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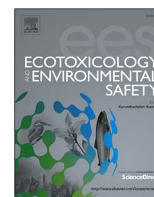

Impacts of discarded coffee waste on human and environmental health

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ABSTRACT

Coffee is one of the most widely consumed beverages throughout the world. So far, many studies have shown the properties of coffee beverages, but little is known about its impacts on human and environmental health from its discard in the environment. So, the present work aims to investigate the mutagenic, genotoxic, cytotoxic and ecotoxic effects of leached (LE) and solubilized (SE) extracts from coffee waste, simulating the disposal of this residue in landfills and via sewage systems, respectively. Chemical analyses were also carried out. LE and SE induced mutagenicity in the TA98 Salmonella strain with and without exogenous metabolization (S9). In the T A100 only SE induced mutagenicity, what was observed without S9. An increase in the frequency of micronuclei was observed in HepG2 cell line after 3 and 24 h of exposure to both extracts. No cytotoxic effects were observed in HepG2 cells by WST-1 assay. The EC50 values for the LE and SE were 1.5% and 11.26% for Daphnia similis, 0.12% and 1.39% for Ceriodaphnia dubia and 6.0% and 5.5% for Vibrio fischeri, respectively. Caffeine and several transition metals were found in both extracts. Coffee waste discarded in the environment may pose a risk to human and environmental health, since this compound can cause DNA damage and present toxicity to aquatic organisms.

1. Introduction

Coffee has been consumed for over 1000 years and is currently one of the most widely consumed beverages around the world. According to the International Coffee Organization more than 8 million tons of this compound were consumed worldwide in 2014 (International Coffee Organization, 2015). Two species are of significant economic importance Coffea arabica (Arabica) providing 75% of the world production and Coffea canephora (Robusta), which provides 25% of the world production (Varnam, Sutherland, 1994; Belitz et al., 2009).

So far, many studies have shown the properties of coffee beverages, such as antioxidant (Gómez-Ruiz et al., 2007), antibacterial (Meckelburg et al., 2014), anti-inflammatory and anti-obesity properties (Jia et al., 2014), and effects on type 2 diabetes mellitus (Akash et al., 2014), amongst several others, but little is known about the impacts on human and environmental health from its disposal in the environment.

During the extraction of the beverage from coffee powder with hot water, a large amount of residue is produced, and considering the worldwide coffee consumption, it can be concluded that tons of coffee waste are generated from cafeterias and domestic production (Tokimoto et al., 2005). The exact values of the quantities discarded have not been established, but can be estimated based on the values generated by industry. This sector of the economy consumes about 50% of the world's coffee in the production of instant coffee, generating about 6 million tons of coffee waste annually (Tokimoto et al., 2005). Thus the amount of coffee waste generated from cafeterias and domestic production must be huge.

Some applications for the use of these residues have been speculated, but most remain unused and are discarded into the environment possibly, endangering human and environment health (Leifa et al., 2000).

Considering cafeterias and domestic production, the waste is mainly discarded in the trash and then sent to a landfill. Another way to discard it is down the sink, where the residue can reach water bodies through the sewage.

Thus in view of the large amount of coffee waste generated from coffee beverage making process and the lack of studies about the impact of this residue on human and environment health, this work aimed to investigate the mutagenic, genotoxic, cytotoxic and ecotoxic effects of

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leached and solubilized extracts from coffee waste, simulating the disposal of this residue in landfills and via the sewage, respectively, besides identifying the physicochemical properties and chemical constitution of these samples.

2. Material and methods

2.1. Sampling

The leached and solubilized extracts were prepared according to the Brazilian Association of Technical Standards 10005 and 10006, respectively (Brazilian Association of Technical Standards, 2004a, 2004b).

To prepare the leached extract, 50.0 g samples of traditional Pilão® coffee waste (about 70% Arabica and 30% Robusta, grain size < 9.5 mm) were transferred to polyethylene bottles and treated with 1.0 L of the extraction solution (5.7 mL of glacial acetic acid, 64.3 mL of 1.0 mol/L NaOH and 930 mL of Milli-Q water). The bottles were closed and shaken for about 18 h using a rotary shaker at 100 rpm (Nova Etica® model 430) at room temperature, and then filtered through a 0.45 µm membrane.

To prepare the solubilized extract, 25 g samples of Pilão® coffee waste were transferred to polyethylene bottles and treated with 100.0 mL of ultra-pure water. The bottles were closed, shaken for 5 min using a rotary shaker at room temperature and then left standing for 7 days before filtering through a 0.45 µm membrane.

2.2. Physicochemical analysis

The physicochemical parameters such as TDS (total dissolved solids), salinity and conductivity were determined in the leached and solubilized extracts using a multi-parameter analyzer (PCS Tester 35 – OAKTON).

2.3. Chemical analysis

2.3.1. Flame atomic absorption spectrometry – FAAS

For the metal analyses, 4 mL samples of leachate and solubilized extract were transferred to microwave vessels and treated with 1.0 mL of HNO_3 (P.A. Sigma) and 1 mL of H_2O_2 (30%, Vetec). The vessels were then placed inside the rotor of a microwave digestion system, sealed, tightened using a torque wrench and finally submitted to a microwave dissolution program for 4 min. After cooling, the digest contents were quantitatively transferred to a polyethylene bottle, diluted to 100 mL with Milli-Q water and stored at 4 ∘C until analyzed. The leached and solubilized extracts were analyzed for their metal contents by standard methods (American Public Health Association, 1998) using flame atomic absorption spectrometry – FAAS (SpectrAA-240, Varian, Australia).

2.3.2. Gas chromatography–mass spectrometry (GC–MS)

For the organic analysis, 100 mL of the leached and solubilized extracts were extracted three times with 30 mL n-hexane (HPLC degree, Tedia, USA). The organic phase was removed and 2 µL injected into the gas chromatograph–mass spectrometer GC–MS (456 GC – MS-TQ, Bruker Daltonics, Inc., Germany). This procedure was carried out in duplicate and with an analytical blank.

Chromatographic separation was achieved using a BR-5MS fused-silica capillary column (30 m × 0.25 mm with a 0.25 µm thick film), using helium as the carrier gas at 0.6 mL/min in the constant flow rate mode. The injections were made in the splitless mode. The MSD was operated by electronic impact (70 eV) in the scanning mode (40–400 *m/z*). The injector port was at 250 °C and the interface temperature was 280 °C. The oven temperature was first maintained at 60 °C, then increased to 240 °C at 3 °C/min, and held at this temperature for 2 min. The components were identified by comparison of their retention times and mass spectra with the corresponding data of reference compounds and by comparison of their mass spectra with those in the NIST libraries. This procedure was carried out in duplicate and with the analytical blank.

2.4. Salmonella/microsome mutagenicity assay

The Salmonella/microsome assay was carried out to evaluate mutagenic effects, using TA98 (hisD3052, rfa, Δbio, ΔuvrB, and pKM101) and TA100 (hisG46, rfa, Δbio, ΔuvrB, and pKM101) Salmonella typhimurium strains in the presence and absence of exogenous metabolization activation (S9), according to the International Organization for Standardization (ISO) protocol no. 16240 (International Organization for Standardization, 2005; Maron, Ames, 1983; Mortelmans, Zeiger, 2000). Briefly, 100 µL of overnight cultures of each strain, 500 µL of 0.1 M sodium phosphate buffer or S9 mix, 1 mL of different dilutions of the extracts in deionized water (100%, 50%, 25%, 12.5% and 6.25%) and 1 mL of molten soft agar were mixed and poured into a minimal agar plate. The plates were incubated at 37 °C for about 70 h and each experiment was carried out in triplicate. Deionized water was used as the negative control. For the TA98 and TA100 strains, the positive controls in the absence of S9 were 1.0 µg/plate of 4-nitroquinoline-1-oxide (4NQO; Sigma, St Louis, MO, USA) and 5.0 µg/plate of sodium azide (AS; Sigma, St Louis, MO, USA), respectively. In the presence of the S9 mix, 3.0 µg/plate of 2-aminoanthracene (2AA; Aldrich, Seelze, Germany) was added.

2.5. Micronucleus (MN) genotoxicity assay

The human hepatocellular carcinoma cell line HepG2 was kindly provided by Dr. Danielle Palma de Oliveira, Faculty of Pharmaceutical Sciences of Ribeirão Preto, São Paulo, Brazil. The cells were maintained in Eagle's Minimum Essential Medium (MEM) with 1.8 mM Ca^{++}, pH 7.4 (Gibco), and supplemented with 1.76 g/L $NaHCO_3$, 0.88 g/L pyruvate, 21.6 mg/L aspartic acid, 16.8 mg/L L-serine, 1% penicillin–streptomycin solution and 10% (v/v) heat-inactivated FBS. The cells were incubated at 37 °C with 5% CO_2 and a relative humidity (RH) of 95%. When the cells reached 80% confluence, the culture medium was removed the cells detached using 0.25% Trypsin-EDTA (Sigma Chemical Co.) for 5 min at 37 °C, centrifuged at 1500 rpm for 5 min, and the supernatant then removed. The cells were suspended in 1 mL medium supplemented with FBS (10%), and 1.0×10^5 cells/mL seeded into a 24well microplate containing a coverslip that had been pretreated with 0.1 M nitric acid, and maintained at 37 °C with 5% CO_2 and 95% RH. After 24 h, the cells were treated with 100 µL each of the different dilutions of the leached or solubilized extracts in MEM supplemented with 10% FBS (6.25%, 12.5%, 25%, 50% and 100%), and the plates incubated for 3 and 24 h. After each incubation period, the cells were rinsed twice with MEM and cultured in fresh culture medium. After 24 h at 37°C, 5% CO_2 and 95% RH, the medium was discarded and the cells fixed with glacial acetic acid–methanol (1:3) fixative (Cornoy's fixative). The fixed cells were rinsed twice with McIlvaine's buffer (MI buffer: 21.01 g/L citric acid and 35.60 g/L Na_2HPO_4, pH 7.5), dried at room temperature and then stained for 40 min with 4′−6-diamidino-2-phenylindole (DAPI) (0.2 µg/mL) dissolved in MI buffer. The number of cells with micronuclei (2000 cells per dilution of the extracts) was determined in a fluorescence microscope (Reichert Univar) with an excitation wavelength of 350 nm to determine the induction of micronuclei. The negative control used was MEM supplemented with FBS (10%) and the positive control was N-methyl-N-nitro-N-nitrosoguanidine (MNNG) at a concentration of 0.5 mM. The MN test was carried out according to OECD guideline no. 487 (OECD, 2010) as described by Fenech (2000). All experiments were carried out in triplicate and repeated twice.

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