Chrysobalanus icaco L. fruits inhibit NADPH oxidase complex and protect DNA against doxorubicin-induced damage in Wistar male rats

Vinicius Paula Venancio a,b, Marcella Camargo Marques b, Mara Ribeiro Almeida a, Lilian Regina Barros Mariutti b, Vanessa Cristina de Oliveira Souza a, Fernando Barbosa Jr. a, Maria Lourdes Pires Bianchi a, Cleni Mara Marzocchi-Machado a, Adriana Zerlotti Mercadante b, and Lusânia Maria Greggi Antunes a

School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil; bSchool of Food Engineering, University of Campinas, Campinas, São Paulo, Brazil

ABSTRACT
Chrysobalanus icaco L. is an underexplored plant found in tropical areas around the globe. Currently, there is no apparent information regarding the effects C. icaco fruits may exert in vivo or potential role in health promotion. This study aimed at providing evidence regarding the in vivo influence of this fruit on antigenotoxicity, antimutagenicity, and oxidative stress in rats. Male Wistar rats were treated with 100, 200, or 400 mg/kg body weight (bw)/d C. icaco fruit for 14 d. Doxorubicin (DXR, 15 mg/kg bw, ip) was used for DNA damaging and as an oxidant to generate reactive oxygen species (ROS). Genomic instability was assessed by the comet assay and micronucleus (MN) test, while antioxidant activity was determined by oxidative burst of neutrophils. Chrysobalanus icaco fruit polyphenols were quantified and characterized by high-performance liquid chromatography coupled to a diode array detector and tandem mass spectrometer (HPLC-DAD-MS/MS). The concentrations of 19 chemical elements were determined by inductively coupled plasma–mass spectrometry (ICP-MS). Significant amounts of polyphenols, magnesium, and selenium were found in C. icaco fruit. This fruit displayed in vivo antioxidant activity against DXR-induced damage in rat peripheral blood neutrophils, antigenotoxicity in peripheral blood cells, and antimutagenicity in bone-marrow cells and peripheral blood cells. Correlation analyses between endpoints examined indicated that the mechanism underlying chemopreventive actions of C. icaco fruit was attributed to inhibition of NADPH oxidase complex manifested as low levels of DNA damage in animals exposed to DXR. Data indicate that phytochemicals and minerals in C. icaco fruit protect DNA against damage in vivo associated with their antioxidant properties.

Chrysobalanus icaco L. (coco plum, guajiru or abajeru) belongs to the Chrysobalanaceae family, which consists of approximately 20 genera and 500 different species (Prance, 1979). Chrysobalanus icaco L. is native to coastal regions such as southern Florida, Brazil, the Bahamas, and the Caribbean. No apparent information regarding the effects of C. icaco fruits was found in the literature; however, leaf extracts were noted to exert biological activities such as decreasing blood sugar levels (Barbosa et al., 2013), insulin sensitivity (White et al., 2016), and angiogenesis (Alves de Paulo et al., 2000). These fruits are rich in anthocyanins (Brito et al., 2007), natural pigments that possess antioxidant capacity and are responsible for many beneficial effects as evidenced by protection of endothelial progenitor cells against angiotensin II-mediated dysfunction (Parzonko et al., 2015) and attenuation of maganese (Mn)-induced oxidative stress in rat astrocytes (da Silva Santos et al., 2014).

Cells in the organism generate reactive oxygen species (ROS), and overproduction of these molecules leads to deleterious interactions with DNA, RNA, proteins, and lipids (Maraldi, 2013; Ghio et al., 2012). Production and elimination of ROS are usually well balanced due to finely regulated systems (Lushchak, 2014; Ghio et al., 2012). Antioxidant compounds obtained from the diet might reduce or prevent excessive production of ROS by promoting endogenous antioxidant
activity and rapidly neutralizing these molecules (Harasym and Oledzki, 2014; Argentin et al., 2015). Therefore, this investigation aimed at providing novel information regarding in vivo anti-genotoxic, antimutagenic, and antioxidant actions induced by C. icaco fruit in rats previously administered a model oxidant drug, doxorubicin (DXR), with the view to potentially use C. icaco fruit in humans suffering from adverse oxidant exposure.

Material and Methods

Chrysobalanus icaco Fruit and Chemicals

Ripe C. icaco L. fruits were harvested at Praia do Farol (1° 7’ 59.98” S, 48° 27’ 33.98” W), Belém, Pará, Brazil. The moisture content of the fresh fruit (pulp + peel) was 81.95 ± 1.48 g/100 g (n = 3). Seeds were removed, and pulp and peel were immediately frozen in liquid nitrogen before lyophilization (–60°C, 50 µm Hg, 7 d, Liotop L101, Liobras, São Paulo, Brazil). The lyophilized fruit was homogenized in a food processor (Walita, Barueri, Brazil), vacuum packed, and stored at –36°C until use.

All-trans-β-carotene (99.9%, CAS 7235-40-7), quercetin (95%, CAS 6151-25-3), gallic acid (GA, 98%, CAS 149-91-7), acridine orange (AO, CAS 10127-02-3), and trypan blue (CAS 72-57-1) were purchased from Sigma-Aldrich (St. Louis, MO). Ellagic acid (EA, 95%, CAS 476-66-4), cyanidin 3-glucoside (97%, CAS 7084-24-4), delphinidin 3-glucoside (95%, CAS 6906-38-3), peonidin 3-glucoside (95%, CAS 68795-37-9), and petunidin 3-glucoside (95%, CAS 6988-81-4) were acquired from Extrasynthèse (Genay, France). All-trans-lutein (98.8%, CAS 38327-39-8) was donated by DSM Nutritional Products (Basel, Switzerland) and all-trans-violaxanthin (95%, CAS 126-29-4) and 9′-cis-neoxanthin (97%, CAS 14660-91-4) were purchased from CaroteNature (Lupsingen, Switzerland). Doxorubicin hydrochloride (DXR, CAS 25316-40-9) was obtained from Laboratório Químico Farmacêutico Bérgamo (São Paulo, Brazil). Low (CAS 39346-81-1) and normal melting point agaroses (CAS 9012-36-6) were purchased from Invitrogen (Carlsbad, CA). Dimethyl sulfoxide (DMSO, CAS 67-68-5) was purchased from Merck Chemicals (Rio de Janeiro, Brazil). GelRed Nucleic Acid Gel Stain 10,000× was purchased from Biotium (Hayward, CA). All other reagents used were of the highest possible purity.

Phytochemical Characterization

Anthocyanins were extracted from 0.05 ± 0.006 g lyophilized sample with 1% (v/v) HCl and identified and quantified by high-performance liquid chromatography coupled to a diode array detector and tandem mass spectrometer (HPLC-DAD-MS/MS) (Rosso and Mercadante, 2007). Phenolic compounds were extracted from 0.1 ± 0.01 g of lyophilized sample by maceration with 5 ml ethyl acetate and 5 ml ethyl ether, filtered and partitioned in a mixture of petroleum ether:ethyl ether (1:1, v/v). The extract was dried in a rotary evaporator (Büchi, Flawil, Switzerland) and dissolved in 10 ml petroleum ether. After saponification, carotenoids were identified and quantified by HPLC-DAD-MS/MS (Rosso and Mercadante, 2007). Phenolic compounds were extracted from 0.1 ± 0.01 g of lyophilized sample with methanol:water (8:2, v/v). The identification and quantification of phenolic compounds were carried out by HPLC-DAD-MS/MS (Chiste and Mercadante, 2012).

All extracts for identification and quantification of bioactive compounds were prepared in triplicate. The chromatographic analyses for identification and quantification of bioactive compounds were carried out in a Shimadzu HPLC (Kyoto, Japan) coupled in series to a diode array detector (DAD, model, SPD-M20A, Shimadzu) and mass spectrometer (Amazon Speed, Bruker Daltonics, Bremen, Germany).

Mineral Characterization

Freeze-dried C. icaco fruit was digested in triplicate using a microwave oven decomposition system (Milestone Ethos D, Italy) in closed vessels, according to Nardi et al. (2009). In total, 19 elements (sodium, potassium, magnesium, calcium, rubidium, iron, manganese, aluminum, zinc, copper, barium, nickel, chromium, selenium, arsenic, lead, cobalt, vanadium, and thallium) were analyzed by inductively coupled plasma–mass spectrometry (ICP-MS) (Elan DRC II, Perkin Elmer, Norwalk, CT).
Animals, Experimental Design, and Dose Selection

The Local Ethics Committee for Animal Use approved the experimental protocol used in this investigation (approval number 11.1.1517.53.0). Male Wistar rats (Rattus norvegicus), 4–5 wk old, weighing 110 ± 10 g, were provided by the animal facility of “Prefeitura do Campus USP de Ribeirão Preto” and kept in polycarbonate cages at 22 ± 2°C, with 12-h light/dark cycle, with ad libitum access to food (Nuvilab, Colombo, Brazil) and fresh water.

Since there are no apparent data regarding C. icaco fruit toxicity, 400 mg/kg body weight (bw)/d was selected as the maximal dose because it mimics consumption of this fruit as a 200-ml daily juice preparation. Animals were divided into 8 groups (n = 6 animals/group) and treated as follows: Controls received water for 14 d. DXR animals alone were intraperitoneally injected (ip) with 15 mg/kg bw 24 h before euthanasia. Groups of animals were administered daily by gavage 100, 200, or 400 mg/kg bw/d C. icaco fruit alone for 14 d. In addition, groups of rats were also administered daily by gavage 100, 200, or 400 mg/kg bw/d C. icaco for 14 d followed by DXR (15 mg/kg bw, ip) 24 h before euthanasia. The freeze-dried C. icaco fruit was dispersed in fresh water and administered daily by gavage to the experimental groups. Twenty-four hours after the last injection, all animals were anesthetized (ketamine/xylazine 100/10 mg/kg bw) and euthanized by cardiac puncture. Blood and bone-marrow cells were collected.

Peripheral Blood Alkaline Comet Assay

In vivo genotoxicity and antigenotoxicity of C. icaco fruit were assessed as previously described by Singh et al. (1988) and Tice et al. (2000). Blood samples were collected in tubes with K2 EDTA preservative (Labtest, Lagoa Santa, Brazil), mixed in 0.5% (w/v) low melting point agarose, and spread on 1.5% (w/v) agarose pretreated microscope slides. After agarose solidification (20 min, 4°C), slides were subjected to lysis (overnight, 4°C), DNA unwinding (20 min, 4°C), electrophoresis (20 min, 0.85 V/cm, 300 mA, 4°C), neutralization (5 min, pH 7.5, 4°C), and fixation (ethanol, 2 min). Immediately before analysis, slides were stained with GelRed (1:10,000 v/v). Tail intensity (percentage of DNA in tail) and tail moment were analyzed in 100 nucleoids per animal using Comet Assay IV software (Perceptive Instruments, Suffolk, UK) and a fluorescence microscope (Axioskop, Zeiss) equipped with 515–560 nm excitation and 590 nm barrier filters. Cell viability was determined in cell suspensions by the trypan blue exclusion method, and all results were greater than 90%.

Micronucleus (MN) Test

The in vivo mutagenicity and antimutagenicity of C. icaco fruit were investigated using the bone-marrow and peripheral blood micronucleus (MN) tests. The bone-marrow cells were collected in fetal bovine serum (FBS) as described by Schmid (1975). Cells were smeared on clean microscope slides, dried, fixed in methanol for 2 min, and stained with Giemsa (pH 5.5). The frequency of micronuclei (MN) in polychromatic erythrocytes (PCE) among 2000 PCE and the ratio between PCE and normochromatic erythrocytes (NCE) among 500 bone-marrow erythrocytes were assessed using light microscopy (Carl Zeiss Axioskop Plus) at 1000× magnification.

The peripheral blood MN test was performed as described by Holden et al. (1997). A drop of peripheral blood from the tail vein was collected and smeared on cleaned microscope slides. The slides were dried, fixed in methanol, and stained with 16 μg/ml AO. The frequency of MN in 1000 reticulocytes (RET) was scored using a fluorescence microscope (Axioskop, Zeiss) equipped with a 488 nm excitation filter.

Oxidative Burst of Neutrophils

Neutrophils were isolated from peripheral blood according to Russo-Carbolante et al. (2002). Cell viability was performed by the trypan blue exclusion method, and purity of neutrophil population was assessed by Giemsa staining. The oxidative burst was measured by chemiluminescence using 1 × 10⁵ neutrophils per reaction, 10⁻⁷ M phorbol 12-myristate 13-acetate (PMA) as stimulus and
10^{-4} M luminol as the luminescent label. The luminescence intensity was followed for 20 min at 37 °C by a luminometer (Autolumat Plus LB 953, EG&G Berthold, Bad Wildbad, Germany) and the area under the curve (AUC) was calculated for each measurement.

**Statistical Analysis**

All results are expressed as mean ± standard deviation (SD). The normality of variable distributions was analyzed by the Kolmogorov–Smirnov test. Data were analyzed by analysis of variance (ANOVA)–Tukey using the software GraphPad Prism 6.0. The *p* values <.05 were considered significant. For the MN test, the percent reduction was calculated according to Waters et al. (1990).

**Results**

**Phytochemical and Mineral Composition**

Phytochemical characterization is presented in Table 1 and described in detail in the Supplemental Data file. Four anthocyanins, namely, delphinidin 3-glucoside, cyanidin 3-glucoside, petunidin 3-glucoside, and peonidin 3-glucoside, were identified based upon the comparison of elution order from C_{18} column and mass spectra features compared to authentic standards analyzed under the same conditions. Cyanidin 3-glucoside and peonidin 3-glucoside were identified for the first time in *C. icaco* fruit. The MS data demonstrate coelution of petunidin 3-glucoside and an acylated anthocyanin tentatively identified as delphinidin 3-(6′′-acetoyl)galactoside or delphinidin 3-(6′′-oxaloyl)arabinoside. These two coeluted anthocyanins corresponded to more than 47% of anthocyanins in this fruit. The assignment of sugar and acid moieties identity in all acylated compounds (Supplementary Table 1) was based upon loss of 204 U in the MS/MS spectra of the molecular ion, which corresponds to loss of one hexose and one acetyl group [M-162-42]^+ or to loss of one arabinose and one oxalyl group [M-132-72]^+ (Wu and Prior, 2005). Thus, it was not possible to differentiate these compounds by MS. The other three anthocyanins found in *C. icaco* were tentatively identified as petunidin 3-(6′′-acetoyl)galactoside or petunidin 3-(6′′-oxaloyl)arabinoside, peonidin 3-(6′′-acetoyl)galactoside or peonidin 3-(6′′-oxaloyl)arabinoside, and petunidin 3-(6′′-acetoyl)glucoside or petunidin 3-(6′′-oxaloyl)arabinoside.

All-trans-lutein was the major carotenoid found in *C. icaco* fruit, representing more than 22% of total carotenoids. Three phenolic compounds were tentatively identified as an EA derivative, a myricetin pentoside, and a quercetin derivative. The concentrations of 19 chemical

### Table 1. Phytochemical and mineral composition of *C. icaco* fruit.

| Compound classes | Major compounds | Non-anthocyanic phenolic compounds | Total anthocyanins | Total carotenoids | Chemical elements |
|------------------|----------------|-----------------------------------|--------------------|------------------|------------------|
|                  |                | 213.33 ± 0.37^{a}                 | 547 ± 44^{c}       | 850 ± 60^{d}    | Sodium (1642 ± 44)^{g}, potassium (1469 ± 35)^{g}, magnesium (282 ± 6.8)^{h}, calcium (216 ± 6.0)^{h}, rubidium (3.8 ± 0.07)^{i}, iron (2.7 ± 0.4)^{i}, manganese (1.3 ± 0.03)^{h}, aluminum (1.2 ± 0.2)^{h}, zinc (1.2 ± 0.04)^{i}, copper (0.9 ± 0.02)^{i}, barium (0.3 ± 0.01)^{i}, nickel (0.3 ± 0.01)^{i}, chromium (0.2 ± 0.02)^{i}, selenium (0.09 ± 0.04)^{i}, arsenic (20.6 ± 1.9)^{i}, lead (9.8 ± 3.6)^{i}, cobalt (6.7 ± 0.7)^{i}, vanadium (2.5 ± 0.9)^{i}, thallium (0.0 ± 0.1)^{i} |
|                  |                | Ellagic acid derivative (133.43 ± 1.04)^{g}, myricetin pentoside (18.68 ± 0.70)^{i}, quercetin derivative (61.22 ± 2.59)^{g} | 547 ± 44^{c}       | 850 ± 60^{d}    | Sodium (1642 ± 44)^{g}, potassium (1469 ± 35)^{g}, magnesium (282 ± 6.8)^{h}, calcium (216 ± 6.0)^{h}, rubidium (3.8 ± 0.07)^{i}, iron (2.7 ± 0.4)^{i}, manganese (1.3 ± 0.03)^{h}, aluminum (1.2 ± 0.2)^{h}, zinc (1.2 ± 0.04)^{i}, copper (0.9 ± 0.02)^{i}, barium (0.3 ± 0.01)^{i}, nickel (0.3 ± 0.01)^{i}, chromium (0.2 ± 0.02)^{i}, selenium (0.09 ± 0.04)^{i}, arsenic (20.6 ± 1.9)^{i}, lead (9.8 ± 3.6)^{i}, cobalt (6.7 ± 0.7)^{i}, vanadium (2.5 ± 0.9)^{i}, thallium (0.0 ± 0.1)^{i} |
|                  |                | Petunidin 3-glucoside + delphinidin 3-(6′′-acetoyl)galactoside or delphinidin 3-(6′′-oxaloyl)arabinoside (257 ± 20)^{i} | 547 ± 44^{c}       | 850 ± 60^{d}    | Sodium (1642 ± 44)^{g}, potassium (1469 ± 35)^{g}, magnesium (282 ± 6.8)^{h}, calcium (216 ± 6.0)^{h}, rubidium (3.8 ± 0.07)^{i}, iron (2.7 ± 0.4)^{i}, manganese (1.3 ± 0.03)^{h}, aluminum (1.2 ± 0.2)^{h}, zinc (1.2 ± 0.04)^{i}, copper (0.9 ± 0.02)^{i}, barium (0.3 ± 0.01)^{i}, nickel (0.3 ± 0.01)^{i}, chromium (0.2 ± 0.02)^{i}, selenium (0.09 ± 0.04)^{i}, arsenic (20.6 ± 1.9)^{i}, lead (9.8 ± 3.6)^{i}, cobalt (6.7 ± 0.7)^{i}, vanadium (2.5 ± 0.9)^{i}, thallium (0.0 ± 0.1)^{i} |
|                  |                | All-trans-lutein (188.6 ± 9.4)^{g}, 9′-cis-neoxanthin (99.8 ± 10.9)^{g}, all-trans-β-carotene (79.2 ± 12.4)^{g}, 9-cis-violaxanthin (76.7 ± 13.4)^{g}, all-trans-violaxanthin (75.7 ± 8.6)^{g} | 547 ± 44^{c}       | 850 ± 60^{d}    | Sodium (1642 ± 44)^{g}, potassium (1469 ± 35)^{g}, magnesium (282 ± 6.8)^{h}, calcium (216 ± 6.0)^{h}, rubidium (3.8 ± 0.07)^{i}, iron (2.7 ± 0.4)^{i}, manganese (1.3 ± 0.03)^{h}, aluminum (1.2 ± 0.2)^{h}, zinc (1.2 ± 0.04)^{i}, copper (0.9 ± 0.02)^{i}, barium (0.3 ± 0.01)^{i}, nickel (0.3 ± 0.01)^{i}, chromium (0.2 ± 0.02)^{i}, selenium (0.09 ± 0.04)^{i}, arsenic (20.6 ± 1.9)^{i}, lead (9.8 ± 3.6)^{i}, cobalt (6.7 ± 0.7)^{i}, vanadium (2.5 ± 0.9)^{i}, thallium (0.0 ± 0.1)^{i} |

*Note.* All results are expressed in dry weight (DW) or fresh weight (FW), as the mean ± SD of triplicate analyses.

^{a}mg ellagic acid equivalent/100 g DW.

^{b}mg cyanidin 3-glucoside equivalent/100 g DW.

^{c}µg all-trans-β-carotene/100 g DW.

^{d}µg all-trans-lutein/100 g DW.

^{e}µg 9′-cis-neoxanthin equivalent/100 g DW.

^{f}µg all-trans-violaxanthin/100 g DW.

^{g}µg/g FW.

^{h}ng/g FW.
elements found in the *C. icaco* fruit are also presented in Table 1.

### Antigenotoxicity of *C. icaco* Fruit in Peripheral Blood by Comet Assay

The comet assay was performed to determine whether *C. icaco* fruit might by itself produce DNA damage or reduce DXR-induced DNA damage in peripheral blood cells. The *C. icaco* fruit did not produce significant genotoxic effects but markedly decreased DXR-induced tail moment and tail intensity in these cells (Figure 1a and Figure 1b). Both DNA damage parameters tail moment and tail intensity were significantly reduced in peripheral blood cells in groups treated with *C. icaco* fruit and DXR compared to DXR alone and the values returned to control levels.

### Chrysobalanus icaco Fruit Antimutagenicity by MN Test

Mutagenicity was also evaluated by MN assay in peripheral blood and bone-marrow cells. The results are shown in Table 2. No marked differences were observed between control and *C. icaco* fruit alone groups for PCE/NCE ratio. The sensitivity of this in vivo experiment was demonstrated by increased MN frequency found in the DXR alone treatment group compared to the control in both peripheral blood and bone-marrow cells. All three *C. icaco* tested doses when administered prior to DXR exerted protective effects compared to DXR alone as evidenced by percent MN reduction in both cell types. For both cell populations analyzed, the higher the dose, the more pronounced was the protective effect.

### Oxidative Burst of Neutrophils

The oxidative burst of neutrophils from peripheral blood was performed to assess the antioxidant activity of *C. icaco* fruit in these cells. The results are illustrated in Figure 2. DXR increased the chemiluminescent response of neutrophils.

![Figure 1](https://via.placeholder.com/150)

**Figure 1.** Antigenotoxicity of *C. icaco* fruit (Ci) on (a) tail moment and (b) tail intensity of peripheral blood cells by alkaline comet assay. Data are mean ± SD, n = 6. DXR: doxorubicin (15 mg/kg bw, ip); Ci100, Ci200, Ci400: *C. icaco* fruit at 100, 200, or 400 mg/kg bw/day. Symbols: a, not significantly different from control; b, significantly different from control *p* < .05; c, significantly different from DXR alone (*p* < .05, ANOVA-Tukey).

![Figure 2](https://via.placeholder.com/150)

**Table 2.** Antimutagenicity of *C. icaco* fruit (Ci) in peripheral blood and bone marrow cells by micronucleus test, *n* = 6. 

| Groups | Peripheral blood | Bone marrow |
|--------|------------------|-------------|
|        | MN/1000 | RET (%) | PCE/NCE ratio | MN/1000 | PCE | Reduction (%) | PCE/NCE ratio |
| Water  | 1.7 ± 1.6 | 0.4 ± 0.1 | 1.5 ± 1.1 | | | | |
| Ci100  | 1.1 ± 1.1<sup>a</sup> | 0.4 ± 0.1<sup>a</sup> | 0.8 ± 0.7<sup>a</sup> | | | | |
| Ci200  | 1.3 ± 0.8<sup>a</sup> | 0.4 ± 0.1<sup>a</sup> | 1.2 ± 0.9<sup>a</sup> | | | | |
| Ci400  | 1.3 ± 0.5<sup>a</sup> | 0.4 ± 0.1<sup>a</sup> | 0.9 ± 0.8<sup>a</sup> | | | | |
| DXR    | 13.3 ± 2.3<sup>b</sup> | 0.4 ± 0.1<sup>a</sup> | 16.0 ± 3.2<sup>b</sup> | | | | |
| Ci100 + DXR | 7.2 ± 2.1<sup>c</sup> | 0.5 ± 0.1<sup>a</sup> | 7.9 ± 1.5<sup>c</sup> | 52.8 | | | 55.9 |
| Ci200 + DXR | 2.2 ± 1.2<sup>c</sup> | 0.4 ± 0.1<sup>a</sup> | 4.4 ± 0.5<sup>c</sup> | 95.7 | | | 80.0 |
| Ci400 + DXR | 2.0 ± 1.4<sup>c</sup> | 0.4 ± 0.2<sup>a</sup> | 4.2 ± 2.2<sup>c</sup> | 97.2 | | | 81.6 |

**Note.** Data are mean ± SD, *n* = 6. MN: micronuclei; RET: reticulocytes; PCE: polychromatic erythrocytes; NCE: normochromatic erythrocytes; Ci100, Ci200, Ci400: *C. icaco* fruit at 100, 200, or 400 mg/kg bw/day; DXR: doxorubicin (15 mg/kg bw, ip).

<sup>a</sup>Not significantly different from control.

<sup>b</sup>Significantly different from control (*p* < .05).

<sup>c</sup>Significantly different from DXR alone (*p* < .05, ANOVA-Tukey).
compared to the control. The C. icaco fruit did not induce a marked oxidative burst in neutrophils but diminished the DXR-induced response at all three tested doses. The oxidative burst inhibition by C. icaco fruit seemed to be dose-dependent since neutrophils exposed to 400 mg/kg bw/d Ci exhibited the lowest chemiluminescent response.

**Correlation Between Oxidative Burst of Neutrophils and DNA Damage Biomarkers**

Taken together, the DNA damage biomarkers assessed by comet assay and the antimutagenicity activity determined by MN test were found to be related to a decrease in oxidative burst. Correlation analysis showed a high correlation between the oxidative burst of neutrophils and comet assay tail moment ($r^2 = .75$), comet assay tail intensity ($r^2 = .81$), bone marrow MN test ($r^2 = .92$), and peripheral blood MN test ($r^2 = .89$).

**Discussion**

This is the first apparent report on the composition of carotenoids and phenolic compounds present in C. icaco fruits. The most abundant carotenoid in C. icaco fruit, all-trans-lutein, was previously shown to modulate the expression of antioxidant genes and decrease DNA damage in mice (Serpeloni et al., 2014). Diets high in β-carotene, another carotenoid found in C. icaco fruit, are associated with reduced risk of type 2 diabetes in healthy men and women (Sluijs et al., 2015). The content of anthocyanins (104 mg cyanidin 3-glucoside equivalents/100 g fresh weight) detected in this study is similar to that previously reported for the same fruit by Brito et al. (2007). These compounds form a complex with the DNA strand, which protects the DNA structure from damage (Sarma and Sharma, 1999). Ellagic acid was the major phenolic compound found in C. icaco fruit. Previous studies demonstrated that EA improved the antioxidant defense system in T cells against lymphoma (Mishra and Vinayak, 2014) and protected cardiomyocytes against DXR-induced toxicity (Lin and Yin, 2013).

In addition to assessing the phytochemical composition of C. icaco fruits, determination of the concentration of certain inorganic chemical elements in these fruits is also important (da Silva Santos et al., 2014). There are no apparent data in the literature concerning the inorganic composition of this fruit. Several “essential trace elements” have an important role in immunological, endocrinological, and antioxidant reactions in the body and their deficiency may lead to diseases (Houston, 1994; Meek et al., 2010; Stern, 2010; Santamaria and Sulsky, 2010).

The chemical element composition indicates C. icaco fruit have as much magnesium (Mg) as avocados (290 µg/g fresh weight) or bananas (270 µg/g fresh weight), as well as three- to sevenfold more selenium (Se) than other Se-rich fruits, such as kiwi fruit (0.031 µg/g fresh weight), grapefruit (0.014 µg/g fresh weight), and tamarinds (0.013 µg/g fresh fruit) (U.S. Department of Agriculture [USDA], 2016). The roles of these elements on health were previously studied: Mg is a micronutrient involved in more than 600 enzymatic reactions, and its supplementation has been recommended for treating migraines, depression, and epilepsy (Baaij et al., 2014). Selenium plays important roles in oxidative stress and antioxidant capacity in humans (Gać et al., 2015).

The phytochemical composition of fruits and vegetables is often responsible for antigenotoxic and antimutagenic effects (Nunes et al., 2013).
Besides C. icaco fruit, other delphinidin- and petunidin-rich fruits or extracts possess in vivo and in vitro antigenotoxic activities (Habermeyer et al., 2005). Both the major phenolic compound and carotenoid found in C. icaco fruit exert antigenotoxic and/or antimutagenic actions as described for EA by Rehman et al. (2012) and for lutein by Serpeloni et al. (2010).

In this investigation, anthocyanins, carotenoids, phenolic compounds, and minerals from C. icaco fruit are probably responsible for decreasing both tail moment and tail intensity comet assay parameters. Since the DNA structure of the C. icaco-treated animals was more intact due to the protective effect of this fruit’s compounds on the genome, this resulted in a significant fall in both bone-marrow and peripheral blood MN frequencies.

The oxidative burst of neutrophils showed that C. icaco fruit decreased ROS production induced by DXR in blood neutrophils after PMA stimulation. It is possible that the phenolic compounds (including anthocyanins) and carotenoids present in this fruit may act either by deactivating the NADPH oxidase complex leading to a decrease in superoxide ion generation by PMA or by increasing the scavenging of ROS generated by neutrophils (Ciz et al., 2012). Other anthocyanin-rich fruits were also shown to act as oxidative stress inhibitors, such as blueberries (Braga et al., 2013) and acai (da Silva Santos et al., 2014).

Correlation analyses provide stronger evidence regarding the chemopreventive mechanism underlying C. icaco fruit-mediated effects. The high correlation coefficients obtained allow us to predict that inhibition of the NADPH oxidase complex or enhanced ROS scavenging rate by C. icaco led to a significant fall in both comet assay biomarkers and reduced frequencies of DNA fragments assessed in both bone-marrow and peripheral blood erythrocytes.

**Conclusions**

In summary, data suggest C. icaco fruit may act as dietary antioxidant and subsequently in vivo protect the DNA from damage induced by DXR. This investigation provides novel information regarding this polyphenol-rich fruit, which may stimulate future research and optimize the use of this underutilized fruit in human health protection.

**Conflict of Interest**

The authors declare that they have no conflict of interest.

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**ORCID**

Vinicius Paula Venancio [http://orcid.org/0000-0003-3239-6380]
Marcella Camargo Marques [http://orcid.org/0000-0002-5917-0843]
Maria Ribeiro Almeida [http://orcid.org/0000-0001-9736-3796]
Fernando Barbosa Jr. [http://orcid.org/0000-0002-498-0619]
Maria Lourdes Pires Bianchi [http://orcid.org/0000-0001-5451-8663]
Clêni Mara Marzocchi-Machado [http://orcid.org/0000-0003-2056-2900]
Adriana Zerlotti Mercadante [http://orcid.org/0000-0003-4547-6064]
Lusânia Maria Greggi Antunes [http://orcid.org/0000-0002-3079-4388]

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