Protective role of tea polyphenol, EGCG, against genotoxic damage induced by anticancer drugs and steroid compounds in cultured human lymphocytes

J Gupta, Y Siddique, G Ara, T Beg, M Afzal

Citation

J Gupta, Y Siddique, G Ara, T Beg, M Afzal. *Protective role of tea polyphenol, EGCG, against genotoxic damage induced by anticancer drugs and steroid compounds in cultured human lymphocytes*. The Internet Journal of Nutrition and Wellness. 2008 Volume 7 Number 1.

Abstract

Tea (Camellia sinensis) is a rich source of polyphenols called flavonoids, effective antioxidants found throughout the plant kingdom. The slight astringent, bitter taste of green tea is attributed to polyphenols. A group of flavonoids in green tea are known as catechins, which are quickly absorbed into the body and are thought to contribute to some of the potential health benefits of tea. The fresh tea leaves contain four major catechins as colourless water soluble compounds. epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG). In this study we seen the antigenotoxic effect of tea polyphenol Epigallocatechin gallate (EGCG), against genotoxic damage induced by a steroid Trenbolone and an anticancer drug Docetaxel in cultured human lymphocytes, both in absence and presence of metabolic activation.

INTRODUCTION

EGCG makes up about 10-50% of the total catechin content and appears to be the most powerful of the catechins. In green tea catechin levels ranged from 51.5 - 84.3 mg/g, with epigallocatechin gallate (EGCG) being the main catechin in Chinese and Indian green teas. (EGCG) epigallocatechin gallate, a major catechin of found in green tea, has possible role in chemoprevention and chemotherapy of various types of cancers mainly prostate cancer ([1], [2]) and colon cancer ([3], [4]). EGCG is found to be the major and most photoprotective polyphenolic component of green tea ([5]). EGCG also induces growth arrest and apoptosis through multiple mechanisms, and can be used for cancer prevention, mainly pancreatic ([6]). Tea catechins and polyphenols are effective scavengers of reactive oxygen species in vitro and may also function indirectly as antioxidants through their effects on transcription factors and enzyme activities ([7]).

Trenbolone is a synthetic steroid used frequently by veterinarians on livestock as a promoter of growth in animal husbandry ([8]). Trenbolone compounds have not yet been approved by the Food and Drug Administration, USA for use by humans due to their considerable negative side effects, although bodybuilders use the drug illegally to increase body mass and strength. Cases of prostate and hepatic cancers have been associated with long term anabolic steroid abuse ([9],[10]). Trenbolone compounds increase nitrogen uptake by muscles after metabolization, leading to increased rate of protein synthesis.

Docetaxel is a clinically well established anti-mitotic chemotherapy medication used mainly for the treatment of breast, ovarian, and non-small cell lung cancer ([11]) Docetaxel has an approved claim for treatment of patients who have locally advanced, or metastatic breast or non small-cell lung cancer who have undergone anthracycline-based chemotherapy and failed to stop cancer progression or relapsed. Docetaxel is marketed worldwide under the name Taxotere by Sanofi-Aventis. Evaluation of docetaxel pharmacokinetics in phase II and III clinical studies were with 100 mg/m² dosages given over one-hour infusions every three weeks Docetaxel is a chemotherapeutic agent and is a cytotoxic compound and so is effectively a biologically damaging drug ([12]). Because docetaxel is a cell cycle specific agent, it is cytotoxic to all dividing cells in the body.

EGCG was studied for its antimutagenic effect on the CAs and SCEs induced by Trenbolone and Docetaxel, both in the presence and absence of metabolic activation system in human lymphocytes in vitro. The readily quantifiable nature of sister chromatid exchanges with high sensitivity for

revealing toxicant-DNA interaction and the demonstrated ability of genotoxic chemicals to induce significant increase in sister chromatid exchanges in cultured cells has resulted in this endpoint being used as indicator of DNA damage in blood lymphocytes of individuals exposed to genotoxic carcinogens ([13]). The above genotoxic endpoints are well known markers of genotoxicity and any reduction in the frequency of these genotoxic endpoints gives us indication of the antigenotoxicity of a particular compound ([14]). Many products protect against xenobiotics either by inducing detoxifying enzymes or by inhibiting oxidative enzymes ([15]).

MATERIALS AND METHODS

CHEMICALS

Docataxel (CAS No. 114977-28-5,Sigma-Aldrich); Trenbolone (CAS No.: 10161-33-8,Sigma-Aldrich); Sodium phenobarbitone (Sigma-Aldrich); Colchicine (Microlab); Dimethyl sulphoxide (Merck); Epigallocatechin-3-gallate (CAS No.: 989-51-5, Sigma-Aldrich); RPMI 1640 (GIBCO, Invitrogen); Phytohaemagglutinin-M (GIBCO, Invitrogen); Antibiotic-antimycotic mixture (GIBCO, Invitrogen); Fetal serum - calf (GIBCO, Invitrogen); 5-bromo-2-deoxyuridine (Sigma-Aldrich); Hoechst 33258 stain (Sigma-Aldrich); Giemsa stain (Merck).

HUMAN LYMPHOCYTE CULTURE

Duplicate peripheral blood cultures were conducted according to Carballo et al.,1993 ([16]). Briefly, 0.5 ml of the heparinized blood samples was obtained from a healthy female donor and was placed subsequently in a sterile flask containing 7 ml of RPMI 1640, supplemented with 1.5 ml of fetal calf serum and 0.1 ml of phytohaemagglutinin. These flasks were placed in an incubator at 37oC for 24 hours. Untreated culture, negative and positive controls were run simultaneously.

SISTER CHROMATID EXCHANGE (SCES) ANALYSIS

For SCE analysis, bromodeoxyuridine (BrdU, 10 µg/ml) was added at the beginning of the culture. After 24 hr of the initiation of culture, treatments were given similarly as described for CAs analysis. Two hours before harvesting, 0.2 ml of colchicines (0.2 µg/ml) was added to the culture flask. Hypotonic treatment and fixation were performed in the same way as in CAs analysis. The slides were processed according to Perry and Wolff (1974) ([17]) with some modification. 200 second divisions metaphases per dose were analysed.

REPLICATION INDEX (RI)

100 metaphases per culture were examined. Each metaphase was classified as being in the first (M1), second (M2), or third (M3) division ([18]). The replication index (RI) was calculated by formula ([19]) as follows:

$RI = [(\%\text{ of cells in } M_1) + 2(\%\text{ of cells in } M_2) + 3(\%\text{ of cells in } M_3)] / 100$

The effect of a genotoxic steroid Trenbolone and an anticancer drug Docetaxel was studied using sister chromatid exchanges (SCE's) and Replication Index as genotoxic end points. Trenbolone and Docetaxel were added at 10, 20, 30 µM each after 24 hr of initiation of the culture, separately. Normal, Positive and the negative control were also run simultaneously. For metabolic activation experiments, 0.5 ml of S9 mix was given with each of the tested dose. Epigallocatechin gallate (EGCG) has been tested for it's possible antigenotoxic effect against Trenbolone and Docetaxel induced genotoxicity. Sister chromatid exchange (SCE) was used as a parameter. Duplicate peripheral blood cultures were done and placed in the incubator at 37ºC for 24 hr and then BrdU (10µg/ml) was added to all cultures, as were, EGCG (tested doses were 20 and 30 µM), Trenbolone (tested doses were 45 and 55 µM) and then with Docetaxel (tested doses were 3 and 6 µM). Both the tested doses of EGCG weretreated with both of the tested doses of Trenbolone and Docetaxel, separately.

STATISTICAL ANALYSIS

Student's two tailed “t” test was used to calculate the statistical significance in CAs and SCEs for antigenotoxicity experiment of tea polyphenol EGCG. Kruskall Wallis test was used for the analysis of the means of frequencies of SCEsinduced by Trenbolone and Docetaxel and cell cycle kinetics was analysed by chi-square test. Student's ‘t' test were also performed. The level of significance was tested from standard statistical table of Fisher and Yates (1963) ([20]).

RESULTS

Genotoxic effect of a genotoxic steroid Trenbolone and an anticancer drug Docetaxel was studied using sister chromatid exchanges (SCE's) and Replication Index as genotoxic end points.A dose dependent increase infrequencies of SCEs and cell cycle kinetics is observed for both Trenbolone and Docetaxel, both in presence as well as absence of S9 mix (Table 1 & 2; Fig 1 & 2). A significant increase in the value of SCEs/cell was observed at 30 µM

(P<0.03) of Trenbolone, in presence of metabolic activation. The cell proliferation kinetics which is the average number of cells that have undergone replication showed a significant differences between the cultures exposed to 30 µM of Docetaxel and the normal control (P<0.03). There has been an increment in M1 cells and decrease of M2 and M3 cells as the doses of Trenbolone and Docetaxel increase.

We found that the genotoxicity induced by Trenbolone and Docetaxel, separately can be countered with 20 and 30 µM of Epigallo catechin gallate (EGCG), a major tea polyphenol. Frequencies of SCEs were reduced when cultures expose to 45 and 55 µM of Trenbolone, were treated with EGCG (20 and 30 µM), both in presence as well as absence of S9 mix (Table 3 & 4; Fig 3 & 4). Similar results were obtained when cultures expose to 3 and 6 µM of Docetaxel, were treated with EGCG (20 and 30 µM), both in presence as well as absence of S9 mix (Table 5 & 6; Fig 5 & 6).

Figure 1

Table 1: Sister chromatid exchanges (SCEs) and Replication Index (RI) in human lymphocytes treated with Trenbolone and Docetaxel, each, in absence of S9 mix.

Treatment (µM)	Cells Scored	SCEs/cell (mean)	Percentage of cells at			RI
			M1	M2	M3	
Trenbolone						
10	100	4.21± 0.31	35	58	7	1.72
20	100	5.04± 0.43	37	57	6	1.69
30	100	5.83 ± 0.46	38	55	7	1.69
Docetaxel						
10	100	3.42 ± 0.32	36	57	7	1.69
20	100	3.67 ± 1.08	38	55	7	1.69
30	100	5.33 ± 1.29	39	55	6	1.67
Untreated	100	2.03 ± 0.12	25	63	12	1.87
Negative control (DMSO, 5 µl/ml)	100	2.51 ± 0.15	24	62	14	1.90
Positive control MMS (6 µM)	100	22.21 ± 0.95*	57#	41#	2	1.45

Significant at *P<0.03 Vs Normal Kruskall-Wallis test
Significant at # P<0.005 Vs Normal Chi-square test.
DMSO: Dimethylsulphoxide, MMS: Methylmethane sulphonate

Figure 2

Table 2: Sister chromatid exchanges (SCEs) and Replication Index (RI) in human lymphocytes treated with Trenbolone and Docetaxel, each, in presence of S9 mix.

Treatment (µM)	Cells Scored	SCEs/cell (mean)	Percentage of cells at			RI
			M1	M2	M3	
Trenbolone						
10	100	5.63± 0.42	37	56	7	1.71
20	100	8.21± 0.82	38	57	5	1.67
30	100	13.67 ± 1.12*	48#	49#	3	1.55
Docetaxel						
10	100	3.74 ± 0.36	39	54	7	1.68
20	100	6.86 ± 0.43	41	53	6	1.65
30	100	9.56 ± 0.49	43#	52#	5	1.62
Untreated	100	2.45 ± 0.14	24	64	12	1.88
Negative control (DMSO, 5 µl/ml)	100	3.01 ± 0.17	25	67	8	1.83
Positive control MMS (6 µM)	100	16.62 ± 0.68**	57#	41##	2	1.45

Significant at *P<0.03; **P< 0.01 Vs Normal Kruskall-Wallis test.
Significant at # P<0.03; ##P < 0.005 Vs Normal Chi-square test.
DMSO: Dimethylsulphoxide, MMS: Methylmethane sulphonate

Figure 3

Table 3: Antimutagenic effect of EGCG on SCEs induced by Trenbolone in cultured human lymphocytes without S9 mix.

Treatment (µM)	Cells scored	SCEs/Cell (Mean ± SE)
Trenbolone		
45	200	6.89 ± 0.66[a]
55	200	7.73 ± 0.69[a]
EGCG		
20	200	2.75 ± 0.30
30	200	2.83 ± 0.31
Trenbolone + EGCG		
45+20	200	3.12 ± 0.33[b]
55+20	200	4.47 ± 0.45[b]
45+30	200	2.72 ± 0.32[b]
55+30	200	4.06 ± 0.42[b]
Untreated	200	2.12 ± 0.23
Negative control (DMSO, 5 µl/ml)	200	2.02 ± 0.21

Significant difference:
[a]P<0.01 with respect to untreated.
[b]P<0.05 with respect to Trenbolone

Figure 4

Table 4: Antimutagenic effect of EGCG on SCEs induced by Trenbolone in cultured human lymphocytes with S9 mix.

Treatment (μM)	Cells scored	SCEs/Cell (Mean ± SE)
Trenbolone		
45	200	7.07 ± 0.67[a]
55	200	7.95 ± 0.71[a]
EGCG		
20	200	2.88 ± 0.32
30	200	2.94 ± 0.33
Trenbolone + EGCG		
45+20	200	3.10 ± 0.31[b]
55+20	200	4.43 ± 0.43[b]
45+30	200	2.98 ± 0.33[b]
55+30	200	4.11 ± 0.40[b]
Untreated	200	2.27 ± 0.26
Negative control (DMSO, 5 μl/ml)	200	2.17 ± 0.23

Significant difference:
[a]P<0.01 with respect to untreated.
[b]P<0.05 with respect to Trenbolone

Figure 5

Table 5: Antimutagenic effect of EGCG on SCEs induced by Docetaxel in cultured human lymphocytes without S9 mix.

Treatment (μM)	Cells scored	SCEs/Cell (Mean ± SE)
Docetaxel		
3	200	6.34 ± 0.59[a]
6	200	7.11 ± 0.63[a]
EGCG		
20	200	2.55 ± 0.26
30	200	2.67 ± 0.29
Docetaxel + EGCG		
3+20	200	4.04 ± 0.41[b]
6+20	200	4.52 ± 0.48[b]
3+30	200	3.92 ± 0.38[b]
6+30	200	4.38 ± 0.43[b]
Untreated	200	2.02 ± 0.22
Negative control (DMSO, 5 μl/ml)	200	1.96 ± 0.20

Significant difference:
[a]P<0.01 with respect to untreated.
[b]P<0.05 with respect to Docetaxel

Figure 6

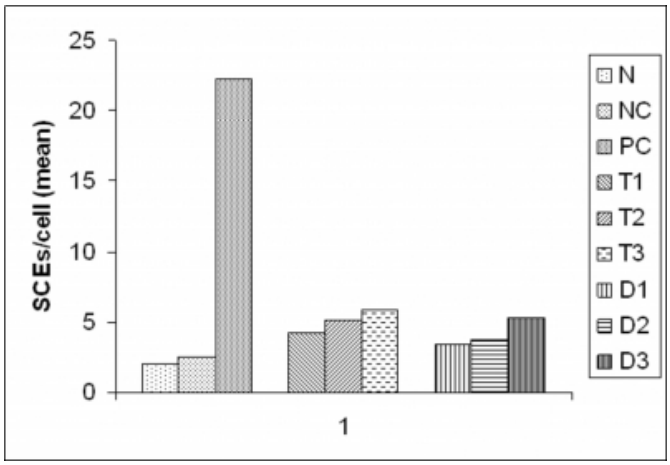
Table 6: Antimutagenic effect of EGCG on SCEs induced by Docetaxel in cultured human lymphocytes with S9 mix.

Treatment (μM)	Cells scored	SCEs/Cell (Mean ± SE)
Docetaxel		
3	200	6.52 ± 0.61[a]
6	200	7.32 ± 0.64[a]
EGCG		
20	200	2.62 ± 0.27
30	200	2.78 ± 0.29
Docetaxel + EGCG		
3+20	200	4.14 ± 0.42[b]
6+20	200	4.44 ± 0.46[b]
3+30	200	3.98 ± 0.40[b]
6+30	200	4.27 ± 0.43[b]
Untreated	200	2.17 ± 0.23
Negative control (DMSO, 5 μl/ml)	200	2.10 ± 0.21

Significant difference:
[a]P<0.01 with respect to untreated.
[b]P<0.05 with respect to Trenbolone

Figure 7

Figure 1: Sister chromatid exchange (SCEs/cell)(mean) in human lymphocytes treated with Trenbolone and Docetaxel, each, in



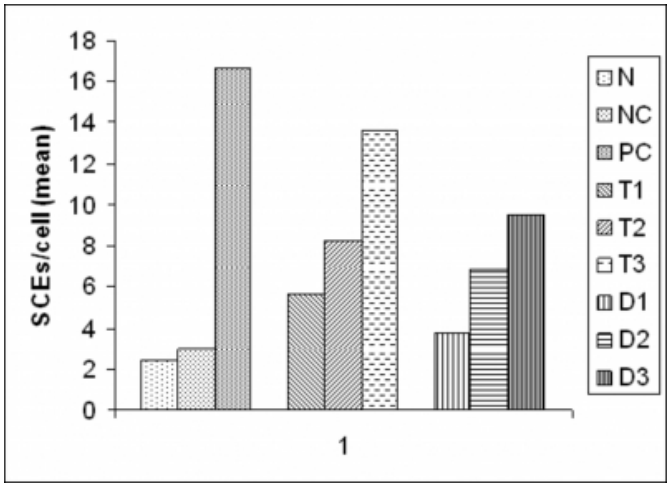
N- Normal; NC- Negative control; PC- Positive control MMS (6 μM)

T1- Trenbolone 10 μM; T2- Trenbolone 20 μM; T3- Trenbolone 30 μM;

D1- Docetaxel 10 μM; D2- Docetaxel 10 μM; D3- Docetaxel 30 μM

Figure 8

Figure 2: Sister chromatid exchange (SCEs/cell)(mean) in human lymphocytes treated with Trenbolone and Docetaxel, each, in



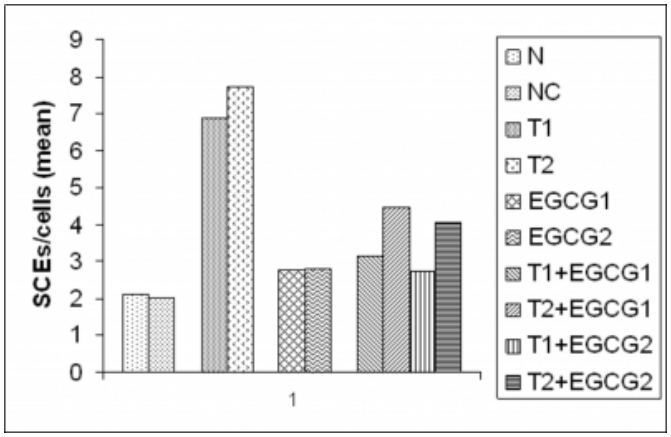
N- Normal; NC- Negative control; PC- Positive control MMS (6 µM)

T1- Trenbolone 10 µM; T2- Trenbolone 20 µM; T3- Trenbolone 30 µM;

D1- Docetaxel 10 µM; D2- Docetaxel 10 µM; D3- Docetaxel 30 µM.

Figure 9

Figure 3: Effect of EGCG on SCEs induced by Trenbolone in cultured human lymphocytes



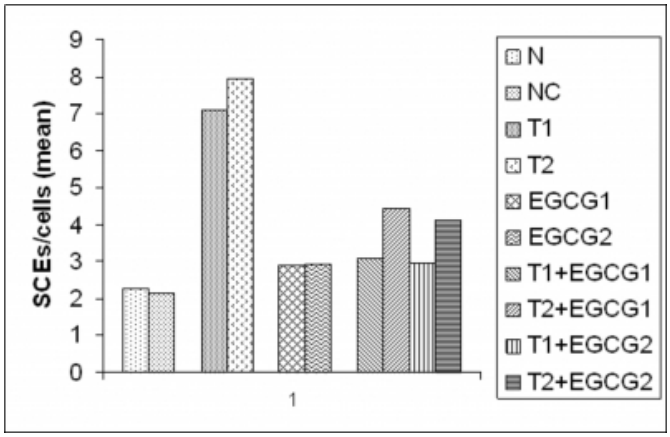
N- Normal; NC-Negative control; T1- Trenbolone 45 µM;

T2- Trenbolone 55 µM; EGCG1- Epigallocatechin gallate 20 µM;

EGCG2- Epigallocatechin gallate 30 µM.

Figure 10

Figure 4: Effect of EGCG on SCEs induced by Trenbolone in cultured human lymphocytes



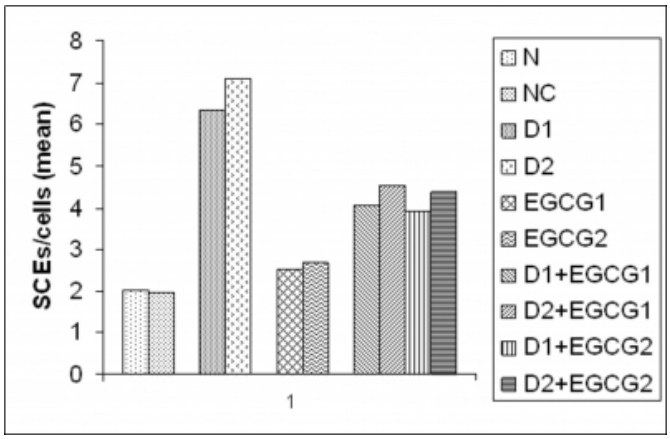
N- Normal; NC-Negative control; T1- Trenbolone 45 µM;

T2- Trenbolone 55 µM; EGCG1- Epigallocatechin gallate 20 µM;

EGCG2- Epigallocatechin gallate 30 µM.

Figure 11

Figure 5: Effect of EGCG on SCEs induced by Docetaxel in cultured human lymphocytes



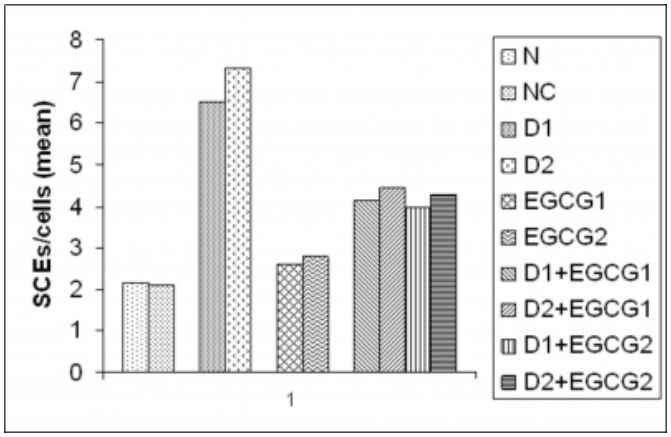
N- Normal; NC-Negative control; D1- Docetaxel 3 µM;

D2- Docetaxel 6 µM; EGCG1- Epigallocatechin gallate 20 µM ;

EGCG2- Epigallocatechin gallate 30 µM.

Figure 12

Figure 6: Effect of EGCG on SCEs induced by Docetaxel in cultured human lymphocytes



N- Normal; NC-Negative control; D1- Docetaxel 3 µM;

D2- Docetaxel 6 µM; EGCG1- Epigallocatechin gallate 20 µM ;

EGCG2- Epigallocatechin gallate 30 µM.

DISCUSSIONS

Plants are the essential source of medicines. Through the advances in pharmacology and synthetic organic chemistry, the dependence on natural products, remain unchanged ([21]). In India, the majority of populations use traditional natural prepartion derived from the plant material for the treatment of various diseases ([23]). The earlier studies have shown that various plant extacts and natural plant products possess protective role against the genotoxic effects of certain estrogens, synthetic progestins and anticancerous drugs in cultured human lymphocytes ([19, 20, 21, 22, 24, 25, 26, 27, 28]). The antigenotoxic potential of the plant extracts have been attributed to their total phenolic content ([29]). It has been shown that, through several mechanisms, tea polyphenols present antioxidant and anticarcinogenic activities, thus affording several health benefits ([30, 31]).The health benefits of catechins have been studied extensively in humans and in animal models. The anticarcinogenic potential of green tea catechins have correlated their cyotoxic effects with the induction of apoptosis, activation of caspases, inhibition of protein kinase, modulation of cell cycle regulation and inhibition of cell proliferation ([32]). For cancer prevention, evidence is so overwhelming that the Chemoprevention Branch of the National Cancer Institute has initiated a plan for developing tea compounds as cancer-chemopreventive agents in human trials ([33]). In this study we have found that a major tea polyphenol, EGCG, can modulte the genotoxic effects of Trebolone and Docetaxel, indicating its protective role. EGCG is widely accepted as an antioxidant. EGCG scavenges superoxide anion radicals (O_2·–), hydrogen peroxide (H_2O_2), hydroxy radicals (HO·), peroxyl radicals, singlet oxygen, and peroxynitrite ([34, 35 36, 37, 38, 39]). The reactive species liberated by genotoxic steroids and anticancer drugs, before or after metabolic activation, may be responsible for their genotoxic action. Formation of endogenous adducts and certain neoplastic change have also been reported earlier ([40, 41]). The suggested mechanism of action of tea polyphenols includes the following ([42]):

- Antioxidant activity and scavenging free radicals.
- Modulating enzymes implicated in the carcinogenic process.
- Modifying the pathways of signal transduction, thereby positively altering the expression of genes involved in cell proliferation, angiogenesis, and apoptosis, all important stages of cancer progression.
- Antimicrobial actions (association between Helicobacter pylori and gastric cancer).

ACKNOWLEDGEMENTS

Thanks are due to the UGC, New Delhi for awarding the project No.-32-482/2006 (SR) to Prof. Mohammad Afzal and to the Chairman, Department of Zoology, A.M.U., Aligarh, for providing laboratory facilities.

References

1. I.A. Siddiqui, M. Saleem , Y.M. Adhami, M. Asim and H. Mukhtar (2007). Tea beverage in chemoprevention and chemotherapy of prostate cancer. Acta Pharmacol Sin., 28(9):1392-408.
2. I. A. Siddiqui, V.M. Adhami, M. Saleem and H. Mukhtar (2006). Beneficial effects of tea and its polyphenols against prostate cancer. Mol Nutr Food Res., 50(2):130-43.
3. H. Xiao, X. Hao, B. Simi, J. Ju, H. Jiang, B.S. Reddy and C.S. Yang (2008). Green tea polyphenols inhibit colorectal aberrant crypt foci (ACF) formation and prevent oncogenic changes in dysplastic ACF in azoxymethane-treated F344 rats, Carcinogenesis., Jan;29(1):113-9.
4. J.H. Yuan, Y.Q. Li and X.Y. Yang (2007). Inhibition of epigallocatechin gallate on orthotopic colon cancer by upregulating the Nrf2-UGT1A signal pathway in nude mice. Pharmacology., 80(4):269-78.
5. S. Katiyar, C.A. Elmets and S.K. Katiyar (2006). Green tea and skin cancer: photoimmunology, angiogenesis and DNA repair. J Nutr Biochem., 18(5):287-96. PMID: 17049833.
6. S. Shankar, G. Suthakar and R.K. Srivastava (2007).

Epigallocatechin-3-gallate inhibits cell cycle and induces apoptosis in pancreatic cancer. Front Biosci.,. 12:5039-51.
7. J.V. Higdon, and B. Frei (2003). Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. Crit Rev Food Sci Nutr., 43(1):89-143.
8. M. Richold, (1988). The genotoxicity of trenbolone, a synthetic steroid. Archives of Toxicology 61, 249-258.
9. J.T. Roberts, and D.M. Essenhigh (1986). Adenocarcinoma of prostate in a 40-year-old body-builder. Lancet 2, 742.
10. K.A. Lyseng-Williamson, and C. Fenton, (2005). Docetaxel: a review of its use in metastatic breast cancer. Drugs. 65(17):2513-31.
11. H.P. Rang, M.M. Dale, J.M. Ritter, and P.K. Moore, (2003) Pharmacology. 5th ed. London: Churchill Livingstone; p. 694-8.
12. R.J. Albertini, D.Anderson, G.R. Douglas, L. Hagmar, K. Heminiki, F. Merelo, A.T. Natrajan, H. Norppa, D.E.G. Shuker, R. Tice, M.D.Walters, and A. Aitio (2000). Mutat. Res., 463, 111-172.
13. M.A. Morse, and G.D. Stoner (1993). Carcinogenesis, 14, 1737-1746.
14. M.A. Carballo, S. Aluarez, A. Boveris (1993) Mutat. Res., 288, 215-222.
15. P. Perry, and S. Wolff (1974), Nature, 251: 156-158.
16. R.A. Fisher, and Y. Yates (1963) Statistical table for biological, agricultural and medical research, 6th edition, Oliver and Boyd, Edinburg, pp 138.
17. E.L. Schneider, Y. Nakanishi, J. Lewis, and H. Sternberg. (1981).Simultaneous examination of examination of sister chromatid exchanges and cell replication kinetics in tumor and animal cells in vivo
Cancer Res, 41:4973-4975.
18. J.L. Ivett, and R.R. Tice (1982). Average generation of time: a new method of analysis and quantitation of cellular proliferation kinetics. Eniron Mutagen 4, 358.
19. Y.H. Siddique, and M. Afzal (2004). Antigenotoxic effects of allicin against SCEs induced by methyl methane sulphonate in cultured mammalian cells. Indian J Exp Biol., 42, 437-438.
20. Y.H. Siddique, and M. Afzal (2005). Antigenotoxic effect of allicin against methyl methanesulphonate induced genotoxic damage. J Environ Biol., 26, 547-550. PMID: 16334295.
21. Y.H. Siddique, and M. Afzal (2005). Protective role of allicin and L-ascorbic acid against the genotoxic damage induced by chlormadinone acetate in cultured human lymphocytes. Indian J Exp Biol., 43, 769-772. PMID: 16187526.
22. T. Beg, Y.H. Siddique, and M. Afzal (2007). Antigenotoxic potential of gingerol in cultured mammalian cells. The Icfai J Life Sci., 1, 39-44.
23. Y.H. Siddique, T. Beg, and M. Afzal (2006). Protective effect of nordihydroguaiaretic acid (NDGA) against norgestrel induced genotoxic damage. Toxicol in vitro 20, 227-233. PMID: 16061348. PMID: 16061348.
24. Y.H. Siddique, G. Ara, T. Beg and M. Afzal (2007). Anti-genotoxic effect of Ocimum sanctum L. extract against cyproterone induced genotoxic damage in cultured mammalian cells. Acta Biol Hung., 58, 4: 397-409. PMID: 18277466.
25. Y.H. Siddique, G. Ara, T. Beg and M. Afzal (2007). Protective role of nordihydroguaiaretic acid (NDGA) against the genotoxic damage induced by ethynodiol diacetate in human lymphocytes in vitro. J Environ Biol., 28, 279-282. PMID: 17915765.
26. Y.H. Siddique, G. Ara, T. Beg and M. Afzal (2008). Anti-genotoxic effect of nordihydroguaiaretic acid (NDGA) against chlormadinone acetate induced genotoxic damage in mice bone marrow cells. J Nat Med., 62: 52-56.
27. Y.H. Siddique, T. Beg, and M. Afzal (2008). Antigenotoxic effect of apigenin against anti-cancerous drugs. Toxicol in vitro., 22: 625-631.
28. Y.H. Siddique, G. Ara, T. Beg, M. Faisal, M. Ahmad and M. Afzal (2008). Antigenotoxic role of Centella asiatica L. extract against cyrpoterone acetate induced genotoxic damage in cultured human lymphocytes. Toxicol in vitro., 22: 10-17. PMID: 17719740.
29. T. Maurich, L. Pistelli and G. Turchi (2004). Anti-clastogenic activity of two structurally related pterocarpans purified from Bituminaria bituminosa in cultured human lymphocytes. Mutat Res., 561, 75-81. PMID: 15238232.
30. E. González de Mejía, (2003). The chemo-protector effects of tea and its components. Arch Latinoam Nutr.,53(2):111-8. PMID: 14528600.
31. M. Afzal, Y.H. Siddique, and T. Beg (2008). Mutagen induced genotoxicity and action of tea polyphenolics in mammalian test system: in vitro and in vivo models and methodological considerations. IN: Recent Trends in Toxicology. (Ed. Y.H. Siddique). Transworld Research Network, 37/661 (2), fort P.O., Trivandrum, Kerala, India
32. C.S. Yang (1999). Tea and health. Nutrition, 15:946-949.
33. I.A. Siddiqui, F. Afaq, V.M. Adhami, N. Ahmad and H. Mukhtar (2004). Antioxidants of the beverage tea in promotion of human health. Antioxid Redox Signal., 6(3):571-82. PMID: 15130283.
34. W. Bors, and M. Saran (1987). Radical scavenging by flavonoid antioxidants. Free Radic. Res. Commun. 2:289-294Carballo, M.A., S. Aluarez, A. Boveris; Mutat. Res., 288, 215-222 (1993).
35. Q. Guo, B. Zhao, S. Shen, J. Hou, J. Hu, and W. Xin (1999). ESR study on the structure-antioxidant activity relationship of tea catechins and their epimers. Biochim. Biophys. Acta 1427:13-23.
36. G.R. Haenen, J.B. Paquay, R.E. Korthouwer, and A. Bast. (1997). Peroxynitrite scavenging by flavonoids. Biochem. Biophys. Res. Commun. 236:591-593.
37. T. Nakagawa, and T. Yokozawa (2002). Direct scavenging of nitric oxide and superoxide by green tea. Food Chem. Toxicol. 40:1745-1750.
38. F. Nanjo, M. Honda, K. Okushio, N. Matsumoto, F. Ishigaki, T. Ishigami, and Y. Hara. (1993). Effects of dietary tea catechins on alpha-tocopherol levels, lipid peroxidation, and erythrocyte deformability in rats fed on high palm oil and perilla oil diets. Biol. Pharm. Bull. 16:1156-1159.
39. J.B. Paquay, G. R. Haenen, G. Stender, S. A. Wiseman, L. B. Tijburg, and A. Bast. (2000). Protection against nitric oxide toxicity by tea. J. Agric. Food Chem. 48:5768-5772.
40. A. Martelli, and G. Brambilla. (2002). Are some progestins genotoxic liver carcinogens? Mutat Res., 512: 155-163.
41. A. Martelli, F. Mattioli, R. Angiola, G. Reimann, and G. Brambilla (2003). Species, sex and inter-individual differences in DNA repair induced by nine sex steroids in primary culture of rat and human hepatocytes. Mutat Res., 536: 69-78.
42. J. Gupta, Y.H. Siddique, T. Beg, ssG. Ara, and M. Afzal (2008. A Review on the beneficial effects of tea polyphenols on human health. Inter. Journ. of Pharmac. 4(5): 314-338.

Author Information

Jyoti Gupta, Ph.D.
Scholar, Human Genetics and Toxicology Lab ,Department of Zoology, Aligarh Muslim University

Yasir Hasan Siddique, Ph.D.
Human Genetics and Toxicology Lab ,Department of Zoology, Aligarh Muslim University

Gulshan Ara, Ph.D.
Scholar, Human Genetics and Toxicology Lab ,Department of Zoology, Aligarh Muslim University

Tanveer Beg, Ph.D.
Scholar, Human Genetics and Toxicology Lab ,Department of Zoology, Aligarh Muslim University

Mohammad Afzal, Ph.D.
Professor, Section of Genetics, Human Genetics and Toxicology Lab ,Department of Zoology, Aligarh Muslim University