 g of acerola pulp/mL (ACE), with and without [131 I]. *The results are significantly different compared to the negative control (CO-). #The results are significantly different compared to DMSO. §The results are significantly different compared to beta-carotene.

Table 3. Mean nuclear division index (NDI) and standard deviation of 1500 control cells compared to cells that were treated with beta-carotene (μM) or acerola (g/mL) and ^{131}I (μCi).

Group	Total cells analyzed	NDI (means \pm SD)
CO-	1500	1.50 \pm 0.05
Iodine 10	1500	1.65 \pm 0.02
DMSO	1500	1.38 \pm 0.01
BETA 0.2	1500	1.57 \pm 0.07
BETA + I 10	1500	1.30 \pm 0.04*#
ACE 0.25	1500	1.68 \pm 0.10
ACE + I 10	1500	1.43 \pm 0.13*

CO- = negative control; DMSO = dimethylsulfoxide; BETA = beta-carotene; I = iodine; ACE = acerola. *The results are significantly different compared to Iodine 10. #The results are significantly different compared to BETA 0.2.

Table 4. Total phenols values and antioxidant activity expressed in IC_{50} concentration in $\mu\text{g/mL}$ needed to capture 50% of the free radical DPPH.

Extracts	Total phenols (mg gallic acid/100 g of pulp)*	IC_{50}
Methanol 50%/acetone 70%	4478.37 \pm 361.36 ^a	260.58 \pm 8.76 ^{bc}
Water	4631.70 \pm 55.00 ^a	257.10 \pm 6.89 ^{bc}
Methanol 80%	6142.65 \pm 15.80	290.33 \pm 27.48 ^{bc}
Acetone 80%	4807.84 \pm 102.58 ^a	579.92 \pm 43.80 ^c
BHT 2500 $\mu\text{g/mL}$	-	24.47 \pm 1.81

*Laboratory data published in Düsman et al. (2012a). ^aStatistically significant result compared to the methanol extract 80% ($P < 0.05$). ^bStatistically significant result compared to the acetone extract 80% ($P < 0.001$). ^cStatistically significant result compared to the BHT ($P < 0.001$).

DISCUSSION

The results showed that only the highest dose of ^{131}I tested (10 μCi) was mutagenic, but it is noteworthy that all of the radioisotope doses resulted in a greater number of MN than the negative control. These data indicate a dose-dependent action, because the increased concentration of radiation was an increased frequency of MN. Dose-dependent results were also found by Gutiérrez et al. (1997), as patients that received low doses of ^{131}I ($\leq 13,513.51 \mu\text{Ci}$), did not have frequencies of MN and binucleated cells with MN that were significantly different than those obtained before treatment. However, in patients receiving doses greater than 13,513.51 μCi ^{131}I , there was an increase in the frequency of MN and binucleated cells with MN, which was similar to what we observed for doses of 10 μCi .

MN, which is a small nuclear mass that is surrounded by membrane and separated from the main nucleus, can be derived from chromatid or chromosome breaks (Fenech, 2000); although Saffi and Henriques (2003) argue that chromatid/chromosome breaks are the main type of radiation-induced DNA damage, MN may also result from single-strand breaks, double-strand breaks, base and sugar damage, and oxidative damage to bases between protein-DNA and DNA-DNA interchange bridges.

Federico et al. (2008) reported negative results for the MN test using blood from 11 patients who received an average of $94,594.59 \pm 10,000 \mu\text{Ci}$ ^{131}I for ablation of thyroidal remnants. According to the authors, this may be due to an early activation of cellular repair machinery that maintains the integrity of the genome and may prevent double-strand DNA

breaks from progressing to chromosomal mutations by either repairing the lesions or by eliminating the damaged cells via apoptosis. It is possible that these mechanisms may also have occurred in our HTC cells that were treated with 0.1, 0.5, 1, and 5 μCi [^{131}I], as these doses of radioisotope appeared less mutagenic than the highest dose of 10 μCi .

It is possible that an induction of these cellular defense mechanisms may have altered cell division; however, the nuclear division indexes (Table 1) indicate no cytotoxic effect toward the HTC cells. Similar results were previously reported by da Silva et al. (2008), where treatment with 100,000 μCi [^{131}I] did not alter the cell proliferation kinetics of blood lymphocytes in Wistar rats.

Our data suggest that high concentrations of [^{131}I] (10 μCi) are mutagenic toward *R. norvegicus* HTC cultured, which agrees with previously published data from Watanabe et al. (2004) and Joseph et al. (2009); these studies also used the MN test to assess DNA damage and showed that there was significant DNA damage in human peripheral lymphocytes at the doses 100,000 and 10,000 to 850,000 μCi [^{131}I], respectively. These data indicate that higher doses cause greater damage to cells and have a dose-dependent effect, as indicated by Gutiérrez et al. (1997). Additional studies also reported an increased frequency of chromosomal alterations in lymphocyte cells (da Silva et al., 2008) and bone marrow of Wistar rats treated with different doses of [^{131}I] (Düsman et al., 2011).

It is noteworthy that although the highest concentration of [^{131}I] may have been mutagenic, it was not significantly different from the other doses tested, which induced more MN compared to the negative control. Moreover, concentrations of [^{131}I] from 0.1 to 5 μCi had a statistically similar average number of MN, similar to the results previously described by Düsman et al. (2011) in which different doses of [^{131}I] resulted in a similar percentage of induced chromosomal aberrations in the bone marrow of Wistar rats.

The results of this study indicate that neither the antioxidants tested (synthetic beta-carotene and acerola fruit pulp) was mutagenic or cytotoxic. It has been shown previously that beta-carotene was not mutagenic toward the bone marrow cells of mice and rats based on the MN test (El-Habit et al., 2000; El-Makawy and El-Ashmaoui, 2003) and was not toxic (2.5, 3.75, and 5.0 mg) when administered to rats (Oliveira et al., 2007). The data from Düsman et al. (2012b) corroborate these results because, *in natura*, acerola pulp (0.2, 0.4 and 5.0 mg/mL) and, frozen pulp (0.2 and 0.4 mg/mL) also showed no mutagenic and cytotoxic activity in bone marrow cells of Wistar rats based on the chromosomal aberration test. In addition, using the comet assay, Nunes et al. (2011) also showed that 0.5, 1, and 2 mg/mL unripe and ripe acerola fruit showed no genotoxicity in rat cells.

Because beta-carotene (0.2 μM) and acerola pulp (0.25 g/mL) do not exert cytotoxic or mutagenic effects, and because we observed a lower number of MN among the concentrations tested, we evaluated the radioprotective activity of these compounds in response to exposure to a high dose of [^{131}I] (10 μCi).

The results showed that treatment with acerola plus [^{131}I] was not mutagenic, and although the data are not significantly different from treatment with only [^{131}I], the simultaneous treatment with acerola decreased the average number of MN induced by the radioisotope by greater than 50% (Figure 3). Nunes et al. (2011) previously showed that unripe acerola fruit demonstrates greater DNA damage protection against hydrogen peroxide damage compared to ripe fruit extracts. Thus, unripe acerola fruit may exert more significant radioprotection compared to the fresh pulp that was tested here.

It is possible that administration of acerola pulp decreased the number of MN that were induced by [¹³¹I] ionizing radiation, as indicated by Wakabayashi et al. (2003) and Hanamura et al. (2005), because certain acerola components (i.e., anthocyanins) can scavenge free radicals. The results of physico-chemical analysis corroborate the beneficial radioprotective effect of acerola in the present study, as we showed that acerola pulp had high levels of antioxidant potential (IC₅₀ about 257.1 to 290.33 µg/mL by 50% methanol/70% acetone, water and 80% methanol extracts) (Table 4) and antioxidant compounds, similar to ascorbic acid (1960.02 ± 2.32 mg/100 g sample), anthocyanins (8.09 ± 0.09 mg/100 g sample), carotenoids (0.615 ± 0.005 mg/100 g sample), yellow flavonoids (11.03 ± 0.13 mg/100 g sample), and total phenols (6142.65 ± 15.80 mg gallic acid/100 g pulp) (Table 4) (laboratory data published in Düsman et al., 2012a).

Nunes et al. (2011) also claim that the protective effect exerted by acerola may be related to vitamin C and the complex mixture of nutrients, especially in the unripe fruit. This mixture of nutrients may interact with the DNA and protect it from oxidative stress. In this sense, Blumenthal et al. (2000) also reported that the combination of vitamins E, C, and A, when administered to mice for 14 days (3 days before and 11 days after radiation exposure), reduced the toxic effect of radioimmunotherapy with 400 µCi [¹³¹I].

According to Calegario (2007), the human body can respond to ionizing radiation by neutralizing oxidizing radicals, repairing structural changes that have occurred in the DNA, inducing apoptosis if there are signs of cell instability, activating immune responses, and by recognizing the damaged cell as foreign by changes in the membrane. All of these mechanisms may have been stimulated during simultaneous treatment with acerola and [¹³¹I], which resulted in a lower number of MN.

Aside from this, significant decreases in NDI after simultaneous treatment with acerola or beta-carotene (Table 3) could be explained by the fact high NDI observed after treatment with 10 µCi [¹³¹I] may have resulted from the cells attempt to rapidly divide in response to damage and quickly replace the damaged cells, as was previously described by Düsman et al. (2011). Thus, acerola and beta-carotene, when administered with [¹³¹I], may have reduced cell damage and decreased the NDI.

However, beta-carotene had no effect on the mutagenic activity of [¹³¹I] in this study and only decreased the number of MN by approximately 16% (Figure 3). Furthermore, treatment with beta-carotene plus [¹³¹I] resulted in a lower NDI than that observed after treatment with beta-carotene only, in agreement with data previously published by Morales-Ramirez et al. (1998) in which the authors observed a synergistic effect of beta-carotene and gamma radiation. Treatment with radiation and the carotenoid appeared to increase the frequency of sister chromatid exchanges induced by gamma radiation in mice bone marrow cells that were treated before and after irradiation.

Although beta-carotene is an antioxidant, previous studies have suggested that it also has pro-oxidant and pro-carcinogenic activity (Young and Lowe, 2001; Lowe et al., 2003) because antioxidant activity depends on the pigment concentration and oxygen tension in the tissue. At high concentrations, this carotenoid may have antagonistic activity (i.e., pro-oxidant) and may induce oxidative stress by increasing free radicals or by failing to decrease the mutagenicity of [¹³¹I] ionizing radiation.

Thus, the consumption of vegetables that contain several antioxidants, such as acerola fruit, which contains vitamin C, carotenoids, anthocyanins, yellow flavonoids, and phenols, may be more beneficial than consumption of individual synthetic carotenoids like beta-car-

otene. Because there are numerous adverse side effects that are associated with radioiodine exposure, and because fresh acerola pulp appears to protect against radiation DNA damage. Future studies should be conducted to determine if acerola consumption can alleviate the harmful side effects of human [¹³¹I] exposure as a result of thyroid diagnostics and therapy.

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