TITLE:

Validation of coffee silverskin extract as a food ingredient by the analysis of cytotoxicity and genotoxicity

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Abbreviations

CGA, chlorogenic acid; CSE, coffee silverskin extract; Endo III, Endonuclease III; Fpg, Formamidopyrimidine-DNA glycosylase

Abstract

The aim of the present study was to validate the food safety of CSE, by studying its effect on cytotoxicity (100 - 20000 µg/ml) and genotoxicity (10, 100 and 1000 µg/ml) and also to investigate its preventive potential (1, 10 and 100 µg/ml) against B(a)P induced DNA damage. Prior to analyses, the antioxidant capacity and the microbiological quality of CSE were tested. DNA damage (strand breaks and oxidized purines/pyrimidines) was evaluated by the alkaline single-cell gel electrophoresis or comet assay. HepG2 cells were pre-treated with CSE (1, 10 and 100 µg/ml) for 24 h followed by the addition of 100 µM B(a)P in presence of CSE for other 24 h. Detection of oxidized purines and pyrimidines was carried out using Formamidopyrimidine DNA glycosylase or Endonuclease III enzymes, respectively. Chlorogenic acid (CGA), the major antioxidant present in coffee, was used as a control. Treatment with 100 µM B(a)P significantly increased ($p < 0.05$) levels of DNA strand breaks and oxidized purine and pyrimidine bases. Treatment of HepG2 cells with CSE did not induce either cytotoxicity or genotoxicity. CSE significantly inhibited ($p < 0.05$) genotoxicity induced by B(a)P and the observed effect may be associated to its antioxidant capacity. CGA alone at the concentration present in CSE was effective against B(a)P. Thus, CGA seems to be a contributor to the preventive effect of CSE against B(a)P induced DNA damage in HepG2 cells. In conclusion, CSE presents potential as a natural sustainable chemoprotective agent against the chemical carcinogen B(a)P.

Highlights

* CSE did not induce either cytotoxicity or genotoxicity

* CSE significantly inhibited genotoxicity induced by B(a)P in HepG2 cells and the observed effect may be associated to its antioxidant capacity

* CGA may be a contributor to the chemopreventive effect of CSE against B(a)P induced DNA damage in HepG2 cells

Keywords: benzo (a) pyrene, chlorogenic acid, coffee silverskin, DNA bases oxidative damage, food ingredient, genotoxicity.

1. Introduction

The validation process for a novel food or ingredient established by the European legislation (EFSA Journal 2016;14(11):4594) comprises chemical characterization (toxic and health promoters), proposal for applications, *in vitro* assays, *in vivo* toxicity, *in vivo* bioactivity and human trials (Figure 1). In 2016, EFSA published a guidance for preparing and presenting scientific data for novel food applications. This guidance indicates the requirements needed when submitting an application for the authorization of novel foods: physicochemical, biochemical properties and microbiological characterization as well as data on the compositional, nutritional, toxicological and allergenic properties of the novel food (EFSA Panel on Dietetic Products, 2016). Information on genotoxicity is a key component in risk assessment of novel foods or ingredients. The purpose of genotoxicity testing for risk evaluation of substances in food is to identify compounds that could cause heritable damage in humans, to predict potential genotoxic carcinogens in cases where carcinogenicity data are not available, and to contribute to understand the mechanism of action of chemical carcinogens (Efsa Scientific Committee, 2011).

Coffee silverskin (CS) is a thin tegument of the outer layer of the two beans present in the coffee cherry. CS is the only by-product generated in the roasting process and it contains phytochemicals with antioxidant character such as chlorogenic acid (CGA). The extract (WO/2013/004873) prepared from Arabica coffee silverskin (CSE) is enriched in CGA and possesses high antioxidant power (del Castillo et al., 2016). The present study provides novel scientific information for supporting the usefulness of CSE as a sustainable natural source of bioactive compounds.

One of the mechanisms that leads to DNA lesions is oxidative stress associated with the activity of metabolic enzymes that can induce DNA strand breaks and oxidized bases (Xue & Warshawsky, 2005). Since CSE comes from a natural source and is known to

possess high antioxidant power, this extract could protect cells from DNA damage when induced by an oxidative agent.

HepG2 cells, a human hepatoblastoma cell line, are widely used in the field of xenobiotic metabolism for the study of cytotoxic or genotoxic agents (Cao et al., 2007). Benzo(a)pyrene (B(a)P) is the most widely used model compound for studying the effects of carcinogenic polycyclic aromatic hydrocarbons (PAHs) (Brinkmann et al., 2013). B(a)P is formed during incomplete combustion or pyrolysis of organic material and it is found in air, water, soils and in thermally processed foods and cigarette smoke (International Agency for Research on Cancer (IARC), 2012). B(a)P is a DNA-reactive chemical that interacts with DNA. Strand breaks or alkali labile sites, including abasic sites, may result from the action of reactive oxygen species (ROS) that arise during metabolism of food mutagens in cells (Haza & Morales, 2013).

The aim of the present study is to give new information related to CSE safety in order to use it as a food ingredient, as well as to provide scientific data to support a cause-effect relationship of the antioxidant power claim made on coffee and their derivatives. We also investigated the chemoprotective potential of CSE against B(a)P induced DNA damage in HepG2 cells and the contribution of CGA in CSE as a chemoprotective agent.

2. Materials and methods

2.1. Chemicals

Chlorogenic acid (CGA), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), benzo(a)pyrene (B(a)P), dimethyl sulfoxide (DMSO) and low melting point agarose (LMP) were purchased from Sigma–Aldrich (St Louis, MO, USA). Formamidopyrimidine-DNA glycosylase (Fpg) and Endonuclease III (Endo III) were

obtained from Trevigen Inc. (Gaithersburg, MD, USA). Culture medium and supplements required for the growth of HepG2 cells were purchased from Lonza (Lonza Group, Basel, Switzerland). MTT Proliferation Kit I was purchased from Roche (Indianapolis, IN, USA).

2.2. Preparation of soluble coffee silverskin extract

Coffee silverskin from Arabica variety was provided by Fortaleza S.A. (Spain). CSE was produced as described in the patent WO 2013/004873 (del Castillo et al., 2013). Briefly, 50 mg of coffee silverskin were added per H₂O milliliter. This mixture was stirred for 10 minutes at 100 °C, filtered and the filtrate was freeze-dried. Powdered CSE was prepared in aqueous solution, sterile filtered and added to medium to achieve final concentrations between 1 and 20000 µg/ml. CSE chemical composition is described in Table S1 (Supplementary Material). CGA is the major antioxidant present in coffee; therefore, it was used as an antioxidant control.

2.3. Overall antioxidant capacity of coffee silverskin extract

The trapping capacity of cationic free radicals was evaluated using the method of radical ABTS^{•+} bleaching described by Re et al., (1999) and modified by (Oki et al., 2006) for its use in a microplate. Aqueous solutions of CGA (0.15–2.0 mmol/l) were used for calibration. Absorbance was measured in microplate using a UV-Visible Spectrophotometer (BioTek Instruments, Winooski, VT, USA). All measurements were performed in triplicate and results were expressed as % CGA eq. (w/w).

2.4. Microbiological analyses

CSE was microbiologically analyzed to evaluate the safety of its use as a food ingredient. Count of (1) total aerobic microorganisms, (2) aerobic microorganisms forming endospores and (3) moulds and yeasts were carried out. All assays were performed in sterile conditions and with previous solubilization of 10 g of CSE in BPW (90 mL) by using a stomacher (230 rpm, 1 min). Different conditions were set for each analysis: (1) pour plate method, PCA medium, incubation at 30°C 72 h; (2) pour plate, BHI agar medium, preincubation (80°C, 10 min) and incubation at 37°C 48h; and (3) spread method, SDA with chloramphenicol and incubation at 25°C 120 h. Results were expressed as colony forming units (CFU)/g.

2.5. Cell culture

Human hepatocellular carcinoma (HepG2) cells were purchased from the Biology Investigation Center Collection (CIB, Madrid, Spain). Only cells of passage 10-17 were used in the experiments. Cells were cultured as a monolayer in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% v/v heat inactivated fetal calf serum (FBS), 50 U/ml penicillin and 50 µg/ml streptomycin and 1% v/v L-glutamine. Cell cultures were incubated at 37°C and 100% humidity in a 5% CO₂ atmosphere.

2.6. Cytotoxicity

Cell viability was determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay (Cell Proliferation Kit I, Roche, Indianapolis, IN, USA) to select non-toxic concentrations of CSE and CGA. First, HepG2 cells were

cultured at a density of 1×10^5 cells per well of a 96-well plate for 24 h. Then, cells were treated with CSE (0 – 20000 $\mu\text{g/mL}$) or CGA (0 – 10000 $\mu\text{g/mL}$) for 24 h. Subsequently, cells were incubated in MTT Labeling Reagent for 4 h at 37 °C and then, 100 μl of solubilization solution were added. After 24 h, the optical density of each well was read at 620 nm (test wavelength) and 690 nm (reference wavelength) using a microplate reader. Experiments were carried out in triplicate ($n = 16$). Results were expressed as the percentage of viability (% SDH) with respect to the control (medium treated cells).

2.7. DNA damage

The comet assay was carried out according to the protocol of Olive *et al.* with minor modifications (Olive et al., 1992). Briefly, HepG2 cells were plated on 24 well plates at a density of 1.5×10^5 cells/ml culture medium. Twenty-four hours after seeding, cells were exposed to CSE (1–1000 $\mu\text{g/ml}$), or CGA (1–1000 $\mu\text{g/ml}$), for another 24h at 37 °C and 5% CO_2 . After incubation, 12 μl of a suspension of 1×10^5 cells were mixed with 70 μl of LMP agarose type VII (0.75% in PBS), distributed on a LMP agarose type VII (0.3% in PBS) pre-coated slide, and left to set on an ice tray. Three slides were prepared for each concentration of the compound tested, one slide for control and the others slides to be treated with Fpg or Endo III. After solidification, cells were lysed in darkness for 1 hour in a high salt alkaline buffer (2.5 M NaCl, 0.1 M EDTA, 0.01 M Tris, 1% Triton X-100, pH 10). Slides were then equilibrated 3 x 5 minutes in enzyme buffer (0.04 M HEPES, 0.1 M KCl, 0.5 mM EDTA, 0.2 mg/ml BSA, pH 8). Then, slides were incubated with 30 μl of Fpg or Endo III at 1 $\mu\text{g/ml}$ in enzyme buffer for 30 minutes at 37 °C in a humid dark chamber. Control slides were incubated with 30 μl enzyme buffer. Following enzyme treatment, slides were placed in electrophoresis buffer (0.3 M NaOH, 1 mM EDTA, pH 13, cooled in a refrigerator) in darkness for 40 minutes. Electrophoresis was performed

in a cold-storage room, in darkness, in a Bio-Rad subcell GT unit containing the same buffer, for 30 minutes at 25 V. After electrophoresis, slides were neutralized using 0.4 M Tris pH 7.5 and fixed in methanol. Subsequently, DNA was stained with ethidium bromide (10 µg/ml) in Tris acetate EDTA (TAE 1X) for 5 minutes and examined in a fluorescence microscope (OLYMPUS BH-2) connected to a computerized image analysis system (Comet Score 1.0). Percent (%) DNA in the tail was used as the parameter for DNA damage analysis using the software. The level of Fpg and Endo III sites was obtained by subtracting the value of % DNA in the tail obtained without enzymes from the value obtained when enzymes were present. B(a)P (100 µM) was used as positive control.

2.8. Analysis of DNA damage induced by simultaneous treatment with B(a)P and CSE or CGA in alkaline comet assay

HepG2 cells were seeded into 24-multiwell plates at a density of 1.5×10^5 cells/ml culture medium. After 24 hours, CSE or CGA (1–100 µg/ml) were added and plates were incubated for 24 hours at 37 °C and 5% CO₂. After incubation, cells were treated simultaneously with CSE or CGA and B(a)P 100 µM for other 24 hours. After the treatments, cells were then processed as described above.

2.9. Statistical Analysis

Images of 50 randomly selected cells per concentration were evaluated and the test was carried out three times. Untreated cells (C₀) were considered as negative controls. The reported Tail DNA is the mean ± standard error (SE) of three independent experiments. One-way analysis of variance (ANOVA) was carried out and statistical comparisons of

the different treatments were performed using Tukey's test. Values of $p < 0.05$ were considered statistically significant. All statistical analyses were performed using the R package software environment Version 3.2.0 (<http://www.r-project.org/>).

3. Results

3.1. CSE overall antioxidant capacity

The *in vitro* antioxidant capacity of the CSE was evaluated by the ABTS^{•+} radical cation decolorization assay in order to assess the reproducibility of CS extraction. An overall antioxidant capacity value of 138.73 ± 14.8 mg CGA equivalents/gram of CSE was obtained for the trapping capacity of cationic free radicals of CSE. This result confirms that the patented CSE possesses *in vitro* antioxidant properties due to the presence of antioxidant molecules such as phenolic compounds, mainly CGAs, and melanoidins (Table S1, Supplementary Material).

3.2. Microbiological analyses of CSE

Results showed values of 3.25×10^5 CFU/g for endospores, 4.3×10^3 CFU/g of total aerobic microorganisms and a content of yeasts and molds lower than 10^2 CFU/g.

3.3. Evaluation of CSE and CGA cytotoxicity

Figure 2 shows data on CSE cytotoxicity in HepG2 cells obtained by the MTT method. The studied concentrations of CSE were 100-20000 µg/ml. Since CGA has been detected in CSE and the antioxidant capacity of this extract has been associated to this compound

among others, its effect on the viability of HepG2 cells was also studied (1-10000 µg/ml). Concentrations of CSE up to 10000 µg/ml did not affect the viability of HepG2 cells (>80%), but a remarkable significant decrease ($p<0.05$) was observed for CSE at 20000 µg/ml. The inhibitory concentration required to reduce cell viability 50% (IC₅₀) after 24 hours of extract incubation was 13158 µg/ml (Figure 2A). On the other hand, CGA also resulted non-cytotoxic at concentrations up to 5000 µg/ml and the IC₅₀ was 8050 µg/ml (Figure 2B). When CGA (1-1000 µg/ml) was added to cells, a significant increase ($p\leq0.05$) in cell viability was observed. Therefore, to evaluate the genotoxicity and the possible protective effect of CSE and CGA on DNA damage, concentrations between 1 and 1000 µg/ml were chosen.

3.4. Analysis of DNA damage induced by CSE or CGA in alkaline comet assay

Figure 3A shows the effect of CSE and CGA on the induction of strand breaks assessed by the comet assay without any enzyme treatment. A significant increase ($p\leq0.05$) in strand breaks was observed when B(a)P 100 µM, used as positive control, was added to HepG2 cells (70% tail DNA). None of the tested concentrations of CSE and CGA (10-1000 µg/ml) resulted genotoxic to HepG2 cells (Figure 3A). There were no significant differences ($p>0.05$) in strand breaks between untreated control cells and cells treated with CSE or CGA (10-1000 µg/ml).

In order to determine if CSE or CGA produced oxidative DNA damage in purines or pyrimidines, DNA was digested with two repair specific enzymes Fpg (Figure 3B) or Endo III (Figure 3C) that recognize and cut oxidized DNA purines or pyrimidines, respectively. Cells treated with B(a)P 100 µM showed a significant increase ($p\leq0.05$) in oxidized purines and pyrimidines (12% and 10% tail DNA, respectively). No significant

oxidative DNA damage ($p > 0.05$) was produced in purines or pyrimidines when cells were treated with CSE or CGA. Therefore, none of the studied concentrations induced relevant strand breaks or oxidized purines or pyrimidines (FPG or Endo III-sensitive sites, respectively) in the cells.

3.5. DNA damage induction by a combined treatment of B(a)P and CSE or CGA in the Alkaline Comet assay

The effect of CSE and CGA in B(a)P induced DNA damage in HepG2 cells is shown in Figure 4. Considering B(a)P induced DNA damage as 100%, the maximum reduction of DNA strands breaks (26% and 29% for CSE and CGA, respectively) was observed at the highest concentration used (100 µg/ml). At all tested concentrations, DNA strand breaks were significantly reduced ($p \leq 0.05$) by the pretreatment with CSE and CGA showing a significant protective effect against B(a)P induced DNA damage.

Both CSE and CGA in addition to decreasing DNA strand breaks, they significantly reduced ($p \leq 0.05$) the formation of Fpg and Endo III sensitive sites. The maximum reduction of Fpg sensitive sites (69% and 76% for CSE and CGA, respectively, compared with the control) was observed at the lowest concentration of CSE (1 µg/ml) but at the highest concentration of CGA (100 µg/ml). However, Endo III sensitive sites were reduced (70% and 77% for CSE and CGA, respectively) in both cases by the highest doses of the tested compounds (100 µg/ml).

4. Discussion

In this work, we proved the microbiological quality and the non-cytotoxic and non-genotoxic character of CSE in order to give more information related to the safety of this extract as a novel food ingredient. CSE possesses high antioxidant power and data obtained in this study are in line with those previously described by other authors (Borrelli et al., 2004; del Castillo et al., 2016; Mesías et al., 2014). With regard to the biological contaminants that could be present in coffee, ochratoxin A (OTA) is a mycotoxin produced by *Aspergillus ochraceus* and *Penicillium verrucosum* that tends to bioaccumulate along the food chain. OTA can induce renal toxicity, nephropathy, and immunosuppression, representing a risk for human safety. Therefore, its content in foods should be determined. Coffee is contaminated with OTA when coffee fruits fall onto the soil or during storage (Commission, 2006). Coffee is considered a secondary source of OTA in the human diet. However, OTA can be destroyed during roasting (Ferraz et al., 2010). Commission Regulation (EC) No 123/2005 defined OTA limits as 5 µg/kg for roasted coffee and 10 µg/kg for soluble coffee (Kyprianou, 2005). Since CSE is an extract from a coffee by-product, there is no specific OTA regulation limit; however, the absence of molds in the extract reduces the risk of OTA contamination. Therefore, CSE presented excellent microbiological quality becoming a safe food ingredient.

The study of the genotoxic potential is a basic component of chemical risk assessment (Louis Bresson et al., 2016). According to EFSA, the aim of genotoxicity testing of novel foods is to identify substances that could cause heritable damage in humans. The Scientific Committee recommends the Comet Assay as one of the suitable *ex vivo* tests for identifying substances that cause gene mutations and/or structural chromosomal aberrations. The *ex vivo* comet assay is easy to conduct and may be applied to any tissues from which single cell suspensions can be prepared. It is considered an indicator test to detect pre-mutagenic lesions and can be used for mechanistic studies (Efsa Scientific

Committee, 2011). No previous research on genotoxicity of CS and its derivatives has been published.

The liver is the main organ of xenobiotic transformation. In consequence, liver cell lines are widely used in the field of xenobiotic metabolism such as genotoxicity studies (Mersch-Sunderman et al., 2004). HepG2 is a human hepatoblastoma cancer cell line that keeps many characteristics of hepatocytes such as the presence of enzymes that play key roles in the activation and detoxification of DNA-reactive carcinogens. Therefore, HepG2 cells represent a relevant *ex vivo* model to detect cytoprotective and non-genotoxic agents (Cao et al., 2007). Our results demonstrate that neither CSE nor CGA were cytotoxic for HepG2 cells up to concentrations of 10000 and 1000 µg/ml, respectively (Figure 2). These results are in agreement with studies using CSE in other human cell lines. For instance, concentrations of CSE and CGA up to 10 µg/ml and 10 µM respectively, did not affect the viability of pancreatic cells (INS-1E) (Fernandez-Gomez et al., 2016). Furthermore, studies using skin keratinocytes (HaCaT) have demonstrated that CSE is not cytotoxic when used at concentrations between 10 and 1000 µg/ml (Iriondo-DeHond et al., 2016). Considering these results, in the present work cells were exposed to non-cytotoxic concentrations of CSE and CGA in the range of 1 to 1000 µg/ml and DNA damage was evaluated. The results obtained in the comet assay indicated that both CSE and CGA did not cause a significant increase in DNA damage (strand breaks) after 24 h of treatment in HepG2 cells (Figure 3A). This is the first time the harmlessness and non-genotoxic effect of CSE is described. On the other hand, our results showed that CGA in concentrations present in CSE did not increase DNA strand breaks. Abraham et al., (2007) also confirmed the non-genotoxic profile of CGA in human promyelocytic leukemia cells (HL-60) when used at 200 µM.

Oxidatively damaged bases were detected by the comet assay by adding two repair specific enzymes, Fpg and Endo III, which are able to recognize the oxidized purines and pyrimidines, respectively. B(a)P (100 μ M), positive oxidative control, induced a significant increase of DNA strand breaks, Fpg and Endo III sensitive sites in HepG2 cells. Burczynski and Penning (2000) showed that during the metabolic process, B(a)P produces ROS that can cause oxidative DNA damage and form adducts with DNA (Burczynski & Penning, 2000). Our results showed that CSE did not induce a significant increase of oxidation of purines and pyrimidines compared to the control (Figure 3B and 3C). However, a non-significant increase ($p>0.05$) can be observed when cells were treated with CSE 1000 μ g/ml for 24 h. Moreover, CGA also did not increase oxidation of the purines and pyrimidines compared to the control in HepG2 cells.

In the present study, we evaluated the protective effect of CSE and CGA against B(a)P induced DNA damage (strand breaks and oxidized purines/pyrimidines) in HepG2 cells. Our results showed a significant decrease ($p \leq 0.05$) in DNA strand breaks when cells were pretreated with CSE and CGA showing a significant protective effect against B(a)P induced DNA damage. Bakuradze et al., (2014) observed and confirmed the protective effect of roasted coffee consumption on DNA integrity in humans, as reported in a previous study. This study concluded that repeated coffee consumption is associated with reduced background DNA strand breakage (Bakuradze et al., 2011). The reduction of spontaneous DNA strand breaks observed in this study may be attributed to the presence of constituents with chemopreventive properties and antioxidants known to be present in coffee (such as chlorogenic acids and roast-associated constituents) (Bakuradze et al., 2014). Considering that CS keeps part of the polyphenolic compounds that are normal constituents of coffee beans, such as CGA (del Castillo et al., 2016), it is likely that this effect described for coffee brews is maintained also in CS.

CSE and CGA showed a higher protective effect against B(a)P induced oxidative DNA damage compared to that observed for the strand breaks. This could be due to the well-known antioxidant capacity of CSE (del Castillo et al., 2016) and CGA (European Food Safety Authority (EFSA), 2011). The mixture of compounds present in the CSE and CGA alone can reduce oxidative damage to DNA and therefore, decrease cellular oxidative stress. In CSE the main free phenolic compounds are CGA and caffeic acid, present in large amounts in green coffee beans. These phenolic compounds may react during roasting with polysaccharides and proteins forming melanoidins, which are rich in phenolic groups able to exert antioxidant activity (Borrelli et al., 2004). Therefore, it is possible that the observed protective effect is attributed to the combination of antioxidant compounds present in CSE. CS also contains flavonoids that could contribute to the observed protective effect (Costa et al., 2014). Delgado et al., (2008) reported that certain flavonoids are active against oxidized pyrimidines induced by B(a)P, but not against oxidized purines.

One mechanism of cellular protection against carcinogens is the activation of phase II detoxifying and antioxidant enzymes (Talalay, 2000). Fernandez-Gomez et al., (2016) provided novel information regarding the antidiabetic mechanism of action of CSE and its components CGA and caffeine. CSE ($\geq 1 \mu\text{g/mL}$) and CGA ($\geq 5 \mu\text{M}$) increased the enzymatic activity of glutathione peroxidase (GPx). These studies concluded that physiological concentrations of pure CGA ($10 \mu\text{M}$) were able to protect pancreatic cells against oxidative stress while same concentrations of caffeine were ineffective. Further research should be conducted to identify which compound/s are responsible for the observed benefits since CGA concentrations in CSE were not effective against induced oxidative stress in pancreatic cells (Fernandez-Gomez et al., 2016).

The induction of antioxidant enzymes is mediated by the antioxidant or electrophile response element (ARE/EpRE), located in the promoter region of many of these phase II genes. The transcription factor NF-E2 p45 subunit-related factor 2 (Nrf2) plays a key role in ARE-mediated gene expression (Rushmore et al., 1991). Volz et al., (2012) have investigated the effect of a coffee extract rich in CGA and N-methylpyridinium (NMP), on the Nrf2-activating properties. In HT29 cells the enriched coffee extract increased nuclear Nrf2 translocation and enhanced the transcription of ARE-dependent genes such as NAD(P)H:quinone oxidoreductase and glutathione-S-transferase (GST)A1. Pure CGA has been identified as a potent activator of the Nrf2/ARE pathway, activating gene expression of different phase II enzymes in the same cell line (Boettler et al., 2011). In addition, in a pilot human intervention study data indicated an association between Nrf2 transcription and a decrease in oxidative DNA damage in peripheral blood lymphocytes observed after 4 weeks of daily coffee consumption (enriched with CGA and NMP), supporting the hypothesis that coffee-mediated effects on gene expression are of chemoprotective relevance (Volz et al., 2012). Since the CSE presents high antioxidant properties due to a mixture of molecules exerting synergic effect including CGA, the observed chemoprotective effect might be associated to its effect on the antioxidant enzymatic defense of the cells.

In conclusion, the present study confirms that treatment of HepG2 cells with CSE did not induce either cytotoxicity or genotoxicity. Our results indicate that CSE protects human cells from DNA strand breaks and oxidative DNA damage effects of B(a)P, and that free CGA or linked to other chemical structures seem to be contributors to the observed chemoprotective effect of CSE. This extract presents potential as a natural and sustainable food ingredient.

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