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Protective role of green tea extract against genotoxic damage induced by anabolic steroids in cultured human lymphocytes

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Abstract
Tea (*Camellia sinensis*) is one of the most popular beverages consumed worldwide as an infusion of leaves and is valued for its medicinal properties. Tea 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). Epidemiologic observations and laboratory studies have indicated that tea polyphenols act as antioxidants in vitro by scavenging reactive oxygen and nitrogen species and chelating redox-active transition metal ions and hence tea may reduce the risk of a variety of illnesses, including cancer and coronary heart disease. In this study we seen the antigenotoxic effect of green tea extract against genotoxic damage induced by two anabolic steroids Trenbolone and Methyltestosterone in cultured human lymphocytes, both in absence and presence of metabolic activation. The results prove the antigenotoxic potential of green tea extract. Because the epidemiologic studies and research findings in laboratory animals have shown the antigenotoxic potential of tea polyphenols, the usefulness of tea polyphenols for various human diseases like cancer and coronary heart disease etc should be evaluated in clinical trials.

Keywords: Green tea extract (GTE); Tea polyphenols; Trenbolone; Methyltestosterone; Antigenotoxicity; Genotoxicity; Chromosomal aberrations; Sister chromatid exchanges.

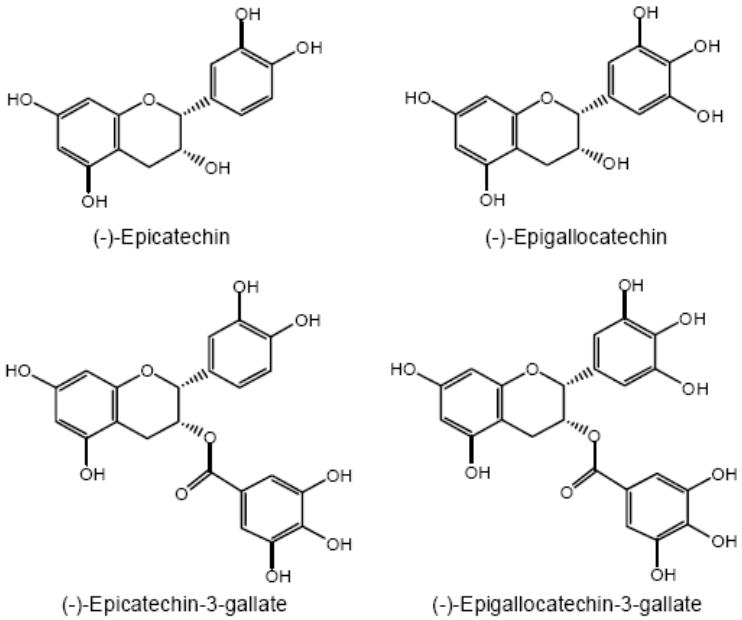
Introduction

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 (Yamamoto *et al*., 1997). Green tea is prepared by picking, lightly steaming and allowing the leaves to dry (Werkhoven, 1978). Catechins are highly potent flavonoids present in tea and serve perhaps as the best dietary source of natural antioxidants. 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 (Gupta *et al*., 2008).Several catechins are present in significant quantities; epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) (Zhu *et al*., 2000) [Figure 1]. EGCG makes up about 10-50% of the total catechin content and appears to be the most powerful of the catechins – with antioxidant activity about 25-100 times more potent than vitamins C and E. 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 (Higdon and Frei, 2003).

Trenbolone is a synthetic steroid used frequently by veterinarians on livestock as a promoter of growth in animal husbandry (Richold, 1988). Trenbolone is not used in an unrefined form, but is rather administered as Trenbolone acetate. Trenbolone acetate is often referred to as "Fina" by users, because injectible trenbolone acetate is often prepared from Finaplix H pellets, an ear-implant used by cattle ranchers to maintain the weight of cattle during shipping to slaughter. 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. 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. It is well suited for the rapid buildup of strength and muscle mass, usually providing the user exceptional results in a relatively short time period. Trenbolone compounds have a binding affinity for the androgen receptor three times as high as that of testosterone (Beg *et al*., 2007). Cases of prostate and hepatic cancers have been associated with long term anabolic steroid abuse (Roberts and Essenhigh ,1986; Overly et al., 1984).

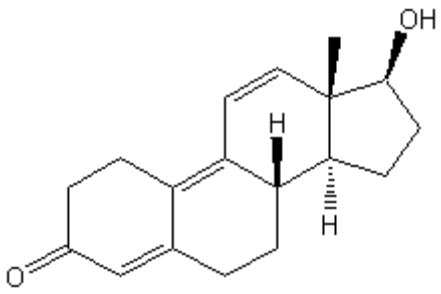
Figure 1. Main catechin components of green tea polyphenols.



Trenbolone

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

17β-Hydroxyestra-4,9,11-trien-3-one



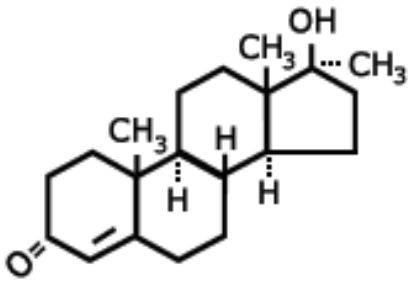
Methyltestosterone is a 17-alpha-alkylated anabolic steroid used to treat men with a testosterone deficiency. It is also used in women to treat breast cancer, breast pain, swelling due

to pregnancy. Methyltestosterone capsules USP 10 mg are red capsules imprinted “ICN 0901” on both sections. People who abuse Methyltestosterone could suffer from acute poisoning and may also be at risk of death from premature heart disease or cancer. A case of night blindness was reported after continuous use of Methyltestosterone (Nisbett *et al*., 1985). For women, long term effects include voice changes and in children, fusion of the epiphyses in children. Androgen ingestion by a pregnant mother can cause virilization of a female fetus (Dewhurst and Gordon, 1984). Orally active (17-alpha substituted) anabolic steroids can cause abnormalities of hepatic function, manifest as abnormally elevated hepatic enzyme activity in biochemical tests of liver function, and sometimes as overt jaundice. The histological abnormality of peliosis hepatis has been associated with anabolic steroid use (Soe et al., 1992). Angiosarcoma (Falk et al, 1979) and a case of hepatocellular carcinoma in an anabolic steroid user has been reported (Overly et al., 1984).

Methyltestosterone

$C_{20}H_{30}O_2$, Molecular Weight. 302.46

17-β-hydroxy-17-methylandrost-4-en-3-one



Materials and Methods

Chemicals

Methyltestosteone (CAS No. 58-18-4, Sigma-Aldrich); Trenbolone (CAS No.: 10161-33-8,Sigma-Aldrich); Sodium Phenobarbitone (Sigma-Aldrich); Colchicine (Microlab); Dimethyl sulphoxide (Merck); Methyl methane sulphonate(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).

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 concentrations 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 according to Carballo *et al*., 1993. Briefly, 0.5 ml of the heparinized blood samples was obtained from a healthy donor and was 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. Untreated culture, negative and positive controls were run simultaneously. Duplicate peripheral blood cultures were done

and placed in the incubator at 37ºC for 24 hr and then Green tea extract (GTE) (tested doses were 1.075X 10^{-4}, 2.127 X 10^{-4} and 3.15 X 10^{-4} g/ml), Trenbolone (tested doses were 40 and 60 µM) and Methyltestosterone (tested doses were 40 and 60 µM) were added, separately (Both the steroids were dissolved in DMSO). Then, all the tested doses of Green tea extract (GTE) were treated with both of the tested doses of Trenbolone and Methyltestosterone, 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 (1983).

Sister chromatid exchange (SCE) 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 hour 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 (1974), 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 (Schneider *et al*., 1981). The replication index (RI) was calculated by formula (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 tea polyphenol EGCG. 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 (1963).

Results

Genotoxic effect of a genotoxic steroid Trenbolone and Methyltestosteone was studied using sister chromatid exchanges (SCEs) and Replication Index as genotoxic end points. A dose dependent increase in frequencies of SCEs and decrease in cell cycle kinetics is observed for both Trenbolone and Methyltestosteone both in presence as well as absence of S9 mix (Table 1 & 2). 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 Methyltestosteone, separately can be countered with 1.075X 10^{-4}, 2.127 X 10^{-4} and 3.15 X 10^{-4} g/ml of Green tea extract (GTE). Frequencies of SCEs were reduced when cultures expose to 40 and 60 µM of Trenbolone, were treated with Green tea extract (GTE), both in presence as well as absence of S9 mix (Table 3 & 4; Fig 1 & 2). Replication Index showed an increase when cultures expose to 40 and 60 µM of Trenbolone, were treated with Green tea extract (GTE), both in presence as well as absence of S9 mix (Table 3 & 4; Fig 3 & 4). Similar results were obtained when cultures expose to 40 and 60 µM of Methyltestosteone, were treated with Green tea extract (GTE), both in presence as well as absence of S9 mix (Table 5 & 6; Fig 1, 2, 3, 4).

Discussion

Anabolic steroids are a class of steroid hormones related to the hormone testosterone. Anabolic steroids have been used by physicians for many purposes as for hypoplastic anemias due to leukemia or kidney failure, especially aplastic anemia,(Basaria et al., 2001) Growth stimulation, to increase lean body mass and prevent bone loss in elderly men etc. (Kenny et al., 2001; Baum and Crespi, 2007; Francis, 2007) They 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 (Lavery and McEwan, 2005) or activate processes that send signals to other parts of the cell (Cheskis, 2004). In the case of anabolic steroids, the receptors involved are called the androgen receptors. The mechanisms of action differ depending on the specific anabolic steroid. Different types of anabolic steroids bind to the androgen receptor with different affinities, depending on their chemical structure (Hartgens and Kuipers, 2004). The earlier studies have shown that various plant extracts and natural plant products possess protective role against the genotoxic effects of certain estrogens, synthetic progestins and anticancerous drugs in cultured human lymphocytes (Siddique and Afzal, 2004; Siddique and Afzal, 2005 a,b; Beg *et al*., 2007a,b; Siddique *et al*., 2006 a,b; Siddique *et al*., 2007 a-c; Siddique *et al*.,2008 a-c) and mice bone marrow cells (Siddique *et al*.,2005; Siddique *et al*., 2006b; Siddique *et al*., 2008a).Green tea extract (GTE) was studied for its antimutagenic effect on the SCEs and RI induced by Trenbolone and Methyltestosterone, 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 (Albertini *et al*., 2000). 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 (Many products protect against xenobiotics either by inducing detoxifying enzymes or by inhibiting oxidative enzymes (Morse and Stoner, 1993). The antigenotoxic potential of the plant extracts have been attributed to their total phenolic content (Maurich *et al*., 2004). It has been shown that, through several mechanisms, tea polyphenols present antioxidant and anticarcinogenic activities, thus affording several health benefits (González de Mejía, 2003; Afzal *et al*., 2008).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 cytotoxic effects with the induction of apoptosis, activation of caspases, inhibition of protein kinase, modulation of cell cycle regulation and inhibition of cell proliferation (Yang, 1999). 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 (Siddiqui *et al*., 2004; Gupta *et al*., 2008).

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

Treatment (μM)	Cells Scored	SCEs/cell (mean)	RI
Trenbolone			
40	200	4.22± 0.31[b]	1.72
60	200	5.84 ± 0.46[b]	1.69
Methyltestosterone			
40	200	4.42 ± 0.32[b]	1.69
60	200	5.34 ± 1.29[b]	1.67
Untreated	200	2.04 ± 0.12	1.91
Negative control (DMSO, 5 μl/ml)	200	2.52 ± 0.15	1.88
Positive control MMS (6 μM)	200	16.62 ± 0.68[a]	1.65

Significant at [a]P<0.03 Vs Normal Kruskall-Wallis test
[b]P<0.05 with respect to Untreated.
SE: Standard Error, DMSO: Dimethylsulphoxide, MMS: Methylmethane sulphonate

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

Treatment (μM)	Cells Scored	SCEs/cell (mean)	RI
Trenbolone			
40	200	5.64 ± 0.42 [b]	1.71
60	200	8.22 ± 0.82 [b]	1.67
Methyltestosterone			
40	200	4.74 ± 0.36 [b]	1.68
60	200	9.56 ± 0.49 [b]	1.62
Untreated	200	2.46 ± 0.14	1.89
Negative control (DMSO, 5 μl/ml)	200	3.02 ± 0.17	1.87
Positive control MMS (6 μM)	200	22.22 ± 0.95[a]	1.45

Significant at [a]P< 0.01 Vs Normal Kruskall-Wallis test.
[b]P<0.05 with respect to Untreated.
DMSO: Dimethylsulphoxide, MMS: Methylmethane sulphonate

Table 3: Effect of GTE on Sister chromatid exchanges (SCEs) and Replication Index (RI) induced by Trenbolone in human lymphocytes without S9 mix.

Treatment (μM)	Cells scored	SCEs/cell (Mean ± SE)	RI
GTE (g/ml)			
$1.075X10^{-4}$	200	2.08 ± 0.21	1.82
$2.127X10^{-4}$	200	2.28 ± 0.23	1.80
$3.15X10^{-4}$	200	2.60 ± 0.25	1.78
T1(μM)+ GTE (g/ml)			
40 + $1.075X10^{-4}$	200	4.06 ± 0.42 [a b]	1.73
40 + $2.127X10^{-4}$	200	3.12 ± 0.33 [a b]	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 b]	1.70
60 + $2.127X10^{-4}$	200	4.44 ± 0.46 [a b]	1.72
60 + $3.15X10^{-4}$	200	4.14 ± 0.42 [a b]	1.74
Untreated	200	2.04 ± 0.12	1.91
Negative control (DMSO, 5 μl/ml)	200	2.52 ± 0.15	1.88

Significant difference:
[a]P<0.01 with respect to untreated
[b]P<0.05 with respect to Trenbolone (Values given in Table1)
T1: 40 μM Trenbolone; T2: 60 μM Trenbolone.
GTE: Green tea extract; DMSO:Dimethylsulphoxide; SE: Standard Error.

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Table 4: Effect of GTE on Sister chromatid exchanges (SCEs) and Replication Index (RI) induced by Trenbolone in human lymphocytes with S9 mix.

Treatment (μM)	Cells scored	SCEs/cell (Mean ± SE)	RI
GTE (g/ml)			
$1.075X10^{-4}$	200	2.32 ± 0.22	1.80
$2.127X10^{-4}$	200	2.44 ± 0.24	1.79
$3.15X10^{-4}$	200	2.70 ± 0.28	1.76
T1 (μM)+ GTE (g/ml)			
$40+1.075X10^{-4}$	200	4.48 ± 0.45 [a b]	1.71
$40+2.127X10^{-4}$	200	4.44 ± 0.43 [a b]	1.73
$40+3.15X10^{-4}$	200	4.28 ± 0.35 [a b]	1.75
T2 (μM)+ GTE (g/ml)			
$60+1.075X10^{-4}$	200	7.32 ± 0.64 [a b]	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
Untreated	200	2.46 ± 0.14	1.89
Negative control (DMSO, 5 μl/ml)	200	3.02 ± 0.17	1.87

Significant difference:
[a]P<0.01 with respect to untreated, [b]P<0.05 with respect to Trenbolone (Values given in Table2). T1: 40 μM Trenbolone; T2: 60 μM Trenbolone. GTE: Green tea extract; DMSO:Dimethylsulphoxide; SE: Standard Error.

Table 5: Effect of GTE on Sister chromatid exchanges (SCEs) and Replication Index (RI) induced by Methyltestosterone in human lymphocytes without S9 mix.

Treatment (μM)	Cells scored	SCEs/cell (Mean ± SE)	RI
GTE (g/ml)			
$1.075X10^{-4}$	200	2.08 ± 0.21	1.82
$2.127X10^{-4}$	200	2.28 ± 0.23	1.80
$3.15X10^{-4}$	200	2.60 ± 0.25	1.78
M1 (μM)+ GTE (g/ml)			
$40+1.075X10^{-4}$	200	3.14 ± 0.30 [a b]	1.70
$40+2.127X10^{-4}$	200	2.36 ± 0.21 [a b]	1.73
$40+3.15X10^{-4}$	200	2.24 ± 0.17 [a b]	1.75
M2 (μM)+ GTE (g/ml)			
$60+1.075X10^{-4}$	200	4.44 ± 0.43 [a b]	1.68
$60+2.127X10^{-4}$	200	4.22± 0.31 [a b]	1.70
$60+3.15X10^{-4}$	200	3.10 ± 0.31 [a b]	1.73

Untreated	200	2.04± 0.12	1.91
Negative control (DMSO, 5 μl/ml)	200	2.52 ± 0.15	1.88

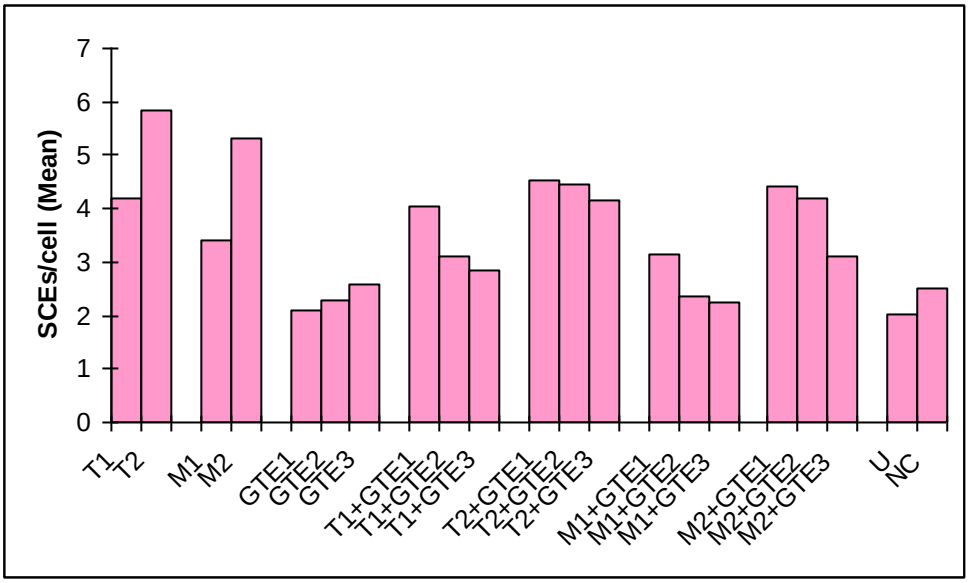
Significant difference:
[a]P<0.01 with respect to untreated
[b]P<0.05 with respect to Methyltestosterone (Values given in Table1).
M1: 40 μM Methyltestosterone; M2: 60 μM Methyltestosterone.
GTE: Green tea extract; DMSO:Dimethylsulphoxide; SE: Standard Error.

Table 6: Effect of GTE on Sister chromatid exchanges (SCEs) and Replication Index (RI) induced by Methyltestosterone in human lymphocytes with S9 mix.

Treatment (μM)	Cells scored	SCEs/cell (Mean ± SE)	RI
GTE (g/ml)			
$1.075X10^{-4}$	200	2.32 ± 0.22	1.80
$2.127X10^{-4}$	200	2.44 ± 0.24	1.79
$3.15X10^{-4}$	200	2.70 ± 0.28	1.76
M1 (μM)+ GTE (g/ml)			
$40+1.075X10^{-4}$	200	4.06 ± 0.42 [a b]	1.70
$40+2.127X10^{-4}$	200	2.67 ± 0.29 [a b]	1.72
$40+3.15X10^{-4}$	200	2.28 ± 0.23 [a b]	1.74
M2 (μM)+ GTE (g/ml)			
$60+1.075X10^{-4}$	200	7.32 ± 0.64 [a b]	1.63
$60+2.127X10^{-4}$	200	6.52 ± 0.61 [a b]	1.64
$60+3.15X10^{-4}$	200	6.34 ± 0.59 [a b]	1.66
Untreated	200	2.45 ± 0.14	1.89
Negative control (DMSO, 5 μl/ml)	200	3.01 ± 0.17	1.87

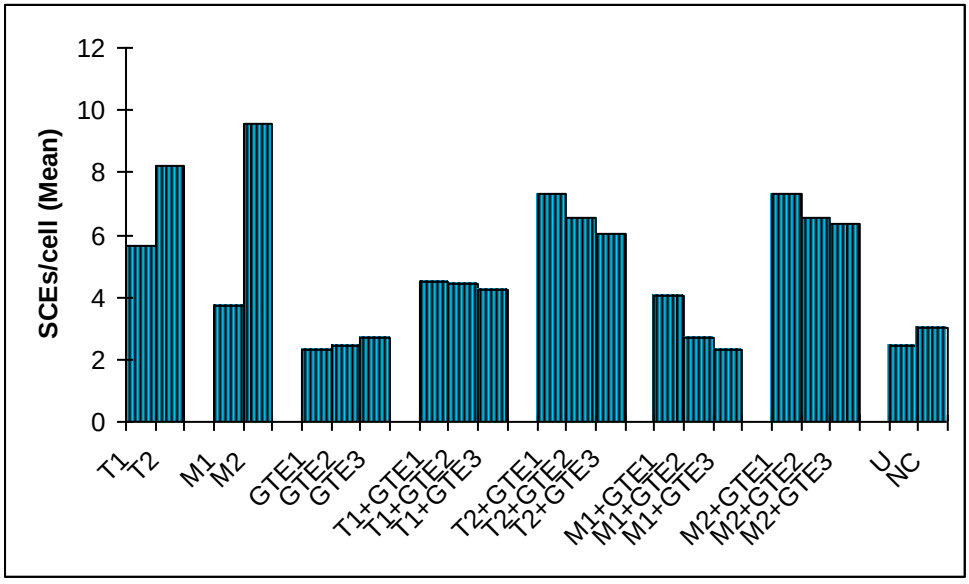
Significant difference:
[a]P<0.01 with respect to untreated
[b]P<0.05 with respect to Methyltestosterone (Values given in Table 2).
M1:40 μM Methyltestosterone; M2: 60 μM Methyltestosterone.
GTE: Green tea extract; DMSO:Dimethylsulphoxide; SE: Standard Error.

FIG.1 Sister chromatid exchange (SCEs/cell)(mean) in human lymphocytes treated with Trenbolone, Methyltestosterone and Green tea extract, in absence of S9 mix.



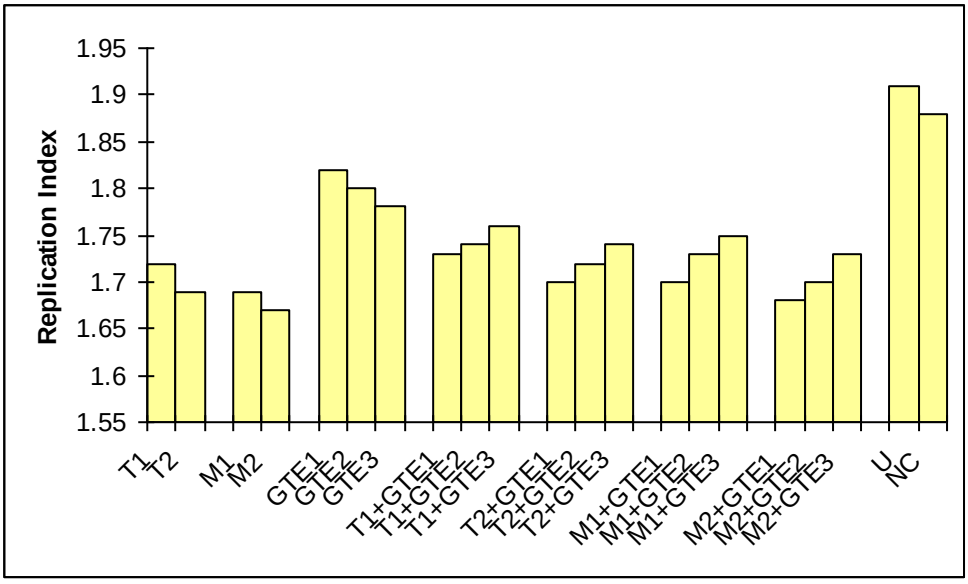
T1 = Trenbolone 40 μM; T2 = Trenbolone 60 μM; M1 = Methyltestosterone 40 μM; M2 = Methyltestosterone 60 μM; GTE1= Green tea extract 1.075X 10^{-4} g/ml; GTE2 = Green tea extract 2.127 X 10^{-4} g/ml; GTE3 = Green tea extract 3.15 X 10^{-4} g/ml; U = Untreated; NC = Negative Control (DMSO 5 μl/ml)

FIG.2 Sister chromatid exchange (SCEs/cell)(mean) in human lymphocytes treated with Trenbolone, Methyltestosterone and Green tea extract, in presence of S9 mix.



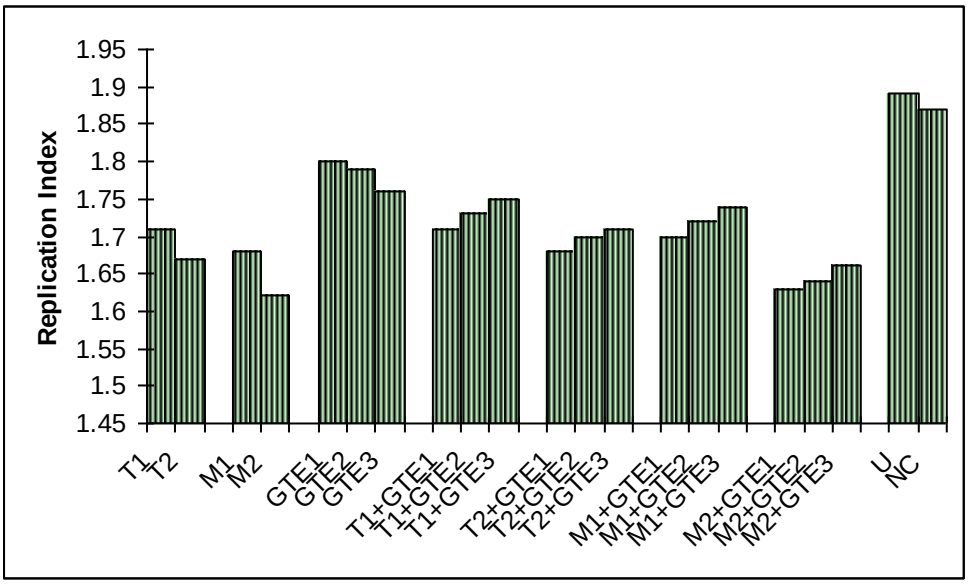
T1 = Trenbolone 40 μM; T2 = Trenbolone 60 μM; M1 = Methyltestosterone 40 μM; M2 = Methyltestosterone 60 μM; GTE1= Green tea extract 1.075X 10^{-4} g/ml; GTE2 = Green tea extract 2.127 X 10^{-4} g/ml; GTE3 = Green tea extract 3.15 X 10^{-4} g/ml; U = Untreated; NC = Negative Control (DMSO 5 μl/ml)

FIG.3 Replication Index (RI) in human lymphocytes treated with Trenbolone, Methyltestosterone and Green tea extract, in absence of S9 mix.



T1 = Trenbolone 40 μM; T2 = Trenbolone 60 μM; M1 = Methyltestosterone 40 μM; M2 = Methyltestosterone 60 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 Replication Index (RI) in human lymphocytes treated with Trenbolone, Methyltestosterone and Green tea extract, in presence of S9 mix.



T1 = Trenbolone 40 μM; T2 = Trenbolone 60 μM; M1 = Methyltestosterone 40 μM; M2 = Methyltestosterone 60 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)