Protective effect of (–)-epigallocatechin-3-gallate on capsaicin-induced DNA damage and oxidative stress in human erythrocytes and leucocytes in vitro

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Abstract The aim of this study is to show that protective effects of the main catechin (–)-epigallocatechin-3-gallate (EGCG) against capsaicin (CAP) induced oxidative stress and DNA damage in human blood in vitro. Superoxide dismutase, catalase, glutathione peroxidase and malondialdehyde (MDA) level were studied in erythrocytes and leucocytes with increased concentrations of CAP. DNA damage in leucocytes was measured by the comet assay. Human blood cells have been administered with doses between 0 and 200 μ M of CAP and/or EGCG (20 μ M) for an hour at 37 °C. Treatment with CAP alone has increased the levels of MDA and decreased antioxidant enzymes in human blood cells. A significant increase in tail DNA%, mean tail length and tail moment indicating DNA damage has been observed at the highest dose of CAP treatment when compared to controls. Treatment of cells with CAP plus EGCG prevented CAP-induced changes in antioxidant enzyme activities and MDA level and mean tail length indicating DNA damage. A significant increase in mean tail length was observed at high doses of CAP. These data suggest that EGCG can prevent toxicity to human erythrocytes and leucocytes caused by CAP, only at low doses.

Keywords CAP · (–)-Epigallocatechin-3-gallate · Human blood cells · Comet assay

Introduction

Pepper fruits (*Capsicum annuum* L.) are important vegetables used as vegetable food and as spices with well-known antioxidant properties (Rice et al., Rice-Evans et al. 1997). Capsaicin (CAP) (trans-8-methyl-N-vannilyl-6-nonemide) is the major pungent principle found in hot red and chili peppers of the plant genus *Capsicum* that has long been used as spices, food additives and drugs (Cordell and Araiyo 1993; Sharma et al. 2013) and is extensively consumed as a condiment in Asian, African and Latin American countries (Banji et al. 2013). It is employed as a condiment and colorant in the cosmetic and pharmaceutical industries (Banji et al. 2013).

While some studies demonstrated a marked mutagenic and genotoxic activity of the compound in the presence or absence of an external metabolic activation system, others failed to provide an evidence for its genotoxic potential (Surh and Lee 1996). Capsaicin has been tested for its effects on experimental carcinogenesis and mutagenesis (Vinayaka et al. 2010). Carcinogenicity studies in mice demonstrated that CAP can induce duodenal adenocarcinomas (Toth et al. 1984) and it acts as a promoter of stomach and liver tumors (Agarwal et al. 1986). Conversely, other studies pointed out the antimutagenic, antigenotoxic

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and anticarcinogenic properties of CAP (Surh 1999; Surh and Lee 1996). CAP is aptly referred as a ‘double edged sword’ as it possesses both a genotoxic and a chemopreventive potential (Surh and Lee 1995). CAP readily evokes the formation of micronuclei and promotes sister chromatid exchange in mice (Diaz Barriga-Areos et al. 1995; Lee and Cho 1997). Excessive and frequent consumption of capsicum leads to organic disturbances in the digestive tract (Sanghvi 1981) characterized by duodenal (Toth and Gannett 1992) and gastric tumors (Agarwal et al. 1986; Banji et al. 2013).

Flavonoids belong to a group of natural substances with variable phenolic structures and are found in fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine. These natural products were known for their beneficial effects on health long before flavonoids were isolated as the effective compounds. More than 4,000 varieties of flavonoids have been identified. The flavones are characterized by a planar structure because of a double bond in the central aromatic ring (Padma et al. 2012). The flavanol epigallocatechin-3-gallate (EGCG) is the most abundant catechin in green tea which is the unfermented extract of the leaves of *Camellia sinensis*. Green tea contains up to 25 % catechins (% dry weight) of which EGCG constitutes 9–13 %, while epicatechingallate, epigallocatechin and epicatechin make up the remainder (IARC 1991). EGCG has demonstrated chemopreventive properties in a number of rodent studies (Crespy and Williamson 2004; Fujiki et al. 1992; Fujita et al. 1989; Yamane et al. 1996; Yang et al. 2002).

EGCG possesses numerous biological functions, including antioxidant, anti-inflammatory, and anticancer effects (Devika and Stanely Mainzen Prince 2008; Kumar and Kumar 2009). Regarding EGCG-induced generation of ROS, hydrogen peroxide production induced by EGCG has been associated with threefold to sixfold enhancement of cisplatin efficacy in ovarian cancer cells, even in some cell lines highly resistant to the treatment with the drug alone (Chan et al. 2006). Glei and Pool-Zobel (2006) have investigated that the continuous presence of EGCG can reduce radical-induced DNA damage in primary leucocytes, possibly due to a combination of different mechanisms. Together the findings support the hypothesis that EGCG acts as a protective in human cells.

The popularity of the comet (single-cell gel electrophoresis) assay has increased recently because of

their ability to detect genotoxic responses in a wide variety of tissues (Kirkland and Speit 2008). Comet assay was used in various in vitro and in vivo studies to evaluate DNA damage (Anderson et al. 1998; Cotelle and Ferard 1999; Fairbairn et al. 1995; McKelvey-Martin et al. 1993; Singh et al. 1988; Celik et al. 2013). In this method, a small number of cells suspended in a thin agarose gel on a microscope slide are lysed, electrophoresed and stained with a fluorescent DNA binding dye. The technique is based on the fact that broken DNA migrates more easily in an electric field than intact molecules. When the slide is visualized with a fluorescence microscope, the observed objects resemble comets with a head region containing undamaged DNA and a tail containing the broken DNA. The amount of DNA is able to migrate and the distance of migration indicates the number of strand breaks present in that cell. Greater migration of the chromosomal DNA from the nucleus is an indication of higher level of DNA damage (Singh et al. 1988).

Anderson et al. (2001) showed that EGCG is the most active antigenotoxic compound of the catechins. The strand break reducing effects were already seen at micromolar concentrations. There is, therefore, an increasing interest in the possible beneficial effects of EGCG, the main tea catechin (Glei et al. 2003), on DNA stability and health (Glei and Pool-Zobel 2006).

The aim of the study was to determine the effect of CAP at several different doses, in combination with EGCG on malondialdehyde (MDA) levels and the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in human erythrocytes and leucocytes and also evaluate the extent of DNA damage in human leucocytes with the comet assay.

Materials and methods

Drugs and chemicals

CAP, EGCG and RPMI 1640 were bought from Sigma-Aldrich (Munich, Germany). All other reagents and chemicals used were of analytical grade. Different concentrations of CAP (10–200 μ M) were determined using the method previously reported by Marques et al. (2002), and EGCG (20 μ M) was determined using the method previously reported by Glei and

Pool-Zobel (2006); and CAP and EGCG were dissolved in DMSO.

Isolation of human erythrocytes

Venous blood samples of approximately 20 ml were obtained in heparinized dry tubes from each of six male volunteers (range 21–26 years). All volunteers were healthy, taking no medication, non-smokers, and none of them were farm or agricultural workers. Plasma was separated by 3,000 rpm for 15 min. Erythrocyte packets were prepared by washing with a cold isotonic saline. Then supernatant was removed and erythrocytes were suspended in phosphate buffer. The concentration of hemoglobin was determined by the method published by Drabkin (1946).

Isolation of human leucocytes

Blood was obtained from six healthy and non-smoking volunteers (aged 25–30 years). Comet assay was performed according to the method previously reported (Singh et al. 1988) with some modifications. Peripheral blood was obtained with heparinized syringe immediately before the performance of the test. Lymphocytes were isolated using the Bioncoll (Source BioScience, Nottingham, U.K.) separating solution (Yilmaz et al. 2014). Cell viability was determined by the trypan blue exclusion technique of Pool-Zobel et al. (1992). Cell viability was ~98 %.

Preparation of incubations with CAP and EGCG

Erythrocytes and leucocytes haemolysate obtained from healthy donors were divided into three groups as follow: control group, CAP group and EGCG group. This procedure was applied according to the method previously reported (Konyalioglu and Karamenderes 2005), and all incubation mixtures were prepared as below:

For erythrocytes:

- Control group; erythrocyte hemolysate 750 µl, PBS 1,000 µl and distilled water 250 µl;
- CAP group; erythrocyte hemolysate 750 µl, CAP (10–200 µM) 50 µl, PBS 950 µl and distilled water 250 µl;

- EGCG group; erythrocyte hemolysate 750 µl, CAP (10–200 µM) 50 µl, EGCG (20 µM) 250 µl and PBS 950 µl.

For leucocytes:

- Control group; leucocyte hemolysate 50 µl and PBS 1,050 µl;
- CAP group; leucocyte hemolysate 50 µl, CAP (10–200 µM) 50 µl and PBS 1,000 µl;
- EGCG group; leucocyte hemolysate 50 µl, CAP (10–200 µM) 50 µl, EGCG (20 µM) 500 µl and PBS 500 µl.

These experimental groups were incubated in a shaking water-bath (60 rpm) for an hour at 37 °C. Following the incubation, antioxidant enzymes activities, LPO, protein and Hb levels were determined (Konyalioglu and Karamenderes 2005).

Assays of antioxidant enzyme activities

SOD activity was measured as the inhibition of autoxidation of pyrogallol, according to the method of Marklund and Marklund (1974). Activity was monitored at 440 nm for 180 s. Data are expressed as U of SOD/mgHb and for leucocytes as U of SOD/mg protein.

CAT activity was measured according to the method of Aebi (1984) as rate constant of hydrogen peroxide (H₂O₂) decomposition. Activity was monitored at 240 nm for 60 s. Data are expressed as U CAT/mgHb and for leucocytes as U CAT/mg protein.

GPx activity was measured according to the method of Paglia and Valentine (1967). Reaction mixtures contained NADPH, reduced glutathione, Tris-HCl and glutathione reductase. Reactions were initiated by the addition of H₂O₂ and GPx activity was measured as the change in absorbance at 340 nm. Data are presented as U GPx/mgHb and for leucocytes as U GBx/mgHb protein.

Absorbance was measured at 532 nm to determine the MDA content. Specific activity was presented as nmol/mgHb and nmol/protein. MDA contents were assayed according to the method previously reported (Ohkawa et al. 1979). MDA reacts with thiobarbituric acid (TBA) to form a colored complex. MDA contents as an indicator of lipid peroxidation were determined after incubation at 95 °C with TBA (1 % w/v).

Determination of total protein concentration

Total protein concentration, in leucocyte hemolysate, was evaluated by using BSA as standard with the method developed by Lowry et al. 1951.

Determination of Hb concentration

Hb concentration was determined by Drabkin's method in erythrocyte hemolysate (Bauer et al. 1974).

Determination of DNA damage (comet assay)

Isolated human lymphocytes were incubated with 0, 10, 30, 50, 100 and 200 μM concentrations of CAP for 1 h at 37 °C. After the incubation, lymphocytes were centrifuged with RPMI 1640 at 1,348g for 5 min, and then the supernatant was removed and resuspended in PBS. Treated cells were suspended in low melting point agarose (0.65 %), and 75 μl of suspension was quickly layered over slides which were precoated with normal melting point agarose (0.65 %), immediately covered with a cover slip and the slides were placed at +4 °C for 10–15 min. After solidification, the cover-slip was gently removed and immersed in cold lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris pH 10 in which 10 % DMSO and 1 % Triton $\times$ -100 were added) at 4 °C for 1 h. The slides were removed and placed on a horizontal gel electrophoresis platform covered with electrophoresis buffer (300 mM NaOH, 1 mM EDTA pH 13). Then, they were left in the solution for 20 min to allow the unwinding of the DNA (Ozkan et al. 2009). Electrophoresis was run at 25 V for 20 min at 4 °C. All procedural steps were performed under yellow light conditions to minimize additional DNA damage. The slides were then placed vertically in a neutralizing tank and gently washed three times for 5 min with neutralizing buffer (0.4 M Tris-HCl buffer, pH 7.5). Ethidium bromide (10 mg in 50 ml of distilled water) was dispensed directly onto slides and covered with a cover slip.

All measurement data were analyzed using BS 200 ProP with software (BAB Bs Comet Assay software) image analysis (BS 200 ProP, BAB Imaging System, Ankara, Turkey). The DNA comets were evaluated by measuring the tail length, tail moment and tail DNA % of 50 comets. All experiments were performed at least three times, each with two parallel slides per data

point. A higher percentage tail DNA indicated a higher level of DNA damage (Behravan et al. 2011).

Data analysis

Differences between groups were evaluated by one-way analysis of variance (ANOVA) followed by the Tukey test. The protective effects of EGCG with different doses of CAP were compared using Student's *t* test for paired samples. Values were expressed as mean $\pm$ standard error of the mean (SEM). Statistical significance was accepted at the $p < 0.05$ level.

Results

Evaluation of biochemical parameters

Tables 1 and 2 show the effect of CAP and/or EGCG on erythrocytes and leucocytes MDA and enzymic antioxidant status. The MDA levels and SOD, CAT and GPx activities in cells treated with 10 μM of CAP were statistically similar to nontreated cells or cells treated with EGCG alone. A significant ($p < 0.05$) increase in MDA with concomitant decrease in the activities of enzymic antioxidants SOD, CAT and GPx were observed at concentrations of 30 and 50 μM of CAP administered groups of erythrocytes and leucocytes compared with the control group. CAP (30 and 50 μM) treatment with EGCG resulted in a free radical quenching effect and thereby significantly ($p < 0.05$) decreased MDA and reinstated the enzymic antioxidant activities to near normalcy in erythrocytes and leucocytes. However, these results suggested that EGCG has no protective effect on CAP-induced changes in MDA levels and antioxidant enzyme activities in erythrocytes and leucocytes at doses of CAP of 100 and 200 μM ($p < 0.05$) (Tables 1, 2).

Image analysis

The results of DNA damage obtained after treatment by CAP were summarized in Fig. 1 and Table 3. There were no differences in DNA damage between cells that were treated with 10 and 30 μM CAP as compared with nontreated control cells or cells that were treated with EGCG alone. DNA strand breaks showed clear dose response between DNA damage and different doses of CAP (50–200 μM). The tail

Table 1 Effects of CAP treatment in absence and presence of EGCG on SOD, GPx, CAT activities and MDA levels in human erythrocytes in vitro (mean $\pm$ SEM)

Groups (each group, n = 6)	MDA (nmol/mgHb)	SOD (U/mgHb)	CAT (U/mgHb)	GPx (U/mgHb)
Control	6.01 $\pm$ 2.12	864 $\pm$ 23.24	376 $\pm$ 21.48	47 $\pm$ 5.23
EGCG (20 μ M)	6.98 $\pm$ 3.1	880 $\pm$ 42.19	365 $\pm$ 21.48	43 $\pm$ 4.45
CAP (10 μ M)	7.23 $\pm$ 1.26	871 $\pm$ 54.36	355 $\pm$ 52.75	41 $\pm$ 6.44
CAP + EGCG (10 + 20 μ M)	6.8 $\pm$ 2.27	834 $\pm$ 53.24	368 $\pm$ 45.65	42 $\pm$ 2.3
CAP (30 μ M)	9.45 $\pm$ 4.09 ^{abcd}	780 $\pm$ 44.27 ^{abcd}	324 $\pm$ 25.34 ^{abcd}	34 $\pm$ 2.45 ^{abcd}
CAP + EGCG (30 + 20 μ M)	6.28 $\pm$ 2.32 ^e	829 $\pm$ 34.29 ^e	368 $\pm$ 45.65 ^e	40 $\pm$ 2.56 ^e
CAP (50 μ M)	12.01 $\pm$ 2.18 ^{abcdef}	728 $\pm$ 42.23 ^{abcdef}	295 $\pm$ 66.23 ^{abcdef}	29 $\pm$ 0.65 ^{abcdef}
CAP + EGCG (50 + 20 μ M)	7.65 $\pm$ 3.36 ^g	844 $\pm$ 28.54 ^g	354 $\pm$ 27.45 ^g	42 $\pm$ 8.91 ^g
CAP (100 μ M)	15.67 $\pm$ 8.22 ^{abcdefgh}	658 $\pm$ 42.19 ^{abcdefgh}	245 $\pm$ 34.3 ^{abcdefgh}	25 $\pm$ 7.5 ^{abcdefgh}
CAP + EGCG (100 + 20 μ M)	14.2 $\pm$ 7.62 ^{abcdefgh}	637 $\pm$ 46.16 ^{abcdefgh}	228 $\pm$ 74.1 ^{abcdefgh}	28 $\pm$ 8.7 ^{abcdefgh}
CAP (200 μ M)	16.22 $\pm$ 4.12 ^{abcdefgh}	624 $\pm$ 22.19 ^{abcdefgh}	231 $\pm$ 63.8 ^{abcdefgh}	23 $\pm$ 4.35 ^{abcdefgh}
CAP + EGCG (200 + 20 μ M)	16.74 $\pm$ 5.02 ^{abcdefgh}	645 $\pm$ 62.86 ^{abcdefgh}	243 $\pm$ 86.8 ^{abcdefgh}	22 $\pm$ 4.8 ^{abcdefgh}

Different letters indicate significant differences between groups ($p < 0.05$)

Table 2 Effects of CAP treatment in absence and presence of EGCG on SOD, GPx, CAT activities and MDA levels in human leucocytes in vitro (mean $\pm$ SEM)

Groups (each group, n = 6)	MDA (nmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)
Control	1.45 $\pm$ 0.06	352 $\pm$ 58.7	276 $\pm$ 20.12	43.2 $\pm$ 16.2
EGCG (20 μ M)	1.98 $\pm$ 0.2	348 $\pm$ 43.04	266 $\pm$ 31.98	41.4 $\pm$ 12.1
CAP (10 μ M)	2.45 $\pm$ 0.08	340 $\pm$ 56.2	261 $\pm$ 12.6	39.3 $\pm$ 10.1
CAP + EGCG (10 + 20 μ M)	1.95 $\pm$ 0.9	349 $\pm$ 63.1	268 $\pm$ 41.8	40.1 $\pm$ 6.3
CAP (30 μ M)	3.75 $\pm$ 1.1 ^{abcd}	320 $\pm$ 46.22 ^{abcd}	255 $\pm$ 58.6 ^{abcd}	35.5 $\pm$ 7.5 ^{abcd}
CAP + EGCG (30 + 20 μ M)	1.38 $\pm$ 0.2 ^e	345 $\pm$ 72.3 ^e	276 $\pm$ 65.2 ^e	42.6 $\pm$ 11.23 ^e
CAP (50 μ M)	4.08 $\pm$ 1.6 ^{abcdef}	308 $\pm$ 26.8 ^{abcdef}	240 $\pm$ 54.3 ^{abcdef}	30.4 $\pm$ 9.6 ^{abcdef}
CAP + EGCG (50 + 20 μ M)	1.92 $\pm$ 0.05 ^g	342 $\pm$ 43.6 ^g	260 $\pm$ 43.1 ^g	40.2 $\pm$ 3.15 ^g
CAP (100 μ M)	5.36 $\pm$ 1.01 ^{abcdefgh}	295 $\pm$ 74.4 ^{abcdefgh}	210 $\pm$ 24.2 ^{abcdefgh}	25.5 $\pm$ 6.75 ^{abcdefgh}
CAP + EGCG (100 + 20 μ M)	5.45 $\pm$ 6.1 ^{abcdefgh}	305 $\pm$ 33.8 ^{abcdefgh}	220 $\pm$ 34.58 ^{abcdefgh}	27.1 $\pm$ 5.7 ^{abcdefgh}
CAP (200 μ M)	5.89 $\pm$ 0.9 ^{abcdefgh}	286 $\pm$ 25.7 ^{abcdefgh}	190 $\pm$ 11.6 ^{abcdefgh}	23.3 $\pm$ 2.2 ^{abcdefgh}
CAP + EGCG (200 + 20 μ M)	6.18 $\pm$ 0.9 ^{abcdefgh}	289 $\pm$ 39.4 ^{abcdefgh}	186 $\pm$ 9.64 ^{abcdefgh}	24.2 $\pm$ 3.65 ^{abcdefgh}

Different letters indicate significant differences between groups ($p < 0.05$)

DNA% and mean tail length induced by increasing doses of CAP were significantly ($p < 0.05$) higher than those of controls. Treatment with 100 and 200 μ M of CAP showed a maximum increase of 94.24 ± 0.37 and 94.67 ± 1.94 , respectively, which were approximately twofold higher, compared to control tail DNA %. The CAP and EGCG groups treated with the same CAP doses showed mean $\pm$ SEM values of 81.88 ± 4.41 and 93.75 ± 2.51 ,

respectively. However, a gradual decrease in tail DNA% and mean tail length in the 50 μ M dose were observed showing a EGCG dependent decrease in DNA damage (Fig. 1g, h; Table 3). No statistically significant changes were observed in the EGCG-plus-CAP (50 μ M) groups compared with the control but not similarity in DNA damage with that at 100 and 200 μ M of doses of CAP was observed.

Fig. 1 Comet appearances of the CAP in absence and presence of EGCG treated lymphocytes and control group **a** DNA of control cells, **b** 20 μ L of EGCG- **c** 10 μ L of CAP- **d** 10 μ L of CAP + 20 μ M of EGCG- **e** 30 μ M of CAP- **f** 30 μ M of

CAP + 20 μ M of EGCG- **g** 50 μ M of CAP- **h** 50 μ M of CAP + 20 μ M of EGCG- **i** 100 μ M of CAP- **j** 100 μ M of CAP + 20 μ M of EGCG- **k** 200 μ M of CAP- **l** 200 μ M of CAP + 20 μ M of EGCG-treatment cells' DNA

Fig. 1 continued**Table 3** Number of cells (lymphocytes) with damaged DNA from twelve different subjects, first treated or untreated with EGCG and subsequently incubated with five different concentrations of CAP

Treatmeant doses	The amount of comets counted	Tail DNA% Mean $\pm$ SEM	Tail lenght Mean $\pm$ SEM
Control	100	56.25 $\pm$ 5.04	4.95 $\pm$ 0.45
EGCG (20 μ M)	100	59.66 $\pm$ 6.23	5.45 $\pm$ 2.25
CAP (10 μ M)	100	60.12 $\pm$ 3.25	8.12 $\pm$ 3.75
CAP + EGCG (10 + 20 μ M)	100	62.25 $\pm$ 6.58	4.03 $\pm$ 0.52
CAP (30 μ M)	100	71.99 $\pm$ 4.83	11.53 $\pm$ 2.81
CAP + EGCG (30 + 20 μ M)	100	64.36 $\pm$ 3.49	11 $\pm$ 1.73
CAP (50 μ M)	100	79.93 $\pm$ 3.48 ^{abcdef}	64.50 $\pm$ 13.44 ^{abcdef}
CAP + EGCG (50 + 20 μ M)	100	64.60 $\pm$ 4.36 ^g	8.37 $\pm$ 1.27 ^g
CAP (100 μ M)	100	94.24 $\pm$ 0.37 ^{abcdefgh}	54.36 $\pm$ 8.24 ^{abcdefgh}
CAP + EGCG (100 + 20 μ M)	100	81.88 $\pm$ 4.41 ^{abcdefgh}	21.84 $\pm$ 6.87 ^{abcdefgh}
CAP (200 μ M)	100	94.67 $\pm$ 1.94 ^{abcdefgh}	80.74 $\pm$ 8.33 ^{abcdefgh}
CAP + EGCG (200 + 20 μ M)	100	93.75 $\pm$ 2.51 ^{abcdefgh}	76.31 $\pm$ 17.17 ^{abcdefgh}

Estimated mean values of tail DNA%, and tail length of comets in lymphocytes. Different letters indicate significant differences between groups ($p < 0.05$)

Values are expressed as median for comet scores in 100 cells from six subjects

Discussion

CAP is the chemical responsible for the pungent, hot properties of *Capsicum*, a vegetable widely consumed

in the diet of many countries in the world (Diaz Barriga-Areos et al. 1995). Genotoxicity and carcinogenicity studies evaluating CAP effects are sparse and contradictory (Marques et al. 2002). To our

knowledge, the International Agency for Research on Cancer (IARC) has no available evaluation on CAP (<http://www.iarc.fr>), but considering its frequent consumption in food, its current therapeutic application and its putative mutagenic–carcinogenic activities, the correct assessment of this compound is crucial from the public health standpoint (Marques et al. 2002). Human blood cells were treated in vitro with doses ranging from 0, 10, 30, 50, 100 and 200 μM of CAP at 1 h. It was observed that at concentrations ranging from 30 to 200 μM , CAP was cytotoxic in human erythrocytes and leucocytes. Therefore, usage of CAP is limited by the induction of toxicity to these cells.

EGCG (5–10 μM) significantly increased γ -radiation-induced apoptosis in MDA-MB-231 cells. Thus, not only EGCG but also its metabolites may potentiate the effects of radiotherapy (Lecumberri et al. 2013; Zhang et al. 2012). EGCG has been shown to exert both antioxidant and pro-oxidant activities. The concentration of EGCG in the cell environment seems to be a major factor to explain this dual role. EGCG increased intracellular hydrogen peroxide in cancer cells and ATO-induced heme oxygenase-1 (HO-1) provided ferrous iron, thus increasing Fenton reaction and, as a consequence, oxidative damage to cells (Lecumberri et al. 2013). But this catechin has been shown to act as an effective antioxidant when used at low doses (within the range of high nanomolar and low micromolar levels) and to induce the production of reactive oxygen species (ROS) and oxidative damage at higher doses (Lambert and Elias 2010; Lecumberri et al. 2013). These relatively low amounts of EGCG as well as higher EGCG concentrations (20 μM) resulted in significantly lower levels of DNA strand breaks in human leucocytes treated in vitro, which was in line with other investigations (Dhawan et al. 2002). Treatment with 20 μM EGCG was most effective in reducing various degrees of DNA degradation, reflected by the shapes of the comets (e.g. long tails), lipid peroxidation, and in augmenting oxidative stress of human blood cells.

Reactive oxygen species generate superoxide anions and its derivatives, particularly highly reactive and damaging hydroxyl radical, induce peroxidation of cell membrane lipids (Mukherjee et al. 2003). Lipid peroxidation, a type of oxidative degeneration of polyunsaturated fatty acids (PUFA), has been linked with altered membrane structure and enzyme

inactivation (Karthikeyan et al. 2007). Consequently, lipid peroxidation is known to induce cellular damage and is responsible for ROS induced organ damage. Lipid peroxidation of membranes is regulated by the availability of substrate in the form of PUFA, the availability of inducers, such as free radicals and excited state molecules and the physical status of membrane lipids (Anandan et al. 1998). Thiobarbituric acid reactive substances (TBARS) are produced by lipid peroxidation and are considered as indicators of oxidative stress (Karthikeyan et al. 2007; Yousef et al. 2009). Erythrocytes and leucocytes are critical targets for natural products and plants as well as many other drugs. Moreover, human erythrocytes and leucocytes are excellent subjects for studies of biological effects of free radicals, since they are both structurally simple and easily obtained. Indeed, they have been used as a model for the investigation of free-radical induced oxidant stress because of several reasons: They are continually exposed to high oxygen tensions, they are unable to replace damaged components, the membrane lipids are composed partly of PUFA side chains which are vulnerable to peroxidation, and they have antioxidant enzyme systems (Bukowska 2003; Konyalioglu and Karamenderes 2005). SOD, which converts superoxide radicals to hydrogen peroxide, is widely distributed in cells having oxidative metabolism and is thought to protect such cells against the toxic effects of superoxide anion (Fridovich 1975). CAT is a heme protein that catalyzes the direct degradation of hydrogen peroxide to water. It protects the cellular constituents against oxidative damage (Shimeda et al. 2005). GPx catalyzes the reduction of hydrogen peroxide and hydroperoxide to non-toxic products and scavenges the highly reactive lipid peroxides in the aqueous phase of cell membranes (Anandakumar et al. 2008; Vijayalakshmi et al. 1997). Incubation with EGCG proved to be capable of protecting against oxidative damage caused by 30 and 50 μM of CAP in human blood cells in vitro but the same effect was not detected at the higher concentrations of CAP (100 and 200 μM). The presence of EGCG with CAP normalized the levels of MDA and the activities of antioxidant enzymes to nearly normal values of control using only low doses of CAP.

Considering high consumption of *Capsicum* fruits and the mutagenic–carcinogen antecedents of CAP, different groups have performed several studies to

further evaluate the genotoxicity of this chemical. In a report, CAP has been shown to be genotoxic and caused micronuclei (MN) formation and sister chromatid exchanges (SCE) during a subchronic treatment in mice (Diaz Barriga-Areos et al. 1995). Richeux et al. (1999) reported the role of CAP in inducing DNA strand breaks in human SHSY neuroblastoma cells. Marques et al. (2002) suggested that CAP can induce sister chromatid exchange, and micronuclei formation. The alkyl moiety in CAP caused DNA methylation (Jones and Buckley 1990; Jones 1984) and led to alteration in the structure of cellular DNA (Banji et al. 2013). These findings suggest that the comet assay is a highly sensitive technique to study DNA damage caused by CAP. The results of microscopic observation on leucocytes showed that tail DNA increased with increasing doses of CAP. With increasing exposure dose, the percentage of DNA in the comet tail rises, whereas the amount in the comet head decreases. EGCG appears capable of protecting human leucocytes against oxidative DNA damage caused by 50 and 100 μM doses of CAP. Cells treated with 10 and 30 μM of CAP showed no tails because there was no DNA damage.

CAP substantially damaged nuclear material and increased oxidative stress. Antioxidant status in erythrocytes and leucocytes as well as DNA fragmentation were assessed in the human blood. This study investigated the possible protective role of EGCG in combination with ameliorating CAP induced DNA damage and oxidative stress in a dose-dependent manner, which is likely due to antioxidant constituents in itself.

Conclusions

Our findings support the hypothesis that different doses of CAP may influence erythrocytes and leucocytes of humans. EGCG has an antioxidative effect and offers a protection against CAP induced toxicity by restoring the altered levels of biochemical parameters. The results encourage further investigation on toxicity induced by CAP using with EGCG.

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Conflict of interest The authors declare that there are no conflicts of interest.

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