Epigallocatechin-3-Gallate (EGCG), An Alternative to Extenuate Occupational Risk Factors Outcomes?—An Interventional Study

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Abstract Introduction: Occupational risk factors are major players for increased risk of cardiovascular diseases and cancer. Studies support a protective role of Epigallocatechin-3-gallate (EGCG) in disease onset, with associated antioxidant properties and reactive oxygen species production. We aimed to evaluate the in vivo effects of EGCG intake on cardiovascular risk factors, DNA damage and oxidative DNA damage. Methods: Voluntaries were enrolled in this interventional study with safeguard of all ethical considerations. Peripheral blood was collected at the beginning and after 90 days of 225 mg EGCG ingestion per day. Lipid profile and liver function parameters were assessed using colorimetric methods. Vitamins A and E in serum were quantified by HPLC–DAD. DNA damage and oxidative DNA damage were assessed through comet assay. Results: Vitamin A, as well as the lipid profile and liver function parameters, were not affected by EGCG intake, whereas serum levels of vitamin E, DNA damage and DNA oxidative damage increased after EGCG consumption. Discussion/Conclusions: EGCG induce low-level oxidative stress which may trigger protective antioxidant systems associated with vitamin E. Further research is crucial to understand the extent of EGCG effects and its potential as an alternative to extenuate occupational risk factors outcomes.

Keywords Occupational risk factors · Epigallocatechin-3-gallate (EGCG) · Hematologic parameters · Oxidative DNA damage · Antioxidant defence systems

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1 Introduction

Occupational risk factors are currently considered major players in the onset of human diseases, as evidence from several cohort studies in Europe, USA and Japan, which included over 600,000 individuals, indicate that work stressors and occupational exposure to chemicals such as lead, are correlated with an increased risk for cardiovascular diseases such as incident coronary heart disease and stroke (Kivimäki et al. 2015; Poręba et al. 2011). Additionally, increased incidence of different types of cancer can also be related to occupational exposures (Pukkala et al. 2009).

Epidemiological and interventional studies support a protective role of green tea in the development of several diseases, including cardiovascular diseases and cancer (Singh and Li 2012). Green tea, prepared from the dried leaves of the plant *Camellia sinensis* (Theaceae), is one of the most popular beverages worldwide, and it is known to contain catechins, dietary polyphenolic compounds associated with a variety of beneficial health effects. The widely renowned biological actions of catechins have been associated with their antioxidant and free radical scavenging properties (Aydin et al. 2015; Singh and Li 2012). Epigallocatechin-3-gallate (EGCG), the major polyphenol in green tea, has been shown to inhibit cancer cell growth and tumorigenesis in vitro (Ahmed et al. 2004) and in animal models (Kaur et al. 2007; Yang et al. 2009), during the initiation, promotion and progression stages, particularly by eliminating cancer cells through induction of apoptosis but also by protecting normal cells against genotoxic hazards (Kuroda and Hara 1999; Lambert and Elias 2010; Roy et al. 2003). Also, interventional human studies using catechins as supplement, observed a decrease of plasma oxidized LDL in comparison with controls (Nami et al. 2007), and in smokers it was verified a reduction of benzo[a]pyrene adducts (Hakim et al. 2003; Schwartz et al. 2005), however the study from Eichenberger et al. (2009), did not find significant effects of green tea extract on oxidative stress markers.

On the other hand, our previous study have demonstrated that EGCG consumption is able to impact plasma molecular profile (Ruben et al. 2020), and several studies have suggested genotoxicity and carcinogenic potentials of EGCG, by showing induction of oxidative stress and DNA damage through the generation of reactive oxygen species (ROS) (Aydin et al. 2015; Kanadzu et al. 2006; Lu et al. 2013) in human lymphocytes (Lu et al. 2001), cancer cell lines, xenograft tumors, and mouse liver. Therefore, EGCG seems to have a dual role as antioxidant and pro-oxidation, and these effects were suggested to be due to H_2O_2 generation by polyphenols in solution.

As previously stated, ROS react with cellular components, causing oxidative damage to biomolecules and specifically oxidative DNA damage which may involve the cleavage of single and double-strands of DNA (Aydin et al. 2015). This type of DNA lesion is able to be detected by comet assay (Collins 2004, 2009), a technique used for the assessment of protective effects of antioxidants on DNA damage in human nutrition studies (Gillian et al. 2008) with or without the addition of the

repair enzymes, such as formamidopyrimidine N-glycosylase (FPG), to characterize DNA lesions due to the repair of oxidized bases (Collins et al. 2001).

The aim of this study was to perform an interventional study to evaluate the effects, in vivo, of EGCG intake during an interventional study with 90 days duration in cardiovascular risk factors, namely lipid profile and liver function parameters, vitamins A and E levels, DNA damage and oxidative damage in individuals' lymphocytes which ingested commercial capsules of green tea extract (225 mg EGCG/capsule), in order to assess its potential as an alternative to minimize occupational risk factors effects in the onset of cardiovascular diseases and cancer.

2 Methods

2.1 Study Population and Design

This is an interventional, uncontrolled, prospective, longitudinal and of individual analysis study, which included 30 healthy individuals (with no previously diagnosed pathologies). Inclusion criteria considered were adult voluntaries (ages superior to 18 years old and less than 65 years old) with no acknowledged previously diagnosed pathology of any type. Exclusion criteria applied were included viral infections, consumption of tea and forgotten capsules on consecutive days during the study. Data was analyzed under blind conditions.

2.2 Supplementation and Questionnaires

Commercial capsules of green tea extract with 225 mg EGCG/capsule (My Protein®) were provided for all the participants in the study with the instructions to take 1 capsule daily. EGCG dosage was selected considering that, 400 mg and 800 mg of EGCG intake, which are considered safe doses previously used in human clinical trials (Identifier:NCT00942422, n.d.) result in peak serum concentrations in the range of 100–400 ng/mL (Chow et al. 2003a, b). Thus, the selected dosage is considered safe. The participants filled up 2 questionnaires, the first in the moment of the first blood collection (T0; June) with questions regarding demographic data such as age, gender, weight (cm), height (kg) and smoking habits. In the second questionnaire, after 90 days (T90; September) of the interventional study, the participants were asked to report adverse effects through the intervention, namely alterations in the nervous system (headaches, migraines, mood swings); gastrointestinal system (heartburn, reflux, diarrhea, cramps, weight loss/gain); cardiac and respiratory effects and information regarding potential missing capsules intake.

2.3 Collection of Biological Samples

Peripheral blood of the volunteers was collected in a tube with anticoagulant ethylenediaminetetraacetic acid (EDTA) VACUETTE® for Comet assay and in a serum tube with no anticoagulant VACUETTE® for assessment of lipid profile and liver function parameters and HPLC determination of Vit E [α-tocopherol]/μM and Vit A [retinol]/μM, using standard blood collection procedures at time 0 (T0) and after 90 days (T90) of the daily ingestion of 225 mg EGCG/capsule. All samples were refrigerated at 4 °C until processed in the laboratory.

2.4 Lipid Profile and Liver Function Parameters

Blood samples (serum tubes) were centrifuged at 5000 rpm for 5 min and lipid profile parameters determination was preformed using colorimetric methods Triglycerides (mg/dL, LPL/GK/GPO-PAP); Cholesterol (mg/dL, LPL/GK/GPO-PAP); HDL (mg/dL, PEG/CHOD-PAP) and Friedewald calculus for LDL (mg/dL). Liver function parameters namely, transaminases GOT (UI/L) and GPT (UI/L) were assessed using colorimetric methods (IFCC com act. P5P) and GGT (UI/L) trough Colorimetry kinetics. All parameters were determined in the Laboratory of Clinical Analyses—General Lab, Lisbon.

2.5 Comet Assay

Lymphocytes were isolated from blood samples collected by use of a Ficoll-Paque® gradient and placed in RPMI1640 medium, cryopreserved in a freezing mix (90% v/v of FBS and 10% v/v DMSO) and stored at −80 °C for comet assay analysis. For the analysis of DNA damage and oxidative DNA damage, a modification of the comet assay (originally described by Singh et al. 1988), was performed to assess the DNA oxidation in these cells (Collins et al. 2012; Collins 2014). Briefly, cells were thawed at 37 °C and centrifuged to eliminate freezing mix; it was diluted 30 μL of cell suspension was diluted in 140 μL of 1% low melting-point agarose (Pronadisa), and 70 μL of this mix was transferred as a drop onto the slide pre-coated with 1% standard agarose (SeaKem®), in a total of 2 drops per slide, and each was then covered with a coverslip to set the gels. As positive control, cells were treated with 100 mM H_2O_2 were used as a control. Afterwards, slides were then placed in a lysis solution (2.5 M NaCl, 0.1 M Na_2EDTA, 10 mM Tris and 1% Triton® X-100, pH 10), for 1 h at 4 °C. For oxidative damage detection, slides were incubated with the FPG (kindly donated by Prof. Andrew Collins) for 30 min at 37 °C. After this treatment, all slides were submerged into an electrophoresis solution (10 M NaOH and 0.5 M EDTA) for 40 min at 4 °C and subsequently electrophoresis was conducted in the

same solution under 20 V for 20 min at 4 °C. After washed and air dried, the gels were stained with DAPI. The scoring of 100 comets per slide was performed with Comet Assay IV Perceptive Instruments® software. The visualization of the slides was performed by a single observer (Collins 2012).

2.6 High Performance Liquid Chromatography (HPLC)

Blood samples (5 mL) were collected in serum tubes and centrifuged at 5000 rpm for 5 min; serum samples were put in 1.5 mL microtubes and stored at −20 °C. Vitamins A (retinol) and E (α-tocopherol) were determined in the serum using reverse-phase high-performance liquid chromatography with a diode array detector (HPLC–DAD; Thermo Scientific Surveyor, USA) adapting the method described in Jaworowska and Bazylak (2014) and Ladeira et al. (2015). Vitamins A and E were separated on a 200 × 4.6 mm Hypersil-BDS C18 column (Thermo Scientific®) with a 10 × 4 mm precolumn Javelin BDS C18 (Thermo Scientific®) using pure methanol as the mobile phase. Tocopheryl acetate was used as internal standard. Detection was performed by Diode Array at 325 and 295 nm for retinol and α-tocopherol, respectively. Chromatograms were integrated automatically by the system software Xcalibur 2.0.

2.7 Statistical Analysis

Data obtained were organized using the IBM SPSS software, through which all the statistical treatment was performed. The level of significance was established at 5%. Kolmogorov–Smirnov test was applied to verify the differences between the values of each variable in T0 and T90, with the aim to access whether there is statistical evidence that they follow a normal distribution. Descriptive statistics, case study analysis and inferential statistics (Student’s t test) was performed.

3 Ethics Statement

This work was developed in a context of a research study for graduate thesis dissertation of Laboratory Biomedical Sciences degree of Escola Superior de Tecnologia da Saúde, Instituto Politécnico de Lisboa, Portugal. All volunteers provided a signed written informed consent before enrolment in the study in accordance with the Helsinki Declaration and Oviedo Convention and in Agreement with the Portuguese Law nº 58/2019 de 8 de agosto regarding data protection.

4 Results

Before enrollment in the study, voluntaries signed a written informed consent and at T0 before blood collection completed a demographic questionnaire with data regarding age, gender, weight (cm), height (kg) and smoking habits from which results are summarized in Table 1. After the 90 days intake of EGCG voluntaries responded to a second questionnaire regarding potential secondary effects including alterations in the nervous system (headaches, migraines, mood swings); gastrointestinal system (heartburn, reflux, diarrhea, cramps, weight loss/gain); cardiac; respiratory. No adverse effects were reported by the volunteers.

4.1 Lipid Profile and Liver Function Parameters Are not Affected by EGCG

Potential effects of 225 mg EGCG daily intake in lipid profile (Triglycerides, cholesterol, HDL, and LDL) and liver function parameters (AST, ALT, GGT) were evaluated trough using standard laboratory colorimetric methods and kinetics. No significant differences between the average values of the analyzed parameters were observed at T0 and T90, as demonstrated in Table 2, which indicated that EGCG 225 mg daily consumption does not affect lipid profile and liver function in vivo.

4.2 Serum Levels of Vitamin E Increase After EGCG Consumption

Serum variations of α-tocopherol (vitamin E) and retinol (vitamin A) potentially associated with EGCG 225 mg capsule consumption for 90 days, were assessed by HPLC. Vitamin E serum levels have significantly increased (31.48 and 35.26 respectively, Student's t test $p = 0.041$), however no significant effects in serum levels of vitamin A were observed between T0 and T90 (2.73 and 2.47 respectively, Student's t test $p = 0.419$), as demonstrated in Fig. 1.

Table 1 Characterization of voluntaries enrolled in the study

Male	Female	Age	Height (cm)	Weight (kg)	Smoker	Non smoker
10	20	19–43	155–195	45–108	1	29

Table 2 Lipid profile and liver function parameters determination prior (T0) and after 90 days (T90) of EGCG 225 mg daily capsule consumption

	Triglycerides (mg/dL)	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	AST/GOT (UI/L)	ALT/GPT (UI/L)	GGT (UI/L)	Ratio LDL/HDL
T0 (mean ± std. dev.)	107.77 ± 45.07	183.4 0 ± 37.88	61.60 ± 13.81	100.30 ± 28.59	24.23 ± 8.9	25.37 ± 8.39	19.13 ± 18.82	1.68 ± 0.49
T90 (mean ± std. dev.)	119.17 ± 41.44	181.67 ± 31.11	63.53 ± 14.85	93.97 ± 22.18	25.40 ± 9.29	30.73 ± 22.01	22.07 ± 25.38	1.55 ± 0.53

Results are presented as (mean ± SD; N = 30)

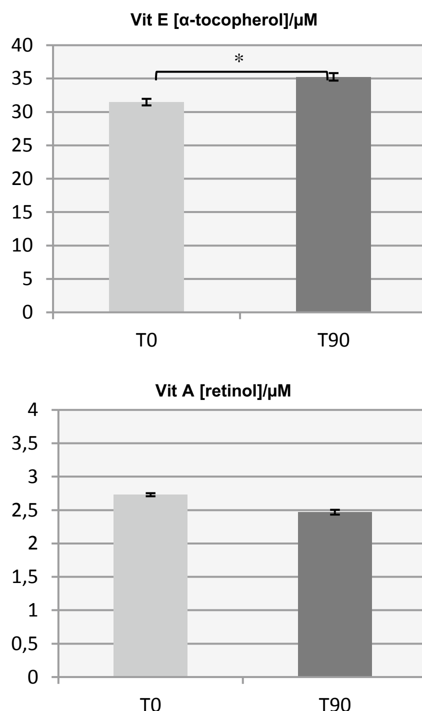

Fig. 1 HPLC determination of Vit E [α-tocopherol]/μM and Vit A [retinol]/μM average prior to EGCG consumption (T0) and after 225 mg EGCG daily capsule for 90 days (T90). Experiments were replicated three times. Student's t test (* $p < 0.05$)

4.3 EGCG Consumption Induces DNA Damage and Oxidative Damage

Global DNA damage and oxidative DNA damage—particular type of DNA damage caused by the oxidation of the nucleotides, were both assessed by comet assay. Descriptive statistics is presented in Table 3, where is possible to verified that regarding both parameters assessed—% DNA damage and % DNA oxidative damage—there is an increase after 90 days of EGCG intake (19.77 ± 12.70 and 19.87 ± 5.65) in comparison with T0 (10.40 ± 11.08 and 13.33 ± 8.17), respectively. In order to observe the distribution of the DNA damage and DNA oxidative damage, Fig. 2 showed that in general T90 samples presented slightly higher increase of DNA damage in comparison with T0 samples.

Table 3 % DNA damage and % DNA oxidative damage means (mean ± SD; N = 30) prior to EGCG consumption (T0) and after 90 days of 225 mg EGCG/daily capsule (T90) (mean ± SD; N = 30)

	% DNA damage mean ± std. dev	% DNA oxidative damage mean ± std. dev
T0	10.40 ± 11.08	13.33 ± 8.17
T90	19.77 ± 12.70	19.87 ± 5.65

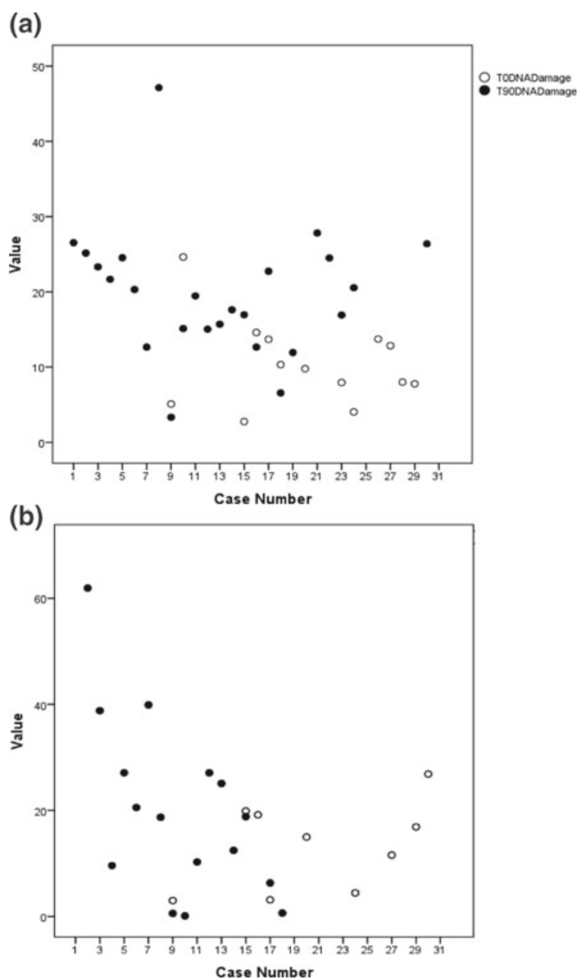

Fig. 2 Graphic representation of % DNA damage (A) and % DNA oxidative damage (B) of the T0 (N = 30) and T90 samples (N = 30) distribution in all analyzed individual samples from the volunteers enrolled in the study. White dots represent T0 samples and black dots T90 samples

5 Discussion

Currently, cardiovascular diseases and cancer are some of the most concerning diseases worldwide, with associated occupational risk factors as key players. In this context, the unquestionable and widespread human exposure to several compounds, including natural products with described health benefits, such as antioxidant properties, raises questions regarding its potential benefic effects for human health.

Green tea, one of the most consumed beverage in the world, has described antioxidant properties which include the ability to limit the amount of free radicals by scavenging ROS, upregulating basal levels of antioxidant enzymes, and increasing the activity of these antioxidant enzymes (Reygaert 2018). However, the pro-oxidant effects of green tea polyphenols have also been suggested as potential mechanisms

for cancer (Lambert and Elias 2010) and cardiovascular diseases prevention associated with reduced total lipid levels and improved LDL to HDL ratio (Bhardwaj and Khanna 2013).

EGCG, the major polyphenol in green tea, has demonstrated antioxidant effects related to anticancer function, particularly associated with decreased cell proliferation and induction of apoptosis in colon carcinoma cells, inhibition of adhesion and invasion of hepatoma cells and downregulation of ROS levels (Min and Kwon 2014). EGCG has been used in human clinical trials, where 400 mg to 800 mg of EGCG intake, which result in peak serum concentrations in the range of 100 to 400 ng/mL, are considered safe doses (Chow et al. 2003a, b). Moreover, a recently published review of toxicological evidence from laboratory studies demonstrated that the liver is a target organ for EGCG, associated with levels and dosing conditions, which can present hepatotoxicity with associated alterations in liver function parameters (Hu et al. 2018).

In our interventional study, we did not observe any alteration in the individual's lipid profile or liver function parameters induced by the daily exposure to 225 mg of EGCG, which agrees with the previously described safe dosage threshold.

On the other hand, although previous studies have demonstrated that EGCG prevented DNA damage at low concentrations in different cells, it also acted as a pro-oxidant at higher concentrations (Johnson and Loo 2000). Additionally, under certain conditions, catechins may be unstable and undergo auto-oxidation and behave like pro-oxidants (Lambert and Elias 2010; Min and Kwon 2014). Studies in vitro showed that 10 μM EGCG increased DNA damage assessed by alkaline comet assay after 1 h and enhanced micronuclei formation after 24 h (Elbling et al. 2005). Results from Aydin et al. (2015) showed that above the concentrations of 0.01 μM EGCG a significant antioxidant capacity is observed and at all concentrations studied above 5 μM of EGCG significant DNA damage was reported (Aydin et al. 2015). However, within the concentrations of 0.01–10 μM EGCG significantly reduced oxidative DNA damage induced by H_2O_2 and at the concentration of 5 μM EGCG induced FPG sensitive sites indicating the increased oxidized purine base levels. Study from Kanadzu et al. (2006) reported that EGCG was inhibitory against DNA breakage stimulated or not by mutagens in lymphocytes at relatively lower concentrations, but increased DNA breakage in lymphocytes at higher concentrations, all experiments measured by comet assay. Considering these findings, it was suggested that the pro-oxidative effects of EGCG at high concentrations might resulted from the generation of radicals, and EGCG is both antioxidant and pro-oxidant. Nevertheless, it is important to emphasize that EGCG effects observed in vitro may not be the same as in vivo due to obvious complexity of living organisms (Kim et al. 2014).

Our results, regarding in vivo genotoxicity assessment, showed an increase of DNA damage % and oxidative DNA damage parameters from T0 to T90, which indicate a pro-oxidant effect of EGCG at the tested concentration. Although 225 mg is considered a low safe dose it is relevant to contemplate that in this study exposure was continued for 90 days, which is not taken into consideration by in vitro studies.

Furthermore, previous studies have described that pro-oxidant effects appear to be responsible for the induction of apoptosis in tumor cells (Furukawa et al. 2003) but

may also induce endogenous antioxidant systems in normal tissues offer protection against carcinogenic insult (Lambert and Elias 2010). Depending on the source, antioxidant defense systems can be endogenous—antioxidant enzymes and non-enzymatic antioxidative system; and exogenous—classified depending to their origin as natural, nature-identical and synthetic (Hevelke et al. 2016). While EGCG is classified as a synthetic antioxidant, nature-identical antioxidants such as Vitamin E, a major lipid-soluble component in the cell antioxidant defense system, also have well described effects in oxidative stress management (Hevelke et al. 2016). Vitamin E, has been described as crucial for anti-inflammatory processes, inhibition of platelet aggregation, immune-enhancing activity associated with prevention and reversal of several disease pathology and complications, including cancer, ageing, arthritis and cataracts (Rizvi et al. 2014).

Here, the increase of vitamin E serum levels associated with increased DNA damage and oxidative DNA damage suggest that moderate/low concentrations of EGCG, can induce low level oxidative stress which may be beneficial cue for the body to initiate induction of protective anti-oxidant systems and boost immune responses (Kim et al., 2014).

Overall, EGCG continuous exposure to low concentrations (225 mg daily exposure for 90 days) endorse some level of oxidative stress that may lead to the induction of endogenous antioxidant systems (Lambert and Elias 2010) with no effects in lipid profile or liver function parameters in vivo.

6 Conclusions

In this study, we demonstrated that the daily consumption of low concentrations of EGCG (225 mg) does not affect lipid profile or liver function parameters in vivo in healthy volunteers, however it increases low level oxidative stress observed by DNA oxidative damage which may act as a beneficial cue for the body to initiate induction of protective antioxidant systems associated with vitamin E, and boost immune responses. Further research is crucial in order to understand the extent and potential effects of EGCG in oxidative stress, DNA damage and DNA oxidative damage and potential interactions with antioxidant defense systems, as well as its potential as an alternative to minimize occupational factors effects in the increased risk of cardiovascular diseases and cancer.

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