PROTECTIVE ROLE OF GREEN TEA EXTRACT AGAINST GENOTOXIC DAMAGE INDUCED BY ANTICANCER DRUG AND STEROID COMPOUND, SEPARATELY, IN CULTURED HUMAN LYMPHOCYTES.

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Summary

Tea (*Camellia sinensis*) is a rich source of polyphenols called flavonoids, effective antioxidants found throughout the plant kingdom. A group of flavonoids in green tea are known as catechins. The fresh tea leaves contain four major catechins as colourless water soluble compounds. epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG). The aim of present work is to study antigenotoxic effect of Green tea extract (GTE), 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, using duplicate peripheral blood cultures. Metaphase plates so obtained were used for Chromosomal aberration (CAs) analysis, Sister chromatid exchange (SCEs) analysis and calculation of Replication index. 40 and 60 µM of Trenbolone, 3 µM and 6 µM Docetaxel (with and without metabolic activation) was used. $1.075X\ 10^{-4}$, $2.127\ X\ 10^{-4}$ and $3.15\ X\ 10^{-4}$ g/ml Green tea extract (GTE), was used to study its antigenotoxic role against above mentioned drugs. A dose dependent increase in frequencies of SCEs, abnormal metaphase were observed for by Trenbolone and Docetaxel treatment, both in presence as well as absence of S9 mix, which was mitigated to a great extent by treatment with Green tea extract (GTE). It is concluded that the genotoxicity induced by Trenbolone and Docetaxel, can be countered with Green tea extract (GTE) doses.

Keywords: Green tea extract (GTE), Tea polyphenols, Trenbolone, Docetaxel, Antigenotoxicity, Genotoxicity.

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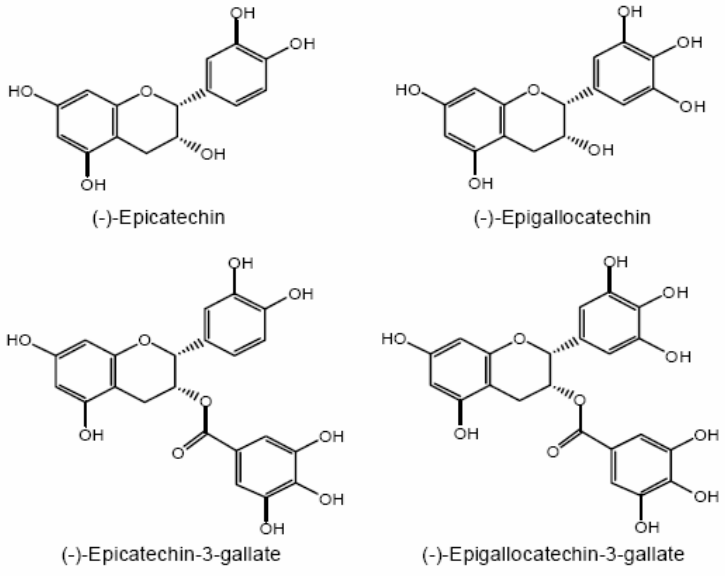
Introduction

Tea is the second-most consumed beverage in the world (water is the first) and has been used medicinally for centuries in India and China. The tea shrub (genus *Camellia*, family Theaceae) [chromosome number (2n=30)] is a perennial evergreen with its natural habitat in the tropical and sub tropical forests of the world. Cultivated varieties are grown widely in its home countries of South and South East Asia, as well as in parts of Africa and the Middle East (1). Green tea is prepared by picking, lightly steaming and allowing the leaves to dry .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. Flavonoids are group of phenolic compounds occurring abundantly in vegetables, fruits, and green plants that had attracted special attention as they showed high antioxidant property (2). The fresh tea leaves contain four major catechins as compounds: epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG) (3) [Figure 1]. EGCG makes up about 10-50% of the total catechin content and appears to be the most powerful of the catechins.(2). In India, the majority of populations use traditional natural prepartion derived from the plant material for the treatment of various diseases (4) and for that reason it has become necessary to assess their antimutagenic potential or mutagenic potential for modulating the action of plant extract when associated with other substances.

Figure 1. Main catechin components of green tea polyphenols.



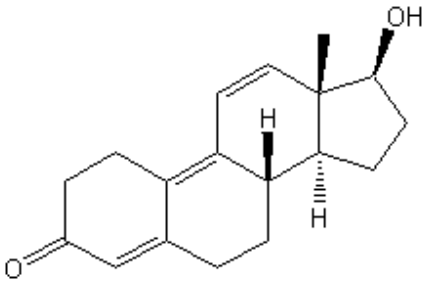
Trenbolone is a synthetic steroid used frequently by veterinarians on livestock as a promoter of growth in animal husbandry (5). Trenbolone is not used in an unrefined form, but is rather administered as Trenbolone acetate. Trenbolone compounds have not

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 (6). Trenbolone compounds increase nitrogen uptake by muscles after metabolization, leading to increased rate of protein synthesis. Trenbolone is a very potent androgen with strong anabolic activity. Trenbolone compounds have a binding affinity for the androgen receptor three times as high as that of testosterone (7).

Trenbolone

$C_{18}H_{22}O_2$ Molecular weight : 270.366

17β-Hydroxyestra-4,9,11-trien-3-one

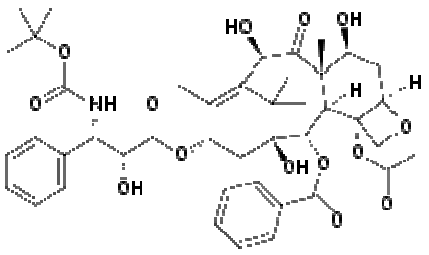


Docetaxel is a clinically well established anti-mitotic chemotherapy medication used mainly for the treatment of breast, ovarian, and non-small cell lung cancer (8). Docetaxel has an approved claim for treatment of patients who have locally advanced, or metastatic breast or non small-cell lung cancer 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. Docetaxel is a chemotherapeutic agent and is a cytotoxic compound and so is effectively a biologically damaging drug (9, 6).

Docetaxel

$C_{43}H_{53}NO_{14}$ Molecular weight :807.879 g/mol

(2*R*,3*S*)-*N*-carboxy-3-phenylisoserine, *N*-tert-butyl ester, 13-ester with 5, 20-epoxy-1, 2, 4, 7, 10, 13-hexahydroxytax-11-en-9-one 4-acetate 2-benzoate, trihydrate



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); 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).

Preparation of leaf extract: *Camellia sinensis* L. leaves were collected from the nursery of Forest Research Institute (FRI), Dehradun (U.A.) and were air dried and grounded to fine powder. Extraction was done by soaking samples (30g of dry weight) in 300 ml of acetone for 8-10 h at 40-60ºC in Soxhlet's apparatus. After filtration, the excess of solvent was removed by rotatory evaporator. The extract is labeled as Green tea extract (GTE). The extract concentration of $1.075X\ 10^{-4}$, $2.127\ X\ 10^{-4}$ and $3.15\ X\ 10^{-4}$ g/ml of culture medium were established.

Human lymphocyte culture: Duplicate peripheral blood cultures were conducted (10). Briefly, 0.5 ml of the heparinized blood samples were obtained from two healthy donors and were placed subsequently in a sterile flask containing 7 ml of RPMI 1640, supplemented with 1.5 ml of fetal calf serum, 1.0 ml antibiotic-antimycotic mixture and 0.1 ml of phytohaemagglutinin. These flasks were placed in an incubator at 37^{o}C for 24 hours.

Preparation of S9 mix: Liver S9 fraction was prepared from Swiss albino healthy rats (Wistar Strain), each weighing about 200g. The rats were given 0.1% Phenobarbitone (1 mg/ml) in drinking water for 1 week for the induction of liver enzymatic activities. The S9 mix was freshly prepared as per standard procedures of Maron and Ames (11). The animals were sacrificed and the livers were collected immediately. The liver slices were then carefully homogenized at 4°C in 0.15 M KCl and centrifuged at 9000 rpm at 4°C for 10 min.

Chromosomal aberration analysis: Untreated culture, negative and positive controls were run simultaneously. Duplicate peripheral blood cultures were done, from blood collected from two different donors. The cultures were placed in the incubator at 37ºC for 24 hr and then Trenbolone (tested doses were 40 and 60 µM) and Docetaxel (tested doses were 3 and 6 µM) were added, separately, (Both the chemicals were dissolved in DMSO). Then, all the tested doses of Green tea extract (GTE) (tested doses were $1.075X\ 10^{-4}$, $2.127\ X\ 10^{-4}$ and $3.15\ X\ 10^{-4}$ g/ml), were treated with both of the tested doses of Trenbolone and Docetaxel, separately. For metabolic activation experiments, 0.5 ml of S9 mix was given with each of the tested dose for 6 h. S9 mix was prepared according to standard protocol of Maron and Ames (11). The cells were collected by centrifugation and washed in prewarmed media to remove traces of S9 mix and drugs. The culture bottles were kept for another 48 h in an incubator at 37º C. At 47^{th} hr, 0.2 ml of colchicine (0.2 µg/ ml) was added to the culture bottle. Cells were centrifuged at 1000 rpm for 10 min. The supernatant was removed and 8ml of prewarmed (37^{o}C) 0.075 M KCl (hypotonic solution) was added.

Cells were resuspended and incubated at 37^{o}C for 15 min. The supernatant was removed by centrifugation, at 1000 rpm for 10 min, and subsequently 5ml chilled fixative (methanol: glacial acetic acid, 3:1) was added. The fixative was removed by centrifugation and the procedure was repeated twice. The slides were stained in 3% Giemsa solution in phosphate buffer (pH 6.8) for 15 min. About 75 cells from each duplicate culture were observed and a total of 300 metaphases were examined for the occurrence of different types of abnormalities. Criteria to classify different types of aberrations were in accordance with the recommendations of Environmental Health 48 for Environmental Monitoring of Human Population (12) IPCS, 1985)

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 above. The cells were collected by centrifugation and washed in prewarmed media to remove traces of S9 mix and drugs. One hours before harvesting i.e. after 46 h, 0.2 ml of colchicines (0.2 µg/ml) was added to the culture flask for mitotic arrest. Cells were centrifuged at 1000 rpm for 10 min. The supernatant was removed and 8ml of prewarmed (37^{o}C) 0.075 M KCl (hypotonic solution) was added. Cells were resuspended and incubated at 37^{o}C for 15 min. The supernatant was removed by centrifugation, at 1000 rpm for 10 min, and subsequently 5ml chilled fixative (methanol: glacial acetic acid, 3:1) was added. The fixative was removed by centrifugation and the procedure was repeated twice The slides were processed according to Perry and Wolff (13), with some modification. The slides were stained in 3% Giemsa solution in phosphate buffer (pH 6.8) for 15 min. 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 (14). The replication index (RI) was calculated by formula (15)Ivett and Tice,1982) as follows:

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

Statistical analysis: Student's two tailed "t" test was used to calculate the statistical significance in CAs and SCEs for antigenotoxicity experiment of Green tea extract (GTE). Kruskall Wallis test was used for the analysis of the means of frequencies of SCEs induced 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 (16). Standard error (SE) for Chromosomal Aberrations is calculated using software STATISTICA.

Results

Genotoxic effect of a genotoxic steroid Trenbolone and an anticancer drug Docetaxel was studied using Chromosomal aberration analysis, sister chromatid exchanges (SCE's) and Replication Index as genotoxic end points. A dose dependent increase in frequencies of CAs, SCEs and cell cycle kinetics is observed for both Trenbolone and Docetaxel, both in presence as well as absence of S9 mix (Table 1- 4; Fig 2 - 7). A significant increase in the value of SCEs/cell was observed at 6 µM ($P<0.05$) of Docetaxel, in presence of

metabolic activation. The cell proliferation kinetics which is the average number of cells that have undergone replication showed a significant decrease between the cultures exposed to Docetaxel and Trenbolone and the normal control. 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 Green tea extract (GTE) (tested doses were $1.075X\ 10^{-4}$, $2.127\ X\ 10^{-4}$ and $3.15\ X\ 10^{-4}$ g/ml). Frequencies of CAs and SCEs were reduced when cultures expose to 40 and 60 µM of Trenbolone and 3 and 6 µM of Docetaxel were treated with Green tea extract (GTE (tested doses)) both in presence as well as absence of S9 mix.(Table 1-4; Fig 2-4) Similar results were obtained for cell cycle kinetics when after treatment with GTE, cultures exposed to Trenbolone and Docetaxel, showed an increase in number of M2 and M3 cells, both in presence as well as absence of S9 mix. (Table 3, 4; Fig 6, 7).

Table 1 Effect of Green Tea extract (GTE) on chromosomal aberrations (CAs) in human lymphocytes induced by Trenbolone and Docetaxel, each, in absence of S9 mix.

Treatment (μM)	Total Cells Scored (75 cells each from duplicate cultures of two donors)	Abnormal cells (Mean ± SE)	Chromosomal Aberrations				
			Gaps	CTB	CSB	CTE	DIC
Treatment (μM)							
GTE (g/ml)							
$1.075X10^{-4}$	300	2 ± 0.40	2	1	1	-	-
$2.127X10^{-4}$	300	3 ± 0.57	2	2	1	-	-
$3.15X10^{-4}$	300	3 ± 0.57	2	2	1	-	-
Trenbolone							
40 (T1)	300	18 ± 0.91 [a]	7	9	2	1	-
60 (T2)	300	21 ± 0.40 [a]	7	6	2	1	1
Docetaxel							
3 (D1)	300	19 ± 1.08 [a]	6	5	3	1	-
6 (D2)	300	22 ± 0.91 [a]	7	8	4	2	1
T1 (μM)+ GTE (g/ml)							
$40+1.075X10^{-4}$	300	15 ± 0.91 [a]	7	7	2	1	1
$40+2.127X10^{-4}$	300	12 ± 0.91 [a b]	6	6	2	1	-
$40+3.15X10^{-4}$	300	9 ± 0.70 [a b]	5	4	1	1	-
T2 (μM)+ GTE (g/ml)							
$60+1.075X10^{-4}$	300	16 ± 0.57 [a b]	7	5	2	1	1
$60+2.127X10^{-4}$	300	13 ± 0.64 [a b]	6	4	1	1	-
$60+3.15X10^{-4}$	300	10 ± 0.91 [a b]	4	3	1	1	-
D1 (μM)+ GTE (g/ml)							
$3+1.075X10^{-4}$	300	15 ± 0.40 [a]	6	4	3	1	-
$3+2.127X10^{-4}$	300	12 ± 0.57 [a c]	6	4	2	1	-
$3+3.15X10^{-4}$	300	8 ± 0.91 [a c]	4	3	1	1	-
D2 (μM)+ GTE (g/ml)							
$6+1.075X10^{-4}$	300	20 ± 0.81 [a]	7	6	4	2	1
$6+2.127X10^{-4}$	300	17 ± 0.40 [a]	5	3	2	1	-
$6+3.15X10^{-4}$	300	13 ± 0.64 [a c]	3	2	2	-	-
Untreated	300	2 ± 0.40	1	1	-	-	-
Negative control (DMSO, 5 μl/ml)	300	3 ± 0.40	2	2	-	-	-

Significant difference:
[a]P<0.01 with respect to untreated; [b]P<0.05 with respect to Trenbolone; [c]P<0.05 with respect to Docetaxel
DMSO:Dimethylsulphoxide; CTB; Chromatid break; CSB: Chromosome break; CTE: Chromatid exchange; DIC: Dicentric: SE: Standard Error.

Table 2 Effect of Green Tea extract (GTE) on chromosomal aberrations (CAs) in human lymphocytes induced by Trenbolone and Docetaxel, each, in presence of S9 mix.

Treatment (μM)	Total Cells Scored (75 cells each from duplicate cultures of two donors)	Abnormal cells (% ± SE)	Chromosomal Aberrations				
			Gaps	CTB	CSB	CTE	DIC
Treatment (μM)							
GTE (g/ml)							
$1.075X10^{-4}$	300	3 ± 0.57	2	1	1	-	-
$2.127X10^{-4}$	300	4 ± 0.40 [a]	2	2	1	-	-
$3.15X10^{-4}$	300	4 ± 0.64 [a]	2	2	1	-	-
Trenbolone							
40 (T1)	300	22 ± 0.91 [a]	7	6	2	1	-
60 (T2)	300	25 ± 0.57 [a]	7	9	2	1	1
Docetaxel							
3 (D1)	300	21 ± 0.40 [a]	6	5	3	1	-
6 (D2)	300	24 ± 0.81 [a]	7	8	4	2	1
T1 (μM)+ GTE (g/ml)							
$40+1.075X10^{-4}$	300	21 ± 0.40 [a]	7	7	2	1	1
$40+2.127X10^{-4}$	300	17 ± 0.57 [a b]	6	6	2	1	-
$40+3.15X10^{-4}$	300	11 ±1.08 [a b]	5	4	1	1	-
T2 (μM)+ GTE (g/ml)							
$60+1.075X10^{-4}$	300	25 ± 0.57 [a]	7	5	2	1	1
$60+2.127X10^{-4}$	300	20 ± 0.40 [a b]	6	4	1	1	-
$60+3.15X10^{-4}$	300	14 ± 0.57 [a b]	4	3	1	1	-
D1 (μM)+ GTE (g/ml)							
$3+1.075X10^{-4}$	300	18 ± 0.91 [a]	6	4	3	1	-
$3+2.127X10^{-4}$	300	14 ± 0.40 [a c]	6	4	2	1	-
$3+3.15X10^{-4}$	300	9 ± 0.70 [a c]	4	3	1	1	-
D2 (μM)+ GTE (g/ml)							
$6+1.075X10^{-4}$	300	20 ± 0.81) [a]	7	6	4	2	1
$6+2.127X10^{-4}$	300	15 ± 0.91 [a c]	5	3	2	1	-
$6+3.15X10^{-4}$	300	12 ± 0.64) [a c]	3	2	2	-	-
Untreated	300	2 ± 0.40	1	1	-	-	-
Negative control (DMSO, 5 μl/ml)	300	3 ± 0.57	2	2	-	-	-

Significant difference:
[a]P<0.01 with respect to untreated; [b]P<0.05 with respect to Trenbolone; [c]P<0.05 with respect to Docetaxel
DMSO:Dimethylsulphoxide; CTB; Chromatid break; CSB: Chromosome break; CTE: Chromatid exchange; DIC: Dicentric: SE: Standard Error.

Table 3 Effect of Green Tea extract (GTE) on Sister chromatid exchanges (SCEs) and Replication Index (RI) in human lymphocytes induced by Trenbolone and Docetaxel, each, in absence of S9 mix.

Treatment (μM)	Cells Scored	SCEs/cell (mean ± SE)	RI
GTE (g/ml)			
$1.075X10^{-4}$	200	2.78 ± 0.31	1.82
$2.127X10^{-4}$	200	2.88 ± 0.32	1.80
$3.15X10^{-4}$	200	3.04 ± 0.35	1.78
Trenbolone			
40 (T1)	200	4.22± 0.31[a]	1.72
60 (T2)	200	5.84 ± 0.46 [a]	1.69
Docetaxel			
3 (D1)	200	3.42 ± 0.32 [a]	1.69
6 (D2)	200	5.34 ± 0.46 [a]	1.67
T1 (μM)+ GTE (g/ml)			
40 + $1.075X10^{-4}$	200	4.06 ± 0.42 [a]	1.73
40 + $2.127X10^{-4}$	200	3.12 ± 0.33 [a]	1.74
40 + $3.15X10^{-4}$	200	2.84 ± 0.31 [a b]	1.76
T2 (μM) + GTE (g/ml)			
60 + $1.075X10^{-4}$	200	4.52 ± 0.48 [a]	1.70
60 + $2.127X10^{-4}$	200	4.44 ± 0.46 [a]	1.72
60 + $3.15X10^{-4}$	200	4.14 ± 0.42 [a b]	1.74
D1 (μM)+ GTE (g/ml)			
3+$1.075X10^{-4}$	200	3.12 ± 0.33 [a]	1.70
3+$2.127X10^{-4}$	200	2.84 ± 0.31 [a c]	1.73
3+$3.15X10^{-4}$	200	2.75 ± 0.30 [c]	1.75
D2 (μM)+ GTE (g/ml)			
6+$1.075X10^{-4}$	200	4.44 ± 0.43 [a]	1.68
6+$2.127X10^{-4}$	200	4.06± 0.42 [a c]	1.70
6+$3.15X10^{-4}$	200	3.10 ± 0.31[a c]	1.73
Untreated	200	2.04 ± 0.12	1.91
Negative control (DMSO, 5 μl/ml)	200	2.58 ± 0.30	1.88
Positive control MMS (6 μM)	200	16.62 ± 0.68[*]	1.65

Significant at *P<0.03 Vs Normal Kruskall-Wallis test. Significant difference: [a]P<0.01 with respect to untreated; [b]P<0.05 with respect to Trenbolone. [c]P<0.05 with respect to Docetaxel. GTE: Green tea extract; T1: 40 μM Trenbolone; T2: 60 μM Trenbolone. D1: 3 μM Docetaxel; D2: 6 μM Docetaxel. DMSO:Dimethylsulphoxide; MMS: Methylmethane sulphonate SE: Standard Error.

Table 4. Effect of Green Tea extract (GTE) on Sister chromatid exchanges (SCEs) and Replication Index (RI) in human lymphocytes tinduced by Trenbolone and Docetaxel, each, in presence of S9 mix.

Treatment (μM)	Cells Scored	SCEs/cell (mean ± SE)	RI
GTE (g/ml)			
$1.075X10^{-4}$	200	3. 13 ± 0.30	1.80
$2.127X10^{-4}$	200	3. 42 ± 0.32	1.79
$3.15X10^{-4}$	200	3.74 0.36	1.76
Trenbolone			
40 (T1)	200	5. 63 ± 0.42 [a]	1.71
60 (T2)	200	8. 21 ± 0.82 [a]	1.67
Docetaxel			
3 (D1)	200	6. 86 ± 0.43 [a]	1.68
6 (D2)	200	9. 56 ± 0.49 [a]	1.62
T1 (μM)+ GTE (g/ml)			
40 +$1.075X10^{-4}$	200	4. 47 ± 0.45 [a b]	1.71
40 + $2.127X10^{-4}$	200	4. 43 ± 0.43 [a b]	1.73
40 + $3.15X10^{-4}$	200	4. 27 ± 0.35 [a b]	1.75
T2 (μM)+ GTE (g/ml)			
60 + $1.075X10^{-4}$	200	7. 32 ± 0.64 [a]	1.68
60 + $2.127X10^{-4}$	200	6. 52 ± 0.61 [a b]	1.70
60 + $3.15X10^{-4}$	200	6. 02 ± 0.54 [a b]	1.71
D1 (μM) + GTE (g/ml)			
3 + $1.075X10^{-4}$	200	4.11 ± 0.40 [a c]	1.70
3 + $2.127X10^{-4}$	200	2. 98 ± 0.33 [a c]	1.72
3 + $3.15X10^{-4}$	200	2. 27 ± 0.26 [a c]	1.74
D2 (μM) + GTE (g/ml)			
6 + $1.075X10^{-4}$	200	7. 11 ± 0.63 [a c]	1.63
6 + $2.127X10^{-4}$	200	6. 34 ± 0.59 [a c]	1.64
6 + $3.15X10^{-4}$	200	4. 44 ± 0.46 [a c]	1.66
Untreated	200	2. 45 ± 0.14	1.89
Negative control (DMSO, 5 μl/ml)	200	3. 01 ± 0.17	1.87
Positive control MMS (6 μM)	200	22. 21 ± 0.95**	1.45

Significant at **P< 0.01 Vs Normal Kruskall-Wallis test. Significant difference: [a]P<0.01 with respect to untreated; [b]P<0.05 with respect to Trenbolone. [c]P<0.05 with respect to Docetaxel. GTE: Green tea extract; T1: 40 μM Trenbolone; T2: 60 μM Trenbolone. D1: 3 μM Docetaxel; D2: 6 μM Docetaxel. DMSO:Dimethylsulphoxide; MMS: Methylmethane sulphonate SE: Standard Error.

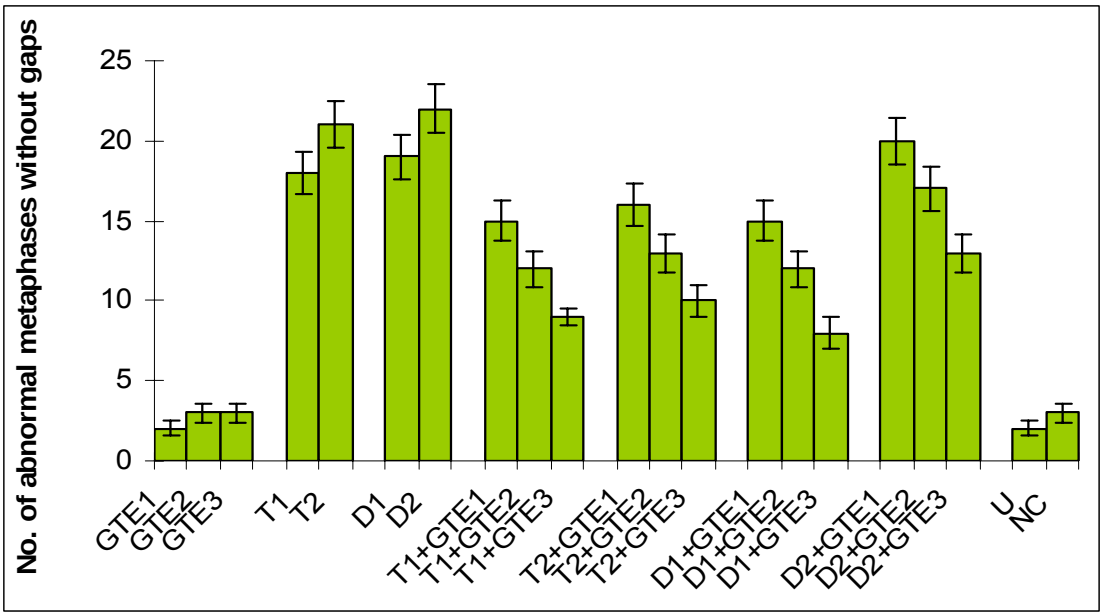


Fig. 2 Effect of Green tea extract on abnormal metaphases induced by Trenbolone and Docetaxel in absence of S9 mix

T1 = Trenbolone 40 µM; T2 = Trenbolone 60 µM; D1= Docetaxel 3 µM; D2= Docetaxel 6 µM; GTE1= Green tea extract 1.075X 10^{-4} g/ml; GTE2 = Green tea extract 2.127 X 10^{-4} g/ml; GTE3 = Green tea extract 3.15 X 10^{-4} g/ml; U = Untreated; NC = Negative Control (DMSO 5 µl/ml)

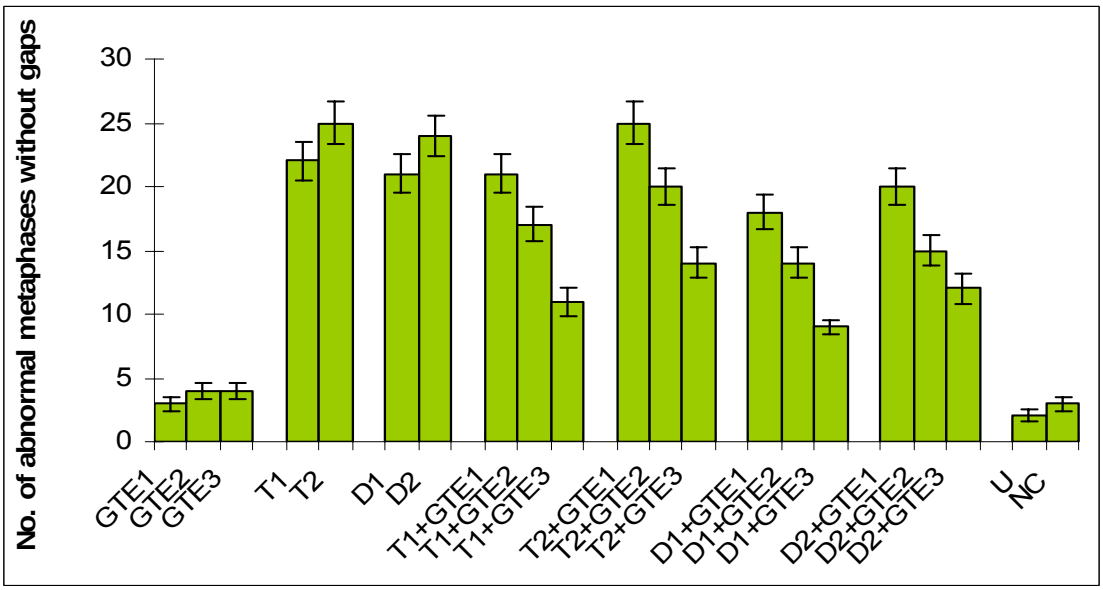


Fig. 3 Effect of Green tea extract on abnormal metaphases induced by Trenbolone and Docetaxel in presence of S9 mix

T1 = Trenbolone 40 µM; T2 = Trenbolone 60 µM; D1= Docetaxel 3 µM; D2= Docetaxel 6 µM; GTE1= Green tea extract 1.075X 10^{-4} g/ml; GTE2 = Green tea extract 2.127 X 10^{-4} g/ml; GTE3 = Green tea extract 3.15 X 10^{-4} g/ml; U = Untreated; NC = Negative Control (DMSO 5 µl/ml)

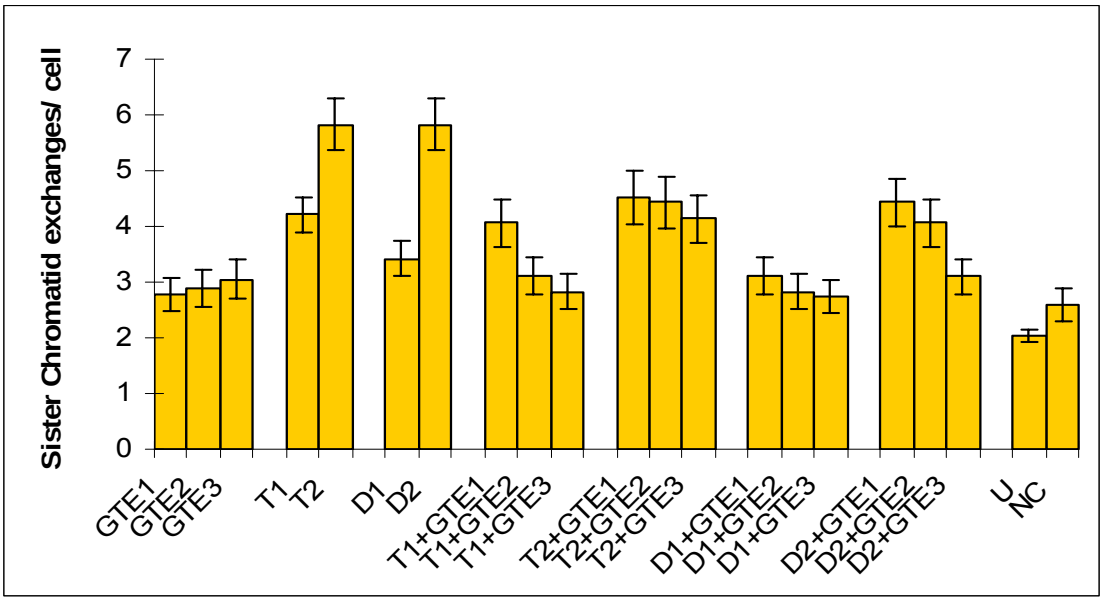


Fig. 4 Effect of Green tea extract on Sister chromatid exchanges induced by Trenbolone and Docetaxel in absence of S9 mix

T1 = Trenbolone 40 µM; T2 = Trenbolone 60 µM; D1= Docetaxel 3 µM; D2= Docetaxel 6 µM; GTE1= Green tea extract 1.075X 10^{-4} g/ml; GTE2 = Green tea extract 2.127 X 10^{-4} g/ml; GTE3 = Green tea extract 3.15 X 10^{-4} g/ml; U = Untreated; NC = Negative Control (DMSO 5 µl/ml)

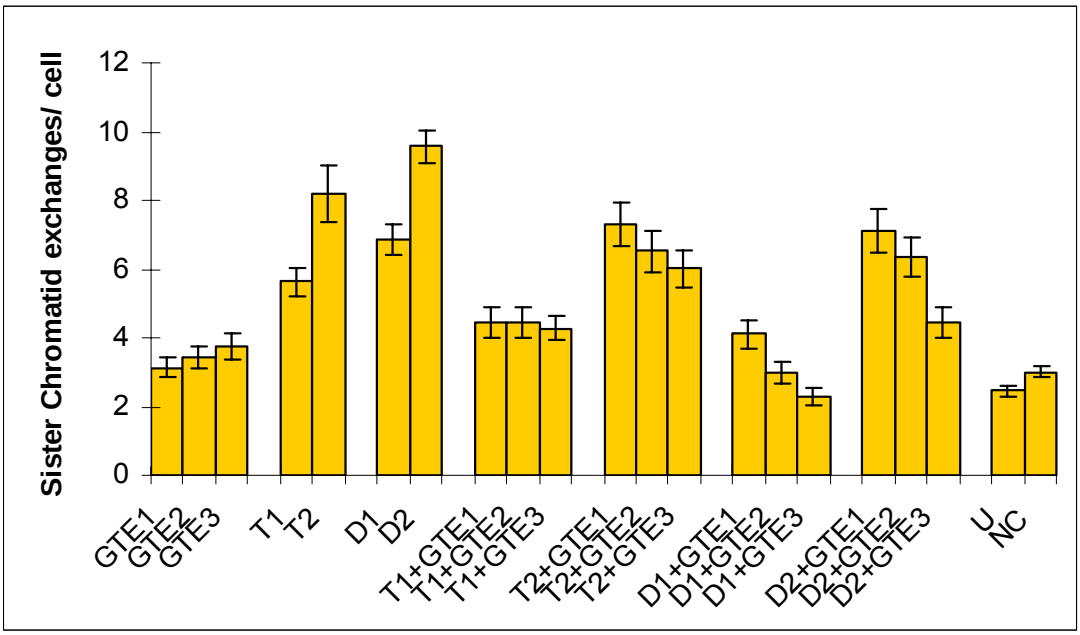


Fig. 5 Effect of Green tea extract on Sister chromatid exchanges induced by Trenbolone and Docetaxel in presence of S9 mix

T1 = Trenbolone 40 µM; T2 = Trenbolone 60 µM; D1= Docetaxel 3 µM; D2= Docetaxel 6 µM; GTE1= Green tea extract 1.075X 10^{-4} g/ml; GTE2 = Green tea extract 2.127 X 10^{-4} g/ml; GTE3 = Green tea extract 3.15 X 10^{-4} g/ml; U = Untreated; NC = Negative Control (DMSO 5 µl/ml)

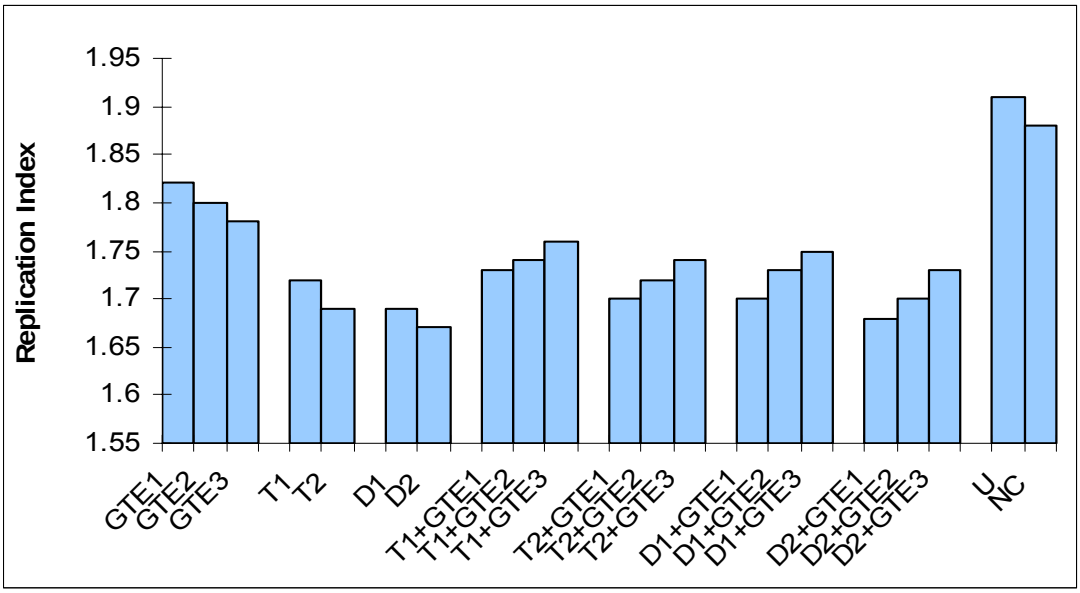


Fig. 6 Effect of Green tea extract on Replication Index (RI) induced by Trenbolone and Docetaxel in absence of S9 mix

T1 = Trenbolone 40 µM; T2 = Trenbolone 60 µM; D1= Docetaxel 3 µM; D2= Docetaxel 6 µM; GTE1= Green tea extract 1.075X 10^{-4} g/ml; GTE2 = Green tea extract 2.127 X 10^{-4} g/ml; GTE3 = Green tea extract 3.15 X 10^{-4} g/ml; U = Untreated; NC = Negative Control (DMSO 5 µl/ml)

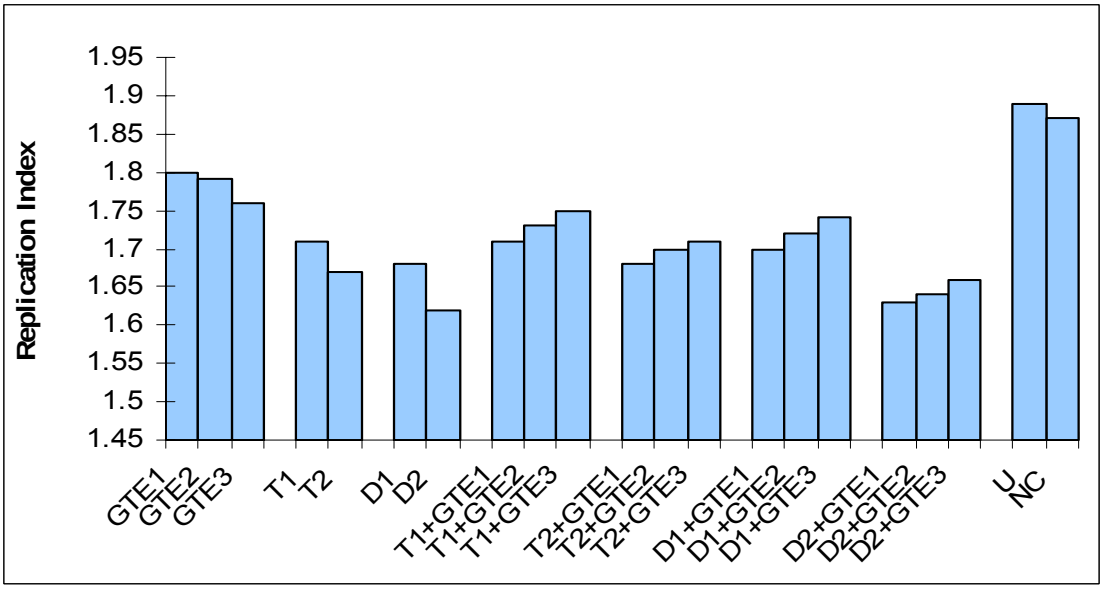


Fig. 7 Effect of Green tea extract on Replication Index (RI) induced by Trenbolone and Docetaxel in presence of S9 mix

T1 = Trenbolone 40 µM; T2 = Trenbolone 60 µM; D1= Docetaxel 3 µM; D2= Docetaxel 6 µM; GTE1= Green tea extract 1.075X 10^{-4} g/ml; GTE2 = Green tea extract 2.127 X 10^{-4} g/ml; GTE3 = Green tea extract 3.15 X 10^{-4} g/ml; U = Untreated; NC = Negative Control (DMSO 5 µl/ml)

Discussion

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 (17,18,19,20,21,4,22,24,25,26,27) and mice bone marrow cells (28,29,30).Our study clearly demonstrates the antigenotoxic potencial of Green tea extract (GTE) both in absence as well as presence of metabolic activation systems.

Genotoxic effects of anti-cancer drugs in non- tumor cells are of special significance due to the possibility that they may induce secondary tumors in cancer patients. Also, the mutagenic and carcinogenic effects of antineoplastic agents on the health care persons handling these drugs also need to be considered carefully (31, 26). Anabolic steroids, as Trenbolone, also show genotoxicity as they cause increase protein synthesis within cells, which results in the buildup of cellular tissue (anabolism), especially in muscles. The main way in which steroid hormones interact with cells is by binding to proteins called steroid receptors. When steroids bind to these receptors, the proteins move into the cell nucleus and either alter the expression of genes (32,6) or activate processes that send signals to other parts of the cell (33, 34).

An increase in the frequency of chromosomal aberrations in peripheral blood lymphocytes is associated with an increased overall risk of cancer (35, 36). Most of the chromosomal aberrations observed in the cells are lethal, but there are many other aberrations that are viable and cause genetic effects, either somatic or inherited (37).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 (38). 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 (38). The antigenotoxic potential of the plant extracts have been attributed to their total phenolic content (39). It has been shown that, through several mechanisms, tea polyphenols present antioxidant and anticarcinogenic activities, thus affording several health benefits (40,41). . Catechins are involve in thiol-dependent activation of mitogenic- activated protein kinases (42). Specifically, EGCG regulates expression of VEGF, matrix metalloproteinases, uPA, IGF-1, EGFR, cell cycle regulatory proteins and inhibits NFk B, PI3-K/Akt, Ras/Raf/MAPK and AP-1 signaling pathways, thereby causing strong cancer chemopreventive effects (43). The galloyl structure on the B ring and the gallate moiety are important for the inhibition (44). Most of the relevant mechanisms of cancer prevention by tea polyphenols are not related to their redox properties, but are due to the direct binding of the polyphenol to target molecules, including the inhibition of selected protein kinases, matrix metalloproteinases, and DNA methyltransferases (2). Activation of Forkhead box O transcription factor (FOXO3a) by the green tea polyphenol epigallocatechin-3-gallate induces estrogen receptor alpha expression reversing invasive phenotype of breast cancer cells (45).

Tea polyphenols act as antioxidants in vitro by scavenging reactive oxygen and nitrogen species and chelating redox-active transition metal ions. (46). They may also function indirectly as antioxidants through, inhibition of the redox-sensitive transcription factors, nuclear factor-kappaB and activator protein-1; inhibition of "pro-oxidant" enzymes, such as inducible nitric oxide synthase, lipoxygenases, cyclooxygenases and xanthine oxidase; and induction of phase II and antioxidant enzymes, such as glutathione S-transferases and superoxide dismutases. (47)). Green tea polyphenols can act as a biological antioxidant in a cell culture experimental model and protect cells in culture (48) and mammalian veins (49) from oxidative stress-induced toxicity. In scavenging assays using a xanthine-xanthine oxidase (enzymatic system), epicatechin gallate (ECG) shows the highest scavenging potential (50). Tea catechins prevent the molecular degradation in oxidative stress conditions by directly altering the subcellular ROS production, glutathione metabolism and cytochrome P450 2E1 activity (51). 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 (17).

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