SENRA Academic Publishers, Burnaby, British Columbia
Vol. 3, No. 3, pp. 917-924, 2009
ISSN: 1715-9997

Canadian Journal of pure&applied sciences

EFFECT OF GREEN TEA ON CYTOGENETIC CHANGES INDUCED BY GIBBERELLIN A_3 IN HUMAN LYMPHOCYTE CULTURE

*Saber A Sakr, Sobhy E Hassab-Elnabi and Dalia A El-Ghonaimy
Department of Zoology, Faculty of Science, Menoufia University, Shebin El-kom, Egypt

ABSTRACT

Gibberllin A_3 is a plant growth regulator hormone used to increase the growth and flowering of fruits and vegetables. In the present work, the cytogenetic effect of different concentrations of gibberellin A_3 (0.1, 0.5, 1 and 2 mg) was studied in human lymphocyte culture. Treating cultures with gibberellin A_3 induced chromosomal aberrations, sister chromatid exchanges and DNA damage. The chromosomal aberrations include gap, break, deletion and centromeric attenuation. DNA damage was detected by comet assay and by total genomic damage method using gel electrophoresis. By increasing the concentration of gibberellin A_3, the number of damage cells and the damage DNA spots increase. Moreover, the gel electrophoresis method showed that there was an increase of released DNA and RNA as the concentration of gibberellin A_3 increase. Green tea (*Camellia sinensis*) is one of the most popular bervages consumed. Green tea and its constituents were found to have a variety of therapeutic effects. The results obtained in the present investigation showed that when green tea and gibberellin A_3 were simultaneously applied in the culture media, the mutagenic changes induced by gibberellin A_3 were significantly reduced.

Keywords: Gibberellin A_3, cytogenesis, green tea, human lymphocytes.

INTRODUCTION

Gibberellic acids are a group of plant growth regulators that have been identified in different plants (Jones and MacMillan, 1984) and they are used in agriculture as plant regulators to stimulate both cell division and cell elongation that affect leaves as well as stems (Liu and Loy, 1976). Gibberellic acid or one of its metabolites if applied to dwarf varieties of peas, broad beans or maize, growth is greatly accelerated (Carpita *et al*., 1979). On the other hand, GA_3 was found to have carcinogenic effect in experimental animals. Feeding toads *Bufo regularis* with gibberellin A_3 induced hepatocellular carcinomas in 16% of the animals (El-Mofty and Sakr 1988). Moreover, El-Mofty *et al*. (1994) showed that gibberellin A_3 induced breast and lung adenocarcinomas in mice. Gibberellic acid was found to induce chromosomal aberrations in human lymphocytes (Arutiunian and Zalinian, 1987) and mice (Bakr *et al*., 1999).

Green tea (*Camellia sinensis*) is one of the most popular beverages consumed. A number of studies have shown that traditional beverages, as represented by teas, are beneficial to human health, suggesting that these beverages might warrant investigation from the viewpoint of nutrition. Green tea or its constituents were found to possess a variety of effects, including antimutation (Kada *et al*., 1985), antibiotic action (Toda *et al*., 1989), antihypercholesterolemia (Muramatsu *et al*., 1986), antihypertension (Hara and Tonooka, 1990), antihyperglycemia (Shimizu *et al*., 1988) and anti-inflammatory action (Sagesaka *et al*., 1996). Various systems were used to confirm anticancer activities of green tea. These included experimental animals in which cancer was induced chemically. Green tea was found to inhibit hepatocarcinogenesis induced in rats by diethylnitrosamine (Klaunig, 1992) and suppresses the growth of urinary bladder tumor in rats induced by N-butyl-N (4-hydroxybutyl) nitrosamine (Sato, 1999). Epidemiological studies revealed that the incidences of stomach cancers all over the world are the lowest among a population that consumes green tea on a regular basis (Fujiki *et al*., 1996). Gibberellic acid is used in Egypt to increase the size of some fruits and vegetables such as strawberries, grapes, tomatoes and cabbages. On the other hand, the exposure to such chemicals, increase the risk of many diseases including cancer in farmers and other agricultural uses. This stimulated us to study the side cytogenetic effect of gibberellic acid in human lymphocytes culture and an attempt to reduce its hazardous effects by green tea.

*Corresponding author email: Sabsak@yahoo.com

MATERIALS AND METHODS

Tested compounds

a. Gibberellic acid (GA_3) was dissolved in the tissue culture media and was applied at doses of 0.1, 0.5, 1 and 2mg/ 5ml culture medium.
b. Green tea aqueous extract was prepared according to Han (1997). It was applied at dose of 0.5mg/5ml culture medium.

Media

Human lymphocytes are easy to culture, and readily available. They do not divide and grow unless artificially

stimulated this is usually activated by adding phytohaemagglutinin (PHA) to the cultures and results in a high mitotic yield. These lymphocyte cultures were used according to the method adopted by (Moorhead *et al.*, 1960). The culture medium consists of RPMI 1640 culture medium with L-glutamine (sigma) supplemented with 20% fetal calf serum; 1% peincillin (5000 IU/ml), streptomycin (5000mg/ml); and 0.005g% phytohaemagglutinine (PHA) (Biochrome).

Applied techniques

a. Chromosomal aberrations

One ml heparinized whole blood from healthy non-smoking donor was mixed with 5 ml culture medium and incubated at 37°C. Gibberellic acid and green tea was added at 72 hours of incubation. Colcimed (10mg/ml; Biochrome) was used to inhibit spindle formation at a final concentration of 0.2µg/ml. Two hours before harvesting and fixation, cells were arrested in metaphase by addition of colcimed. After 72 hrs, cells were centrifuged (1000rpm; 10 min) and the supernatants were discarded. The cell pellets were then resuspended in approximately 5 ml of prewarmed hypotonic solution (KCl) and incubated for 15 min. Tubes were then centrifuged at 1000 rpm for 10 min. The supernatant was removed and the pellets were thoroughly mixed with 5ml of cold fixative (1 part acetic acid to 3 parts methanol) added drop by drop; this step was repeated twice. The tubes were spanned at 900 rpm for 10 min. The cells pellets were resuspended in equal small volumes of fresh fixative to form a milky suspension. Slides were prepared by pipetting 4 drops of cells suspension on to clean, grease free slides and air dried. The slides were then stained with 10% Giemsa for 12 min; immersed in distilled water for washing; air dried for 3 days and mounted in DPX. For each treatment, at least 250 well spread metaphases were examined for chromosomal aberrations. For the mitotic index, minimum of 1000 cells per dose were counted.

b. Sister chromatid exchanges (SCEs)

In this experiment SCE's technique was used, which based on the fact that after two rounds of replication in the presence of Bromodeoxyuridine (BrdU), the chromosomes contain one chromatid in which the DNA is unifilarly substituted with BrdU and one chromatid in which the DNA is bifilarly substituted. These two chromatids are stained differently with Giemsa. For this purpose, cultures were used as previously described in the detection of chromosomal aberrations.

Staining technique of SCE's

Slides from BrdU-treated cultures were air-dried and kept in the dark. Three days old slides were stained using the fluorescence plus giemsa technique according to Pery and Wolff (1974). With this technique a light-dark contrast between sister chromatids can be presented giving the chromosomes a "harlequin-like" appearance (unifiliarly BrdU substituted chromatid: dark: bifilarly substituted chromatid: light).

Fluorescence-plus Giemsa Staining

A stock solution of Hoechst 33258 (bisbenzimide) was prepared at a concentration of 0.5mg/ml in sterile H_2O. This can be stored for up to 1 month in the dark at 4°C. The stock Hoechst solution was diluted 1:200 with distilled water immediately prior to use. The slides were immersed in diluted Hoechst for 10 min, washed one time with distilled water (Immersing). This was followed by exposure of the slides to UV rays (254 nm) at a distance of approximately 15 cm for 60 min, covered with 2 × SSC (sodium salt citrate) – solution (pH 7.0). After that the slides remained in a solution of 2 × SSC (pH 7.0) at 60°C for 90 min. The cells were stained with 10% Giemsa in phosphate buffer solution (pH 6.8) for 15 min, left to air dry and mounted in DPX.The SCE frequency was scored of 50 metaphases (2N = 46) in the second cycle of division (MII) per dose.

c. Total damage of DNA (double strand breaks)

Double strand breaks of DNA were detected according to Wlodek *et al*. (1991). Human lymphocytes were isolated according to Boyum (1968) from whole blood by ficoll separating solution (Sigma). The cells were washed in a medium of TGD of DNA. The isolated lymphocytes were incubated with the tested agents for 2 hr. The viability of the cells was determined by trypan blue. From the stock of lymphocytes, 0.5ml (1×10^6 lymphocytes) to 0.2 ml (4×10^5) were transferred to 1.5 ml ependorf tube and completed to 1 ml with medium, then treated with tested substances. Viability of treated cells was measured. Treated cells were centrifuged for 1 min by Ependorf microcentrifuge. The pellets were suspended in 15 micro-liter medium and loaded directly in the well of gel.

Gel preparation

Gels were prepared with 1.5% electrophoretic grade agarose (BRL) and 0.2% polyvinylpyrolidine (PVP; Sigma). The agarose and PVP were boiled with tris borate EDTA buffer (1 × TBE buffer; 89mM Tris, 89mM boric acid, 2mM EDTA, pH 8.8). 0.5 micro-gram/ml ethidium bromide was added to gel at 40°C. then gels were poured and allowed to solidify at room temperature for 1hr. before the samples were loaded. Treated human lymphocytes from 0.5 ml blood were loaded in wells. 15 micro-liter of lysing buffer (50mM NaCl, 1 mM Na_2 EDTA, 1% SDS, pH 8.3) was added on the cells for 15min. 5 µl from 6X loading buffer was added on the lysis cells. Electrophoresis was performed for 2hrs at 50 volt using 1X TBE buffer as running buffer. Gel was photographed using a Polaroid camera while the DNA

was visualized using a 312 nm UV light under a transilluminator.

Damage scoring

The intensity of intact, fragmented (released DNA) and RNA were measured as optical density by gel pro analyzer program.

d. Comet assay (single strand breaks of DNA)

DNA stranded breaks were measured in the alkaline comet assay using the method described by and Singh *et al*. (1988).The isolation and treatment of human lymphocytes with GA_3 and green tea in this technique are similar to the steps of total genomic technique. Viability of the cells was determined by trypan-blue exclusion before using the cells for studies (Boyum, 1968). Scoring was performed according to Hassab El-Nabi and Sallam (2002). Examinations were done with a fluorescent microscope equipped with an excitation filter of 510 nm and barrier filter of 590 nm. The migration was evaluated by observing and measuring the nuclear DNA, where the rounded spot of DNA was considered as a normal DNA spot, while the nuclear DNA, migrating towards the anode, appeared as comet spot and considered as damaged DNA spot. Five hundred spots of DNA were examined and classified into three types: (1) normal spots; round shape, (2) damaged spots; in which the length of the migrated fragments is less than or equal to the diameter of the basal nuclear DNA, and (3) strongly damaged spots; where the length of the comet was greater than the diameter of the basal nuclear DNA.

e. Statistical Analysis

In the present work, the chromosomal aberrations and the number of damaged cells (single cell gel electrophoresis assay) are represented in tables as percentage (%) while, SCE's are represented as mean ± standard deviation. The significance of difference between the treated and control were calculated according to χ^2 test and Student's t-test.

RESULTS

1. Chromosomal aberrations

Different types of chromosomal aberrations were observed in human lymphocyte cultures treated with gibberellic acid. These aberrations included chromatid gap, dicentric, chromatid break, chromatid deletion, fragment, and centromeric attenuation (Figures 1-3). An increase in the percentage of these aberrations was observed with the increase of the dose. The percentage of total chromosomal aberrations showed significant increase at doses 0.5, 1 and 2 mg of GA_3 and the percentages were 5.6, 9.2 and 11.6, respectively (Table 1). Conversely, cultures treated with GA_3 and green tea showed a decrease in the percentage of the chromosomal aberrations when compared with those treated with GA_3 alone. Green tea induced no chromosomal abnormality.

2. Sister chromatide exchange

Data in table 2 showed the frequencies of sister chromatid exchanges in human lymphocyte cultures treated with different doses of GA_3 and green tea. The data showed that there was highly significant ($p<0.01$) increase in SCE's frequencies in cultures treated with different doses of GA_3. Simultaneous application of GA_3 at the used doses and green tea caused reduction of the high SCE values induced by GA_3. This reduction did not reach the SCE's frequencies of control.

3. DNA damage

DNA detection by comet assay showed that strong damage spots of DNA (migration towards the anode) were observed in human lymphocyte cultures treated with different doses of GA_3 (Fig. 4). The tested doses of GA_3 induced a significant increase in the percentage of total damaged spots with values of 24.7, 34.8, 47.4 and 58.6 % when compared with control (5.1%). Simultaneous treatments with green tea and GA_3 decrease the percentage of total damage spots (Table 3).

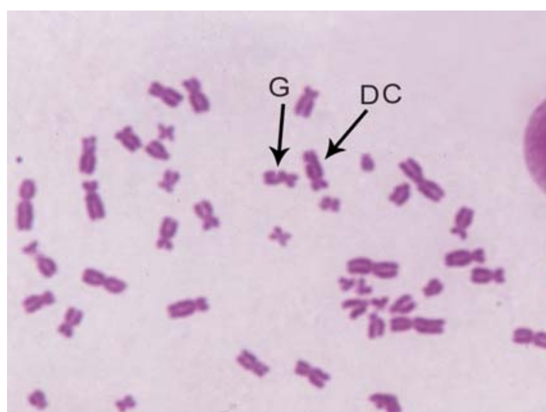

Fig. 1. Chromosomes with gab (G), dicentric chromosome (DC) in human lymphocyte cultures treated with gibberellic acid. (X 1000).

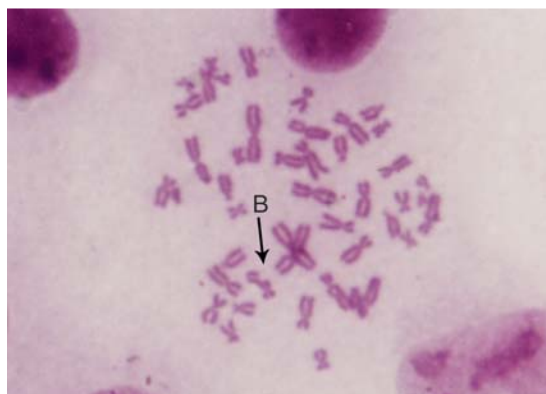

Fig. 2. Chromosomes with break (B) in human lymphocyte cultures treated with gibberellic acid. (X1000).

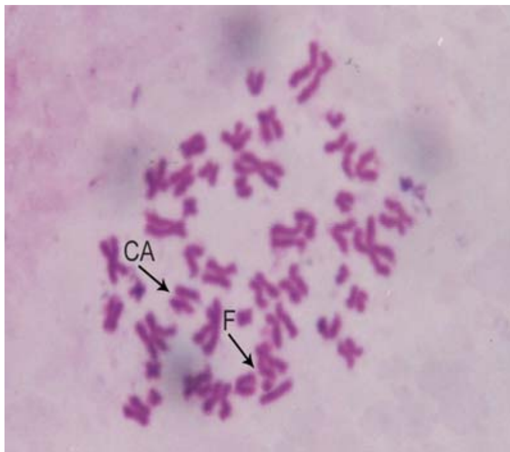

Fig. 3. Chromosomes with centromeric attenuation (CA) and fragment (F) in human lymphocyte cultures treated with gibberellic acid. (X 1000).

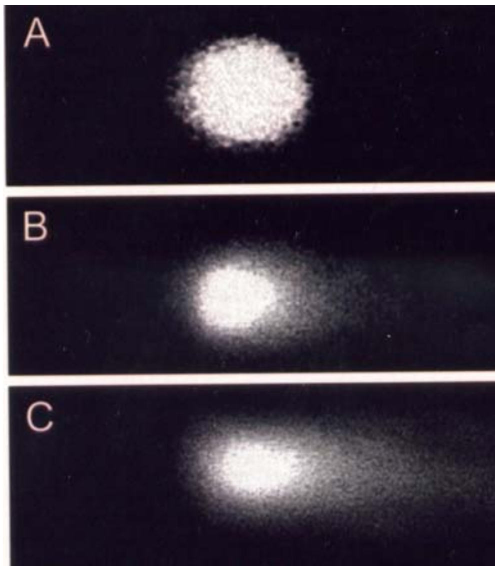

Fig. 4. Photomicrograph showing single strand breaks (comet assay) of DNA of human lymphocyte cultures treated with gibberellic acid. (A) Normal DNA spots (no migration). (B) Damaged DNA spots (migration towards the anode). (C) Strong damaged DNA spots (more migration towards the anode).

Electrophoretic pattern of nucleic acids of lymphocytes appeared as three main bands in gel. RNA area located at up 150 bp, nucleoprotein area located at 300-350 bp and DNA area located near the wells of gel to nucleoprotein area (Fig. 5). The DNA and RNA have orange color with ethidium bromide, while nucleoprotein has a purple color. When the gel was exposed to UV by UV transilluminator for 5 min. the color of nucleoprotein changed from purple to white. After staining the gel with commassi blue the DNA and RNA not stained while, nucleoprotein stained darkly with blue color (Hassab El-Nabi and Elhassaneen, 2008). Figure 5 revealed that the intensity of DNA fragmentation increased in GA_3-treated cultures. GA_3 induced strong and obvious damage at concentrations of 1 and 2mg as represented in lanes 4 and 5. On the other hand, treatment with GA_3 and green tea decreased the amount of fragmented DNA. Similarly, GA_3 increased the intensity of total RNA in a dose dependent manner. The optical density of released DNA is shown in figure 6. There was an increase in the intensity of released (fragmented) DNA in human lymphocyte cultures treated with GA_3. The values of the optical density of released DNA were increased with values of 36.242, 50.028, 65.003, and 126.44 at the dose levels of 0.1, 0.5, 1 and 2 mg GA_3 respectively, when compared with control. Simultaneous treatment with gibberellic acid at the previous doses, and green tea decreased the amount of fragmented DNA and the optical density values were 33.28, 38.494, 45.908, and 125.03. The optical density of total RNA was increased with values of 51.992, 55.243, 57.986, and 74.091 when compared with control value of 45.496. Treatment with GA_3 and green tea decreased the intensity of total RNA with values of 49.196, 54.42, 55.337, and 58.702 when compared with that treated with GA_3 (Fig. 7).

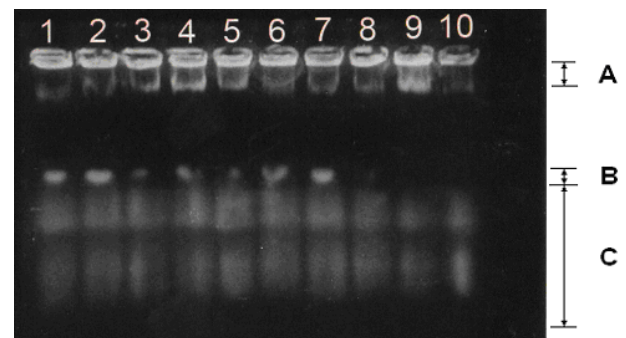

Fig. 5. Gel electrophoresis showing the effect of GA_3 and green tea on total DNA (section A), nucleoprotein (section B), and RNA pattern (section C) in human lymphocyte cultures; lane 1 control; lanes 2, 3, 4, 5 represent human lymphocyte cultures treated with 0.1, 0.5, 1, and 2 mg/ml respectively; lanes 6, 7, 8, 9 represent human lymphocyte cultures treated with the same doses of GA_3 and green tea .Lane 10 : green tea .

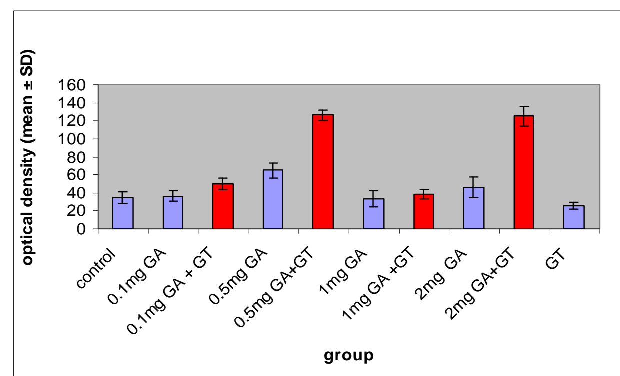

Fig. 6. Optical density of released fragments of DNA.

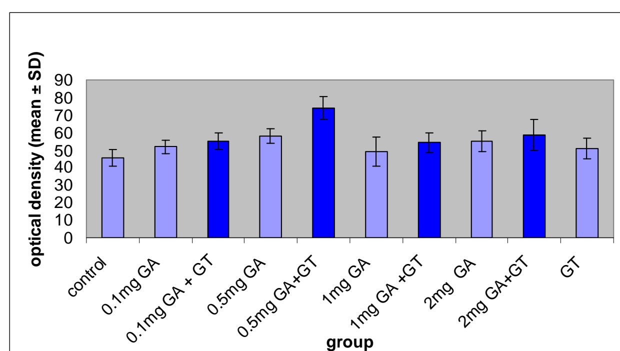

Fig. 7. Optical density of released RNA.

DISCUSSION

The present results showed that gibberellin A_3 induced chromosomal aberrations in human lymphocyte culture.Similar result was obtained by Zalinian *et al.* (1990). Gibberellic acid caused highly significant increase ($p<0.001$) in the percentage of total damage spots at all tested doses when detected with comet assay. Using total genomic damage of DNA method, gel pro analyzer charts have shown that there was an increase in the intensity of released DNA and that of RNA as the dose of gibberellic acid increased. In this concern, Hassab-Elnabi and Sallam (2002) reported that gibberellic acid induced total genomic damage of DNA especially at high doses.

The effect of growth hormones, especially gibberellins on plants was studied by many investigators Fath *et al.* (1999) reported that endogenous barley aleuronic nucleases degraded aleuronic DNA to 180 base pairs. When the aleuronic cells were incubated with gibberellic acid, nucleases enzymes were accumulated in the cells causing DNA fragmentation or damage. So, the DNA damage may results from direct attack of DNA by gibberellic acid causing alkali labile and single strand breaks and total genomic damage and this may be due to accumulation of nucleases. Jacqmard (1968) has proposed that one of the effects of GA_3 is to promote the onset of DNA synthesis in cells which are arrested in the G1 phase of the cell cycle. This notion is supported by the data of Liu and Loy (1976) on the effect of GA_3 on the cell cycle of watermelon seedlings. GA_3 reduces the duration of the cell cycle by nearly 30% and it does so primarily by reducing the length of G1 by 30% and that of S by 36%. It was well established that plant hormones and growth regulators affect the synthesis of RNA and protein (Jacobsen 1977; Bewley and Black, 1978).

Chemical carcinogens - by themselves or after activation - interact with cellular macromolecules such as DNA, RNA, and proteins, and these interactions result in the development of neoplasia (Williams and Weisburger, 1991). It has also been reported that replication of DNA with carcinogen-induced lesions is an essential step in the initiation of carcinogenesis. Gibberellin A_3 was found to have carcinogenic effects in amphibians (El-Mofty and Sakr, 1988) and mammals (El-Mofty *et al*., 1994). Thus, the present results may explain some of the events led to carcinogenicity of GA_3.

The present results indicated that green tea has a curative effect against mutagenicity of gibberellic acid. Green tea was found to reduce chromosomal aberrations induced by gibberellic acid. Similar effects of green tea or its constituents were studied by different investigators. Sasaki *et al*. (1993) reported that green tea decreased the frequency of chromosome aberrations induced by benzo[a]pyrene or mitomycin C in cultured Chinese Hamster Ovary (CHO) cells and mice. They suggested

Table 1. Frequency of chromosomal abnormalities observed in 250 metaphase spreads of human lymphocyte cultures treated with gibberellic acid (0.1, 0.5, 1 & 2mg) and green tea (0.5mg).

Dose	% of chromosomal abnormalities						% of TCA
	Gap	Break	Deletion	Fragment	Dicentric	Centromeric Attenuation	
Control	0.8	0	0	0.4	0	0	1.2
0.1mg GA	0.8	0.4	0.4	0.8	0	0	2.4
0.1mg GA +GT	0.8	0	0.4	0.4	0	0	1.6
0.5mg GA	1.6	0.8	0.8	1.6	0.4	0.4	5.6*
0.5mg GA +GT	1.2	0.4	0.4	1.2	0	0	3.2
1mg GA	2.4	1.2	1.2	2.8	0.8	0.8	9.2**
1mg GA +GT	1.6	0.4	0.8	1.6	0.4	0.4	5.2
2mg GA	2.8	1.6	1.6	3.2	1.2	1.2	11.6**
2mg GA +GT	2.4	0.8	1.2	2.4	0.4	0.4	7.6

GA: gibberellic acid; GT: green tea; TCA: Total chromosomal aberrations
* Statistically significant ($p<0.01$) , ** highly significant ($p<0.001$)

that catechins, well-known antimutagens in tea samples, might account for the observed inhibitory effect green tea extract. Ito *et al*. (1989) recorded the suppression of chromosomal aberrations induced by aflatoxin-B_1 in rat bone marrow cells *in vivo* by green tea.

Table 2. Sister Chromatid exchanges (SCE's) of human lymphocyte cultures treated with gibberellic acid and green tea.

Treatment	SCE's (mean +/- SD)
Control	3.4 +/- 2.12
0.1 mg GA	4.84 +/- 2.49
0.1 mg GA+GT	3.68 +/- 1.93
0.5 mg GA	5.56 +/- 3.02**
0.5 mg GA+GT	3.84 +/- 2.13
1mg GA	6.28 +/- 2.6**
1mg GA+GT	4.2 +/- 1.68
2mg GA	6.56 +/- 2.88**
2mg GA+GT	4.72 +/- 1.49

** Statistically highly significant ($p<0.01$)

Simultaneous treatment with gibberellic acid and green tea revealed a reduction in the frequency of sister chromatid exchange. Cheng (1989) reported that green tea decreased the frequency of sister chromatid exchanges induced by oxygen radical in IAR 20 liver cells treated with hypoxanthine and xanthine oxidase.Wang *et al*. (1989) reported that water extracts of green tea and their major constituents, green tea polyphenols , have antimutagenic activity. They found that green tea polyphenols inhibited gene forward mutation in V79 cells treated with benzo[alpha]pyrene (BP)and aflatoxin-B_1 and also decreased the frequency of sister-chromatid exchanges and chromosomal aberrations in V79 cells treated with AFB_1. Fujie *et al*. (1993) reported that crude catechin extracted from green tea suppressed sister-chromatid exchanges induced by trihalomethanes in rat erythroblastic cells *In vitro*. Tanaka (2000) observed that epigallocatechin gallate, the major constituent of green tea and catechin a minor constituent, equivalently decreased the frequencies of sister-chromatid exchanges induced by paraquat which is a generator of reactive oxygen species. Green tea strongly inhibited the increase in sister chromatid exchanges and micronuclei induced by Fried fish in V79 and IAR20 cells (Liu, 1990).

The present work revealed that human lymphocyte cultures treated with gibberellic acid showed strong damage cells using comet assay. After treating with green tea the percentage of the total damage spots decreased. Similarly, Roy *et al*. (2003) reported that the green tea extract, polyphenol epigallocatechin gallate had a curative effect on the cytogenetic change and DNA damage induced by toxicants $H_{(2)}O_{(2)}$ and carcinogen N-methyl-N'-nitro-N-nitrosoguanidine in Chinese hamster V-79 cells in culture. Zhang *et al*. (2002) recorded modification of lung cancer susceptibility by green tea extract as measured by the comet assay. Lin *et al.* (2003) studied the induction of apoptosis in HL-60 cells by tea extracts: green tea, oolong tea, black tea, and pu-erh using DNA fragmentation ladder and flow cytometry. The results showed that the ability of tea extracts to induce apoptosis was in the order green tea > oolong > black tea > pu-erh tea. Chen *et al*. (2004) reported that epigallocatechin gallate, a major constituent of green tea polyphenols, induced a GO/G1 arrest and apoptosis in NBT-II bladder tumor cells.

Parshad *et al*. (1998) found that addition of green or black tea extracts, their polyphenols or curcumin to cultures of human skin fibroblasts or PHA-stimulated blood lymphocytes significantly reduced the frequencies of radiation-induced chromatid breaks. The protective action of these plant polyphenols seems to result from their

Table 3. The percentage of DNA damage detected by comet assay in human lymphocyte cultures treated with gibberellic acid and green tea.

Treatment	% of Normal Spots	% of Damage Spots	% of Strong damage spots	% of total damage spots
Control	94.9	4	1.1	5.1
GT	93.8	5	1.2	6.2
0.1 mg GA.	75.3	14.3	10.4	24.7**
0.1 mg GA.+GT	90.8	5	4.2	9.2
0..5 mg GA.	65.2	18.4	16.4	34.8**
0..5 mg GA. +GT	86.3	8.2	5.5	13.7
1 mg GA.	52.6	19.1	28.3	47.4**
1 mg GA. +GT	81.4	10.3	8.3	18.6*
2 mg GA.	41.4	21.2	37.4	58.6**
2 mg GA. +GT	77.8	15	7.2	22.2**

* Statistically significant ($p<0.01$); ** Statistically highly significant ($p<0.001$)

known antioxidant properties, particularly the scavenging of hydroxyl free radicals. Feng *et al*. (2002) reported that black tea polyphenols, theaflavins, prevent cellular DNA damage using the comet assay by inhibiting oxidative stress and suppressing cytochrome P450 1A1 in cell cultures.

The present results proved that green tea had a curative effect against cytogenicity of GA_3. A growing body of research has demonstrated green tea polyphenols to be powerful components with anticarcinogenic and antimutagenic properties. The mechanism of these green tea components is attributed to: 1- Antioxidant and free radical scavenging activity, 2- Stimulation of detoxification systems, especially selective induction or modification of phase I and phase II metabolic enzymes with increasing the formation and excretion of detoxified metabolites of carcinogens, 3- Inhibition of biochemical markers of tumor initiation and promotion, including lowering the rate of cell replication and thus the growth and development of neoplasm, and 4- Prevention of mutagenicity and genotoxcity (Weisburger,1999). Thus in the present work, it is speculated that one or more constituents of green tea may be responsible for prevention of mutagenic effects of gibberellic acid through one or more of these mechanisms.

REFERENCES

Arutiunian, RM. and Zalinian, GG. 1987. Cytogenetic effect of natural mutagenesis modifiers in a human lymphocyte culture. The action of caffeine during the induction of chromosome aberrations by gibberellic acid. Tsitol. Genet. 21(2):101-105.

Bakr, SM., Moussa, EM. and Khater, E. Sh. 1999. Cytogenetic evaluation of gibberellin A_3 in Swiss albino mice. J Union Arab Biol. 11(A):345-351.

Bewley, JD. and Black, M. 1978. Physiology and Biochemistry of Seeds in Relation to Germination, Vol. 1. Development, Germination, and Growth. Springer-Verlag, Berlin. 1:306.

Boyum, A. 1968. Separation of leukocytes from blood and Bone marrow. Scand. J. Clin. Lab. Invest. 21 (Suppl. 97):77-89.

Carpita, NC., Nabors, MW., Ross, CW. and Petrie, NL. 1979. The growth physics and water relations of red-light induced germination in lettuce seeds. III. Changes in the osmotic and pressure potential in the embryonic axes of red- and far-red-treated seeds. Planta. 144:217-224.

Chen, JH., Tipoe, GL., Long, EC., SH So, H., Ka-Man Leung., Wai-Ming Tom, Fung, CW. and Nanji, AA. 2004. Green tea polyphenols prevent toxin- induced hepatotoxicity in mice by down-regulating inducible nitric oxide-derived prooxidants. American J Clin Nutrition. 80(3):742-751.

Cheng, SJ. 1989. Inhibitory effect of green tea extract on promotion and related action of TPA. Zhongguo Yi Xue Ke Xue Yuan Xue Bao. 11(4):259-264.

El-Mofty, MM. and Sakr, SA. 1988. Induction of neoplasm in the Egyptian toad *Bufo regularis* By gibberellin-A_3.Oncology. 45:61-64.

El-Mofty, MM., Sakr, SA., Rizk, AM. and Moussa, EA. 1994. Carcinogenic effect of gibberellin A_3 in Swiss albino mice. Nutr Cancer. 21:183-190.

Fath, A., Bethke, PC. and Jones, RL. 1999. Barley aleurone cell death is not apoptotic: characterization of nuclease activities and DNA degradation. Plant J. 20(3): 305-315.

Feng, Q., Torii, Y., Uchida, K. Nakamura, Y. and Osawa, T. 2002. Black tea polyphenols, theaflavins, prevent cellular DNA damage by inhibiting oxidative stress and suppressing cytochrome P450 1A1 in cell cultures. J. Agric. Food. Chem. 50(1): 213-220.

Fujie, K., Aoki, T., Ito, Y. and Maeda, S. 1993.Sister-chromatid exchanges induced by trihalomethanes in rat erythroblastic cells and their suppression by crude catechin extracted from green tea. Mutat. Res. 300(3-4):241-246.

Fujiki, H., Suganuma, M., Okabe, S., Komori, A., Sueoka, E., Sueoka, N., Kozu, T. and Sasaki, Y. 1996. Japanese green tea as a cancer preventive in humans. Nutr. Rev. 54:S67-S70.

Han, C. 1997. Screening of anticarcinogenic ingredients in tea polyphenols. Cancer letters. 114:153-158.

Hara, Y. and Tonooka, F.1990. Hypotensive effect of tea catechin on blood pressure of rats. Nippon Eiyo Shokuryo Gakkaishi. 43:345-348.

Hassab El-Nabi, SE. and Sallam, FA. 2002. The protective effect of ellagic acid against the mutagenic potential of berelex in human lymphocyte cultures. J. Egypt. Ger. Soc. Zool. 37(C):77-98.

Hassab El-Nabi, SE. and Elhassaneen, YA. 2008. Detection of DNA damage, molecular apoptosis and production of home-made ladder by using simple techniques. Biotechnology. 7(3):514-522.

Ito, Y. Ohnishi, S and Fujie, K. 1989.Chromosome aberrations induced by aflatoxin B1 in rat bone marrow cells *in vivo* and their suppression by green tea. Mutat. Res. 222(3):253-261.

Jacobsen, JV. 1977. Regulation of ribonucleic acid metabolism by plant hormones. Ann. Rev. Plant Physiol. 28:537-564.

Jacqmard, A. 1968. Early effects of gibberellic acid on mitotic activity and DNA synthesis in the apical bud of *Rudbeckia bicolor*. Physiol. Veg. 6: 409-416.

Jones, RL. and MacMillan, M. 1984. Gibberellins. In: Advanced Plant Physiology. Ed. Wilkins, MB. Pitman, London. pp.21-52.

Kada, T., Kaneo, K., Matsuzaki, S., Matsuzaki, T. and Hara, Y. 1985. Detection and chemical identification of natural bioantimutagens. Mutat. Res. 150:127-132

Klaunig, JE. 1992. Chemopreventive effects of green tea components on hepatic carcinogenesis Prev. Med. 21(4):510-519.

Lin, YS., Tsai, YJ., Tsay, JS. and Lin, JK. 2003. Factors affecting the levels of tea polyphenols and caffeine in tea leaves. J. Agric. Food Chem. 51(7):1864-1873.

Liu, PB. and Loy, JB. 1976. Action of gibberellic acid on cell proliferation in the subapical shoot meristem of watermelon seedlings. Am. J. Botany. 63:700-704.

Liu, XL. 1990. Genotoxicity of fried fish extract, MelQ and inhibition by green tea antioxidant. Zhonghua Zhong Liu Za Zhi. 12(3):170-173.

Moorhead, PS., Nowell, PC., Mellraan, WJ., Battips, DM. and Hungerford, DA. 1960. Chromosome preparations of leukocytes cultured from human peripheral blood. Exp. Cell Res. 20:613-616.

Muramatsu, K. Fukuyo, M. and Hara, Y. 1986. Effect of green tea catechins on plasma cholesterol level in cholesterol-fed rats. J. Nutr. Sci. Vitaminol. 32:613-622.

Parshad, R., Sanford, KK., Price, FM., Steele, VE., Tarone, RE., Kelloff, GJ. and Boone, CW. 1998. Protective action of plant polyphenols on radiation-induced chromatid breaks in cultured human cells., J.Anticancer Res. 18(5A):3263-3266.

Perry, P. and Wolff, S. 1974. New Giemsa method for the differential staining of sister chromatids. Nature (London). 251:156-158.

Roy, M., Chakrabarty, S., Sinha, D., Bhattacharya, RK. and Siddiqi, M. 2003. Anticlastogenic, antigenotoxic and apoptotic activity of epigallocatechin gallate: a green tea polyphenol. Mutat. Res. (523-524):33-41.

Sagesaka, YM., Uemura, T., Suzuki, Y., Sugiura, T., Yoshida, M., Yamaguchi, K. and Kyuki, K. 1996. Antimicrobial and anti-inflammatory actions of tea-leaf saponin. Yakugaku Zasshi. 116:238-243.

Sasaki, YF., Yamada, H., Shimoi, K., Kator, K. and Kinae, N. 1993. The clastogen-suppressing effects of green tea, Po-lei tea and Rooibos tea in CHO cells and mice. Mutat. Res. 286(2):221-232.

Sato, D. 1999. Inhibition of urinary bladder tumours induced by N-butyl-N-(4-hydroxybutyl)-nitrosamine in rats by green tea. Int. J. Urol. 6:93-99.

Shimizu, M., Wada, S., Hayashi, T., Arisawa, M., Ikegaya, K., Ogaku, S., Yano, S. and Morita, N. 1988. Studies on hypoglycemic constituents of Japanese tea. Yakugaku Zasshi. 108:964-970

Singh, NP., McCoy, MT., Tice, RR. and Schneider, EL. 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell Res. 175: 184-191.

Tanaka, R. 2000. Protective effects of (-)-epigallocatechin gallate and (+)-catechin on paraquat-induced genotoxicity in cultured cells. J. Toxicol. Sci. 25(3):199-204.

Toda, M., Okubo, S., Ohnishi, R. and Shimamura, T. 1989. Antibacterial and bactericidal activities of Japanese green tea. Jpn. J. Bacteriol. 44:669-672.

Wang, ZY., Cheng, SJ., Zhou, ZC., Athar, M., Khan, WA., Bickers, DR. and Mukhtar, H. 1989. Antimutagenic activity of green tea polyphenols. Mutat. Res. 223(3):273-285.

Weisburger, JH.1999. Tea and health: the underlying mechanisms. Proc Soc Exp Biol Med. 220:271-275.

Williams, GM. and Weisburger, JH. 1991. Chemical Carcinogenesis. In: Casarett and Doull's Toxicology: the basic science of poisons. Eds. Klaassen, CD., Amdur, MO. and Doull, J. (4th ed.). Pergamon Press, New York, pp. 127-200.

Wlodek, D., Banath, J. and Olive, PL. 1991. Comparison between pulsed-field and constant-field gel electrophoresis for measurement of DNA double-strand breaks in irradiated Chinese hamster ovary cells. Int. J. Radiat. Biol. 60(5):779-790.

Zalinian, GG. , Arutiumian, RM. and Sarkisian, GG. 1990. The cytogenetic effect of natural mutagenesis modifiers in a human lymphocyte culture. The action of amino benzamide during the gibberellic acid induction of chromosome aberrations. Tsitol Genet. 24(3):31-34.

Zhang, H., Spitz, MR., Tomlinson, GE., Schabath, MB., Minna, JD. and Wu, X. 2002. Modification of lung cancer susceptibility by green tea extract as measured by the comet assay. Cancer. Detect. Prev. 26(6):411-418.

Received: May 14, 2009; Revised: August 2, 2009;
Accepted: August 5, 2009