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Organically produced coffee exerts protective effects against the micronuclei induction by mutagens in mouse gut and bone marrow

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ABSTRACT

While researchers have extensively evaluated the beneficial effects of coffee consumption in reducing the frequency of certain diseases, studies examining the differences between organic and conventional coffee intake are still needed. Therefore, this paper aims to investigate the functional effects of organic and conventional coffee by examining both its chemical composition and its mutagenic/antimutagenic properties.
Infusions of 10% or 20% (w/v) of organic and conventional coffee were administered by gavage (10 mL/kg b.w., once or twice a day) to male Swiss mice against doxorubicin (DXR) and 1,2-dimethylhydrazine dihydrochloride (DMH)-induced mutagenicity. The levels of chlorogenic acids, caffeine and trigonelline from the coffee infusions and oxidative stress analysis from the liver were measured by HPLC. Gut and bone marrow micronucleus assays were used as mutagenic/antimutagenic endpoints, as well as the crypt measurements and gut apoptosis index. The *in vivo* tests revealed that only organic coffee exerted protective effects, despite oxidative stress analysis and crypt measurements not showing differences among treatments. Intriguingly, the low dose (10% w/v mL/kg) displayed a robust protective effect that showed a significant reduction in bone marrow micronuclei (26.8%), gut micronuclei (11.5%) and apoptosis (27.8%), whereas the higher coffee dose (2 × 20% w/v) only showed a protective effect against bone marrow micronucleus (43.7%). These results highlight that organic coffee could be considered to have beneficial functional effects, although it is still a challenge to define conclusions from analytical data and all the possible interactions from this complex food matrix.

1. Introduction

There are many controversies over the effects of organic coffee, especially in regards to their effects on human health of its chemical contaminants and nutritional composition. Previous studies have demonstrated few and inconsistent differences in the nutrient composition of organically produced foods when compared to foods produced by conventional methods (Bourn & Prescott, 2002; Herencia, García-Galavís, Dorado, & Maqueba, 2011). Despite these controversies, the perception among consumers is that organically produced crops possess higher nutritional quality; this added value results in prices that are minimally 20% higher than crops produced on non-organic farms (Dos Santos, Dos Santos, & Conti, 2009). Currently, Brazil is the major world producer of coffee, and incorporates the three different agricultural methods for coffee cultivation, *i.e.*, organic, traditional and technological procedures (ICO, 2012).

Coffee is the third most widely consumed beverage in the world, after water and tea (Villanueva et al., 2006). It is a complex mixture of bioactive compounds that contain the original coffee constituents, such as caffeine, caffeoyl quinic acids (CQAs) and trigonelline, along with compounds formed during roasting, such as N-methylpyridinium (NMP), nicotinic acid, nicotinamide and melanoidins (Lang, Yagar, Eggers, & Hofmann, 2008). These compounds act as radical scavengers, inducing the expression of antioxidant enzymes, in addition to exhibiting metal chelating activity, observed in different *in vitro* and *in vivo* bioassays (Bakuradze et al., 2010). Evidence is gradually revealing that a high coffee consumption may reduce the risk of some types of

Abbreviations: DXR, doxorubicin; bw, body weight; i.p., Intraperitoneally; DMH, 1,2-dimethylhydrazine dihydrochloride; MN, micronuclei; PS, physiological saline; BMG, body mass gain; SGR, specific growth rate; FCR, feed conversion ratio; OC, organic coffee; CC, conventional coffee; PCE, polychromatic erythrocytes; HE, hematoxylin–eosin; AI, apoptosis index; TBA, thiobarbituric acid; TEP, 1,1,3,3-tetraethoxypropane; MDA, malondialdehyde; DNPH, dinitrophenylhydrazine; MNPCE, micronucleated polychromatic erythrocytes; PCO, protein carbonyl content; HDL, high density lipoprotein; non-HDL, non-high density lipoprotein.

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human cancer, and this risk reduction is mainly associated with its antioxidant activities (Nkondjock, 2009).

Coffee consumption has also been associated with a variety of adverse effects that cannot be ignored. Major health concerns are the addiction to caffeine and potential for withdrawal syndrome, increased central nervous system activity, increased anxiety, insomnia, and potential for lower birth weight in pregnancy (Dorea & Da Costa, 2005). Additionally, carcinogenic compounds, such as polycyclic aromatic hydrocarbons, can also be formed by the incomplete combustion of organic matter during roasting. Fortunately, these carcinogenic compounds have been detected only at insignificant quantities in brewed coffee (Orecchio, Ciotti, & Culotta, 2009). Furthermore, many studies enumerate the potentiating effect that coffee and caffeine have on mutagenesis mediated by both chromosomal aberrations and the shortened repair time of chromosomal damage induced by other mutagenic agents (Nehlig & Debry, 1994). Subsequently, while mutagenic studies on conventional coffee are contradictory, such studies are non-existent for the effects of organic coffee and should be performed.

Among the various techniques used to detect genetic and genotoxic effects, the micronucleus assay is widely applicable for different cell types with potential for detection of both aneugens and clastogens (Kirsch-Volders et al., 2011). Although the micronucleus test is most frequently used to evaluate bone marrow, the gut micronucleus assay considers the gastrointestinal tract and its contact with food. For these assays, the potent mutagens and carcinogens DXR (Dhawan, Kayani, Parry, Parry, & Anderson, 2003) and DMH (Poul, Jarry, Elhkim, & Poul, 2009; Suzuki et al., 2009; Vanhauwaert, Vanparys, & Kirsch-Volders, 2001) are widely used. DXR is a cytotoxic and mutagenic agent that induces micronucleus formation in mammalian system through its clastogenic and aneugenic effects (Dhawan et al., 2003). This micronuclei formation is mediated through the accumulation of reactive oxygen species (Kiyomiya, Matsuo, & Kurebe, 2001), the stabilization of the topoisomerase II–DNA complex, and the enzymatic inhibition of DNA-dependent protein (Guano et al., 1999; Wassermann, 1996). In contrast, the colon carcinogen DMH is considered a potent alkylating and clastogenic compound that induces point mutations, micronuclei formation, sister chromatid exchanges, methyl adducts of DNA bases and apoptosis in the colonic epithelial cells (Newell & Heddle, 2004).

Because of the health implications of coffee drinking, as well as the scarcity of data on the difference between organic and conventional production, our research evaluates the functional effects, chemical composition and mutagenic/antimutagenic aspects of organic *versus* conventional coffee production.

2. Materials and methods

2.1. Coffee samples and coffee infusions

For assays, we used samples of roasted ground *Coffea arabica* L. cv. Mundo Novo from the 2009/2010 crop, which were naturally processed, rated as hard grade (78 points), and medium roasted (55# agtron) in a commercial fluidized bed roaster (i-Roast model no. 40009, USA, Hearthware, 210–220 °C) for 8 min. The organic and conventional samples were provided by the Associação de Pequenos Produtores de Poço Fundo, Minas Gerais, Brazil, and the organic coffee was certified (BCSOKO Garantie Master Certificates nº A-2007-00308/2010-02629). To characterize these coffee powders, an analysis of the coffee powder was performed in triplicate as follows: moisture content was determined by exposure to infrared radiation at 120 °C for 8 min (IAL, 2008); fat was measured using the Bligh and Dyer (1959) method; protein was assessed using the Kjeldahl procedure (conversion factor 6.25); and ash content was determined by incineration at 550 °C in a muffle furnace, and carbohydrates were calculated from the remainder (the difference using the fresh weight-derived) by AOAC (2005). The infusions used in the present study were prepared by adding 5 g or 10 g of coffee powder to 50 mL of water heated at 90 °C and then filtered through a paper filter (pore diameter 14 μm).

2.2. Analysis of chlorogenic acid, caffeine and trigonelline

Coffee infusions and chemical standards (*i.e.*, chlorogenic acids, caffeine and trigonelline) were dissolved in methanol (2.0 mg/mL) (n = 3). The analysis was performed using a SHIMADZU PROMINENCE high-performance chromatograph coupled to a UV–visible (UV/vis) spectrophotometric detector (model SPD-M20A), a SIL-20A power injector and a C-18 VC-ODS RP18 175 column of 25 cm. The mobile phase consisted of H_2O/CH_3COOH (95/5 v/v) (A) and acetonitrile (B) and used the following gradient elution from A/B: 0 min, 95/5; 5 min, 95/5; and 10 min, 87/13; the phase also had a flow rate of 0.7 mL/min. The UV/vis signal detection was programmed as follows: 0–15 min, 272 nm; 15–23 min, 320 nm; and 23–40 min, 272 nm (Alves, Dias, Benassi, & Scholz, 2006).

2.3. Animals, treatment and growth performance

The animals used in this study were handled in accordance with the Ethical Principles for Animal Research adopted by the Brazilian College of Animal Experimentation (COBEA) with a protocol approved by the University's Ethical Committee for Animal Research (protocol nº. 316/2010). Male Swiss mice were obtained from CEMIB (UNICAMP Campinas, SP, Brazil) and were approximately 4–5 weeks of age. Mice were fed *ad libitum* with a commercial pellet diet (Fri-lab Ratos II®) and water. The mice were divided into 10 ten groups of 10 or 20 animals per group (Table 1). The groups received different infusions of coffee or water by oral gavage of 10 mL/kg twice a day for 15 days. Before the end of the experiment (24 h), half of the animals from groups 1 to 7 (n = 10 animals) received Doxorubicin chloridate i.p. (DXR — Rubidox®, Bergamo Laboratory; 30 mg/kg), and the other half (n = 10 animals) received an oral gavage of 1,2-dimethylhydrazine dihydrochloride (DMH, Sigma, St. Louis, MO, USA) (30 mg/kg). Both treatments comprised a single dose with a volume of 10 mL/kg. Groups 8 to 10 (n = 10 animals) received only a physiological solution (PS, NaCl 0.9% w/v). The animals that received DXR treatment (n = 10) were used for the bone marrow MN analysis, and the animals (n = 10) that received DMH treatment were used for the gut MN test, apoptosis analysis and morphometric measurements. At the end of the study, all animals were anesthetized with ketamine and xylazine and then euthanized by exsanguination. During necropsy, the bone marrow

Table 1
Experimental design for the evaluation of the mutagenic/antimutagenic effects of organic/conventional coffee beverages.

Groups (n)	Treatments (first gavage)	Treatments (second gavage)	*In vivo* test	
			Bone marrow assay**	Gut assay**
G1*	Water	Water	DXR	DMH
G2*	OC 10% (w/v)	Water	DXR	DMH
G3*	OC 20% (w/v)	Water	DXR	DMH
G4*	OC 20% (w/v)	OC 20% (w/v)	DXR	DMH
G5*	CC 10% (w/v)	Water	DXR	DMH
G6*	CC 20% (w/v)	Water	DXR	DMH
G7*	CC 20% (w/v)	CC 20% (w/v)	DXR	DMH
G8**	Water	Water	PS	
G9**	OC 20% (w/v)	Water	PS	
G10**	CC 20% (w/v)	Water	PS	

DMH: 1,2-dimethylhydrazine dihydrochloride(30 mg/kg b.w.); DXR: doxorubicin (30 mg/kg b.w.); PS: physiological solution (NaCl 0.9% w/v); OC: organic coffee; CC: conventional coffee; bone marrow assay: MN test; Gut assay: MN test, apoptosis and morphometric measurements.
* n = 20.
** n = 10.